Metric 3.4.5

Updated list with additional information (shared for the first time) in highlighted aqua blue w.r.t. Books and chapters in edited volumes published

3.4.5 **Number of books and chapters in edited volumes published per teacher during the last five years**

S. No.	Name of the teacher	Title of the book published	Title of the chapters published	Year of publication	Number	Whether at the time of publication Affiliating Institution was same Yes/No	Name of the publisher
1	Dr. Anuradha Goel	Fundamentals of Plant Physiology	Not Applicable	2024	978-93-5540-680-4	Yes	Kalyani Publishers, H.O. B-1 1292 Rajinder Nagar Civil Line Ludhiana 141008
2	Dr. Moly Saxena	Seed technological association for self-sufficiency in oilseeds and pulses	Seed health management in major oilseed crops: Challenges and wayforward	2024	9788119105243	Yes	Satish Serial Publishing House
3	Dr. D. R. Saxena	Seed technological association for self-sufficiency in oilseeds and pulses	Current challenges and wayford for seed health management of pulse crops	2024	9788119105243	Yes	Satish Serial Publishing House
4	Rao, S. K., Dr. M. Yasin and Mahvash khaleel	Seed technological association for self-sufficiency in oilseeds and pulses	Critical seed planning, intensive monitoring and awareness campaign: A Need	2024	9788119105243	Yes	Satish Serial Publishing House
5	S. K. Rao, S. K. Sharma, R. R. Hanchinal, Dr. M. Yasin	Seed technological association for self-sufficiency in oilseeds and pulses	Not Applicable	2024	9788119105243	Yes	Satish Serial Publishing House
6	Rajesh Aarwe, Deepali Vishwakarma, Deependra Singh Rajput, Shrikant Patidar, Sanju Singh and Neelesh Raipuria	Not Applicable	MAJOR INSECT PESTS OF MUSTARD AND THEIR MANAGEMENT	2024	978-81-967311-9-9 (Book chapter)	Yes	Empyreal Publishing House, New Delhi, India
7	Dr SK Dwivedi	National Education Policy-2020 for Incredible India	Not Applicable	2024	978-81-969823-2-4	Yes	Genic Books Publishers Pvt. Ltd. AGRA
8	A. Sharma, Jyoti Rani, Pavneet Kaur, S K Dwivedi and Munisha Sharma	Handbook of Nutraceuticals	Potential Food Nutraceutical Ingredients	2024	978-3-030-69677-1	Yes	Springer Nature Switzerland

9	S K Dwivedi, Kanika Issar and Vivek Tiwari	Handbook of Nutraceuticals	Nutrient requirements in Health and Disease	2024	978-3-030-69677-1	Yes	Springer Nature Switzerland
10	Devraj, Shorya Raj, S K Dwivedi and Avnish K Pandey	Innovations in Food Processing Industry	Food Concentration	2024	9789358875669	Yes	NIPA, New Delhi
11	Dr Sneha Pandey	Innovative Strategies in Agribussiness Management.	Farm Management Principles	2023	Pp-88-108. No.978-93-58990-09-6.	Yes	Elite Publishing House.
12	Dr. Sneha Pandey	Modern Approaches in Agricultural Economics	Farm Management and Efficiency Analysis.	2023	Pp- 102-134. No. 978-93-5899-058-4	Yes	Elite Publishing House.
13	Dr. Sneha Pandey	Modern Approaches in Agricultural Economics.	Agricultural Marketing and Consumer Behaviour.	2023	Pp-272-285. No. 978-93-5899-058-4.	Yes	Elite Publishing House.
14	Singh Nisha, Sharma Janmejay, Singh Ajay, Abhishek	Fundamentals of Agronomy	Not Applicable	2023	978-93-92953-90-3.	Yes	Vital Biotech Publication, Kota Rajashthan
15	Sharma Amita, Sharma Janmejay, Shrivastav S.C. and Mohaniya Lakhan Singh	Introduction of Agro-forestry.	Not Applicable	2023	978-93-5540-998-0.	Yes	Kalyani Publication
16	Singh Shailendra, Singh Ajay, Sharma Janmejay and Chicham Sudarshan	New horizon in climate smart agriculture.	Not Applicable	2023	978-93-92953-99-6.	Yes	Vital Biotech Publication, Kota Rajashthan ISBN No. (Under publication)
17	Mohaniya Lakhan Singh, Sharma Janmejay, Sharma Amita, Sasode Rajni Singh and Bagri Uma Shankar	Fundamental of Agronomy (In Hindi)	Not Applicable	2023	. 978-93-95182-10-2	Yes	S.R. Scientific Publicaiton, Agra
18	Mohaniya Lakhan Singh, Sharma Janmejay and Sharma Amita	Advanced Research and Review in Agronomy	Weed Science and Weed Management System.	2023	9789392804700	Yes	Bright Sky Publication, New Delhi Vol - 4
19	Mohaniya Lakhan Singh, Sharma Amita and Sharma Janmejay	Advanced Research and Review in Agronomy.	Farming and cropping system	2023	9789392804700	Yes	Bright Sky Publication, New Delhi Vol - 4
20	Mohaniya Lakhan Singh, Sharma Janmejay and Sharma Amita	Advances in Agronomy.	Water Management / irrigation in agronomy.	2023	9789355708168	Yes	Akinik Publi-cation, New Delhi Vol - 26

21	Balkrishan Nisha Singh Singh Janmejay Sharma; Ajay Bhadauria Jaideep Bhadauria and Sudhir Bhadauria	Advance innovation in sustainable agriculture	Constraints and Prospect in Rainfed Region.	2023	978-93-92953-23-1 Page No. 229-238	Yes	Vital Biotech Publication
22	Balkrishan Singh, Nisha Singh,Ritu Sharma and Shweta Sharma	Emerging trend in agriculture sciences	Production technology of cabbage(Brassica oleraceae var. capitata)	2023	978-93-92953-74-3 Page No. 177-184	Yes	Vital Biotech Publication
23	Priyadarshani A. Khambalkar, Shashi S. Yadav, Akhilesh Singh, S. K. Trivedi	A Glossary of Soil Science	Not Applicable	2023	978-93-91383-29-9	Yes	Nipa Genx Electronic Resources & Solutions P. Ltd.
24	Khambalkar PA, Yadav, Shashi S., Singh, Akhilesh, Trivedi, S. K. and Gurjar Narendra.S.	A Practical Manual on Soil Testing.	Not Applicable	2023	978-93-94023-04-8	Yes	International books and periodical supply service. 38, Nishant Kunj, Pitampura Road, Delhi 110034
25	Priyadarshani A. Khambalkar, Murlidhar Sadawarti, Ekta Joshi and Shashi S. Yadav	Climate Smart Approaches towards Sustainable Crop Production	Climate Smart Agriculture and Carbon Sequestration	2023		Yes	Edited by An International Publishers VITAL BIOTECH get Accredited by following International organization Publisher: VITAL BIOTECH PUBLICATION
26	Dr. Neelam Bunkar	Futuristic Trends in Agriculture and Allied Sciences	Status of DTPA-Extractable Micronutrient Cations in Major Rapeseed-Mustard Growing Area of Madhya Pradesh.	2023	978- 93- 100- 0218- 8	Yes	Rajut B. K., Kirar N.S, Yadav R. and Bunkar N.
27	Tripathi MK, Tiwari S., Tripathi N., Malviya R. K., Bhatt D., Tiwari P. N. and Tiwari S	Advances in Agricultural Biotechnology	In vitro morphogenesis in gladiolus (Gladiolus hybridus Hort.) from corm slice	2023	978-93-5570-680-5	Yes	AkiNik Publication
28	Tripathi MK, Tiwari G, Patidar SL, Tiwari S, Tripathi N and Tiwari S	In book:Advances in Agricultural Biotechnology	Optimization of an effectual and reproducible plantlet regeneration protocol from embryogenic cell suspension culture in Plumbago zeylanica Linn	2023	978-93-5570-680-5	Yes	AkiNik Publication

29	Tripathi M.K., Tripathi N, Tiwari S, Payasi D K., Bhatt D, Tomar Y S and Tiwari S	In Book: Recent Advances in Molecular Biology Plant Physiology	Effect of plant growth regulators on in vitro morphogenesis and regeneration of Amaryllis belladonna L	2023	978-93-5570-680-5	Yes	AkiNik Publication
30	Tripathi MK, Tripathi N, Tiwari S, Malviya RK, Tiwari PN and Tiwari S	In book: Recent Advances in Molecular Biology Plant Physiology	Plant regeneration from cultured cormel explants in gladiolus (Gladiolus hybridus Hort.)	2023	978-93-5570-680-5	Yes	AkiNik Publication
31	M.K. Tripathi, G. Tiwari, Sushma Tiwari, Niraj Tripathi, Mohini Sharma, Nishi Mishra and Sharad Tiwari	Research Trends in Multidisciplinary Research	Effect of Diverse Plant Growth Regulator Concentrations and Amalgamations in Plantlet Regeneration in Rauvolfia serpentina (L.) Benth from Cell Suspension Culture	2023	978-93-5570-680-5	Yes	AkiNik Publication
32	Adlak T., Tiwari S., Rathore M. S., Tripathi N., Tiwari P.N. and Tripathi M. K	In Book: Cutting Edge Research in Biology	Biotechnological approaches for genetic improvement of crops	2023	978-81-19217-40-3	Yes	B P International
33	Lalita Nargawe and Intjar Singh Dawar	Olericulture in Modern Era,	Basics of Learning and Teaching	2023	978-81-7 622-532-8	Yes	Biotech Books
34	Intjar Singh Dawar, Lalita Nargawe, Nilesh Sharma and Damodar Jatav	Smart Horticultural Technologies,	Layering in fruit crops	2023	978-81-7622-533-5	Yes	Biotech Books
35	Govinda Bihare, Ankit Malviya, Anushka Khare and Lalita Nargawe	A Text Book of E-Extension	Not Applicable	2023	978-81-19778-27-0	Yes	ELPHIONE
36	Lalita Nargawe, Akanksha Sharma, Ramesh Chand Bunkar, Ms. Laksheeta Chauhan and Kabita Mondal	Fundamtntal of Extension Education	Not Applicable	2023	978-81-19906-63-5	Yes	Golden Leaf Publishers (Lucknow)
37	Dr. Rajesh Aarwe, Dr. Shrikant Patidar and Dr. Neelesh Raypuriya	Population Dynamics and Chemical Control of Major Insect Pests of Okra (Abelmoschus esculentus L. Moench).	Not Applicable	2023	978-620-6-79253-6 (Book)	Yes	LAMBERT Academic Publishing, Dodo Books Indian Ocean Ltd. and Omni Scriptum S.R.L (United Kingdom)

38	Rajesh Aarwe Deependra Singh Rajput, ShrikantPatidar	A text book on Insect Morphology	Not Applicable	2023	978-93-95182-35-5 (Book)	Yes	SR Scientific Publication 8, Gandhi Nagar, Near Paliwal Park, Agra, Uttar Pradesh
39	Biswajit Pramanick, Rajiv Dubey, Amit Kesarwani, Anurag Bera, K.L. Bhutia, Mukesh Kumar and Sagar Maitra	Not Applicable	Advancemenyt in mitigating the effects of waterlogging stress in wheat- Abiotic Stresses in Wheat-Unfolding the Challenges	2023	978-0-323-95368—9	Yes	ELSEVIER Academic Press-
40	Kanwar, Jyoti, Kanpure, R.N. and Ausari, Praveen Kumar	Karonda Enthobotanical	Not Applicable	2023	978-93-95319-60-7	Yes	
41	Bindesh Arya, K.C. Meena, D.K. Patidar, Nitin Soni and B.K. Patidar	Nutrient Management in Organic Cultivation of Coriander (Coriandrum sativum L.). Possibilities of Organic Farming in India	Not Applicable	2023	978-93-5461-678-5	Yes	Daya Publishing House
42	Akanksha Sikarwar, Debesh Singh, Pankaj Maida ,Dr. J. Jyothsna and Nazmeen Khanam	Precision Farming and Protected Cultivation in Digital Era	Not Applicable	2023	9788119821686 (Book)	Yes	Royal Book Publishing 21/11, K M Nagar, Ayodhiyapatinam, Salem. Tamil Nadu-636103
43	Girish Patidar, Pankaj Maida, Teena Patidar and Suresh Barde	Mode of Insect-Plant Interaction	Not Applicable	2023	978-93-95118-80-4.(Book chapter)	Yes	Integrated Publications New Delhi
44	Bindesh Arya, K.C. Meena, D.K. Patidar, Nitin Soni and B.K. Patidar	Nutrient Management in Organic Cultivation of Coriander (Coriandrum sativum L.). Possibilities of Organic Farming in India	Not Applicable	2023	978-93-5461-678-5	Yes	Daya Publishing House
45	Dr. Nitin Soni	Physiological Disorders of Commercial fruit crops	Not Applicable	2023	978-93-58991-60-4	Yes	Elite publishing house, New Delhi
46	Kanwar, Jyoti, Kanpure, R.N. and Ausari, Praveen Kumar	Horticulture for Nutrition and Income Security	Karonda Enthobotanical	2023	978-93-95319-60-7	Yes	NIPA Genx Electronic Resources & Solutions P.LTD New Delhi-110034

47	Pragya Singh and Nikhil Parihar	Recent Trends in Vegetable Scinece-unleashing the future directions	Biotic stress management in vegetable crop production (Book Chepter)	2023	978-93-58992-62-5	Yes	Elite publishing house, New Delhi
48	Dhan Singh Mandloi, Manoj Kumar Kureel, Karan Vir Singh, Santosh Kumar Maida	Production technology of Ornamental Plants	Not Applicable	2023	978-81-964111-3-8	Yes	Elphinstone Publication & Distributors, New Delhi-110062
49	Dhan Singh Mandloi, Manoj Kumar Kureel, Karan Vir Singh, Santosh Kumar Maida	नर्सरी प्रबंधनः एक परिचय एवं इसकी उपयोगिता	Not Applicable	2023		Yes	Elphinstone Publication & Distributors, New Delhi-110062
50	Dr.O.P.Daipuria Dr. Shobhana Gupta, Dr. Yagya Dev Mishra	Training Mannual ICAR-Winter School on Entrepreneurial Development in Agriculture for Sustainable Growth and Reliance	Not Applicable	2022	ISO Certified 90012008	YES	Rajmata Vijayraje Scindia Krishi Vishwa Vidyalaya, College of Agriculture, Gwalior (M.P.)
51	Nisha Singh and Balkrishan Singh	Trend in agriculture science	Integrated farming system (IFS) for sustainable livelihood security.	2022	978-93-92953-04-0 Page No. 237-251	Yes	Vital Biotech Publication
52	R.S. Sikarwar	Natural Resource Management in terms of crop germplasm in India:	Natural Resource Management in terms of crop germplasm in India:	2022	Page no. 102-107.	Yes	S.R.Scientific publication, Agra (U.P.)
53	Dr. Shashi S. Yadav	Climate Change: Perspectives and Impact on Indian Agriculture	Not Applicable	2022		Yes	*Published byMahima Research Foundation and Social Welfare194, Karaundi, Banaras Hindu Univarsity, Varanasi-221 005, UP*
54	M.K. Tripathi, G. Tiwari, Sushma Tiwari, Niraj Tripathi, Devendra K. Payasi and Sharad Tiwari	In book: Advances in Agricultural Biotechnology	In vitro Regeneration of Sandalwood (Santalum album Linn.) Employing Different Explants	2022	978-93-5570-680-5	Yes	AkiNik Publication
55	Tripathi M. K., Tiwari G., Tiwari S, Tripathi N, Sharma M, Bhargav S, Tomar YS, Patidar S. L. and Tiwari S	In book: Research Aspects in Biological Science	Influence of Plant Growth Regulators on In vitro Morphogenesis in Plumbago Zeylanica Linn	2022	978-81-19217-40-3	Yes	BP International

56	Tripathi M. K., Tiwari G., Tiwari S, Tripathi N, Mishra N, Tomar YS, Uikey D S and Tiwari S	In book: Research Aspects in Biological Science	Plant Regeneration in Rauvolfia serpentina (L.) Benth via Organogenic Mode	2022	978-81-19217-40-3	Yes	BP International
57	RS Tomar, Prabha Singh, Sushma Tiwari, Manoj Kumar Tripathi, Sanjay Singh, K Bhojaraja Naik, Chandan Kumar Singh, Shailesh Kumar Singh	Agro-biodiversity and Agri-ecosystem Management	Genomics Approaches for Restoration and Conservation of Agro-Biodiversity. Agro-biodiversity and Agri-ecosystem Management	2022		Yes	
58	Vibhute M., Tripathi M. K., Tiwari R., Tiwari S., Tripathi N., Sharma M., Tomar Y S and Tiwari S.	In book: Research Aspects in Biological Science	Massive in vitro propagation from cultured nodal segment of three Citrus species	2022	978-81-19217-40-3	Yes	BP International
59	Bhawar PC, Tiwari S, Tripathi M. K., Sikarwar R. S., Tomar R. S. and Tripathi N,	In book: Research Developments in Science and Technology	Assessment of Groundnut Germplasm Lines for Foliar Fungal Diseases and Population Structure Analysis using Gene Based SSR Markers	2022	978-93-5570-680-5	Yes	AkiNik Publication
60	Deepa Bhatt, M. K. Tripathi, M. Vidhya Sankar, Sushma Tiwari, Mohini Sharma, Niraj Tripathi and Sharad Tiwari	In book: Current Topics in Agricultural Sciences	Standardization of in vitro regeneration protocol in Gerbera jamesonii Bolus Ex Hooker F.	2022	978-81-19217-40-3	Yes	BP International
61	M. K. Tripathi, D. Bele, Sushma Tiwari, Nishi Mishra, Niraj Tripathi, G. Tiwari and Sharad Tiwari	In book: Research Developments in Science and Technology	Plantlet regeneration from cultured nodal segments in sandalwood (Santalum album Linn.)	2022	978-81-19217-40-3	Yes	BP International
62	Sonali Singh, M. K. Tripathi, Sushma Tiwari, Niraj Tripathi, G. Tejovathi, Ashok Ahuja and Sharad Tiwari	In book: Research Developments in Science and Technology	Production of synthetic seed from encapsulated nodal segments of giloe (Tinospora cordifolia Willd.)	2022	978-81-19217-40-3	Yes	BP International

63	Chitralekha Shyam, M. K. Tripathi, Sushma Tiwari, Niraj Tripathi and R. S. Sikarwar	In book: Research Developments in Science and Technology	Morpho-physiological variations and genetic components analysis in Brassica juncea (Linn.) Czern & Coss	2022	978-81-19217-40-3	Yes	BP International
64	Shikha Upadhyay, A. K. Singh, M. K. Tripathi, Sushma Tiwari cand Niraj Tripathi	In book: Current Topics in Agricultural Sciences	Biotechnological iinterventions to combat against ccharcoal rot and Rhizoctonia root rot ddiseases of ssoybean [Glycine max (L.) Merrill].	2022	978-81-19217-40-3	Yes	BP International
65	Chitralekha Shyam, M. K. Tripathi, Sushma Tiwari, Niraj Tripathi and R. S. Sikarwar	In book: Research Developments in Science and Technology	Analysis of genetic differences in fatty acids and oil contents among Brassica juncea (Linn.) Czern & Coss genotypes.	2022	978-81-19217-40-3	Yes	BP International
66	Mishra N, Tripathi MK, Tiwari S, Tripathi N, Gupta N, Sharma A, Solanki RS and Tiwari S.	In book: Innovations in Science and Technology	Characterization of soybean genotypes on the basis of yield attributing traits and SSR molecular markers.	2022	978-81-19217-40-3	Yes	BP International
67	Mishra N, Tripathi MK, Tripathi N, Tiwari S, Gupta N and Sharma A.	In book: Innovations in Science and Technology	Screening of Soybean Genotypes against Drought on the Basis of Gene-Linked Microsatellite Markers	2022	978-81-19217-40-3	Yes	BP International
68	Chitralekha Shyam, M. K. Tripathi, Sushma Tiwari, Niraj Tripathi and Ashok Ahuja	In book: Recent Progress in Plant and Soil Research	Identification of low and high erucic acid containing genotype(s) in Indian mustard employing molecular markers	2022	978-81-19217-40-3	Yes	BP International
69	Arjun Kashyap, R. Lekhi, Hina Manjhi and Brajraj Singh Kasana	Falo Ke Dehik Vikar Avam Nivaran	Not Applicable	2022	978-9383774-371	Yes	S.R. Scientific Publication
70	Dr. P.K.S. Gurjar, Dr. R. Lekhi	Sabji beej Utpadan Prodhyogiki (Book)	Not Applicable	2022	ISSN 978-81-951068-4-4	Yes	Madhya Bharat, Krishak Bharti

71	Kumar R., Krishan B., Sundaram P.K., Kumawat N., Jeet P. and Singh A.K. (2022Pavan Kumar, A.K. Pandey, Susheel Kumar Singh, S.S. Singh, V.K. Singh (eds.) DOI: 10.1002/	Structure and Function of Pedospre	Crop Diversification: An Approach for Productive and Climate-Resilient Production System Sustainable Agriculture System and Technologies. .	2022	9781119808565.ch5	Yes	Springer Nature Singapore Pte Ltd.
72	Deepak Kumar Verma and H. K. Balai	Modern Perspectives in Social Science and Extension Education	Communication: Meaning, Purpose, Importance and Purpose	2022	978-93-94243-19-4	Yes	The Society of Academic Research For Rural Development
73	Deepak Kumar Verma and H. K. Balai	Modern Perspectives in Social Science and Extension Education	Data Collection: Method, Tools and Importance	2022	978- 93-94243-19-4	Yes	The Society of Academic Research For Rural Development
74	Rakesh Kumar Singh	Innovative approaches in diagnosis and management of crop disease Volume 2 Field and horticultural crops	Not Applicable	2022	978-00318-783-7	yes	CRC Press/ apple academic press
75	S.S. Chouhan. G.S. Ghatiye, Swati Barche, Bhupendra Kurmi, d s Mandloi and M P Nayak and S.S. Dhakad	Handbook of IPM	Not Applicable	2022	978-93-90757-30-5	yes	Brillion Publishing,New Delhi
76	Rakesh Kumar Singh	Innovative approaches in diagnosis and management of crop disease Volume 3: Nano molecules and Biocontrol Agents	Not Applicable	2022	987-1-77463-027-3	yes	CRC Press/ apple academic press
77	Jadav M L, Kumawat, N., Bhargav, K.S., Rajesh Gupta, Bhagat D V, and S K Choudhary	"Basics of Soil and Water Conservation Engineering"	Not Applicable	2022	978-81-7622-536-6 (2022)	Yes	Biotech Books, New Delhi
78	Dr. Rohan Sharma, Dr. Abilasha Sharma, Dr. K. N. Pathak	Practical Manual on : Fundamental of Agricultural extension education	Not Applicable	2022	Publication no. 138/2022	Yes	R.A.K.College of Agriculture, Sehore, M.P.

79	Anil K. Singh, Minakshi Padhi, Anjana Sisodia, Vandana Sisodia, Vishwa Mohan Dev Chouhan and Anuj Kumar	Disease Sepectrum in carnation crop (Dianthus carophyllus L.) and Management strategies. Diseases of horticultural crops Dignosis and management	Not Applicable	2022	978- 1-77463- 969-6(pbk) 978-1-00316-046-5 (ebk	Yes	Apple Academic press, USA & CRC Press, Boca Raton, Florida, USA.
80	Karan Vir Singh and Anuj Kumar	Not Applicable	Economic Upliftment of Farmers Through Floriculture. (Edt.) Evergreen technologies for Indian Agriculture. Page 225-233	2022	978-93-92851-26-1	yes	Narendra Publishing House, Delhi
81	Dr SK DWIVEDI	HORTICULTURE: Basic Principles and Recent Approaches.	Post Harvest Management of Horticultural Crops	2022	978-93-91383-96-1	Yes	NIPA GENX ELECTRONIC
82	Dr SK DWIVEDI	Nanotechnology Horizons In Food Process Engineering: Trends, Nanomaterials, and Food Delivery	Post Harvest Physiology, Value Chain Advancement, and Nano Technology in Fresh-Cut Fruits and Vegetables	2022	978-93-91383-96-1	Yes	NIPA GENX ELECTRONIC
83	Joshi Ekta, Khambalkar Priyadarshni A., SIngh Akhilesh, Agarwal Pragati, Singh Neelam, Yadav Shashi Singh, Sasode D.S., Popiha Bordoloi and Bhadauria S.S.	Recent Advances in Agriculture Science & Technology for sustainable India.	Climate change : perspectives and impact on Indian agriculture.	2021	978-81-953029-5-6	yes	Mahima Research Foundation and Social Welfare
84	Joshi Ekta, Agarwal Pragati, Sasode D.S., Khambalkar Priyadarshini, Bordoloi Popiha, Ginger Dinesh and Joshi Neeshu	Recent Advances in Agriculture Science & Technology for sustainable India.	Aquaponics : an innovative sustainable food production farming system.	2021	978-81-953029-5-6	yes	Mahima Research Foundation and Social Welfare

85	M. L. Choudhary , M. K. Tripathi , Sushma Tiwari , R. K. Pandya, Neha Gupta , Niraj Tripathi and Prerana Parihar	In book: New Visions in Science and Technology	Morpho-physiological and molecular characterization of pearl millet [Pennisetum glaucum (L.) R. Br.] germplasm lines for drought tolerance	2021	978-81-19217-40-3	Yes	BP International
86	Mishra N, Tripathi MK, Tripathi N, Tiwari S, Gupta N, Sharma A and Shrivastav MK.	In book: Current Topics in Agricultural Sciences	Role of biochemical and antioxidant enzymes activities in drought tolerance in soybean: A recent study	2021	978-81-19217-40-3	Yes	BP International
87	Chitralekha Shyam, M.K. Tripathi, Sushma Tiwari, Ashok Ahuja, Niraj Tripathi and Neha Gupta	In book: Current Topics in Agricultural Sciences	Plant Regeneration in Indian mustard [Brassica juncea (Linn.) Czern & Coss]: Experimental iinvestigation.	2021	978-81-19217-40-3	Yes	BP International
88	M. K. Tripathi, Sushma Tiwari, Niraj Tripathi, Gyanendra Tiwari, Deepa Bhatt, Megha Vibhute, Neha Gupta, Nishi Mishra, Prerana Parihar, Purnima Singh, Akash Sharma, Ashok Ahuja and Sharad Tiwari	In book: Current Topics in Agricultural Sciences	Plant tissue culture techniques for conservation of biodiversity of some plants appropriate for propagation in degraded and temperate areas	2021	978-81-19217-40-3	Yes	BP International
89	Gyanendra Tiwari, M.K. Tripathi, Sushma Tiwari, Niraj Tripathi, Devi Singh Uikey and R.P. Patel	In book: Current Aspects in Pharmaceutical Research and Development	In vitro production of secondary metabolites reserpine and ajmalicine in Rauvolfia serpentina (L.) Benth	2021	978-81-19217-40-3	Yes	BP International
90	Sushma Tiwari, M.K. Tripathi, R.S. Tomar and Ashok Ahuja,	In book: Recent Progress in Plant and Soil Research	Plant Genomic DNA Isolation: An Important Technology for Marker Assisted Selection	2021	978-81-19217-40-3	Yes	BP International

91	Ashok Ahuja, M.K. Tripathi, Sushma Tiwari, Niraj Tripathi, Gyanendra Tiwari, Nishi Mishra, Shashank Bhargav and Sharad Tiwari	In book: Current Aspects in Pharmaceutical Research and Development	Recent advancements on callus and cell ssuspension ccultures: An eeffectual rreserve for the pproduction of ppharmaceutically ssignificant mmetabolites.	2021	978-81-19217-40-3	Yes	BP International
92	Phundan Singh, Sushma Tiwari, Sharda Chaudhary	Objective molecular biology and plant biotechnology	Not Applicable	2021	9.78939E+12	Yes	Daya publishing house A division of Astral International Pvt. Ltd
93	Phundan Singh, Sushma Tiwari, Pratibha Bisen	Plant Molecular Biology and Biotechnology at a glance	Not Applicable	2021	9.78939E+12	Yes	Daya publishing house A division of Astral International Pvt. Ltd
94	Khushboo Tandon, PKS Gurjar and R Lekhi	Dragon Fruit: A Super Food in India. (Book Chapter)	Not Applicable	2021	9.78939E+12	Yes	*Research Trends in Horticulture Science* New Delhi. P: 97-99.
95	Dr. Devendra, Dr. V.K. Singh, Dr. Sudhir Singh, Dr. Bharat	Adhunik Falutpadan	Not Applicable	2021	978-93-8895-024-4	Yes	SSDN, Publisher and Distributor, New Delhi
96	Dr Sushma Tiwari	Not Applicable	Genomic DNA Isolation: An Important Technology for Marker Assisted Selection	2021	978-93-5547-361-5	Yes	Recent Progress in Plant and Soil Research
97	Kumar R., Saurabh K., Kumawat N., Sundaram, P.K., Mishra J.S, Singh D.K., Hans H., Krishna, B. and Bhatt B.P.	Role of Microbial Communities for Sustainability. Microorganisms for Sustainability,	Sustaining Productivity Through Integrated Use of Microbes in Agriculture.	2021	/978-981-15-9912-5_5	Yes	Springer Nature Singapore Pte Ltd.
98	K.S.Kirad, S.S. Chouhan. G.S. Ghatiye, Swati Barche, Bhupendra Kurmi and M.P. Nayak	Hand Book of INM	Not Applicable	2021	978-93-86658-24-1	Yes	Brillion Publishing,New Delhi
99	Rakesh Kumar Singh	Innovative approaches in diagnosis and management of crop disease Volume 1: The Mollucutes	Not Applicable	2021	978-1-00318-760-8	yes	CRC Press/ apple academic press

100	Mohan LalJadav	"Effect of Drip Irrigation and Mulching on Pigeonpea Productivity"	Not Applicable	2021	978-620-4-19723-4 (2021)	Yes	LAP (Lambert Academic Publishing), Chisinau-2068, Republic of Moldova Europe,
101	Dr. M. Yasin, Dr. I. S. Tomar, Dr. Chandan Kumar, Dr. Jagdish Maurya	Samudayik beej bank	Not Applicable	2021	Not Applicable	Yes	Krishi vigyan kendra, Jhabua, Rajmata Vijayaraje Scindhia Krishi Vishwavidyalay, G walior, M.P.
102	Dr. Moly Saxena and Dr. D. R. Saxena	Practical Manual on detection and diagnosis of diseases of horticultural crops	Not Applicable	2021	Publication no. 132/2021	Yes	Plant Pathology, RAK, College of Agriculture Sehore, M.P.
103	Mishra YK, Rajesh Aarwe and Sharma AK.	Not Applicable	Book title: Insect Pest Management: Concept and Approaches. Chapter title: Insecticides Resistance, Mechanism and Their Management Strategies.	2021	978-93-90846-48-1 (Book chapter)	Yes	AkiNik Publications, New Delhi, 1(1):30-36.
104	Rajesh Aarwe and Mishra YK	Not Applicable	Book title: Insect Pest Management: Concept and Approaches. Chapter title: Integrated Pest Management Tools in Soybean (Glycine max (L.) Merrill) Crop.	2021	978-93-90846-48-1 (Book chapter)	Yes	AkiNik Publications, New Delhi, 1(1):67-76.
105	Dr. Anuj Kumar and Dr. Jyoti Kanwar	KNK Series of Agriculture (Volume I)	Not Applicable	2021	RVSKVV Pub.No. 123/2021	yes	RVVSKVV
106	Dr. Anuj Kumar and Dr. Jyoti Kanwar	KNK Series of Horticulture (Volume I)	Not Applicable	2021	RVSKVV Pub.No. 124/2021	yes	RVVSKVV
107	Rajiv Dubey, Vinay Kumar Gautam, Deepak Sharma, L.L. Somani, R.C. Dadheech	Soil and Water Conservation Practices (As per 5th deans committee syllabus) Soil and Water Conservation Practices (As per 5th deans committee syllabus)	Not Applicable	2021	978-81-8321-535-0	Yes	Agrotech Publishing Academy, Udaipur- (2021)

108	K. C. Meena, K Alam Khan, Nitin Soni and D.K. Patidar	Harvesting, Post-harvest, Processing and Value addition of Medicinal and Aromatic plants for Entrepreneurship development	Not Applicable	2021	978-93-91342-42-5	Yes	JPS Scientific Publication, Tiruvannamalai, Tamil Nadu
109	Dr. Pramod Kumar Fatehpuria	Key to Success in Plant Pathology	Not Applicable	2021	978-8188805-198	Yes	S.R.Scientific
110	Jyoti Kanwar and Priyamvada Sonkar	Chapter: KNKN Series on Horticulture for Competitive Examinatios. pp. 34-78.	Not Applicable	2021	RVSKVV Pub. No. 124/2021	Yes	RVSKVV, Gwalior
111	Nitin Soni, D.K. Patidar, Er. A. K. Khan and K. C. Meena	Opportunities in Agriculture & Animal husbandry for Sustainable Entrepreneurship & Livelihood Security	Entrepreneurship Skills Development Through Commercial Production and Value addition of Grapes.	2021	978-93-91342-42-5	Yes	JPS Scientific Publication, Tiruvannamalai, Tamil Nadu, India
112	Joshi Ekta, Vyas Abhay Kumar and Sasode Deep Singh	Nutrient omission studies in maize-wheat cropping system of India	Not Applicable	2020	No. 978-620-0-44022-8	yes	LAMBERT Academic Publishing, Germany
113	Singh Neelam and Joshi Ekta	Nutrient management in kharif groundnut	Not Applicable	2020	NO. 978-3-659-52632-9	yes	LAMBERT Academic Publishing
114	Gupta, R., Tiwari, S., Tripathi, M.K. and Pooniya, S.K.	In book:Recent Trends in Molecular Biology and Biotechnology.	Bioinformatics and its applications in crop improvement.	2020		Yes	Integrated Publications
115	Shashi S. Yadav, S. K. Verma, Priyadarshani A. Khambalkar, Akhilesh Singh	Methods of Soil and Water Analysis	Not Applicable	2020	978-93-88020-71-8	Yes	Satish Serial Publishing House, 403, Express Tower, Commercial Complex, Azadpur, Delhi-110033
116	Dr Sushma Tiwari	Fundamentals of Molecular Biology and Plant biotechnology	Not Applicable	2020	978-93-89719-36-9(PB)	Yes	Daya publishing house A division of Astral International Pvt. Ltd.
117	Rajni Singh Sasode and Pramod Kumar Fatchpuria	Research trends and innovations in Plant Pathology	Foliar Diseases of Cluster Bean: A Consortium Approach for Disease Management to Improve Productivity	2020	978-81-947809-1-5	yes	Integrated Publications

118	R.K.Singh	DISEASE OF FIELD AND HORTICULTURE CROPS AND THEIR MANAGEMENT	Not Applicable	2020	978-93-89996-31-9	yes	Jaya publishing house
119	D.H. Ranade, M.L.Jadav, InduSwarup, O.P. Girothia, D.V. Bhagat, Akhilesh Singh and SharadChoudhary	Rain Water Management in Rainfed Areas	Not Applicable	2020	978-81-7622-458-1 (2020)	Yes	Biotech Books, New Delhi
120	दीपक हरी रानडे, ,एम. एल. जादव,इंदुस्वरुप,दुष्यंत विजय भगत, ओम प्रकाश गिरोठिया ऐस.के. चौधरी,आशीष उपाध्याय	अपवाहित वर्षा जल एकत्रीकरण व संचित जल का उपयोग	Not Applicable	2020	978-81-7622-464-2 (2020)	Yes	Biotech Books, New Delhi,
121	Dr. M.Yasin	People's Biodiversity Register, Panchkhairiya	Not Applicable	2020	Not Applicable	Yes	R.A.K.College of Agriculture Sehore and Krishi Vigyan Kendra Jhabua,
122	Dr. M.Yasin	People's Biodiversity Register, Sajeli Malji sath	Not Applicable	2020	Not Applicable	Yes	R.A.K.College of Agriculture Sehore and Krishi Vigyan Kendra Jhabua,
123	Dr. M.Yasin	(People's Biodiversity Register- PBR)), Kakanvani	Not Applicable	2020	Not Applicable	Yes	R.A.K.College of Agriculture Sehore and Krishi Vigyan Kendra Jhabua,
124	D.K. Tiwari, Rajiv Dubey, R.K. Dwivedi, Sharvan Kumar Yadav	Principles of Organic Farming (As per 5th dean's committee syllabus)	Not Applicable	2020	978-93-90647-03-3	Yes	Agrotech Publishing Academy, Udaipur- (2020)
125	Rajiv Dubey, Shailendra Singh Kushwah and Aakash Mishra	Not Applicable	Recent advances in organic farming with speacial reference to weed management- Advanced Agriculture	2020	978-93-88879-99-6 (HB)	Yes	New Delhi Publishers, New Delhi
126	Aakash Mishra, Rajiv Dubey, Pawan Pant, Punit Mehta, Rajlumar Shinde and Sandip Rathod	Not Applicable	Nano clay: a boon for enhancing agriculture productivity- Advanced Agriculture	2020	978-93-88879-99-6 (HB)	Yes	New Delhi Publishers, New Delhi

127	S.K. Ghoshi, K.C. Meena and Ajay Halder	Garlic (Allium sativum L.) Germplasms : A breeding Research Studies	Not Applicable	2020	978-620-2-52509-1	Yes	Lambert Academic Publication
128	Surendra Singh Ghoshi, Meena K C, Shakthi Prasad N and I S Naruka	Not Applicable	Not Applicable	2020	978-81-926009-9-4	Yes	JNKVV Jabalpur
129	Pankaj Maida and Souvick Banik	'Advance Production Technology in Aloe Vera: Its Pharmacological & Phytochemistry Properties'	Not Applicable	2020	978-93-90541-54-6(Book chapter)	Yes	AkiNik Publications
130	Dr. Pramod Kumar Fatehpuria	A Text book Diseases Of Field and Horticultural Crops& Their Management-I	Not Applicable	2020	978-8188805-181	Yes	S.R.Scientific
131	Dr. Pramod Kumar Fatehpuria	A Text book Diseases Of Field and Horticultural Crops & Their Management-II	Not Applicable	2020	978-8188805-167	Yes	S.R.Scientific
132	Dr. Pramod Kumar Fatehpuria	Current Research and Innovations in Plant Pathology	Management Prospects against Stem Canker of Mustard	2020	978-9383774-302	Yes	AkiNik Publications
133	Dr. Pramod Kumar Fatehpuria	Advances in Horticulture	Production Technology and Disease Management of Chilli Crop	2020	978-93-90217-92-2	Yes	AkiNik Publications
134	Dr. Pramod Kumar Fatehpuria	Not Applicable	A Consortium Approach for Disease Management to Improve Productivity	2020	978-81-947809-1-5	Yes	Integrated Publications
135	Billore, Mridula, Phundan Singh & Sushma Tiwari	Fundamentals of Molecular Biology and Plant Biotechnology	Not Applicable	2020	978-93-89719-376	Yes	Daya Publishing House A divivsion of Astral International Pvt Ltd, New delhi
136	Phundan Singh, Sushma Tiwari & Mridula Billore	Fundamentals of Molecular Biology and Plant Biotechnology	Not Applicable	2020	978-93-89719-36-9(PB)	Yes	Daya Publishing House A divivsion of Astral International Pvt Ltd, New delhi
137	Dr Ram Avadh Prajapati, Dr Roopesh Chaturvedi	Recent Trends in Fiction writing (Themes, Contexts & Techniques)	Not Applicable	2020	978-1-64951-463-9	Yes	Notion Press, India

138	Dr Roopesh Chaturvedi & Dr O. P. Singh	An Oath to Fire	Not Applicable	2020	978-1-64760-487-5	Yes	Notion Press, India
139	Arjun Kashyap, R. Lekhi, Niharika Rathore, Ajay Dangi and B.S. Kasana	Objective Questions on Fruit Crops (Book)	Not Applicable	2019	/ISSN No. 978-9383774-326	Yes	S.R. Scientific Publication
140	M.K. Kureel, D.S. Mandloi, K.V. Singh and Rajesh Lekhi	Post Harvest Management and Value Addition of Fruits and Vegetables	Not Applicable	2019	978-81-7622-399-7	Yes	Bio Tech Books Publication.
141	Joshi Ekta, Prajapat Kailash, Jinger Dinesh, Sasode Deep Singh and Singh Neelam	Precision crop and resource management	Nutrient omission plot technique and its role in determining the nutrients rate and limiting nutrient in SSNM (Page No. 41 - 46)	2019	978-93-83168-45-3	yes	ICAR -IARI, New Delhi
142	KumawatN., KumarR., KhandkarU.R., YadavR.K., DotaniyaM.L., MishraJ.S. and Hans H.	Biofertilizers for Sustainable Agriculture and Environment	Silicon (Si) and Zinc (Zn) Solubilizing Microbes: Role in Sustainable Agriculture	2019	978-3-030-18933-4_6	Yes	Springer Nature Singapore Pte Ltd.
143	KumarR., KumawatN., SaurabhK., HansH., Mishra J.S., KhandkarU.R., MeenaR.S. and Bohra J.S.	Sustainable Management of Soil and Environment	Conservation Agriculture: Perspectives on Soil and Environmental Management in Indo-Gangetic Plains of South Asia.	2019	978-981-13-8832-3_4	Yes	Springer Nature Singapore Pte Ltd.
144	Saurabh S., Kumar R., Mishra J.S., Hans H., Kumawat N., Meena R.S., Rao K.K., Kumar M., Dubey A.K. and Dotaniya, M.L.	Carbon and Nitrogen Cycling in Soil	Carbon and Nitrogen Mineralization Dynamics: A Perspective in Rice-Wheat Cropping System	2019	978-981-13-7264-3_14	Yes	Springer Nature Singapore Pte Ltd.
145	Lalita Nargawe and Anil Kumar	Advances in Computer Science and Information Technology,	Effective Classroom Communication Skills in Teaching	2019	2393-9907	Yes	Krishi Sanskriti

146	Kamal.S.Kirad, G.S.Gathia, S.S.Chauhan, S.K.Badaiya, J.S.Rajput, S.Barche and M P Nayak	Hand Book of DAESI	Not Applicable	2019	978-93-87445-08-6.	Yes	Brillion Publishing. NewDelhi.
147	Dr. S.B. Singh, Dr. A.K Badaya and Dr. S.N. Upadhayay	Toxicology of Insecticides	Not Applicable	2019	978-81-7622-459-8	yes	BIOTECH BOOKS
148	पटेल, राजेश प्रसाद, कानपुरे, आर.एन. एवं हलदार, अजय	मशरूम उत्पादन, प्रबंधन एवं विपणन	Not Applicable	2019	978-93-89907-0-63	Yes	न्यू इंडिया पब्लिशिंग एजेन्सी नई दिल्ली - 110034
149	Dr. Pramod Kumar Fatehpuria	A Competitive Approach of Plant Pathology	Not Applicable	2019	978-9383774-302.	Yes	S.R.Scientific
150	Dr. R.P.Patel	Mushroom Utpadan pravandan evam viprand	Not Applicable	2019	928-93-89907-06-3	Yes	New India Publishing
151	Dr. S. B. Singh	Toxicology of Insecticides	Not Applicable	2019	978-81-7622-459-8	Yes	BIOTECH BOOKS
152	Misra AK, Yadav SB, Mishra SK and Tripathi MK	Not Applicable	Impact of Meteorological variables and climate change on plant diseases.	2019	978-0-42905-721-2	Yes	Apple Academic Press Inc. 1265, Golden rod circle NE, Palm Bay, Florida 32905, USA pp. 313-327
153	Phundan Singh, Mridula Billore, D.S. Mishra	Introduction To Maintenance Plant Breeding	Not Applicable	2019	978-93-88982-450	Yes	Daya Publishing House A divivsion of Astral International Pvt Ltd, New delhi
154	Khursheed Alam Khan, Megh R. Goyal, Abhimannyu A Kalne	Processing of Fruits and Vegetables: From Farm to Fork (Innovations in Agricultural & Biological Engineering)	Not Applicable	2019	978-17-74634-035	Yes	Apple Academic press, USA & CRC Press, Boca Raton, Florida, USA.
155	Manoj Kumar Kureel, Dhan Singh Mandloi, Karan Vir Singh, Rajesh Lekhi	Postharvest Management and Value Addition of Fruits and Vegetables	Not Applicable	2019	978-81-7622-399-7	Yes	Biotech Books, 4762-63/23, Ansari Road, Daryaganj, New Delhi-110002

156	Rumana Khan, A. *Nishant Bhanu, Sharwan Kumar Shukla, Ashutosh Srivastava, A.R.S.S.H. Gupta, A.D.S.S. Ajay Nikhil, H. Sirisha, N. Aneesha)	Recent Trends of Production, Protection and Improvement in Agriculture	Role of Secondary Metabolites in Stress and	2023	978-1-80433-963-3	Yes	Rubicon Publications, London, WC1A 2RP, England
157	*Rumana Khan, A. Nishant Bhanu, Sharwan Kumar Shukla, Ashutosh Srivastava, R.V. Charitha Reddy, Ajay Nikhil A.D.S.S., Gupta A.R.S.S.H., Sirisha H., Aneesha N.	Emerging Trends in Crop Improvement	Crop Improvement Strategies for Climate Resilient Agriculture	2023	978-1804339510	Yes	Rubicon Publications, London, WC1A 2RP, England
158	Sweta Mishra, Shailesh Kumar, R C Srivastava	Genetic Improvement of Small Millets	Floral Biology, Pollination, Genetics, Origin, and Diversity in Barnyard Millet	2024	978-981-99-7231-9	Yes	Springer
159	Akhouri Nishant Bhanu, Hem Raj Bhandari, Pragya Shukla, Kartikeya Srivastava, Mahendra Narain Singh & Sushil K. Chaturvedi	Sustainable Utilization and Conservation of Plant Genetic Diversity. Sustainable Development and Biodiversity	Innovations in Assessment Approaches of Plant Genetic Diversity	2024	978-981-99-5244-1	Yes	Springer, Singapore
160	Dr. Manoj Kumar Tarwariya, Ravi Kumar Richhariya, Debesh Singh. Rohit Pandey and Akanksha Silarwar	Organic Agriculture : Moving Towards Sustainability		2023	9.78812E+12	Yes	Royal Book Publishing
161	M. K. Tarwariya, Richa Pyasi, C.B.S Jatav and	Organic Farming	Application of organic farming practices	2022	ISBN :978-93-81247-14-3	Yes	Shriyanshi Prakashan, Agra
162	M. K. Tarwariya and Richa Pyasi	Hand Book of Horticulture	Organic Production Technology of Tomato & Brijnal region	2023	ISBN :978-93-81247-20-4	Yes	Shriyanshi Prakashan, Agra
163	Neha Singh Kirar, M. K. Tarwariya and D. K. Raidas	Recent Advances in Agriculture	Organic Farming: Need of the Hour	2022	ISBN: 978-93-5627-835-6	Yes	Self-Published, Department of Agricultural Entomology, Shri Vaishnav Institute of Agriculture, Shri Vaishnav Vidyapeeth Vishwavidyalaya, Gram-Baroli Ujjain Road, Indore- 453 111. (M.P.), India

164	Neha Singh Kirar, M. K. Tarwariya and D. K. Raidas	Recent Advances in Agriculture	Sustainable Agriculture and Environment Conservation	2022	ISBN: 978-93-5607-484-2	Yes	Self-Published, Department of Agricultural Entomology, Shri Vaishnav Institute of Agriculture, Shri Vaishnav Vidyapeeth Vishwavidyalaya, Gram-Baroli Ujjain Road, Indore- 453 111. (M.P.), India
165	Gurjar Priyanka, Uikey Varsha	Current trends in agriculture & allied sciences	Description, uses and value added products of moringa	2023	978-93-5967-599-2 page no. 369-376	yes	S.P. Publication
166	Veena Rathore, Anvita Sharma, and Rajul Soni	Advances in Agricultural and Horticultural Sciences	Extension and their various forms in Agricultural Programs and their implications	2022	978-93-5607-484-2	Yes	Editor- Dr. Yuvraj A. Shinde, Self published
167	Bhavna Tomar	Modern Concept of Agricultural Sciences	Utilization of Plant Genetic Resources for Food, Feed and Nutritional Security	2023	978-93-92953-84-2	Yes	Vital Biotech Publication
168	Bhavna Tomar	Modern Concept of Agricultural Sciences	Resource Conservation Technologies	2023	978-93-92953-84-2	Yes	Vital Biotech Publication
169	Bhavna Tomar	Novel Technologies in Modern Agriculture	Concept, Importance and Principles of Organic Farming	2023	978-93-92953-45-3	Yes	Vital Biotech Publication
170	Bhavna Tomar	Novel Technologies in Modern Agriculture	Importance, Components and Principles of FarmingSystem	2023	978-93-92953-82-8	Yes	Vital Biotech Publication
171	Bhavna Tomar	Frontier in Food Processing and Preservation	Food Safety and quality assurance in Processing operations	2023	978-81-19651-43-6	Yes	BS Global Publication House
172	J Himanshu Rao	Role of Modern Technology in Agriculture	Monitor and Control Crop Irrigation System through Smart Sensors	2023	978-93-9397465-5	Yes	Blue Duck Publications Srinagar, J&K
173	J Himanshu Rao	Role of Modern Technology in Agriculture	Application of Remote Sensing and Geographic Information System in Modern Agriculture Technology	2023	978-93-9397465-5	Yes	Blue Duck Publications Srinagar, J&K
174	J Himanshu Rao	Land and Water Management Engineering	Watershed Analysis using Remote Sensing and GIS Techniques	2023	978-93-58998-90-0	Yes	Elite Publishing House, New Delhi
175	J Himanshu Rao And M. L. Jadav	Practical Manual On Protected Cultivation And Secondary Agriculture	Not Applicable	2024	978-93-58994-16-2	Yes	Elite Publishing House, New Delhi
176	Ayushi Trivedi, Vikram Parmar, J Himanshu Rao And Shrankhla Mishra	Advances In Soil And Water Conservation Engineering	Not Applicable	2024	978-93-95185-12-7	Yes	Elite Publishing House, New Delhi

177	Sitaram Seervi, Dr. Pradyumn Singh, Dr. Rajni Singh Sasode and Sandeep Patidar	Plant Protection Insights Diverse Perspective on Plant Pathology and Entomology	Integrated pest management strategies	2024	978-81-976479-7-0	Yes	Stella International Publication, Kurukshetra, Haryana
178	Sitaram Seervi, Pradyumn Singh, Naveen, Neha Tomar and Sandeep Patidar	FUNDAMENTALS OF ENTOMOLOGY	METHODS OF COLLECTION AND PRESERVATION OF INSECTS INCLUDING IMMATURE STAGES.	2024	978-81-19674-82-4	Yes	National Press Associates, Malviya Nagar, New Delhi
179	Prince Mahore, Nikki Bhardwaj, Pradyumn Singh, Deerendra Mahor	Agricultural Insecticide and Their Properties	Not Applicable	2024	978-81-19677-93-1	Yes	Discovery Publishing House, Darya Ganj, New Delhi
180	S.S. Bhatnagar and M. Yasin	Seed technological association for self-sufficiency in oilseeds and pulses	Seed Societies and New Seed Scenario in Madhya Pradesh	2024	9788119105243	Yes	Satish Serial Publishing House
181	Sanjay Sharma, D.K. Shrivastava	Seed technological association for self-sufficiency in oilseeds and pulses	Sustainable Cotton Production is a Better Alternate of Decreasing Yield and Increasing Cost of Bt Cotton in India	2024	9788119105243	Yes	Satish Serial Publishing House
182	M.K. Saxena, Usha Saxena and Shudhanshu Jain	Seed technological association for self-sufficiency in oilseeds and pulses	New Hope of Safflower Crop in Madhya Pradesh	2024	9788119105243	Yes	Satish Serial Publishing House

Metric 3.4.5

Books & Book Chapters

As per ICAR New Syllabus

पादप कार्यिकी के मूल सिद्धान्त

[Fundamentals of Plant Physiology]

अनुराधा गोयल



KALYANI

As per ICAR New Syllabus

पादप कार्यिकी के मूल सिद्धान्त

[Fundamentals of Plant Physiology]

डॉ. अनुराधा गोयल
मृदा विज्ञान विभाग
कृषि महाविद्यालय
राजमाता विजयाराजे सिंधिया कृषि विश्वविद्यालय
ग्वालियर–474 002

कल्याणी पब्लिशर्स
लुधियाना • नई दिल्ली • नोएडा (यू०पी०) • हैदराबाद
चेन्नई • कोलकाता • कटक • गुवाहाटी • कोचि • बैंगलूरु

विषय–सूची

क्र.सं.	अध्याय	पृष्ठ संख्या
1.	पादप कार्यिकी विज्ञान का परिचय और कृषि में इसका महत्व (Introduction to Plant Physiology and Its Importance in Agriculture)	1–8
2.	पादप कोशिका : एक अवलोकन (Plant Cell : An Overview)	9–25
3.	विसरण और परासरण : पौधों द्वारा जल का अवशोषण (Diffusion and Osmosis : Absorption of Water by Plants)	26–41
4.	वाष्पोत्सर्जन और रंध्रों की कार्यिकी (Transpiration and Stomatal Physiology)	42–56
5.	पौधों का खनिज पोषण : पोषक तत्वों के कार्य और कमी के लक्षण (Mineral Nutrition of Plants : Functions and Deficiency Symptoms of Nutrients)	57–70
6.	प्रकाश संश्लेषण : प्रकाशिक और अप्रकाशिक अभिक्रियाएं, सी3, सी4 और कैम पौध (Photosynthesis : Light and Dark Reactions, C3, C4 and CAM Plants)	71–91
7.	श्वसन : ग्लाइकोलाइसिस, टीसीए चक्र और इलेक्ट्रॉन परिवहन शृंखला (Respiration : Glycolysis, TCA Cycle and Electron Transport Chain)	92–107
8.	वसा का उपापचयन : वसीय अम्लों का संश्लेषण और अपघटन (Metabolism of Lipids : Fatty Acid Synthesis and Breakdown)	108–116
9.	पादप वृद्धि नियामक : कार्यिकी भूमिकाएं और कृषि में उपयोग (Plant Growth Regulators : Physiological Roles and Uses in Agriculture)	117–132

10.	प्रमुख फसलों के वृद्धि और विकास के कार्यिकीय पहलू : विकास विश्लेषण, फसल उत्पादकता में कार्यिकीय वृद्धि मानकों की भूमिका (Physiological Aspects of Growth and Development of Major Crops : Growth analysis, Role of Physiological Growth Parameters in Crop Productivity)	133–14
11.	प्रयोग (Practicals)	145–16

अध्याय

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पादप कार्यिकी विज्ञान का परिचय और कृषि में इसका महत्व

(Introduction to Plant Physiology and Its Importance in Agriculture)

1.1. परिचय (Introduction)

पादप कार्यिकी विज्ञान (Plant Physiology) वनस्पति विज्ञान की वह शाखा है जिसमें पौधों में होने वाली विभिन्न प्रकार की जैविक क्रियाओं जैसे – प्रकाश संश्लेषण (photosynthesis), श्वसन (respiration), जनन (reproduction), वृद्धि (growth) तथा गति (movement) आदि का अध्ययन किया जाता है। स्टीफन हेल्स (Stephen Hales) ने पहली बार अपने भौतिकी (Physics) व सांख्यिकी (Statistics) के ज्ञान के आधार पर प्रयोगात्मक विधियों द्वारा पौधों में होने वाले परिवर्तनों जैसे पौधों में वाष्पोत्सर्जन दर (transpiration rate), रसों की गति (movement of saps), पौधों में रसारोहण (ascent of sap) क्रिया में मूलदाब (root pressure) व केशिका बल (capillary force) की भूमिका का स्पष्ट रूप से अध्ययन किया। इसलिए स्टीफन हेल्स (Stephen Hales) को पादप शरीर क्रिया विज्ञान का जनक कहा जाता है।

1.2. पादप कार्यिकी विज्ञान के अध्ययन का उद्देश्य (Objectives of Studying Plant Physiology)

कोशिका सजीवों की संरचनात्मक (structural) एंव कार्यात्मक (functional) इकाई है। पौधों में बीज अंकुरण से लेकर वृद्धि (growth), विकास (development), प्रजनन (reproduction), जीर्णावस्था (ageing) तक पूरे पौधे में बहुत सी प्रक्रियाएं होती हैं। इनमें होने वाली विभिन्न प्रक्रियाओं को पादप कार्यिकी के अंतर्गत अध्ययन किया जाता है। पादप शरीर क्रिया विज्ञान के माध्यम से पौधो में होने वाले सभी जैविक, रासायनिक तथा भौतिक परिवर्तनों का अध्ययन किया जा सकता है। पौधे के जीवन में इन प्रक्रियाओं का क्या महत्व है और कौन–2 से बाहरी तथा आंतरिक कारक हैं जो इन प्रक्रियाओं को कैसे और क्यों प्रभावित करते हैं उसका भी अध्ययन पादप कार्यिकी विज्ञान के अंतर्गत किया जा सकता है। पौधों में होने वाले विभिन्न जैविक, रासायनिक तथा भौतिक परिवर्तन इस प्रकार हैं–

1.2.1. जैविक क्रियाएं (Biological Reactions)

पौधों की कोशिकाओं में होने वाले सभी रासायनिक एवं भौतिक परिवर्तन तथा पौधों की कोशिकाओं एवं वातावरण के बीच सभी प्रकार की गैसों का आदान – प्रदान जैविक क्रियाओं के अन्तर्गत आते हैं

1.2.2. रासायनिक परिवर्तन (Chemical Changes)

रासायनिक परिवर्तनों के अंतर्गत प्रकाश संश्लेषण (photosynthesis), पाचन (digestion), श्वसन (respiration), प्रोटीन्स, वसा तथा हॉरमोन्स पदार्थों का संश्लेषण आदि प्रक्रियाएं आती हैं।

Seed

Technological Innovations for Self-Sufficiency in Oilseeds and Pulses













S.K. Rao
S.K. Sharma
R.R. Hanchinal
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Chapter 10

Seed Health Management in Major Oilseed Crops: Challenges and Wayforward

Moly Saxena

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The importance of quality seed was recognized since times immemorial, human practiced crop husbandry. In recent years, the awareness for seed health has increased among growers, traders and consumers. Indian agriculture has witnessed many outbreaks of plant disease during past decades in different agro-climatic zones of the country under different farming systems.

Disease free plants ensure a healthy crop stand in the field. This provides better outputs at farm level. However, seed-borne pathogens are known to affect seed health and its vigour. A number of crop diseases are disseminated to distinct areas through infected seeds. As a result, new diseases are easily established in those environments. Seed contamination with such pathogens occurs in many ways like direct infection on the field and improper storage conditions. High relative humidity, suitable temperature and higher moisture content enhance pests and diseases establishment in seeds. Infestation by these fungi has been observed in all parts of the seeds as it harms seed tissues externally or internally causing seed rot, necrosis and seedling diseases.A large number of fungal pathogens are known to be seed borne and seed transmitted. Major seed-borne fungal pathogens causing disease on major oilseed crops are give in Table 1.

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Chapter 9

Current Challenges and Wayforward for Seed Health Management of Pulse Crops

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Introduction

Pulses are the important source of proteins for a large number of vegetarian population and helps in improving soil fertility by fixing nitrogen in soil. The cultivated are under pulses in India is 27.99 m ha (2019-20). The country has achieved phenomenal growth in pulses production during last decade and crossed over 25 million tons, this helped in reducing import of pulses from 6.6 million tons to 2.46 million tons in 2020-21. In order to boost pulses production, availability of quality healthy seed is the most important input. The International seed trade of field crops has crossed 8,000 million USD in 2019. India had a market of 175 million USD (International Seed Federation Seed Statistics, 2019). Important pulse crops grown in India are chickpea (*Cicer arietinum* L.), black gram [*Vigna mungo* (L.) Hepper], green gram (*Vigna radiata* (L.) Wilczek), pigeonpea [*Cajanus cajan* (L.) Millsp.], pea (*Pisum sativum* (L.) and lentil (*Lens culinaris* Medik.). Because of their adaptability to various climatic conditions, pulses are also considered climatic resilient crops. In spite of their ability to adopt different climatic conditions, they are prone to various diseases caused by fungi, bacteria, viruses and nematodes. These pathogens are associated with the seeds internally and externally or sometimes transmitted as concomitant contaminants, resulting in poor plant stand, as well as, contributes in introduction and spread of the diseases in various parts of the country. Thus, seed health is of great concern to agro-food industry and regulatory authorities like quarantine and seed certification agencies.

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Seed

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Chapter 17

Critical Seed Planning, Intensive Monitoring & Awareness Campaign: A Need

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In Madhya Pradesh, public seed sectors M.P. Seed and Farm Corporation, National Seed Corporation, Agriculture Universities, OILFED, KRIBHICO, state Directorate of Agriculture and more than 900 seed co-operatives societies involved in seed sector. Share of total public sector in seed production was 40% and remaining 60% was covered by private sector .The private sectors includes more than 75 active companies which are involved in seed production and marketing businesses (Table 1& 2). Seed replacement and varietal replacement rate is multiplied manifolds during two decades due to involvement of seed co-operative societies and private partners. But it is also true that seed production as well as marketing in private seed sector is limited to few crops and limited number of varieties .It indicates that the Private seed producing agencies are producing the seed of only profitable varieties and crops and they are also not taking any interest in increasing seed awareness among the farmers. Slow spread of new varieties because of low less attention of extension planning work is one of the major causes for poor productivity and low profitability of several crops in the state. Intensive planning is needed to workout block-wise economically viable, assured marketable and adjustable with traders, millers and exporters. Identification of gaps in seed business formulation of optimum planning and their monitoring for successful implementation issues are highlighted.

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Seed

Technological Innovations for Self-Sufficiency in Oilseeds and Pulses













S.K. Rao
S.K. Sharma
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Seed Technological Innovations
for
Self-Sufficiency in Oilseeds and Pulses

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Innovative Strategies in Agribusiness Management



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6 Farm Management Principles

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Abstract

Nobody can deny the fact that it is the endeavor of the farming community only that helps realize the higher farm production. Farm management, includes making and implementing of the decisions involved in organizing and operating a farm for maximum production and profit. Farm management draws on agricultural economics and fall under microeconomics. In this chapter we studied about eight principles of farm management viz., comparative advantage, opportunity cost, theory of cost, product substitution, factor substitution, diminishing marginal return, equi-marginal returns and time comparison. These principles provide the farmer to find the practical solution of farm related problems such as production, marketing as well as management of on farm resources. Along with efficient resource management these principles guide to achieve profit maximization level at farm. Thus, farm management tools help farmer in solving farm related problems for successful farm business.

Keywords: Costs, Diminishing Return, Farm management, Input, Principles, Rational, Output.

Introduction

The prosperity of any country depends upon the prosperity of farmers, which in turn depends upon the rational allocation of resources among various uses and adopting

Modern Approaches in Agricultural Economics



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Chapter - 9

Farm Management and Efficiency Analysis

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Abstract

The present chapter entitled 'Farm Management and Efficiency Analysis' is intended to present the management related decisions of the farm resources and farm efficiency measuring tools for sound farm planning by any individual. The chapter includes various farm related decisions to be achieved by the principles of farm managing at the most profitable levels, types of farm, farm resource management, farm production economics, risk and uncertainty in farming, and finally the various farm efficiency analysis tools. The theory of optimal decision making in the organization and management of a farm for profit maximization is the foundational theory of farm management. In the context of increased commercialization there is a greater need to improve the managerial abilities of the farmer. So far, the managers in general have responded admirably to the technological changes that accrued in Indian agriculture. All farm management tools as well as farm efficiency tools are helpful to the farmers in solving their managerial problems for successful operation on the farm business. In concluding that the farmers need of decision making to evaluate the farm businesses will definitely be achieved on the guidelines of resources management and efficiency measures for the profit maximization of farm.

Keywords: Efficiency, Farm, Farm Management, Production Economics, Risk and Uncertainty.

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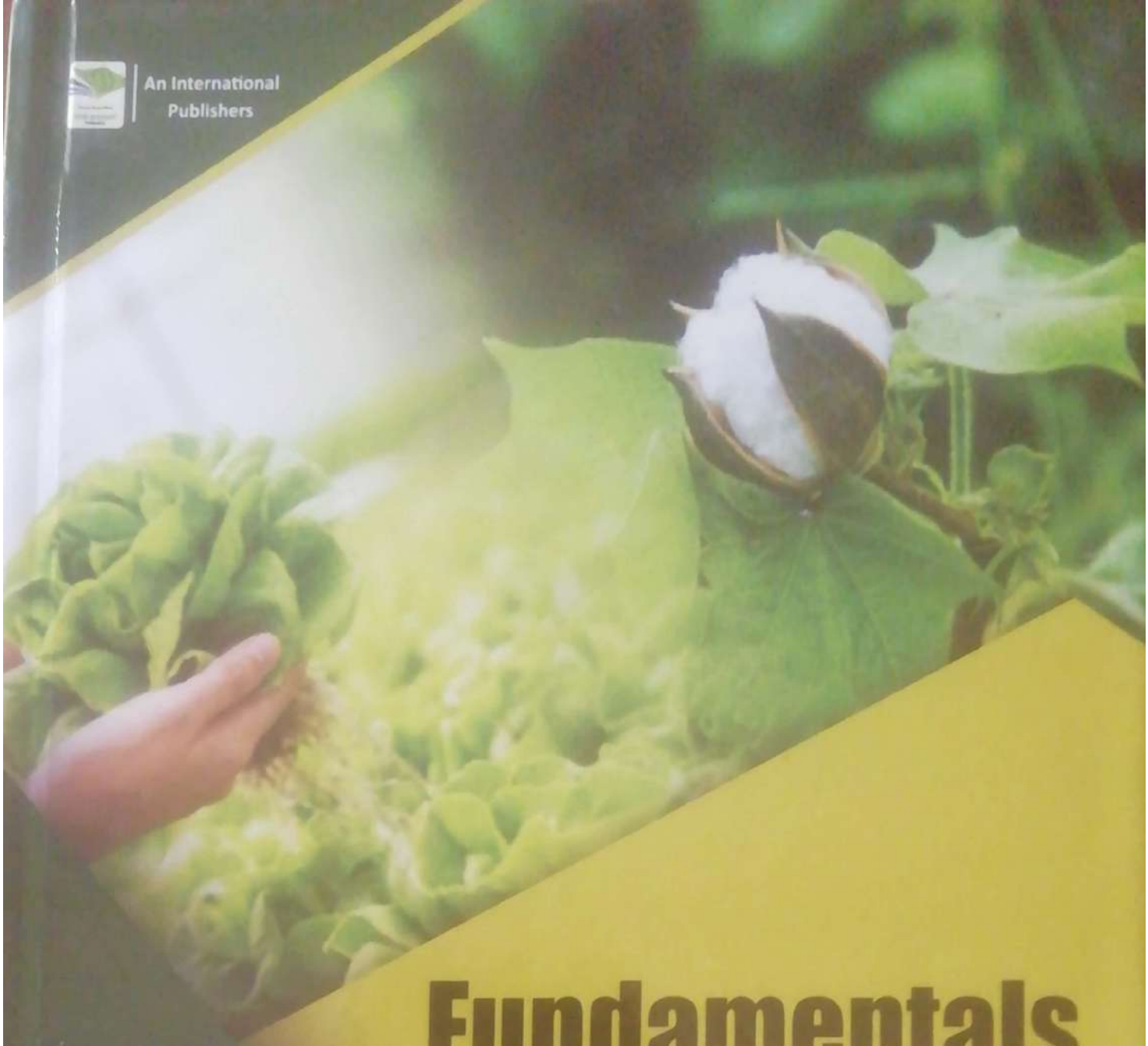
Agricultural Marketing and Consumer Behaviour

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Abstract

The interaction between buyers and sellers is called Market. A Market is an institution or a process that allows and sellers to interact. A market is not necessarily a **Marketplace,** which is a physical location where buyers and sellers go to exchange goods. In marketing, understanding consumer behavior has become very important for businesses. Consumer behavior refers to the study which analyzes how consumers make decisions about their wants, needs, buying or act with respect to a product, service or organization. It is very critical to understand the behavior of consumers to analyze the behavior of potential consumers towards a new product or service. It is also very useful for companies to identify opportunities which have not yet been met. In marketing, understanding consumer behavior has become very important for businesses. Consumer behavior refers to the study which analyzes how consumers make decisions about their wants, needs, buying or act with respect to a product, service or organization. It is very critical to understand the behavior of consumers to analyze the behavior of potential consumers towards a new product or service. It is also very useful for companies to identify opportunities which have not yet been met. In this chapter we are going to understand about agricultural marketing, Characteristics of market, classification of market, functions of market and market functionaries. This chapter also describes consumer behavior, types of consumer behavior, importance of consumer behavior and factors affecting consumer behavior.



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कृषि वानिकी का परिचय

(INTRODUCTION TO AGROFORESTRY)

अमिता शर्मा
जन्मेजय शर्मा
एस. सी. श्रीवास्तव
लाखन सिंह मोहनिया



KALYANI

लेखक-परिचय



डॉ. अमिता शर्मा, राजमाता विजयाराजे सिंधिया कृषि विश्वविद्यालय ग्वालियर में वैज्ञानिक (कृषि वानिकी) के पद पर पदस्थ हैं। इन्हें शिक्षण, अनुसंधान एवं प्रसार में 20 वर्षों का अनुभव है। इन्होंने एम.एस.सी. व पीएच.डी. के शोध कार्य मे 20 से अधिक छात्रों का मार्गदर्शन किया। साथ ही 40 से अधिक शोध पत्र एवं 15 लोकप्रिय लेख प्रकाशित किए। इनके द्वारा 2 अन्य पुस्तकें भी प्रकाशित की गई हैं।



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डॉ. शरद चंद्र श्रीवास्तव, राजमाता विजयाराजे सिंधिया कृषि विश्वविद्यालय ग्वालियर में वरिष्ठ तकनीकी अधिकारी (कृषि अर्थशास्त्र) के पद पर पदस्थ हैं। इन्हें शिक्षण, अनुसंधान एवं प्रसार में 20 वर्षों का अनुभव है। इन्होंने एम.एस.सी. व पीएच.डी के शोध कार्य मे 29 छात्रों का मार्गदर्शन किया। साथ ही 47 शोध पत्र प्रकाशित किए। इनके द्वारा 2 अन्य पुस्तकें भी प्रकाशित की गई हैं।



लाखन सिंह मोहनिया, राजमाता विजयाराजे सिंधिया कृषि विश्वविद्यालय ग्वालियर में अनुसंधान विद्वान (पीएच.डी.) शोधार्थी के रूप में कार्यरत है। इन्होंने राजमाता विजयाराजे सिंधिया कृषि विश्वविद्यालय ग्वालियर से कृषि में स्नातक और सस्य विज्ञान में स्नातकोत्तर किया। साथ ही कृषि वैज्ञानिक चयन मंडल द्वारा आयोजित होने वाला नेट क्वालीफाई किया एवं 7 शोध पत्र और 10 लोकप्रिय लेख प्रकाशित किए हैं। इनके द्वारा 2 अन्य पुस्तकें भी प्रकाशित की गई हैं।

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About the Book

This book entitled "New Horizon in Climate Smart Agriculture" considers the most typical of the expected impacts of climate change on crop production, and the opportunities that exist for adapting to these changes and mitigating climate change through the sustainable intensification of crop production. This book aims to address post-graduate students and research scholars.



Dr. Shailendra Singh has done M.Sc. (Ag) from University of Agricultural Sciences, Dharwad, Karnataka and Ph. D. from Maharana Pratap University of Agriculture and Technology, Udaipur Rajasthan in the discipline of Agronomy with ICAR fellowships. He has more than 8 years experience of research and teaching. He started his carrier as Assistant Professor in 2015 from Navsari Agricultural University, Gujarat. Presently he is serving S.K.N. Agriculture University, Jobner, Rajasthan. During his professional journey he has published 12 research papers, 2 books and several extension publications. He has guided two masters students and acted as minor advisor for five research scholars.



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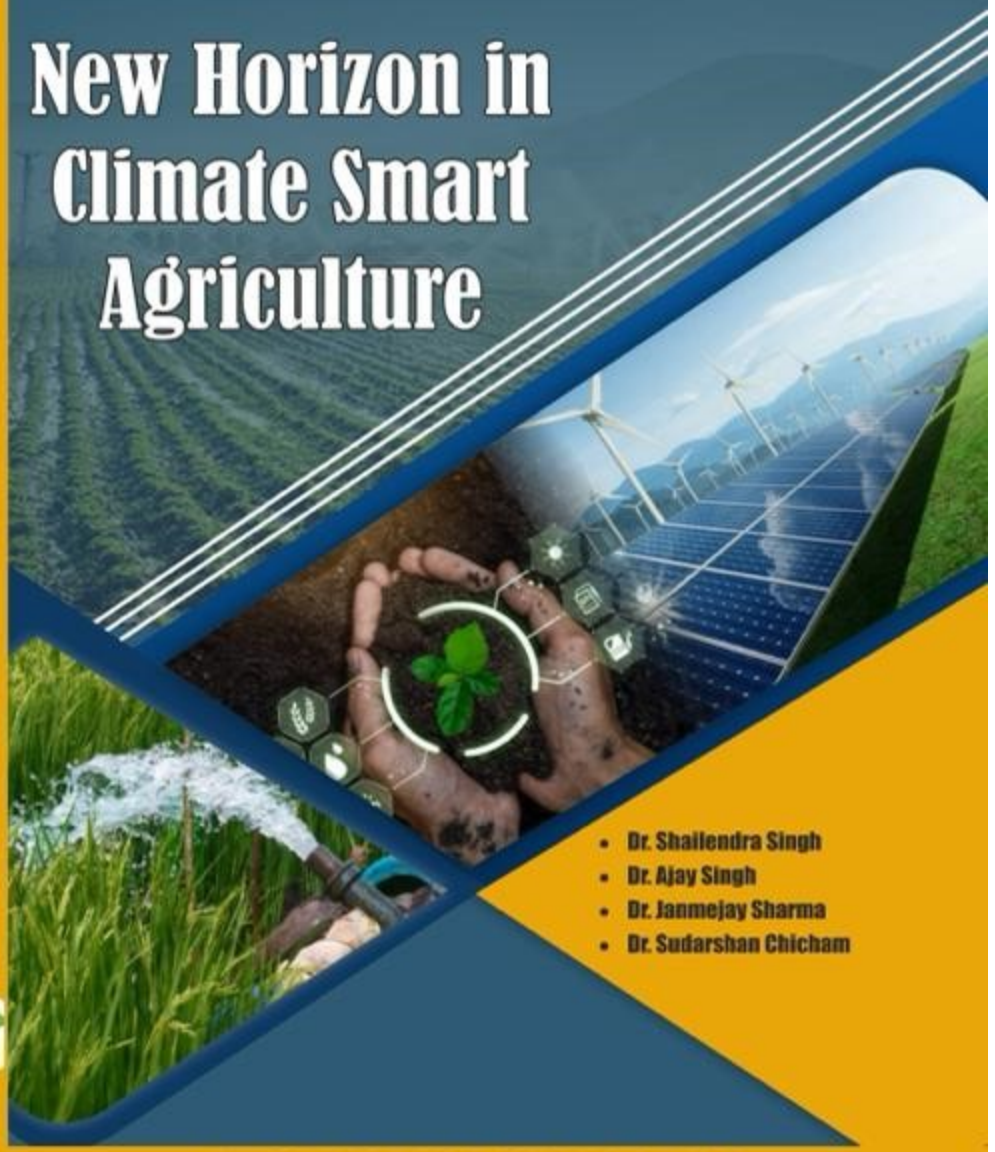
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Chapter 17

Constraints and Prospect in Rainfed Region

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Rainfed farming is a significant agricultural practice globally, providing sustenance to millions of farmers in regions where irrigation is limited. However, it faces numerous challenges and offers both promising prospects and potential risks. The problem lies in its heavy reliance on rainfall, making crops vulnerable to erratic and insufficient precipitation patterns, leading to crop failures, food insecurity, and economic distress for farmers. Climate change exacerbates these challenges, causing unpredictable weather patterns, frequent droughts, and more intense storms. As a result, farmers struggle to adapt their traditional practices to cope with the changing climate, hindering agricultural productivity and income stability.

Despite these issues, rainfed farming also holds prospects. Implementing sustainable water management practices, such as rainwater harvesting and conservation techniques, could mitigate water scarcity. Additionally, embracing climate-resilient crop varieties and modern agricultural technologies can enhance productivity and income. Furthermore, governmental support, infrastructural investments, and farmer training programs can create a conducive environment for rainfed farming's growth. In brief, addressing the challenges through sustainable practices and technological advancements could unlock the potential of rainfed farming,

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Chapter 19

Integrated Farming System (IFS) for Sustainable Livelihood Security

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INTRODUCTION

Integrated farming systems (IFS) is an eco-friendly approach in which waste of one enterprise becomes the input of another thus its make more efficient use of resources from the farm. IFS as a mixed farming system that consists of at least two separate but logically interdependent parts of a crop and livestock enterprises. IFS helps in improving the soil health, weed and pest control, increase water use efficiency and maintains water quality. In integrated farming system the use of harmful chemical fertilizers, weed killers and pesticides should be minimized and also provide safeguards to the environment from the adverse effects. Integrated farming system improves economic condition of the small and marginal farmers which enhanced the education, health and social obligations and overall improvement in livelihood security. Though IFS approach the use of chemicals (fertilizers and pesticides) can be reduced to provide chemical free healthy food to the society.

To meet the multiple objectives of poverty reduction, food and nutritional security, competitiveness and sustainability, several researchers have recommended the farming systems approach to research and development. A farming system is the result of complex interactions among a number of inter-dependent components, where an individual farmer allocates certain quantities and qualities of four factors of production, namely land, labour, capital and management to which he has access (Mahapatra, 1994). Farming systems research is considered as a powerful tool for natural and human resource management in developing countries including India. This

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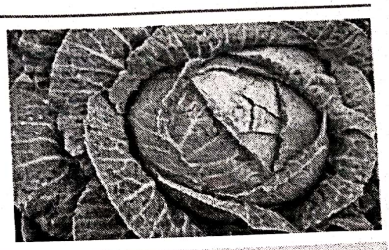
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Chapter 15

Production Technology of Cabbage (*Brassica oleracea* var. Capitata)

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Botanical name: *Brassica oleracea* var. capitata
Family : Brassicaceae/ Cruciferae
Origin: Mediterranean region
Chromosome no. 2n=2x:18
Type of fruit: Siliqua



INTRODUCTION

It is an important Cole crop. From nutrition point of view, it ranks very high. It is used alone or mixed with other vegetables like pea or potato etc. Young green heads contain more vitamin A than old ones. The peculiar flavour in cabbage head is because of a glycoside sinigrin, which carries sulphur. Cabbage contains vitamin U, which is very useful in ulcer in stomach.

The word cabbage is introduced from the French word *Cobache* meaning head. The head of cabbage is ended with various thick overlapping smooth leaves, which envelop a smooth terminal bud. Sometimes-small heads of 5 or 7.5 cm diameter are formed, which are known as cabbage sprouts, having no commercial importance. The heads of cabbage are classified as round or ball head, flat or drumhead and conical head *etc.*

Climatic requirement:

It grows generally in cool and moist weather, and is very hardy to frost. In hot and dry weather, its quality becomes poor due to the enlargement of fibres and bitter taste, and much of the flavour is lost due to excessive high temperature. The cabbage plants do not form heads at a temperature above 30°C. Germination is best at a soil temperature of 13-16°C. Well-hardened seedlings tolerate -2 to -5°C temperature.

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A Practical Manual on Soil Testing

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Chapter - 5

Effect of Plant Growth Regulators on *in vitro* Morphogenesis and Regeneration of *Amaryllis belladonna* L. from Different Explants Cultures

M.K. Tripathi, Niraj Tripathi, Sushma Tiwari, Devendra K. Payasi, Deepa Bhatt, Yashi Singh Tomar and Sharad Tiwari

Abstract

Bulb scale, leaf disc and mature embryos explants *viz*., were inoculated on diverse fortifications of MS medium to accomplish the best *in vitro* response. For bulb scale explant culture, induction medium MS2N.5B evidenced better for callus initiation. Nutrient medium MS.4Td displayed higher shoot proliferating competence, while number of shoot (s) per explants and shoot of higher length were recognized on culture media MS2N.5B/ MS3N.5B. In respect to leaf disc culture, induction medium MS2D.5B/ MS3D.5B encouraged higher degree of callus induction. Whereas, higher morphogenic calli formation was showed by inoculation medium MS2N.5B/MSN.5B. However, plantlets in higher frequencies were attained on regeneration medium MS.5Td/MSB. Cultured mature embryos initiated calli in higher frequencies on culture medium MS2D.5B, while nutrient medium MSN.5B revealed higher number of shoot proliferating explants. While, number of shoots per explant in higher proportions were attained on nutrient medium MSN.5B/MSB. Nevertheless, shoot of higher length was recovered on culture medium MSB. Higher *in vitro* rooting response (root proliferating efficiency, number(s) of roots and mean root length) was investigated on rooting medium MS2IB. The plantlets were transferred to pots and hardened in Environmental Growth Cabinet followed by Net House during initially weaning period and subsequently transferred to field efficaciously. Phenotypic normal plants were obtained.

Keywords: Amaryllis belladonna, bulb scale, mature embryo, leaf disc, organogenesis, somatic embryogenesis and plantlet regeneration

Abbreviations: MS-Murashige and Skoog medium; Wh-White's medium; B_5: Gamborg's medium; NAA-α-Naphthalene acetic acid; 2,4-D-2,4-dichlorophenoxyacetic acid; 2,4,5-T-2,4,5-trichlorophenoxyacetic acid;

Chapter - 6
Plant Regeneration from Cultured Cormel Explants in Gladiolus (*Gladiolus hybridus* Hort.)

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Chapter - 6

Plant Regeneration from Cultured Cormel Explants in Gladiolus (*Gladiolus hybridus* Hort.)

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Abstract

Cormel of gladiolus (*Gladiolus hybridus*) was inoculated on diverse modifications of MS medium supplemented with various auxins and cytokinins in different concentrations as alone along with in diverse combinations. Regarding *in vitro* response, culture medium MS2D.5B/MS2N.5B (MS + 2.0 mgl^{-1} 2,4-D + 0.5 mgl^{-1} BA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar/MS + 2.0 mgl^{-1} NAA + 0.5 mgl^{-1} BA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) encouraged higher callus initiation. Inoculation medium MS2N.5B/MSN.5B(MS + 2.0 mgl^{-1} NAA+ 0.5 mgl^{-1} BA+ 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar/MS + 1.0 mgl^{-1} NAA+ 0.5 mgl^{-1} BA+ 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) displayed higher shoot proliferating efficiency and shoot of higher length, Whilst, higher number of shoot (s) per explant showed by regeneration medium MS3B/MS2B (MS + 3.0 mgl^{-1} BA+ 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar/MS + 2.0 mgl^{-1} BA+ 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar). For *in vitro* rooting, higher root proliferating ability was recognized with rooting medium MS.5IB.5Kn (MS + 0.5 mgl^{-1} IBA + 0.5 mgl^{-1} Kinetin + 15.0 gl^{-1} sucrose + 7.5 gl^{-1}agar), whereas number of root (s) with higher length were convalesced on rooting medium MS.5IB (MS+0.5 mgl^{-1} IBA+15.0 gl^{-1} sucrose+ 7.5 gl^{-1} agar). Phenotypically normal plantlets were attained and subsequently transferred to pots and hardened in Environmental Growth Cabinet and Net House and transferred to field efficaciously.

Keywords: *Gladiolus hybridus*, cormel, direct organogenesis, indirect organogenesis, plantlet regeneration, cormlet formation

Abbreviations

MS: Murashige and Skoog's medium; B_5: Gamborg's medium; Wh: White's medium; BA: 6-benzylaminopurine; TDZ: Thidiazuron; 2-ip: N-isopentenyl amino purine; Kn-Kinetin; NAA: α-Naphthalene acetic acid; 2,

Foreword by
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Horticulture for Nutrition and Income Security



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Production Technology of Ornamental Plants



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डॉ. प्रवीण बर्डे • डॉ. संतोष कुमार मईडा
डॉ. प्रवीण कुमार सिंह गुर्जर

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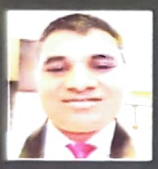
डॉ. धन सिंह मंडलोई वर्ष 2003 से राजमाता विजयाराजे सिंधिया कृषि विष्वविधालय, ग्वालियर--के,एन,के उधानिकी महाविधालय, मंदसौर (म.प्र.) में प्रक्षेत्र विस्तार अधिकारी के पद पर उन्होंने 14 वर्षों तक कार्य किया हैं। इन्होने इसके पष्चात वर्तमान में वर्ष 2016 से राजमाता विजयाराजे सिंधिया कृषि विष्वविधालय, ग्वालियर में तकनीकी अधिकारी (उधानिकी) के रूप में कृषि विज्ञान केंद्र, धार (म.प्र.) पिछले छः वर्षों से है। इन्होने अपनी बी.एससी. (कृषि) आर.ए.के. कृषि महाविद्यालय, सीहोर वर्ष 2000 में जे.एन.के.वी.वी., जबलपुर (म.प्र.) तथा एम.एससी. (उधानिकी) फल विज्ञान से उधानिकी महाविधालय, मंदसौर (राजमाता विजयाराजे सिंधिया कृषि विष्वविधालय), ग्वालियर (म.प्र) से की है। उन्होंने कृषि वैज्ञानिक भर्ती बोर्ड, नई दिल्ली से उधानिकी (फल विज्ञान) में नेट पास किया है। उन्होंने आदिवासी किसानों, विस्तार कर्मियों और विभिन्न लाइन विभागों के साथ कृषि/बागवानी में काम करने का 19 से अधिक वर्षों का अनुभव किया है। डॉ. धन सिंह मंडलोई को आई सी.ए.आर, नई दिल्ली से सर्वश्रेष्ठ कृषि विज्ञान केन्द्र पुरस्कार (राष्ट्रीय 2020) से सम्मानित किया गया। उन्हें सोसाइटी ऑफ ट्रॉपिकल एग्रीकल्चर, नई दिल्ली से साइंटिस्ट ऑफ द ईयर 2020, एटीडीएस, उत्तर प्रदेश से बेस्ट कृषि विज्ञान केन्द्र एक्सटेंशन साइंटिस्ट अवार्ड 2020 से सम्मानित किया गया है। एवं उन्होंने राष्ट्रीय और अंतर्राष्ट्रीय पत्रिकाओं में 04 पुस्तकें, 15 शोध पत्र, 04 पुस्तक अध्याय और 21 लोकप्रिय लेख, 5 विस्तार बुलेटिन प्रकाशित किए हैं।



डॉ. मनोज कुमार कुरील ने जे.एन.के.वी.वी, जबलपुर से कृषि में स्नातक और फल विज्ञान में विशेषज्ञता में उधानिकी में स्नातकोत्तर उपाधि उसी विश्वविद्यालय द्वारा उन्हें प्रदान की गई। उन्होंने 2004 में राष्ट्रीय पात्रता परीक्षा (नेट) उत्तीर्ण की। वर्ष 2003 में वरिष्ठ उधानिकी विकास अधिकारी के रूप में अपना पेशेवर करियर शुरू किया और 2007 में सहायक निदेशक, उधानिकी के रूप में पदोन्नत हुए, लेकिन शिक्षण में रुचि के कारण उन्होंने आकर्षक पद छोड़ दिया और कृषि महाविद्यालय, खंडवा, राजमाता विजयाराजे सिंधिया कृषि विश्वविधालय, ग्वालियर में सहायक प्रोफेसर (उधानिकी) के रूप में शामिल हो गए। वर्ष 2017 में और तब से वहां सेवा दे रहे हैं। इनकी दो पुस्तकें, हैं। और उन्होंने अंतरराष्ट्रीय और राष्ट्रीय पत्रिकाओं में लगभग 21 शोध पत्र प्रकाशित किए हैं और विभिन्न पत्रिकाओं में विभिन्न लोकप्रिय लेख लिखे हैं। शिक्षा के अलावा वह महाविधालय के सहयोगी एनसीसी अधिकारी के रूप में भी कार्यरत हैं। उन्हें 2014 में अधिकारी प्रशिक्षण अकादमी, कैम्पटी (महाराष्ट्र) में एनसीसी (महानिदेशक के बैटन ऑफ ऑनर) के राष्ट्रीय पुरस्कार से सम्मानित किया गया था।



डॉ. प्रवीण बर्डे का जन्म 20 मार्च 1981 को ग्राम गढ़वा पोस्ट बिसनूर तहसील मुलताई जिला बैतूल मध्य प्रदेश में हुआ था। उन्होंने बी.एस.सी. (बागवानी) और एम.एससी। उद्यानिकी फल विज्ञान के.एन.के. उद्यानिकी महाविद्यालय मंदसौर (जे.एन.के.वी.वी., जबलपुर) मध्य प्रदेश और पीएच.डी. उद्यानिकी (फल विज्ञान) डॉ. बी.आर. अम्बेडकर सामाजिक विज्ञान विश्वविद्यालय महू (म.प्र.) वे वर्तमान में राजमाता विजयाराजे सिंधिया कृषि विष्वविधालय, ग्वालियर में फल अनुसंधान स्टेशन, इटखेड़ी, भोपाल (म.प्र.) में उद्यानिकी सहायक के पद पर हूँ। उन्होंने उद्यानिकी अनुसंधान और शैक्षणिक क्षेत्र में व्यापक अनुभव है। प्रासंगिक अनुशासन में उनके उत्कृष्ट योगदान के लिए उन्हें जनपरिषद सोसाइटी से युवा वैज्ञानिक पुरस्कार मिला। 7 शोध पत्र प्रकाशित, 10 लेख, 9 पर्चे, कई राष्ट्रीय और अंतर्राष्ट्रीय सम्मेलन में भाग लिया और प्रशिक्षण कार्यक्रम में दिए गए कई व्याख्यानों ने प्रशिक्षण समन्वयक के रूप में प्रशिक्षण आयोजित किया।



डॉ. संतोष कुमार मईडा पुत्र श्री नानूराम मईडा का जन्म 1 जनवरी 1986 को रतलाम मध्य प्रदेश में हुआ था। उन्होंने बीएससी उद्यानिकी डिग्री और एमएससी उद्यानिकी (सब्जी विज्ञान) से के.एन.के. कॉलेज उद्यानिकी महाविद्यालय मंदसौर, राजमाता विजयाराजे सिंधिया कृषि विश्वविद्यालय ग्वालियर मध्य प्रदेश से डॉक्टर ऑफ फिलॉसफी (पीएचडी) उद्यानिकी (सब्जी विज्ञान) से महात्मा गांधी चित्रकूट ग्रामोदय विश्वविद्यालय चित्रकूट सतना मध्य प्रदेश से वर्ष 2022 में डॉक्टरेट डिग्री पूरी की और दो बुक प्रकाशित हुई हैं। और तीन रीसर्च पैपर प्रकाशित हुआ। और वह वर्तमान में राजस्थान सरकार में सहायक प्रोफेसर उद्यानिकी के पद पर कृषि महाविद्यालय बोरवट, बांसवाड़ा राजस्थान में पदस्थ हैं।



डॉ. प्रवीण कुमार सिंह गुर्जर वैज्ञानिक उधानिकी पद पर राजमाता विजयाराजे सिंधिया कृषि विश्वविधालय, ग्वालियर कृषि विज्ञान केंद्र, लहार (म.प्र.) में वर्ष 2007 से हूँ। इन्हीनें जे.एन.के.वी.वी, जबलपुर से कृषि में स्नातक और फल विज्ञान में विशेषज्ञता में उधानिकी में स्नातकोत्तर उपाधि उसी विश्वविद्यालय द्वारा उन्हें प्रदान की गई। इन्हीनें स्नातक स्नातकोत्तर उपाधि एवं पीएचडी विधार्थीयो को पढाया तथा लगभग 20 विधार्थीयो गाईड किया गया। इनकी तीन प्रयोगिक पुस्तकें, 7 विस्तार बुलेटिन हैं। और उन्होंने अंतरराष्ट्रीय और राष्ट्रीय पत्रिकाओं में लगभग 40 शोध पत्र प्रकाशित किए हैं और विभिन्न पत्रिकाओं में विभिन्न लोकप्रिय लेख 45 लिखे हैं।

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Natural Resource Management in terms of Crop Germplasm in India

Jaya Rathore, Pramod Kumar and R.S. Sikarwar

INTRODUCTION:

The sums total of hereditary material i.e. all the alleles of various genes, present in a crop species and its wild relatives are referred to as germplasm. This is also known as genetic resources or gene pool or genetic stock. Germplasm can be a seed or another parts of the plant e.g., a leaf, a piece of stem, pollen or even just a few cells that can be turned into a whole plant. Germplasm contains the information for a species, genetic makeup a valuable natural resource of plant diversity which plays a vital role for sustainable crop production.

NEED FOR MANAGEMENT/CONSERVATION OF CROP GERMPLASM:

A wide genetic base is essential for the development of new genotypes capable of surviving under heterogeneous environment. Selecting, collecting, exchanging, and preserving crop germplasm resources are not new activities or issues. They are in fact as old as agriculture itself, as old as our knowledge of growing crops for food. But what is new, and pressing, is how to make national and international decisions about managing these activities for the future.

There is a global recognition that biodiversity at all levels e.g., gene pools, species and biotic communities is important for many reasons but it is being rapidly diminished by habitat destruction and other damaging influences resulting from human population growth, climate change, pollution and economic expansion. Habitat destruction, genetic homogeneity in farming systems and alien species invasion are some of the causes of genetic erosion. Loss of genetic diversity has serious implications on economic and social development of any nation.

Therefore, conservation, management and sustainable use of crop germplasm are the fundamental to ecologically sustainable.development and food security of a nation.

STRATEGIES FOR CONSERVATION OF CROP GERMPLASM

There are two broad strategies for conservation of crop germplasm

1. IN-SITU CONSERVATION-

rmplasm is maintained in the natural habitats where they occur.

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CLIMATE CHANGE: PERSPECTIVES AND IMPACT ON INDIAN AGRICULTURE

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CLIMATE CHANGE: PERSPECTIVES AND IMPACT ON INDIAN AGRICULTURE

Ekta Joshi[1], Priyadarshni A. Khambalkar[1], Akhilesh Singh[1], Pragati Agarwal[1], Neelam Singh[1], Shashi S. Yadav[1], D.S. Sasode[1], Popiha Bordoloi[2] and S. S. Bhadauria[1]

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The Intergovernmental Panel on Climate Change (IPCC) reported that humankind is causing global warming through the emission of greenhouse gases (GHGs), particularly carbon dioxide (CO_2) and methane (CH_4) (Stocker *et al.,* 2013). The GHGs play a vital role on the climate system by absorbing the long wave infrared radiation and causes warming the earth's atmosphere. Thus, radiation emitted from the surface through the atmospheric window is higher compared to radiation from the stratosphere (Schmithusen *et al*., 2015). CO_2 levels have been consistently increasing since preindustrial time and its daily mean value reached 400 ppm in May 2013 at the reference site of Mauna Loa, Hawaii (Monastersky, 2013). Most regions in the world are warming because of increasing concentrations of atmospheric CO_2 (Sid Perkins, 2015). This increase is caused by human activities, which is subsequently contributing to increase the earth's surface temperature (Huang *et al.,* 2015). Arid and semi-arid areas comprise about 30% of the Earth's land surface. Climate change and climate variability will likely have a significant impact on these regions. The variability of environmental factors may result in significant effects on regional climate and global climate (Wang *et al.,* 2010), especially the radiative forcing, via the biogeochemical pathways affecting the terrestrial carbon cycle. Global climate change has serious impacts on humans and ecosystems.

This climate change altered the atmospheric concentration which leads to warming of the global temperature by 0.7° C till the recent past. It has been estimated that the Climate Change will lead to increase in temperature between 1.8 and 4° C in the years to come. This increasing temperature has caused global warming and created consequent environmental changes (Singh *et al*., 2011) which lead to decreased precipitation and increased temperature. This climate change will have a significant impact in developing countries like India because of their low adaptation capacity. The agriculture sector is primarily vulnerable and may create risk to the rural population (IPCC, 2007). As it has been estimated by the World Bank that, if climate change continues unhindered, then average temperatures in India is likely to reach as high as 29.1° C by the end of the century (up from 25.1 °C currently). Increase in temperature of the earth may lead to decrease in the length of growing season and yield of most crops (Saseendran *et al.,* 2008; Lal *et al.*, 2020; Sharma *et al.*, 2020; Sagar *et al.*, 2019; Parth *et al.*, 2019; Sagar *et al.*, 2019; Rao, 2019; Sharma and Rao, 2019; Dharbale *et al.*, 2019; Nyath *et al.*, 2018; Singh *et al.*, 2018). The anticipated negative impact of global warming on the climate of India is large; influence livestock population, agriculture and related allied sectors and ecosystem. A wide range of studies have substantiated that agroforestry systems have unique opportunity to augment the carbon stocks in the terrestrial biosphere (IPCC, 2000; Albrecht and Kandji, 2003). It has been estimated that 63 million ha of land areas are suitable for agroforestry and making this system a quantitatively important carbon stock (Kandji *et al.,* 2012).

This article reviews the understanding of consequence of climate change on Indian agriculture, various environmental parameters, livestock's population and health parameter etc. to highlight some important aspects in particular. As the agriculture and animal production system which is vulnerable to climate change is itself a large contributor to potential global warming through emission of CO_2, methane and nitrous oxide. The paper also presents a comprehensive review of the effect of climate change and GHG emissions in Indian scenario.

Is Climate Change Really Happening?: What are the indicators? Climate Change is the long-term average of the weather in a given place. While the weather can change in minutes or hours, a change in climate is something that develops over longer periods of decades to centuries. Climate is defined not only by average temperature and precipitation but also by the type, frequency, duration, and intensity of weather events such as heat waves, cold spells, storms, floods, and droughts (Source: https://www.epa.gov/climate-indicators). Global Climate Change indicators The Earth's climate is



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Chapter 7
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Influence of Plant Growth Regulators on *In vitro* Morphogenesis in *Plumbago Zeylanica* Linn.

M. K. Tripathi [a, b*], G. Tiwari [c, d], Sushma Tiwari [b], Niraj Tripathi [e], Mohini Sharma [b], Shashank Bhargav [b], S. L. Patidar [c] and Sharad Tiwari [f]

DOI: 10.9734/bpi/rabs/v7/3679A

ABSTRACT

The Present investigation was aimed to recognize the most responding explants, plant growth regulators and calculate their optimal concentrations and other physical aspects unveiling *in vitro* morphogenesis in higher rates *via* culturing nodal sections and leaf discs explants. Leaf disc and nodal section explants of *Plumbago zeylanica* were inoculated on basal media amended with diverse concentrations and combinations of different auxins and cytokinins as alone as well as in amalgamations. For inoculated nodal section, nutrient medium MS2N.5B (MS+2. 0 mgl^{-1} NAA+ 0. 5mgl^{-1}BA) evidenced appropriate for higher degree of callus initiation (91.69%), whereas culture medium MS2B (MS+2.0 mg l^{-1} BA) displayed higher shoot proliferating competence (84.14%). While, nutrient medium MS2B.5N (MS+2.0 mg l^{-1} BA+0.5 mgl^{-1}NAA) formed shoot(s) in higher numbers (11.12) along with of bigger length (7.11 cm). For cultured leaf disc, inoculation medium MS2N (MS+ 2.0 mgl^{-1}NAA) induced callus in higher percentage (91.12%), nevertheless greater morphogenic calli formation (45.58%) and plantlets regeneration were attained on culture medium MSN.5Td (MS+1.0mgl^{-1}NAA + 0.5 mgl^{-1} TDZ). In current investigation, MS medium amended with either IBA or NAA at the concentration of 0.1 mg l^{-1} was proved to be optimal for inducing higher *in vitro* rooting response *i.e*., root proliferation, number(s) of roots and root length. Regenerants were established efficaciously under the field conditions after hardening with normal phenotypic characters.

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Keywords: Plumbago zeylanica; nodal section; leaf disc; direct and indirect organogenesis and plantlet regeneration.

ABBREVIATIONS

MS: Murashige and Skoog medium; WH: White's medium; B_5: Gamborg's medium; BA- 6-benzylaminopurine; TDZ-Thidiazuron; Kn-Kinetin; NAA- α-Naphthalene acetic acid; 2, 4-D- 2, 4- dichlorophenoxyacetic acid; GA_3: Gibberellic acid and IBA-Indole-3-butyric acid.

1. INTRODUCTION

Plumbago zeylanica L., a member of the Plumbaginaceae family, is a rambling subscandent perennial herb or under shrub [1-10]. Phytochemical analysis of Plumbago zeylanica extracts showed the presence of many constituents, including plumbagin, linoleic acid, palmitic acid, nonylnonanoate, stigmasterol acetate, lupeol acetate, friedelinol, lupeol, lupanone, sitosterone, and stigmasterol. Plumbagin (5-hydroxy, 2-methyl, 1-4 naphtho-quinone) is an important naphthoquinone which displays a broad range of pharmaceutical activities such as anticancer (Krishnaswamy and [5-6,4]., leishmanicidal [10], anti-microbial [11], antifertile [12], antimutagenic and insecticidal [13]. Plumbagin has also been shown to have glucogenic properties. *Plumbago zeylanica* L roots are widely used in China and other Asian countries to treat cancer, rheumatoid arthritis, dysmenorrhea, and extremity contusion [14, 7]. The root stimulates the secretion of sweat, urine and bile and has a stimulant action on the nervous system. Extract of the root is given inside or applied to the sodium uteri, causes abortion [15,12].

Propagation of chitrak by means of seed is very problematic owing to poor seed setting and quality, erratic germination and seedling mortality as under natural field circumstances [16]. Commercial exploration of wild grown plants has led to the speedy decline of this plant in its natural environment demanding alternate tactics for cultivation and propagation [17]. *In vitro* culture is progressively being employed for the propagation of medicinal plants for continuous conservation and propagation [18-40]. Numerous in *vitro* protocols have been used in efforts to supplement plumbagin production *in P. indica* including the induction of adventitious roots [41] and establishment of cell suspension cultures [42,6].

In chitrak*,* an array of explants has been employed proficiently to establish regenerable cultures *via* somatic embryogenesis and/or organogenesis are shoot tips [43-46], nodal segments [47-52,16,46,5], auxiliary bud [53-54,46,5], hypocotyls [43] and leaf disc [55-56,32,16,43,50,1,5] with fluctuating notch of accomplishment.

Even though, some studies have been steered in some laboratories throughout the world to attain fruitful *in vitro* culture system along with secondary metabolites production in *Plumbago zeylanica* with different successes rate, no work has been done so far in M.P. on these aspects. Further, there is no study under taken

so for to quantity the plumbagin magnitude in the callus and cell suspension of *Plumbago zeylanica.* Considering these points, an effort has been made to recognize the most responding explants, plant growth regulators and calculate their optimal concentrations and other physical aspects unveiling *in vitro* morphogenesis in higher rates *via* culturing nodal sections and leaf discs explants.

2. MATERIALS AND METHODS

To instigate with an initial experimentation, several fortifications of three basal media *viz*., MS [57], WH [58] and B_5 [59] were employed to explore better *in vitro* response of Local cultivar of *Plumbago zeylanica*. During the initial investigations, MS basal medium was found to be more effective compared to B_5 and WH medium. Subsequently, MS media was employed as basal medium for advance investigation. In addition to MS basal micro and macro salts, vitamins, and agar, two different auxins, *viz*., 2, 4-D and NAA and three cytokinins *i.e.*, BAP, kinetin and TDZ in variable concentrations in addition to BAP and TDZ in combination with NAA and 2, 4-D, 30,0 gl^{-1} sucrose and 7.5 gl^{-1} agar were supplemented to augment MS media. Readymade MS basal medium, plant growth regulators and other components were procured from Hi-Media® Laboratories, Mumbai, India.

Explant leaf discs were excised from *in vitro* grown plantlets, whereas, nodal sections were obtained from plants propagated in orchard. For *in vitro* grown plantlets, no surface sterilization was espoused; while for plants collected from orchard an intensive surface sterilization protocol was standardized before inoculation. The indeterminate shoots were excised and collected in distill water from orchard planted plants. Top 6 to 12 nodal segments were trimmed to 0.5-1.0-centimeter length and washed under running tap water for 30 minutes to eradicate adhering grime particles. Then nodal segments were placed into double distilled water comprehending 2% Tween 20 (v/v) for 30 minutes to eliminate the sticky fine particles. The cleaned segments were then subjected to 70% (v/v) ethanol for 1 minute tracked by treatment with diverse concentrations of Bavistin® (BASF, Germany) and aqueous solution of two dissimilar surface sterilizing agents *i.e.,* $HgCl_2$ and Ca $(OCl)_2$ in diverse concentrations and combinations for varied durations with initial vacuum of 100 psi. Lastly, the segments were rinsed 4-5 times with sterile double distilled water.

In inoculated baby food bottles, one piece of nodal section was inoculated. To acquire leaf disc explant, first and second leaves of tender shoots were cut into small square pieces (5-8 mm) with sharp edged scalpel and then inoculated in 100 x17 Petri dishes encompassing nutrient media. In each Petri dish, 4-5 pieces of leave discs were plated. Cultured baby food bottles and Petri dishes were sealed with Parafilm® and incubated under complete darkness at 25±2°C for a week. Later *in vitro* cultured explants were subjected to photoperiod regime of 16 hours light and 8 hours dark at an intensity of 2000-lux luminance provided by PAR lamps.

After 4-5 weeks of preliminary culturing, explants showing sign of organ formation directly on explant surface was subcultured on same medium (initial medium) for regeneration of plantlets. When shoots of proper length were not accomplished, they were afterward transferred to MS elongation medium amended with 1.0 mgl^{-1} GA_3, 20.0gl^{-1} sucrose and 7.5gl^{-1} agar. Inoculated baby food bottles /culture tubes were transferred to 25±2^oC temperature and photoperiod regimes of 60 mmol m^{-2} s^{-1} luminance provided by cool fluorescent tubes for 16 hr. When rooting of regenerants was not attained properly, shootlets were consequently transferred to MS rooting medium fortified with diverse concentrations of auxins: IBA and NAA, cytokinins BA and Kn as sole in addition to IBA in amalgamation with BA and Kn, 15gl^{-1} sucrose and 7.5 gl^{-1} agar. For elongation and rooting, reduced concentration of sucrose was employed based on work conducted by various scientists along with earlier experience of this laboratory [60-79].

Rooted plantlets were systematically splashed with running tap water to eradicate the sticking agar and were planted in 2.5 cm root trainers filled with 1:1:1 sand, soil and FYM sterilized mixture. Root trainers with transplanted plants were transferred to net house for adaptation. Lastly, acclimatized plants were subjected under field conditions. Experiment was laid out in Completely Randomized Design with two replications. Per replication approximately 100-120 explants were inoculated on each media. The arc-sine transformation was made before the analysis of data when data were recorded in percentage. The data were analyzed as per method recommended by Snedecor and Cochran [80].

3. RESULTS AND DISCUSSIONS

The elementary goal of *in vitro* culture of *Plumbago zeylanica* is to standardize consistent protocol for accomplishing plant regeneration in higher occurrences from assorted explants cultures with the intention of use them for mass *in vitro* propagation of required genotypes/cultivars or exclusive clones, secondary metabolites production and advance biotechnological works. Even though hypothetically each and every somatic cell of a plant is totipotent, callus initiation and regeneration competence of a cell is affected by many aspects *viz*., nature of explants, *in vitro* culture circumstances *i.e.,* establishment of aseptic culture, nutritional requirements of basal medium, types and relative concentrations of plant growth regulators supplemented in medium and other culture surroundings.

The proportion of growth and difference speckled significantly with the nutritional composition of basal medium. For chitrak tissue culture experiments, MS basal medium has been employed extensively former by numerous investigators [81-83,1, 5,16,42,43,47,46,53.54,55,56]. During current study basal MS medium was employed throughout the investigation, as this was proved more receptive as compared to WH and B_5 in progression of initial studies. On the basis of prior accomplishments in regenerating plants, we tested more than 100 different combinations of auxins and cytokinins with MS basal media as selection of concentration and combination of plant growth regulators is critical to shoot regeneration.

In current study, inoculated explants tracked either indirect organogenesis *i.e*., plantlets originated *via* callus formation or direct organogenesis: plantlets regenerated directly from inoculated explants alleyways of regeneration. The initial response of inoculated nodal sections was identical afterward 4-7 days and mostly sovereign from explant and nutrient media combinations. All explants became swollen and no callus proliferation was investigated during first few days. In direct organogenesis, adventitious structures were shaped on explant surface. In nodal segment cultures, the nodal segment excised from top 2-5 nodes could not induce higher bud break, which may be owing to tender nature of buds. The acquaintance of explants with sterilant/antioxidants may kill these soft buds. Maximum bud break was evidenced from 6-14 nodes, perhaps attributable to juvenile state and capacity to endure the toxic effect of sterilant/antioxidants. These findings are in harmony to the results of Jose et al [44] and Patidar et al [5-7] who also reported higher morphogenic response tracked by plantlet regeneration that seems to be exceedingly dependent on position of explant in chitrak. Studies have revealed that lower or mid portion of the branch are easier to establish *in vitro* than upper part of the branch [84-87]. Adventitious formation started almost 3-5 days from initial culturing (Fig. 1A). Nevertheless, the period speckled from culture to culture and in a few cases adventitious structures fashioned after 10- 15 days. With time, these adventitious structures designed single shoot (Fig. 1 A) and multiple shoots (Fig. 1 B). In indirect slant, plantlets were regenerated from callus mass. Callus proliferation habitually happened after 4-7 days of inoculation (Fig. 1C). In indirect organogenesis, shootlets formed from the nodules arising on the surface of the callus (Fig. 1D). Shoot initiation started around 7 days from primary culturing, nevertheless, the interval differed from culture to culture and in some instances shootlets shaped after 15-20 days of primary culture (Fig. 1 E).

In inoculated leaf disc, generally phenomenon of indirect organogenesis was documented. Callus proliferation began from the cut boundaries afterward 7-10 days of inoculation and spread around the edges (Fig. 1F). Most of the calli, after prolonged inoculation on the culture media gave rise to plantlets (Fig. 1G- H). Nevertheless, transfer into elongation medium permitted plantlet formation in higher frequencies with elevated growth rate (Fig. 1I-J). When root formation was not accomplished, shootlets were subsequently transferred to the rooting medium. Rooted plants were transferred under $28\pm2^{o}C$ and 60±5% Relative Humidity for 20-25 days in an Environmental Growth Cabinet for initial hardening. Well along these regenerants were transferred to the nethouse/ poly house (Fig. 1K) for 25-30 days before transferring under the field conditions (Fig. 1 L). The plants, after survival in the net house conditions, were evaluated visually based on their phenotypic appearance. Even though the characters were not counted quantitatively, regenerated plants were found phenotypically normal and true to the type.

The analysis of variance accessible in Tables 1-6 revealed highly significant ($p<0.01$) differences among the response of diverse inoculation media combinations in terms of overall callus induction, shoot proliferating efficiency, number (s) of shoot(s) of higher length for nodal segment culture and for callus

induction, morphogenic calli formation and plantlet regeneration for cultured leaf discs for the most of examined parameters. It designates the incidence of substantial magnitude of variation amongst the diverse nutrient media combinations. Moreover, the response of culture media combinations varied significantly ($p<0.01$) for the various *in vitro* features. Nevertheless, non-significant differences were also investigated among different nutrient media combinations fortified with auxins alone for number (s) of shoot(s) and culture media combinations amended with auxins in addition to cytokinins alone for mean shoot length.

For cultured nodal segment, nutrient media MS2N.5B (fortified with comparatively higher magnitude of an auxin in association with a lower amount of a cytokinin) amenities higher notch of callus initiation. Nutrient media MS2D and MSD (comprehending a modest concentration of 2,4 -D) also induced callus in higher incidences than nutrient medium supplemented with other auxin like NAA and cytokinins in similar concentration which advised that 2,4-D is better for callus induction from inoculated nodal segments. In contest to shoot proliferating capability, numbers of shoots and mean shoot length, inoculation media encompassing auxins (alone) in differing concentrations produced shoot proliferation in lower efficiency, *i.e*., lesser number of explants induced shootlets, lesser numbers of shoot (s) per explant and shoots of minimum length. Shoot proliferation has not evinced on medium amended with 2,4-D supplied as alone, nevertheless medium supplemented with NAA responded up to some extent which is the opposing to the results of who attained higher *in vitro* responses on medium amended with NAA as alone.

In previous investigations, Skoog and Miller [88] and Evans *et al.* [89] reported that cytokinin is essential in shoot organogenesis. Hereafter, to achieve the best *in vitro* response basal medium was also amended with diverse types of cytokinins in varying concentrations. In current investigation, culture media amended with cytokinin as sole in moderate concentrations (BAP in rage of 2.0-3.0 mgl^{-1} and Kinetin in range of 1.0-2.0 mgl^{-1}) were responded well, (more than 80% explants induced shootlets) advised that a moderate concentration of cytokinin is essential for this determination. Previous studies also confirmed that lower level of BAP induced early bud sprouting in many. Multiple shoot induction required the attendance of a cytokinin in the nutrient medium, which is in meticulously pertinent to results of other investigations in chitrak [5, 46,50, 54]. Nevertheless, number(s) of shoot (s) with higher length were improved by adding auxins and cytokinins into nutrient medium in amalgamations. Culture media MS2B.5N/ MSB.5N amended with relative higher concentrations of a cytokinin (BAP) in association with a lower concentration of an auxin (NAA), displayed higher *in vitro* response (more than11.12 shoots/explant and mean shoot length > 6.68 cm) than nutrient medium containing a higher concentration of cytokinins in addition to an auxin as alone. Parallel results have also been addressed by Rout et al. [90], Wei et al. [43], Mallikadevi et al. [1] and Sivanesan and Jeong [46] in chitrak.

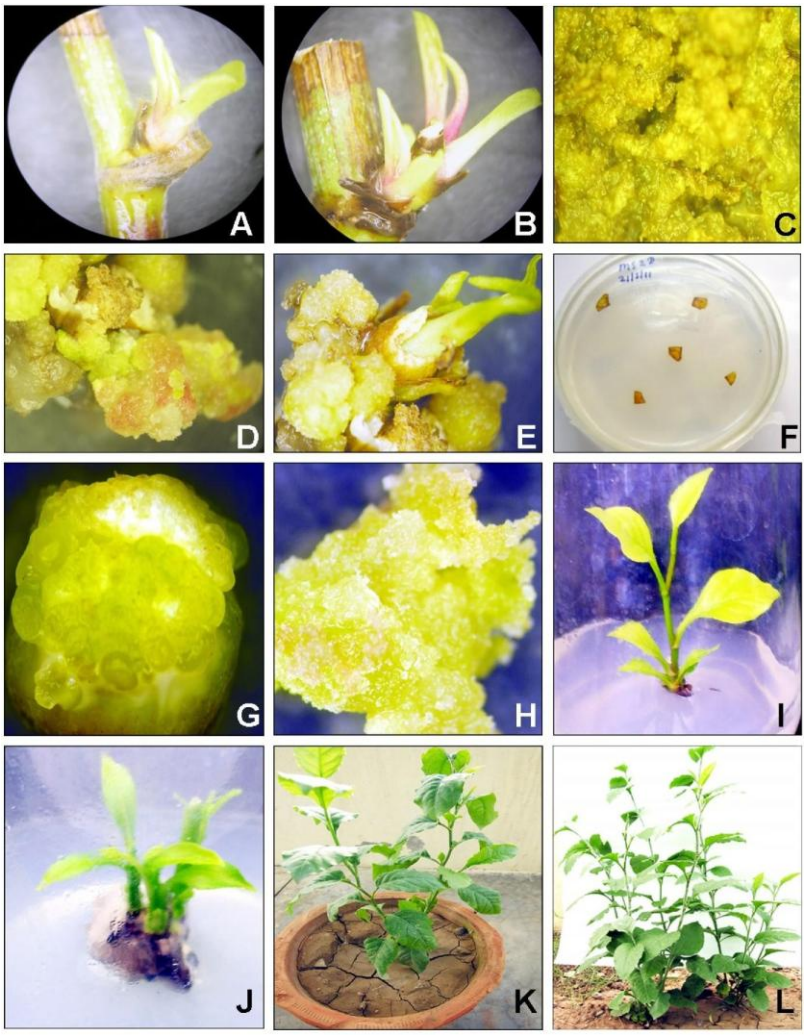


Fig. 1. Plant regeneration in *Plumbago zeylanica*: A. Formation of single shoot directly from cultured nodal segment after 10-15 days; B. Formation of multiple shoots directly from cultured nodal segment after 15-20 days; C. Formation of callus from cultured nodal segments; D-E. Shoot initiation from callus mass from cultured nodal segment after 25-30 days; F. Cultured leaf discs after 7-10 days; G. Initiation of protuberances on surface of callus mass; H. Multiple shoot initiation via indirect organogenesis from cultured leaf discs; I-J. Elongated shoot after 35-40 days in culture; K. Hardening of regenerant in Net House; and L. Regenerants transferred in field

Table 1. Effects of different auxins (alone) in varying concentrations on *in vitro* response of nodal segment cultures

Culture media	Plant growth regulators mgl^{-1}		*In vitro* response			
	2,4-D	**NAA**	**Callus induction (%)**	**Shoot proliferating explant (%)**	**No. of shoots/explant**	**Mean shoot length (in cm)**
MS.5D	0.5	-	81.31^{d}(64.36)	No response	No response	No\response
MSD	1.0	-	88.22^{b}(69.92)	No response	No response	No response
MS2D	2.0	-	91.23^{a}(72.77)	No response	No response	No response
MS3D	3.0	-	84.61^{c}(66.89)	No response	No response	No response
MS4D	4.0	-	76.33^{ef} (60.87)	No response	No response	No response
MS5D	5.0	-	74.53^{f}(59.67)	No response	No response	No response
MS.5N	-	0.5	79.22^{de} (62.86)	67.42^{b} (55.18)	2.94 (9.72)	3.07 (9.95)
MSN	-	1.0	81.25^{d} (64.32)	71.30^{a} (57.63)	2.92 (9.69)	3.67 (10.94)
MS2N	-	2.0	85.32^{c} (67.46)	62.13^{c} (52.00)	2.68 (9.25)	3.50 (10.67)
MS3N	-	3.0	78.13^{e} (62.10)	55.22^{d} (47.98)	2.60 (9.09)	2.76 (9.40)
MS4N	-	4.0	76.27^{f} (60.83)	49.62^{e} (44.76)	2.46 (8.81)	2. 92 (9.69)
MS5N	-	5.0	74.17^{f} (59.43)	45.53^{f} (42.42)	1.98 (8.09)	3.96 (11.38)
Mean			**80.88 (64.29)**	**58.55 (49.99)**	**2.60 (9.11)**	**3.31 (10.34)**
CD $_{0.05}$			**2.36**	**2.07**	**NS**	**NS**

Values within column followed by different letters are significantly different at 1% probability level

Table 2. Effects of different cytokinins (alone) in varying concentrations on *in vitro* response of nodal segment cultures

Culture media	Plant growth regulators mgl^{-1}		*In vitro* response			
	BA	**Kn**	**Callus induction (%)**	**Shoot Proliferating explant (%)**	**No. of shoots/explant**	**Mean shoot length (in cm)**
MS.5B	0.5	-	36.12^{c}(36.92)	66.12^{e} (54.38)	4.22^{c} (11.76)	3.03(9.88)
MSB	1.0	-	45.10^{a}(42.17)	75.09^{c} (60.04)	5.43^{b} (13.41)	4.46(12.11)
MS2B	2.0	-	30.23^{d} (33.34)	84.14^{a} (66.51)	8.23^{a} (16.63)	3.87(11.24)
MS3B	3.0	-	25.24^{e} (30.14)	83.79^{a} (66.24)	7.67^{b} (16.04)	5.54(13.55)
MS4B	4.0	-	19.42^{g} (26.13)	71.12^{d} (57.47)	6.34^{b} (14.53)	2.97 (9.77)
MS5B	5.0	-	15.20^{h}(22.93)	65.13^{e} (53.79)	5.69^{b} (13.74)	2.83 (9.53)
MS.5kn	-	0.5	31.55^{d} (34.16)	75.61^{c} (60.39)	4.32^{c} (11.91)	2.27 (8.44)
MSKn	-	1.0	41.58^{b} (40.14)	79.83^{bc} (63.31)	4.41^{c} (12.04)	3.28(10.30)
MS2kn	-	2.0	26.14^{e} (30.73)	82.12^{b} (64.97)	4.45^{c} (12.10)	4.61(12.32)
MS3kn	-	3.0	21.59^{f}(27.67)	71.12^{d} (57.47)	5.69^{b} (13.74)	5.71(13.77)
MS4kn	-	4.0	15.98^{h} (23.54)	65.15^{e} (53.80)	3.12^{c} (10.03)	4.95(12.78)
MS5kn	-	5.0	11.45^{i} (19.75)	55.63^{f} (48.21)	2.39^{c} (8.69)	3.35(10.42)
Mean			**26.63 (30.64)**	**72.90 (58.88)**	**5.16 (12.89)**	**3.91(11.18)**
$CD_{0.05}$			**2.13**	**4.27**	**2.34**	**NS**

Values within column followed by different letters are significantly different at 1% probability level

Table 3. Combined effects of different auxins and cytokinins on *in vitro* response for cultured nodal segments

Culture media combinations	Plant growth regulators mg^{-1}				*In vitro* response			
	BA	TDZ	2,4-D	NAA	Callus induction (%)	Shoot proliferating explant (%)	No. of shoots/explant	Mean shoot length (in cm)
MS.5B.5N	0.5	-	-	0.5	83.4^{b}(65.95)	71.62^{b}(57.79)	3.43^{e} (10.55)	6.24^{a}(14.44)
MSB.5N	1.0	-	-	0.5	79.16^{c}(62.82)	76.28^{a}(60.83)	10.21^{ab} (18.61)	6.68^{a}(14.93)
MS2B.5N	2.0	-	-	0.5	78.12^{c}(62.09)	79.23^{a}(62.87)	11.12^{a} (19.45)	7.11^{a}(15.42)
MS3B.5N	3.0	-	-	0.5	77.66^{d}(61.77)	75.58^{a}(60.37)	8.92^{b} (17.34)	6.29^{a}(14.47)
MS4B.5N	4.0	-	-	0.5	65.63^{f}(54.09)	67.93^{b}(55.49)	7.96^{c} (16.35)	6.68^{a}(14.93)
MS5B.5N	5.0	-	-	0.5	63.15^{f} (52.60)	51.21^{d}(45.68)	4.78^{d} (12.55)	5.15^{b}(13.05)
MSN.5B	0.5	-	-	1.0	89.23^{a} (70.83)	69.23^{b}(56.29)	3.29^{e} (10.32)	5.19^{b}(13.10)
MS2N.5B	0.5	-	-	2.0	91.69^{a} (73.25)	62.32^{c}(52.11)	7.96^{c} (16.35)	4.10^{b}(11.60)
MS3N.5B	0.5	-	-	3.0	85.17^{b} (67.33)	51.25^{d}(45.70)	6.32^{cd} (14.51)	5.12^{b}(13.01)
MS4N.5B	0.5	-	-	4.0	81.63^{d} (64.60)	48.55^{d}(44.15)	4.59^{d} (12.30)	4.76^{b}(12.53)
MS5N.5B	0.5	-	-	5.0	77.66^{d} (61.77)	40.38^{e}(39.44)	3.24^{e} (10.24)	3.29^{c}(10.32)
MS.5D.5Td	-	0.5	0.5	-	68.69^{e} (55.96)	61.13^{c}(51.41)	3.21^{e} (10.19)	2.67^{c} (9.23)
MSD.5Td	-	0.5	1.0	-	79.68^{c} (63.19)	51.13^{d}(45.63)	4.63^{d} (12.35)	1.77^{c} (7.64)
MS2D.5Td	-	0.5	2.0	-	81.24^{c} (64.32)	50.27^{d}(45.14)	3.16^{e} (10.10)	3.21^{c}(10.19)
MS3D.5Td	-	0.5	3.0	-	74.15^{d} (59.42)	40.28^{e} (39.38)	2.96^{e} (9.76)	3.57^{c}(10.78)
MS4D.5Td	-	0.5	4.0	-	68.24^{e} (55.68)	30.39^{f} (33.44)	2.68^{e} (9.25)	3.07^{c} (9.950
MS5D.5Td	-	0.5	5.0	-	58.23^{g} (49.72)	24.10^{g} (29.38)	2.12^{e} (8.12)	2.67^{c} (9.23)
MSTd.5D	-	1.0	0.5	-	78.23^{c}(62.17)	61.27^{c} (51.49)	7.19^{c} (15.51)	3.11^{c}(10.02)
MS2Td.5D	-	2.0	0.5	-	73.34^{d} (58.89)	51.25^{d} (45.70)	5.94^{d} (14.05)	2.41^{c} (8.72)
MS3Td.5D	-	3.0	0.5	-	61.23^{g} (51.47)	41.29^{e} (39.97)	3.12^{d} (10.03)	3.45^{c}(10.59)
MS.5Td.5N	-	0.5	-	0.5	79.24^{c} (62.88)	71.49^{b}(57.71)	8.10^{c} (16.50)	2.62^{c} (9.13)
MSTd.5N	-	1.0	-	0.5	71.72^{g} (57.85)	61.27^{c} (51.49)	6.54^{c} (14.77)	3.12^{c}(10.03)

Culture media combinations	Plant growth regulators mg^{-1}				*In vitro* response			
	BA	TDZ	2,4-D	NAA	Callus induction (%)	Shoot proliferating explant (%)	No. of shoots/explant	Mean shoot length (in cm)
MS2Td.5N	-	2.0	-	0.5	70.32^{g} (56.97)	58.69^{c} (49.99)	4.16^{d} (11.68)	2.41^{c} (8.72)
MS3Td.5N	-	3.0	-	0.5	65.32^{f}(53.90)	50.25^{d}(45.13)	2.18^{e} (8.25)	3.45^{c}(10.59)
Mean					**75.09(60.40)**	**56.10 (48.61)**	**5.33(12.88)**	**4.09 (11.36)**
CD $_{0.05}$					**4.42**	**4.23**	**1.78**	**2.06**

Values within column followed by different letters are significantly different at 1% probability level

Table 4. Effects of different auxins (alone) in varying concentrations on *in vitro* growth of cultured leaf discs

Culture media	Plant growth regulators $mg.l^{-1}$		*In vitro* response		
	2,4-D	NAA	Callus induction (%)	Morphogenic calli (%)	Plantlet regeneration (%)
MS.5D	0.5	-	66.10^{e} (54.37)	22.29^{d}(28.16)	$4.19e^{e}$ (11.720
MSD	1.0	-	81.12^{bc} (64.23)	25.13^{c} (30.07)	8.23^{d} (16.63)
MS2D	2.0	-	91.92^{a} (73.49)	16.84^{e} (24.21)	6.29^{de} (14.47)
MS3D	3.0	-	78.15^{c} (62.11)	14.34^{ef} (22.23)	3.11^{f} (10.02)
MS4D	4.0	-	74.61^{d} (59.72)	9.67g (18.09)	2.19^{f} (8.27)
MS5D	5.0	-	48.62^{g} (44.19)	5.11^{h} (13.00)	1.16^{f} (6.18)
MS.5N	-	0.5	71.25^{de} (57.56)	22.27^{d}(28.14)	11.16^{c} (19.49)
MSN	-	1.0	78.35^{c} (62.25)	26.23^{c} (30.79)	16.46^{b} (23.92)
MS2N	-	2.0	78.30^{c} (62.22)	39.13^{a} (38.70)	22.93^{a} (28.59)
MS3N	-	3.0	83.66^{b} (66.14)	35.14^{b}(36.34)	12.54^{c} (20.72)
MS4N	-	4.0	68.62^{e}(55.91)	22.14^{d}(28.05)	3.33^{f} (10.39)
MS5N	-	5.0	57.61^{f} (49.36)	12.81^{f} (20.95)	2.19^{f} (8.27)
Mean			**73.19 (59.30)**	**20.93 (26.56)**	**7.82 (14.89)**
CD $_{0.05}$			**4.08**	**2.48**	**2.18**

Values within column followed by different letters are significantly different at 1% probability level.

Table 5. Effects of different cytokinins (alone) in varying concentrations on *in vitro* growth of cultured leaf discs

Culture media	Plant growth regulators mgl^{-1}		*In vitro* response		
	BA	TDZ	Callus induction (%)	Morphogenic calli (%)	Plant regeneration (%)
MS.5B	0.5	-	20.23^{d} (26.71)	1 5.12^{c} (22.86)	26.13^{c} (30.73)
MSB	1.0	-	28.46^{a} (32.22)	24.23^{a} (29.47)	28.16^{c} (32.03)
MS2B	2.0	-	22.26^{cd} (28.13)	18.42^{b} (25.39)	23.22^{d} (28.79)
MS3B	3.0	-	11.47^{e} (19.77)	10.55^{d} (18.93)	19.85^{e} (26.44)
MS4B	4.0	-	10.68^{e} (19.05)	9.64^{d} (18.06)	11.68^{f} (19.96)
MS5B	5.0	-	7.33^{f} (15.67)	5.55^{e} (13.57)	4.32^{g} (11.91)
MS.1Td	-	0.1	19.77^{d} (26.38)	18.35^{b} (25.34)	28.44^{bc} (32.21)
MS.2Td	-	0.2	25.12^{b} (30.06)	24.52^{a} (29.66)	30.59^{b} (33.56)
MS.5Td	-	0.5	25.98^{b} (30.63)	24.98^{a} (29.97)	40.36^{a} (39.42)
MSTd	-	1.0	20.25^{d} (26.73)	18.59^{b} (25.52)	22.58^{d} (28.35)
MS2Td	-	2.0	18.35^{d} (25.35)	15.48^{c} (23.15)	19.66^{e} (26.30)
MS3Td	-	3.0	12.15^{e} (20.38)	9.18^{d} (17.61)	10.89^{f} (19.24)
Mean			**18.50 (25.09)**	**16.22 (23.29)**	**22.16 (27.41)**
CD $_{0.05}$			**2.45**	**2.63**	**2.47**

Values within column followed by different letters are significantly different at 1% probability level.

Table 6. Combined effects of different added auxins and cytokinins on *in vitro* response for cultured leaf discs

Culture media combinations	Plant growth regulators mg. l-1				*In vitro* response		
	2,4- D	NAA	BA	TDZ	Callus induction (%)	Morphogenic calli (%)	Plant regeneration %
MS.5D.5B	0.5	-	0.5	-	63.39^{h}(52.75)	28.50^{f} (32.25)	26.66^{g}(31.07)
MSD.5B	1.0	-	0.5	-	69.16^{g}(56.25)	25.32^{g} (30.19)	34.18^{d}(35.76)
MS2D.5B	2.0	-	0.5	-	85.58^{a}(67.67)	35.44^{d} (36.52)	31.13^{f} (33.90)
MS3D.5B	3.0	-	0.5	-	80.67^{b}(63.90)	32.12^{e} (34.51)	24.56^{gh}(29.69)
MS4D.5B	4.0	-	0.5	-	76.85^{c}(61.22)	21.19^{h} (27.39)	17.46^{i} (24.68)
MS5D.5B	5.0	-	0.5	-	55.61^{k}(48.20)	17.12^{i} (24.42)	5.96^{l} (14.08)
MS.5D.5Td	0.5	-	-	0.5	61.58^{l} (51.68)	32.62^{e} (34.81)	34.86^{d} (36.17)
MSD.5Td	1.0	-	-	0.5	68.43^{g} (55.79)	35.23^{d} (36.39)	31.13^{f} (33.89)
MS2D.5Td	2.0	-	-	0.5	76.48^{cd} (60.97)	38.86^{c} (38.55)	41.49^{c} (40.08)
MS3D.5Td	3.0	-	-	0.5	72.18^{e} (58.15)	28.68^{f} (32.36)	34.20^{d} (35.77)
MS4D.5Td	4.0	-	-	0.5	54.32^{k} (47.46)	25.62^{g} (30.39)	23.17^{h} (28.76)
MS5D.5Td	5.0	-	-	0.5	52.17^{l} (46.23)	19.89^{h} (26.47)	14.56^{k} (22.41)
MS.5N.5B	-	0.5	0.5	-	59.26^{j} (50.32)	28.58^{f} (32.30)	39.83^{c} (39.12)
MSN.5B	-	1.0	0.5	-	62.43^{hi} (52.18)	39.14^{c} (38.71)	41.31^{c} (39.98)
MS2N.5B	-	2.0	0.5	-	74.39^{d} (59.58)	39.45^{c} (38.89)	34.43^{d} (35.51)
MS3N.5B	-	3.0	0.5	-	71.46^{ef} (5.69)	32.67^{e} (38.84)	32.34^{ef} (34.64)
MS4N.5B	-	4.0	0.5	-	63.61^{h} (52.88)	25.23^{g} (30.14)	23.84^{h} (29.21)
MS5N.5B	-	5.0	0.5	-	61.46^{i} (51.61)	11.62^{j} (19.91)	7.71^{l} (16.08)
MS.5N.5Td	-	0.5	-	0.5	58.58^{j} (49.92)	41.39^{b} (40.02)	44.58^{b} (41.87)
MSN.5Td	-	1.0	-	0.5	63.48^{h} (52.80)	45.68^{a} (42.50)	51.12^{a} (45.62)
MS2N.5Td	-	2.0	-	0.5	72.10^{e} (58.10)	44.63^{a} (41.90)	41.49^{c} (40.08)

Culture media combinations	Plant growth regulators mg. l-1				*In vitro* response		
	2,4- D	NAA	BA	TDZ	Callus induction (%)	Morphogenic calli (%)	Plant regeneration %
MS3N.5Td	-	3.0	-	0.5	69.68^{fg} (56.57)	39.83^{bc} (39.12)	33.20^{de} (35.17)
MS4N.5Td	-	4.0	-	0.5	54.32^{k} (47.46)	24.23^{g} (29.47)	20.59^{l} (26.97)
MS5N.5Td	-	5.0	-	0.5	51.17^{l} (45.65)	12.62^{j} (20.79)	14.59^{k} (22.43)
Mean					**65.77 (54.38)**	**30.24 (33.04)**	**29.35 (32.22)**
CD $_{0.05}$					**1.81**	**1.90**	**2.06**

Values within column followed by different letters are significantly different at 1% probability level

For cultured leaf disc, findings evidently designated fluctuating response of plant growth regulators for callus proliferation. At lower levels both the auxins 2, 4-D (up to 2.0 mg. l^{-1}) and NAA (up to 3.0 mg. l^{-1}) have been found to initiate callus proliferation in higher frequencies. A similar result has also been documented by Mallikadevi et al. [1] for chitrak leaf disc culture. At higher levels of 2,4-D (≥2.0 mg. l^{-1}) callus turned blackish with retarded growth whereas cell mortality was observed at ≥4.0 mg. l^{-1} of 2,4-D. Auxin 2, 4-D initiated morphogenic calli and plantlet regeneration in very lower frequencies in all tested concentrations (0.5 - 5.0 mg. l^{-1}). At the higher concentration (≥5.0 mg. l^{-1}) of NAA, most of the callus were non-morphogenic and with the ageing changed colour from cream to dark brown without any further growth. Maximum callus initiation was documented on nutrient medium containing 2,4-D at 2. 0mgl^{-1}. Inoculation media comprising NAA twisted fast growing yellow and green compact callus, which exhibited advantageous consequence on the cell growth of morphogenic cultures. Plant regeneration efficiency decreased with the increased proportion of NAA.

Diverse concentrations of BAP and TDZ (as sole) added in the medium showed very poor callus induction and morphogenic calli formation. Nevertheless, nutrient medium amended with BAP and TDZ enhanced morphogenic and plantlet regeneration competence as compared to media added with NAA and/or 2, 4-D. Earlier investigations for establishing plant growth regulator type, combination and concentration discovered that barely an auxin or a cytokinin was not much operative for morphogenic calli initiation. Generally, all the plant growth regulators formed calli of diverse sizes and texture. Proportion of recurrence of callus was higher when an auxin or a cytokinin as sole were supplemented in culture medium. However, in both the cases, frequency of morphogenic calli was found to be low. Consequently, final experimentations were determined considering combination of an auxin and a cytokinin for realizing the best *in vitro* response.

Maximum morphogenic callus formations in addition to plantlet regeneration were documented on nutrient media amended with 1.0 mgl^{-1} NAA in association with lower concentration (0. 5mgl^{-1}) of a cytokinin TDZ. Analogous findings were also testified by Wei et al. [43]. Much lower results with 2, 4-D, NAA or BAP alone on Media MS5D, MS5N or MS5B (even in higher concentrations) as compared to combination of NAA with TDZ exposed that cytokinins as well as an auxin (alone) were insufficient for higher *in vitro* response. Nevertheless, nutrient medium amended with auxins (alone) initiated calli in higher frequencies as compared to culture medium comprehending cytokinins (alone). *In vitro* produced shoots were treated with GA_3 at the concentration of 1.0 mgl^{-1} gave the maximum shoot length. However, further increase in the concentration of GA_3 (≥ 1.0 mg. l^{-1}) decreased the elongation of shoots (data not presented).

In current research, rhizogenic incidence was investigated to be higher after transferring shootlets into rooting medium. In general, auxins like IBA [48,16,43,52,5,6], IAA [91,1,50,52] along with NAA [52,55] were effective in inducing *in vitro* rooting. In current investigation, MS medium added with 0.1-0.5 mg. l^{-1}IBA/NAA was found to be optimal for displaying higher *in vitro* rooting

response (data not presented). Maximum *in vitro* root proliferation, number of roots of higher length were documented on nutrient media MS.1IB/MS.1N. The findings evidently designated that rooting of *in vitro* shoots of chitrak necessitate lower concentrations of auxins (IBA/NAA) at higher nutrient status. Auxins encouraged adventitious root development on intact plants in addition to excised stems. Of these, IBA was the most operative than any other plant growth regulators in the most of the cases deceptively owing to it is not demolished by IAA oxidase or other enzymes and therefore continues longer [92-107]. The above findings are in conformity with the former results of Selvakumar et al. [48], Chaplot et al. [16], Wei and Yuan [43] and Lubaina et al. [52].

Both direct and indirect shoot organogenesis necessitate plant cells to undergo dedifferentiation and redifferentiation. These two processes are determined not only by exogenous PGRs supplemented in the culture medium, but also endogenous plant hormone levels [108]. Diverse tissues have different levels of endogenous plant hormones; therefore, the basis of explants has a critical impact on plant tissue culture. In this work, we first compared the nodal segment and leaf disc as explant sources, and found that nodal segments were a better source. Although, comparison between these two explants cultures were empirical as culture media employed were different. Nevertheless, trend shown in the present investigation proposed that explant (s) with higher regeneration potential may be employed for micropropagation and advance biotechnological work.

4. CONCLUSION

During the current study, we used the novel technique for nodal segment cultures, which may be attained from plants propagated in orchard as well as *in vitro* regenerated plantlets. The protocol developed for production of plantlets of *Plumbago zeylanica* may be employed consistently for propagation in a commercial scale and *ex situ* conservation of this imperative medicinal plant species. Leaf derived callus cultures may be exploited further for isolation of protoplast and genetic transformation. Root could be employed as explant source for deriving cell suspension cultures for producing and enhancing important secondary metabolites of chitrak by elicitation and immobilization procedures.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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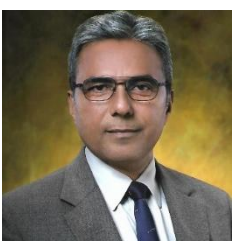
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Chapter 8
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Plant Regeneration in *Rauvolfia serpentina* (L.) Benth *via* Organogenic Mode

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ABSTRACT

An effectual protocol for rapid propagation of *Rauvolfia serpentina* has been standardized in present investigation. Leaf discs and nodal segments were inoculated on MS medium supplemented with diverse auxins and cytokinins in variable concentrations as individual along with in different amalgamations. to recognize the most responding explants, plant growth regulators and calculate their optimum concentrations and other culture conditions revealing *in vitro* morphogenesis tracked by plantlet regeneration in higher frequencies by means of nodal segments and leaf discs culture of locally adapted cultivar. For nodal segment culture, basal MS media amended with 2, 4-D/ NAA at the concentration of 2.0 mgl^{-1}as alone or in association with 0.5mgl^{-1} TDZ were evidenced meaningly better for callus initiation. However, higher shoot proliferating competence, numbers of shoots per explant with maximum length were recognized on inoculation media reinvigorated with TDZ in the range of 0.2-0.5 mgl^{-1} as sole as well as in association with 0.5mgl^{-1}NAA (more than 92% nodal segments initiated shoots, >10.5 shoots/explant and mean shoot length>3.85 cm). Nevertheless, for inoculated leaf disc, maximum callus and morphogenic callus formation along with plantlet regeneration were investigated on culture media supplemented with 2.0 mgl^{-1} auxins: 2,4-D and/or NAA in amalgamation with 0.5$mgl^{-1}$of a cytokinin BA and/or TDZ (more than 76% leaf discs initiated morphogenic calli with >80% regeneration frequencies). Rooting

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medium MS modified with 0.1 mgl-^{1}IBA demonstrated a higher in vitro rooting response (root proliferating efficiency, number of roots with higher length).

Keywords: Rauvolfia serpentina; nodal segment; leaf disc; direct and indirect organogenesis; plantlet regeneration.

ABBREVIATIONS

MS: Murashige and Skoog medium; WH: White's medium; $B_{5:}$ Gamborg's medium; BA: 6- benzyl aminopurine; TDZ: Thidiazuron; NAA: α-Naphthalene acetic acid; 2,4-D: 2, 4- dichlorophenoxyacetic acid; GA_3: Gibberellic acid; IBA: Indole-3-butyric acid; FYM: Farm Yard Manure; CD: Critical difference and M.P.: Madhya Pradesh.

1. INTRODUCTION

Rauvolfia serpentina L. Benth. ex Kurz belonging to family: Apocynaceae is a woody perennial shrub, usually known with diverse names *viz*., sarpgandha, snake root plant, chotachand, Chandrika, *etc*. and is a native to India and other tropical nations of Asia. Only a few drugs have engrossed such a worldwide attention as the roots of *R. serpentina*. 50 indole alkaloids are found in the roots, along with the therapeutically significant compounds reserpine, deserpidine, rescinnamine, and yohimbine. The roots and the entire plant, according to Ayurveda, are used to treat cardiovascular disease, snake bite, rheumatism, hypertension, insanity, epilepsy, and hypochondria [1]. India, which has nearly a world monopoly, has been threatened by the reduction of the plant's wild resource due to rising demand [2-7]. Contempt their extensive geographical distribution and edaphic tolerance, *Rauvolfia* species have not lent themselves to laidback cultivation because an array of factors that affect their propagation, growth and development including their alkaloid content [8-9].

The plant is vegetatively propagated by means of root cutting owing to poor seed viability, low germination percentage, parthenocarpy and somatoplast sterility that may be mostly attributed to the incidence of cinnamic acid and derivatives in the seed [10]. Further *Rauvolfia* is endangered with extinction attributable to its limited cultivation and over exploitation by local people, government agencies and several pharmaceutical industries [11]. This has led to listing of this species as "endangered" by the International Union for Conservation of Nature and Natural Resources (IUCN) [11]. Because of the predominant motives, there is a vast requirement for *in vitro* propagation of *Rauvolfia serpentina* to gratify the growing commercial mandate of the plant for the production of life supporting alkaloids and conservation of this valuable endangered plant itself. Henceforward, improvements in plant tissue culture techniques for the mass propagation of *R. serpentina* are highly wanted.

In Sarpgandha, an array of explants has been employed competently to establish regenerable cultures *via* somatic embryogenesis and/or organogenesis are shoot tips [12,13-20], nodal segments [21-28,6], auxiliary bud [29,17], cell suspension

culture [30,7], auxiliary meristem [31], leaf disc [32-33,20,6], cotyledon [34] and root [35] with fluctuating notch of accomplishment. Even though, a few studies have been steered in some laboratories world-wide to attain prolific *in vitro* culture system and secondary metabolites production in *Rauvolfia serpentina* with varying regeneration competence, very diminutive work has been carried out so far in Madhya Pradesh on these features. In light of this, an exertion has been made to recognize the most responding explants, plant growth regulators and calculate their optimal concentrations and other physical factors revealing *in vitro* morphogenesis followed by plantlet regeneration in higher frequencies by means of nodal segments and leaf discs culture of locally adapted cultivar.

2. MATERIALS AND METHODS

2.1 Culture Media

To commence with an initial experiment, several modifications of three basal media *viz*., MS [36], WH [37] and B_5 [38] were employed to discover better *in vitro* response. Through the initial experimentations, MS basal medium was investigated to be more responsive compared to rest of two basal media. Subsequently, MS media was used for further investigation. Besides MS basal macro and micro salts, vitamins, and agar, two diverse auxins, *viz.,* 2,4-D and NAA and two cytokinins *i.e.,* BAP and TDZ in differing concentrations along with BAP and TDZ in amalgamation with NAA and 2,4-D, 30.0 gl^{-1} sucrose and 7.5gl^{-1} agar were supplemented to strengthen MS media. Readymade MS basal medium, plant growth regulators and other components were acquired from Hi-Media® Laboratories, Mumbai, India.

2.2 Preparation of Explants

RS-1 cultivar of sarpgandha was employed in the current study. Explant leaf discs were attained from *in vitro* grown regenerants, whereas, nodal segments were obtained from plants propagated in orchard. For *in vitro* grown regenerants, no surface sterilization was adopted; whereas for plants collected from orchard a comprehensive surface sterilization protocol was standardized preceding to culture establishment. The indeterminate shoots were excised and placed in distilled water from orchard planted plants. Top 10 to 20 nodal sections were trimmed to 0.5-1.0-centimeter length and were positioned into double distilled water supplemented with 2% Tween 20 (v/v) for 30 minutes to eliminate the sticked fine particles. The eviscerated segments were then treated with 70% (v/v) ethanol for 1 minute tracked by handling with different concentrations of Bavistin® (BASF, Germany) and aqueous solution of two diverse surface sterilizing agents *i.e.,* $HgCl_2$ and Ca $(OCl)_2$ in dissimilar concentrations and amalgamations for varied periods with initial vacuum of 100 psi (Table 1). Lastly, the segments were rinsed 4-5 times with sterile double distilled water.

Table 1. Effect of surface sterilizing and antifungal agent on recovery of aseptic culture

Treatments	Concentration (%)	Exposure time (in minute)	Aseptic culture (%)	Survival of explants (%)
Ca (OCl) 2	5	10	12.34^{r}	22.28^{n}
Ca (OCl) 2	5	15	15.36^{r}	29.12^{lm}
Ca (OCl) 2	5	20	36.46^{no}	42.17^{jk}
Ca (OCl) 2	10	10	25.08^{q}	47.08^{ij}
Ca (OCl) 2	10	15	29.11^{p}	54.31^{h}
Ca (OCl) 2	10	20	38.15^{mn}	62.37^{e}
Ca (OCl) 2	15	10	37.19^{n}	31.19^{l}
Ca (OCl) 2	15	15	43.18^{kl}	44.17^{j}
Ca (OCl) 2	15	20	45.22^{k}	23.42^{n}
Ca (OCl) 2	20	10	63.32^{g}	66.24^{cd}
Ca (OCl) 2	20	15	49.28^{j}	64.16^{d}
Ca (OCl) 2	20	20	46.39^{jk}	62.14^{f}
HgCl2	0.1	2	38.42^{m}	58.32^{g}
HgCl2	0.1	5	42.07^{l}	25.18^{mn}
HgCl2	0.2	2	50.28^{ij}	71.29^{b}
HgCl2	0.2	5	52.28^{i}	34.34^{kl}
HgCl2	0.2	10	78.06^{b}	62.15^{ef}
Bavistin + Ca (OCl) 2	0.5+10	30	71.37^{de}	28.18^{m}
Bavistin + Ca (OCl) 2	0.5+15	30	73.11^{c}	37.17^{k}
Bavistin + Ca (OCl) 2	0.5+20	30	64.09^{f}	48.44^{i}
Bavistin+ HgCl2	0.5+0.1	20	56.02^{h}	67.42^{c}
Bavistin+ HgCl2	0.5+0.2	20	82.22^{a}	74.94^{a}
Bavistin + HgCl2	0.5+0.1	30	78.95^{b}	62.12^{fg}
Bavistin+ HgCl2	0.5+ 0.2	30	68.46^{e}	63.16^{de}
Mean			49.85	49.22
$CD_{0.05}$			**2.96**	**2.91**

Ca (OCl) 2: Calcium hypochlorite, HgCl2: Mercuric chloride.
Values within column followed by different letters are significantly different at 5% probability level

2.3 Culture Establishment

In cultured baby food bottles, one piece of nodal section was inoculated. To acquire leaf disc explant, first and second leaves of tender shoots were cut into small square fragments (5-8 mm) with sharp edged scalpel and inoculated in 100 x17 Petri dishes comprehending nutrient media. In each Petri dish, 4-5 pieces of leave discs were inoculated. Cultured baby food bottles and Petri dishes were sealed with Parafilm® and incubated under complete darkness at 25±2°C for a week. Later *in vitro* inoculated explants were transferred to photoperiod management of 16 hours light and 8 hours dark at an intensity of 2000-lux luminance providing PAR lamps. Afterward 4-5 weeks of initial inoculation, explants displaying sign of organ formation directly on explant surface was subcultured on same medium (initial medium) for regeneration of plantlets. When shoots of proper length were not attained, they were subsequently transferred to MS elongation medium amended with 1.0 mgl^{-1} GA_3, $20gl^{-1}$ sucrose and $7.5gl^{-1}$

agar. Cultured baby food bottles /culture tubes were subjected to 25±2^{o}C temperature and photoperiod regimes of 1200 lux provided by cool fluorescent tubes for 16 hr. When rooting of regenerants was not accomplished accurately, shootlets were then transferred to MS rooting medium augmented with diverse concentrations of auxins *viz*., IBA and NAA, cytokinins *namely* Kn as sole in addition to IBA in association with BA and Kn, 15gl^{-1} sucrose and 7.5g l^{-1} agar. For elongation and rooting, reduced level of sucrose was employed based on work conducted by numerous scientists [39- 60] along with previous experiences of this laboratory [61-81].

2.4 Hardening of Regenerants

Rooted plantlets were systematically splashed with running tap water to eradicate the adhering agar and were placed in 2.5 cm root trainers occupied with 1:1:1 sand, soil and FYM sterilized combination. Root trainers with transplanted plants were transferred to nethouse for adaptation. Lastly, accustomed plants were subjected under field conditions.

2.5 Statistical Analysis

Experiment was arranged in Completely Randomized Design with two replications. Per replication about 100-120 explants were inoculated on each media. The arc-sine transformation was made before the analysis of data when data were in percentage and were analyzed as per method recommended by Snedecor and Cochran [82].

3. RESULTS AND DISCUSSION

The elementary target of *in vitro* culture of *sarpgandha* is to advance dependable procedure for realizing plant regeneration in higher frequencies from various explants cultures to apply them for mass *in vitro* propagation of required genotypes/cultivars or selected clones, secondary metabolites production and advance biotechnological applications. Even though hypothetically each and every somatic cell of a plant is totipotent, callus induction and regeneration competence of a cell is affected by many factors like nature of explants, *in vitro* culture conditions *viz*., establishment of aseptic culture, nutritional composition of basal medium, types and relative concentrations of plant growth regulators supplied in medium and other culture circumstances.

In current study, inoculated explants tracked either direct or indirect organogenic alleyways of regeneration. The initial response of inoculated nodal segments was identical after 4-7 days and generally sovereign from explant and nutrient media combinations. All explants became swollen and no callus initiation was evident in first few days. In direct organogenesis, adventitious structures were shaped on surface of explant approximately 7-10 days from initial inoculation. Nevertheless, the period differed from culture to culture and in a few instances these structures formed even after 20 days. Within time, these structures formed two shoots (Fig. 1A) and multiple shoots (Fig. 1B-D). While in indirect approach plantlets were

regenerated from mass of callus. Callus initiation generally happen from the portion in contact with the medium and spread upward after 7-10 days of inoculation (Fig. 1E). In indirect organogenesis, shootlets fashioned from the nodules arising on the callus surface (Fig. 1F) and started approximately 10 days from inoculation. However, the duration varied from culture-to-culture and in a few cases shootlets formed after 30 days. In cultured leaf disc, plantlets were mostly regenerated *via* indirect organogenesis. Callus proliferation started from the cut edges of cultured leaf discs after 7-10 days of culture (Fig. 1G-H) and shootlets developed from the nodules arising on the surface of the callus (Fig. 1I-J) subsequently. Shoot formation started approximately 10 days from inoculation. Nevertheless, the duration varied from culture to culture and in a few cases, shoots formed even after 45 days. Most of the calli, after prolonged culturing on the induction media gave rise to plantlets. However, transfer into elongation medium permitted plant formation in higher frequencies with elevated growth rate (Fig. 1K). Under circumstances, where, no root formation was evident, shoots were afterward transferred to the rooting medium. Rooted plants were transferred for 20-25 days in an Environmental Growth Cabinet followed by in nethouse (Fig. 1L) for 25-30 days for hardening before transferring under field conditions. The plants, after survival in the nethouse conditions, were evaluated visually based on their appearance. Though the traits were not scored quantitatively, regenerated plants were found phenotypically normal and true to the type.

In tissue culture experiments with sarpgandha, a wide range of basal medium have been used former for instance MS [83-86,20,23,32,2-9], B_5 [87], WH [15] and LS [88]. In accordance with studies steered so far, compositions of nutrient medium do not appear to play key part in *in vitro* response as much as the type and relative concentration of plant growth regulators added in medium. In current study, basal MS medium was employed during the experimentation for both explants cultures, as this has been evidenced more effective than WH and B_5 during preliminary experiments. Nevertheless, for both explants cultures, three diverse sets of nutrient media were verbalized by adding different auxins and cytokinins as alone in addition to an auxin NAA and 2,4-D in combinations with cytokinins BAP and TDZ to basal MS media to realize the best *in vitro* response.

Analysis of variance presented that the mean sum of squares owing to the different surface sterilizing agents were highly significant at 1% probability level (Table 1). The highest percentage of aseptic culture (82.22%) and survival percentage (74.94%) of explants were recorded with application of 0.5% bavistin in association with 0.2% $HgCl_2$ when nodal segment explants were exposed for 20 minutes. Henceforward, this concentration and combination was employed for surface sterilization of nodal explant of *Rauvolfia* in current study.

The analysis of variance presented in Table 2-8 revealed highly significant ($p<0.05$) differences among the response of different culture media combinations in terms of overall callus induction, shoot proliferating efficiency, number (s) of shoot(s) with higher length for nodal segment culture and for callus induction, formation of morphogenic calli and plantlet regeneration for cultured leaf discs. It indicates the presence of considerable amount of variability amongst the different culture media amalgamations.

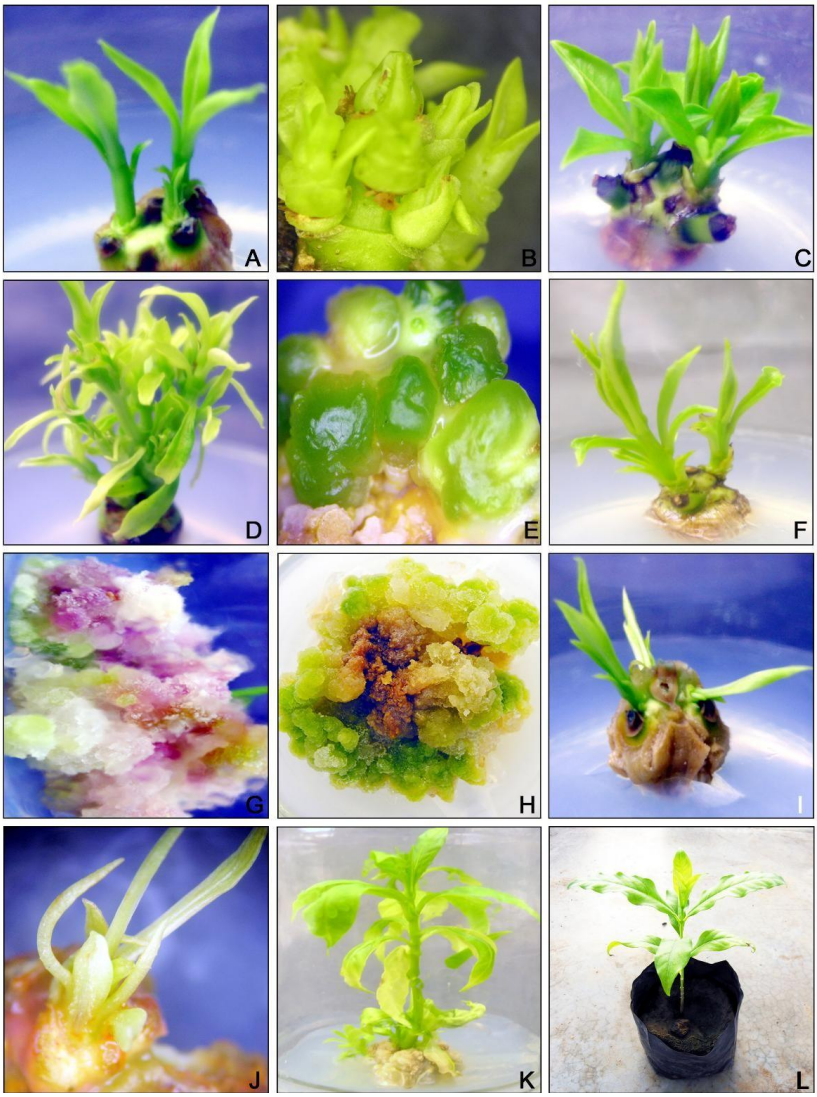


Fig. 1. Plant regeneration in *Rauvolfia serpentina*: A. Shoot formation *via* direct organogenesis from cultured nodal segments; B. Multiple shoot proliferation *via* direct organogenesis from cultured nodal segments; C-D. Multiple shoot formation *via* direct organogenesis from cultured nodal segments; E. Nodal segments derived callus; F. Formation of shoots from nodal segment derived callus; G-H. Leaf discs derived callus; I-J. Formation of shoots from leaf disc derived callus; K. Elongated shootlets and L. Regenerants transferred in Net house for hardening

Table 2. Effect of different auxins (alone) in varying concentrations on *in vitro* response of cultured nodal segments

Culture media	Plant growth regulators mgl^{-1}		*In vitro* response			
combinations ▼	**2,4-D**	**NAA**	**Callus induction (%)**	**Shoot proliferating explant (%)**	**No. of shoots/explant**	**Mean shoot length (in cm)**
MS.5D	0.5	-	77.32^{de}	55.10^{e}	1.56^{d}	1.65^{g}
MSD	1.0	-	88.22^{b}	57.10^{d}	1.88^{b}	2.72^{e}
MS2D	2.0	-	92.10^{a}	60.20^{c}	1.79^{c}	2.94^{cd}
MS3D	3.0	-	81.62^{c}	42.46^{gh}	1.42^{de}	2.54^{ef}
MS4D	4.0	-	74.26^{f}	31.21^{i}	1.29^{ef}	1.85^{f}
MS5D	5.0	-	71.52^{g}	26.45^{j}	0.80^{g}	1.34^{gh}
MS.5N	-	0.5	76.12^{e}	55.22^{de}	1.65^{cd}	3.08^{c}
MSN	-	1.0	77.36^{d}	66.11^{b}	2.90^{b}	3.67^{b}
MS2N	-	2.0	74.72^{ef}	70.37^{a}	3.84^{a}	3.96^{a}
MS3N	-	3.0	82.22^{c}	67.42^{b}	1.23^{f}	3.55^{b}
MS4N	-	4.0	72.48^{fg}	49.52^{f}	1.30^{e}	2.93^{d}
MS5N	-	5.0	70.42^{g}	42.62^{g}	1.23^{f}	2.76^{de}
Mean			**78.19**	**51.98**	**1.74**	**2.74**
CD $_{0.05}$			**2.63**	**2.82**	**0.28**	**0.42**

- *Values within column followed by different letters are significantly different at 5% probability level*

Table 3. Effect of different cytokinins (alone) in varying concentrations on *in vitro* response of cultured nodal segments

Culture media	**Plant growth regulators mgl^{-1}**		***In vitro* response**			
combinations ▼	**BA**	**TDZ**	**Callus induction (%)**	**Shoot Proliferating explant (%)**	**No. of shoots/ explant**	**Mean shoot length (in cm)**
MS.5B	0.5	-	20.10^{b}	82.06^{de}	4.22^{f}	2.97^{c}
MSB	1.0	-	18.16^{c}	87.09^{cd}	6.23^{d}	2.83^{ef}
MS2B	2.0	-	15.18^{d}	84.14^{d}	5.26^{e}	2.87^{e}
MS3B	3.0	-	12.27^{e}	81.08^{e}	6.16^{d}	2.23^{ij}
MS4B	4.0	-	10.35^{ef}	76.07^{ef}	4.19^{fg}	2.94^{b}
MS5B	5.0	-	9.81^{fg}	66.12^{h}	2.25^{h}	3.03^{b}
MS.1Td	-	0.1	22.20^{a}	88.35^{c}	8.23^{c}	2.37^{g}
MS.2Td	-	0.2	19.35^{bc}	92.22^{a}	10.21^{b}	2.27^{i}
MS.5Td	-	0.5	15.33^{cd}	94.06^{a}	12.26^{a}	3.13^{a}
MSTd	-	1.0	13.29^{de}	90.63^{bc}	8.10^{c}	3.03^{b}
MS2Td	-	2.0	10.16^{f}	75.17^{f}	4.22^{f}	2.34^{gh}
MS3Td	-	3.0	5.45^{g}	70.09^{g}	2.24^{h}	2.96^{cd}
Mean			**14.30**	**82.25**	**6.13**	**2.74**
CD $_{0.05}$			**2.76**	**3.05**	**0.19**	**0.03**

- *Values within column followed by different letters are significantly different at 5% probability level.*

Table 4. Combined effect of different auxins and cytokinins on *in vitro* response for cultured nodal segments

Culture media	Plant growth regulators mgl^{-1}				*In vitro* response			
combinations ▼	2,4-D	NAA	BA	TDZ	Callus induction (%)	Shoot proliferating explant (%)	No. of shoots /explant	Mean shoot length (in cm)
MS.5D.5Td	0.5	-	-	0.5	81.24^{d}	81.25^{gh}	5.22^{l}	2.96^{lk}
MSD.5Td	1.0	-	-	0.5	74.68^{gh}	80.25^{h}	6.24^{k}	2.69^{lm}
MS2D.5Td	2.0	-	-	0.5	90.67^{a}	$79.51^{hı}$	6.32^{lk}	3.38^{i}
MS3D.5Td	3.0	-	-	0.5	68.69^{j}	77.80^{ij}	6.54^{j}	3.86^{c}
MS4D.5Td	4.0	-	-	0.5	68.96^{ij}	75.12^{kl}	5.42^{kl}	3.73^{e}
MS5D.5Td	5.0	-	-	0.5	56.36^{m}	75.50^{k}	5.13^{m}	3.08^{ij}
MS.5N.5Td	-	0.5	-	0.5	76.93^{ef}	94.40^{a}	11.78^{a}	4.03^{a}
MSN.5Td	-	1.0	-	0.5	81.93^{cd}	75.78^{i}	10.24^{de}	2.92^{kl}
MS2N.5Td	-	2.0	-	0.5	89.32^{a}	79.35^{i}	9.88^{e}	3.59^{fg}
MS3N.5Td	-	3.0	-	0.5	79.77^{e}	82.20^{fg}	9.66^{f}	3.65^{f}
MS4N.5Td	-	4.0	-	0.5	76.24^{g}	75.68^{jk}	9.83^{ef}	2.63^{m}
MS5N.5Td	-	5.0	-	0.5	69.57^{i}	72.72^{m}	9.13^{h}	3.38^{i}
MS.5B.5N	-	0.5	0.5	-	85.26^{bc}	92.14^{a}	10.58^{a}	3.87^{bc}
MSB.5N	-	0.5	1.0	-	81.13^{de}	84.17^{e}	10.38^{d}	3.46^{h}
MS2B.5N	-	0.5	2.0	-	63.36^{l}	82.08^{g}	10.43^{cd}	3.55^{g}
MS3B.5N	-	0.5	3.0	-	76.44^{fg}	86.18^{d}	9.37^{g}	2.95^{k}
MS4B.5N	-	0.5	4.0	-	72.27^{hi}	84.77^{de}	9.24^{gh}	3.05^{j}
MS5B.5N	-	0.5	5.0	-	67.15^{k}	83.26^{f}	8.37^{hi}	3.44^{hi}
MS.1Td.5N	-	0.5	-	0.1	73.42^{h}	90.23^{b}	10.46^{a}	3.84^{d}
MS.2Td.5N	-	0.5	-	0.2	68.54^{jk}	92.30^{a}	10.62^{a}	3.85^{cd}

Culture media	**Plant growth regulators mgl^{-1}**				***In vitro* response**			
combinations ▼	**2,4-D**	**NAA**	**BA**	**TDZ**	**Callus induction (%)**	**Shoot proliferating explant (%)**	**No. of shoots /explant**	**Mean shoot length (in cm)**
MS.4Td.5N	-	0.5	-	0.5	76.68^{f}	94.14^{a}	11.26^{a}	3.98^{b}
MSTd.5N	-	0.5	-	1.0	82.73^{c}	88.44^{cd}	9.48^{fg}	3.67^{ef}
MS2Td.5N	-	0.5	-	2.0	64.82^{kl}	84.05^{ef}	8.15^{l}	3.52^{gh}
MS3Td.5N	-	0.5	-	3.0	62.27^{lm}	74.58^{i}	7.22^{ij}	2.75^{l}
Mean					**74.51**	**82.74**	**8.78**	**3.40**
CD $_{0.05}$					**3.91**	**2.87**	**2.90**	**0.04**

- *Values within column followed by different letters are significantly different at 5% probability level.*

Table 5. Effect of different auxins (alone) in varying concentrations on *in vitro* response of cultured leaf discs

Culture media	**Plant growth regulators mgl^{-1}**		***In vitro* response**		
combinations ▼	**2,4-D**	**NAA**	**Callus induction**	**Morphogenic calli**	**Plant regeneration**
			(%)	**(%)**	**(%)**
MS.5D	0.5	-	72.64^{ef}	20.29^{d}	8.23^{b}
MSD	1.0	-	81.23^{c}	22.19^{cd}	5.14^{cd}
MS2D	2.0	-	86.45^{ab}	25.42^{c}	3.24^{e}
MS3D	3.0	-	88.19^{a}	18.84^{e}	3.10^{ef}
MS4D	4.0	-	79.37^{cd}	10.45^{f}	2.10^{f}
MS5D	5.0	-	71.25^{f}	5.13^{g}	1.50^{g}
MS.5N	-	0.5	68.60^{fg}	27.69^{bc}	12.45^{a}
MSN	-	1.0	74.35^{e}	32.32^{ab}	10.16^{ab}
MS2N	-	2.0	83.66^{bc}	35.14^{a}	6.42^{bc}
MS3N	-	3.0	85.54^{b}	30.14^{b}	5.83^{c}
MS4N	-	4.0	78.30^{d}	20.24^{de}	3.24^{e}
MS5N	-	5.0	68.60^{fg}	14.80^{ef}	1.98^{fg}
Mean			**78.18**	**21.88**	**5.28**
CD $_{0.05}$			**2.89**	**3.46**	**2.78**

- *Values within column followed by different letters are significantly different at 5% probability level.*

Table 6. Effect of different cytokinins (alone) in varying concentrations on *in vitro* response of cultured leaf discs

Culture media combinations ▼	Plant growth regulators mgl^{-1}		*In vitro* response		
	BA	**TDZ**	**Callus induction (%)**	**Morphogenic calli (%)**	**Plant regeneration (%)**
MS.5B	0.5	-	37.73^{c}	35.02^{cd}	36.13^{cd}
MSB	1.0	-	43.12^{ab}	40.23^{b}	42.37^{b}
MS2B	2.0	-	45.18^{a}	42.41^{a}	44.22^{a}
MS3B	3.0	-	30.47^{d}	28.32^{d}	29.54^{de}
MS4B	4.0	-	22.85^{e}	20.47^{e}	21.68^{e}
MS5B	5.0	-	10.27^{f}	9.24^{f}	9.73^{f}
MS.1Td	-	0.1	39.33^{bc}	37.19^{c}	38.17^{c}
MS.2Td	-	0.2	40.07^{b}	38.43^{bc}	39.66^{bc}
MS.5Td	-	0.5	44.68^{ab}	41.26^{ab}	43.17^{ab}
MSTd	-	1.0	30.26^{de}	28.28^{de}	29.77^{d}
MS2Td	-	2.0	20.25^{ef}	19.22^{ef}	19.85^{ef}
MS3Td	-	3.0	9.77^{fg}	8.48^{fg}	8.44^{fg}
Mean			**31.16**	**29.04**	**30.22**
$CD_{0.05}$			**3.08**	**3.02**	**3.08**

- *Values within column followed by different letters are significantly different at 5% probability level*

Table 7. Combined effect of different added auxins and cytokinins on *in vitro* response for cultured leaf discs

Culture media	Plant growth regulators mgl^{-1}				*In vitro* response		
combinations ▼	2,4-D	NAA	BA	TDZ	Callus induction (%)	Morphogenic calli (%)	Plant regeneration %
MS.5D.5B	0.5	-	0.5	-	72.18^{h}	64.31^{h}	67.46^{gh}
MSD.5B	1.0	-	0.5	-	81.46^{a}	72.44^{d}	79.69^{ab}
MS2D.5B	2.0	-	0.5	-	85.69^{a}	76.29^{ab}	75.19^{d}
MS3D.5B	3.0	-	0.5	-	80.67^{a}	72.94^{bc}	75.58^{c}
MS4D.5B	4.0	-	0.5	-	77.58^{e}	61.49^{l}	72.67^{e}
MS5D.5B	5.0	-	0.5	-	68.06^{jk}	57.89^{k}	64.06^{j}
MS.5D.5Td	0.5	-	-	0.5	74.42^{fg}	64.78^{gh}	67.42^{hi}
MSD.5Td	1.0	-	-	0.5	72.32^{gh}	66.29^{fg}	69.32^{fg}
MS2D.5Td	2.0	-	-	0.5	86.28^{a}	77.64^{a}	80.06^{ab}
MS3D.5Td	3.0	-	-	0.5	81.06^{a}	72.81^{c}	62.58^{jk}
MS4D.5Td	4.0	-	-	0.5	76.14^{ef}	68.78^{ef}	71.28^{ef}
MS5D.5Td	5.0	-	-	0.5	69.72^{i}	62.44^{ij}	65.19^{ij}
MS.5N.5B	-	0.5	0.5	-	73.86^{g}	65.79^{g}	68.38^{g}
MSN.5B	-	1.0	0.5	-	75.34^{f}	67.46^{f}	70.13^{f}
MS2N.5B	-	2.0	0.5	-	84.38^{a}	72.54^{cd}	75.72^{bc}
MS3N.5B	-	3.0	0.5	-	81.13^{a}	70.76^{de}	75.39^{bc}
MS4N.5B	-	4.0	0.5	-	72.11^{hi}	73.62^{b}	73.43^{de}
MS5N.5B	-	5.0	0.5	-	70.49^{ij}	59.32^{jk}	66.17^{i}
MS.5N.5Td	-	0.5	-	0.5	71.58^{i}	63.89^{hi}	66.86^{h}
MSN.5Td	-	1.0	-	0.5	78.43^{de}	63.62^{i}	60.13^{k}
MS2N.5Td	-	2.0	-	0.5	86.48^{a}	77.68^{a}	80.49^{a}
MS3N.5Td	-	3.0	-	0.5	82.18^{a}	69.86^{e}	76.20^{b}

Culture media combinations ▼	**Plant growth regulators mgl^{-1}**				***In vitro* response**		
	2,4-D	**NAA**	**BA**	**TDZ**	**Callus induction (%)**	**Morphogenic calli (%)**	**Plant regeneration %**
MS4N.5Td	-	4.0	-	0.5	64.32^{k}	55.23^{kl}	58.17^{kl}
MS5N.5Td	-	5.0	-	0.5	62.17^{kl}	52.62^{l}	36.57^{l}
Mean					**76.16**	**67.10**	**69.08**
CD $_{0.05}$					**7.09**	**3.01**	**2.92**

- *Values within column followed by different letters are significantly different at 5% probability level*

Table 8. Combined effect of different plant growth regulators added in MS basal media for induction of *in vitro* rooting

Culture media combinations ▼	**Plant growth regulator mgl^{-1}**				***In vitro* rooting**		
	IBA	**NAA**	**BAP**	**Kn**	**Root proliferating shootlets (%)**	**No. of root per shootlets**	**Root length (in cm)**
MS.1IB	0.1	-	-	-	81.91^{a}	6.86^{a}	3.43^{a}
MS.5IB	0.5	-	-	-	79.91^{ab}	6.08^{ab}	3.26^{b}
MSIB	1.0	-	-	-	79.14^{b}	6.32^{ab}	2.86^{c}
MS2IB	2.0	-	-	-	43.36^{h}	5.45^{a}	2.84^{c}
MS3IB	3.0	-	-	-	38.92^{i}	4.12^{e}	1.63^{n}
MS.1N	-	0.1	-	-	35.25^{ij}	3.18^{h}	2.24^{e}
MS.5N	-	0.5	-	-	61.28^{f}	4.68^{c}	1.02^{l}
MSN	-	1.0	-	-	40.08^{hi}	3.38^{g}	1.11^{k}
MS2N	-	2.0	-	-	57.31^{g}	2.86^{hı}	2.13^{f}
MS3N	-	3.0	-	-	34.75^{j}	2.58^{i}	1.05^{kl}
MS.1Kn	-	-	-	0.1	34.18^{jk}	2.40^{ij}	1.35^{i}
MS.5Kn	-	-	-	0.5	31.36^{kl}	4.72^{bc}	2.23^{ef}

Culture media combinations	**Plant growth regulator mgl^{-1}**				***In vitro* rooting**		
▼	**IBA**	**NAA**	**BAP**	**Kn**	**Root proliferating shootlets (%)**	**No. of root per shootlets**	**Root length (in cm)**
MSKn	-	-	-	1.0	31.48^{k}	4.20^{d}	2.24^{e}
MS2Kn	-	-	-	2.0	76.85^{cd}	3.35^{gh}	1.93^{m}
MS3Kn	-	-	-	3.0	65.36^{ef}	2.29^{j}	1.64^{h}
MS.5IB.5B	0.5	-	0.5	-	79.93^{ab}	3.88^{ef}	1.72^{g}
MSIB.5B	1.0	-	0.5	-	77.02^{c}	4.12^{e}	1.18^{j}
MS2IB.5B	2.0	-	0.5	-	71.25^{de}	2.28^{jk}	1.07^{kl}
MS.5IB.5Kn	0.5	-	-	0.5	77.79^{bc}	4.32^{cd}	2.32^{d}
MSIB.5Kn	1.0	-	-	0.5	72.32^{d}	3.46^{f}	2.29^{d}
MS2IB.5Kn	2.0	-	-	0.5	68.86^{e}	3.42^{fg}	1.12^{jk}
Mean					**58.96**	**3.99**	**1.93**
CD $_{0.05}$					**2.92**	**1.79**	**0.02**

- *Values within column followed by different letters are significantly different at 5% probability level*

For callus induction, either nutrient media MS2D or MSD encompassing a moderate concentration of 2,4–D were evidenced suggestively superior (more than 88% explants induced callus) to culture medium encompassing other auxin NAA (Table 2) and cytokinins as sole (Table 3) in similar concentration which advised that 2,4-D either alone (Table 2) or in combination with lower concentration of TDZ (Table 4) is better for callus proliferation from cultured nodal segments. A similar result has also been addressed by Bhatt et al. [20] in sarpgandha.

In respect to shoot proliferating aptitude, numbers of shoots with higher length, nutrient media comprehending auxins as alone in fluctuating concentrations displayed lower shoot proliferation competence, *i.e.,* lesser number of explants proliferated shoots, lesser numbers of shoot (s) per explant and shoots of minimum length. In former investigations, Skoog and Miller [89] and Evans et al. [90] investigated that cytokinin is obligatory unavoidably in shoot organogenesis, henceforward to accomplish the best *in vitro* response basal medium was also invigorated with two cytokinins in differing concentrations. Nutrient media amended with BAP in range of 1.0-3.0 mgl^{-1} and TDZ in range of 0.1- 1.0 mgl^{-1} were responded well (more than 80% and 92% respectively explants proliferated shootlets) proposed that a moderate concentration of cytokinin is essential for this determination. Previous investigations also confirmed that lower level of BAP persuaded early bud sprouting in several species. Multiple shoot proliferation required the attendance of cytokinin in the nutrient medium, which is meticulously pertinent to result of Mishra et al. [25] in other investigations in sarpgandha. Even though shoot proliferating competence and number of shoot (s) in higher percentage acquired on nutrient media reinvigorated with TDZ as alone in the range of 0.2-0.5 mgl^{-1} and BA at 0.5 mgl^{-1}, nevertheless, elevated response was recognized with supplementation of 0.5mgl^{-1}NAA with either TDZ in an array of 0.2- 0.5mgl^{-1} or BA at the concentration of 0.5 mgl^{-1} (more than 92% nodal segments proliferated shoots on culture media MS.5Td.5N, MS.4Td.5N, MS.2Td.5N and MS.5B.5N). Nutrient media MS.5Td.5N, MS.4Td.5N, MS.2Td.5Nand MS.5B.5N also proliferated shootlets in higher numbers of higher length (~10.5 shoots/explant and mean shoot length > 3.85 cm) than nutrient medium comprehending a higher concentration of cytokinin BA in addition to an auxin as alone. Retrieval of shoots in higher numbers on medium enhanced with combination of an auxin with a cytokinin compared to supplementation of an auxin as well as a cytokinin separately, perhaps owing to incidence of direct along with indirect organogenic mode of regeneration simultaneously. Furthermore, the improved proportion of multiple shoot initiation on nutrient media added with BA/TDZ and NAA may be ascribed to increased rate of cell division and production of several primordial outgrowths induced by BA/TDZ in the auxiliary meristematic zone of the explant tissues. Analogous discoveries have also been testified in sarpgandha for shoot tips [13,14], shoot apices [91], lateral buds [14], nodal segments [92,19,24,6] and cotyledons [34]. Moreover, TDZ required in lower level compared to BA for all studied parameters. Efficacy of TDZ at lower level possibly, was by reason of induced accumulation of endogenous cytokinins, as reported former by Murthy et al. [93]. TDZ also affected auxin transport in hypocotyl tissues of *Pelargonium*

species and others [94] and encouraged regeneration frequency by altering the levels of abscisic acid, ethylene and perhaps proline [94].

For cultured leaf disc, consequences evidently designated fluctuating response of plant growth regulators on callus proliferation. At lower levels both the auxins 2, 4-D and NAA have been evidenced to initiate callus proliferation, however, at higher levels of 2,4-D (≥3.0 mgl^{-1}) callus turned in blackish colour with retarded growth, whereas cell mortality was experimented at ≥4.0 mgl^{-1} of 2,4-D. Auxin 2, 4-D initiated morphogenic calli and plantlet regeneration in very lower frequencies regardless of all tested concentrations *i.e*., 0.1-5.0 mgl^{-1}. At the higher concentration (≥5.0 mg l^{-1}) of NAA, most of the calli was non-morphogenic and with the ageing the color altered from cream to dark brown without any further growth. Maximum callus initiation was observed on nutrient medium comprising 1.0-3.0mgl^{-1} 2,4-D either alone or 2,4-D in range of 1.0-3.0mgl^{-1} and NAA at the level of 2.0-3.0 mgl^{-1} in amalgamation with 0.5 mgl^{-1} cytokinins BA/TDZ (more than 81% leaf discs initiated callus). Additionally, nutrient media comprehending NAA fashioned fast growing yellow and green compact callus, which displayed advantageous consequence on the cell growth of morphogenic cultures. Plant regeneration efficacy diminished with the increased level of NAA (Table 5). Diverse concentrations of BAP and TDZ (as alone) added in the medium displayed very poor callus induction and morphogenic calli formation. Nevertheless, nutrient medium amended with BAP and TDZ improved plantlet regeneration competence compared to media fortified with NAA and/or 2, 4-D (Table 6).

Preliminary investigations for launching plant growth regulator type, combination and concentration exposed that barely an auxin or a cytokinin was not much operative for formation of morphogenic calli. Mostly all the plant growth regulators formed calli of different sizes and texture. Rate of reappearance of callus was higher when an auxin was added to the cytokinin in nutrient medium. However, in both the cases, frequency of morphogenic calli was found to be low. Consequently, final experimentations were strongminded seeing combination of an auxin and a cytokinin for realizing the best *in vitro* response (Table 7).

Maximum callus and morphogenic callus formation in addition to plantlet regeneration were recorded on nutrient media amended with 2.0 mgl^{-1} 2,4-D and/or NAA in amalgamation with 0.5mgl^{-1} BA and/or TDZ (more than 76% leaf discs induced morphogenic calli and >80% regenerated plantlets). Analogous findings were also reported by Sehrawat et al*.* [33], Bhatt et al*.* [20] and Pandey et al*.* [32]. In present study, generally explant cultures showing higher number of morphogenic calli regenerated more plantlets. Nevertheless, the progression of morphogenesis was not a predication of higher regeneration incidences. For explants cultures, nutrient medium demonstrating higher morphogenic callus formation may regenerate lesser plants compared to persuading morphogenic calli in lower occurrences. Such deviations happened as a single morphogenic calli produced none or many plantlets (up to 15 from nodal segment culture in clusters). Furthermore, not all the shootlets produced into complete plants *i.e.,* shoots with roots. Sometimes shoot could not develop

after initiation, in a few cases they were deformed and not all the shootlets developed without roots that were able to produce roots even on rooting medium.

In current investigation, rhizogenic frequency was found to be higher after transferring shootlets into rooting medium. Overall, auxins like IBA [12,6-9], IAA [14] in addition to NAA [15,16,19] were effective in inducing *in vitro* rooting. In present study, MS medium amended with 0.1 -1.0 mgl^{-1}IBA was documented to be optimum for displaying higher *in vitro* rooting response (Table 8). Maximum *in vitro* root proliferating efficiency, number of roots per shootlets and root of higher length were recognized on rooting media MS.1IB. The findings undoubtedly designate that rooting of *in vitro* shoots of sarpgandha necessitated lower concentrations of IBA with higher nutrient status. Higher *in vitro* rooting response was also documented on rooting medium comprehending IBA in range of 0.5-1.0 mgl^{-1} in association with 0.5 mgl^{-1} BA/Kn. Nevertheless, rooting media fortified with IBA in lower concentration as alone responded better compared to nutrient media amended with NAA, BA and Kn even in higher concentrations along with in amalgamations that advised that IBA promotes *in vitro* rooting in sarpgandha more swiftly. Auxins promoted adventitious root development on intact plants in addition to excised stems. Of these, IBA was recognized the most operative than any other plant growth regulators in the most of the incidences deceptively since it is not wrecked by IAA oxidase or other enzymes and hence persists longer [95-112]. The above findings are in orthodoxy with the previous results of Roy et al. [14] and Ahmad et al. [18].

For regeneration aptitude, both explants responded contrarily. Maximum plantlets were recovered from nodal segment tracked by leaf disc culture. Though, evaluation between these two explants cultures were empirical as nutrient media employed were dissimilar. Nevertheless, inclination shown in the current study proposed that explant (s) with higher regeneration potential may be used for micropropagation and advance biotechnological studies.

4. CONCLUSION

The findings of current investigation established that under circumstances of these experimentations, *in vitro* response of both explant cultures was under genetic control. As between two assorted explants cultured on diverse nutrient media combinations noteworthy differences were documented for all the culture phases. In conclusion in current study, we employed the novel technique in nodal segment and leaf disc cultures, that may be attained from plants propagated in orchard along with plantlets regenerated *in vitro*. The established protocol may be used for production of plantlets of *sarpgandha* on a commercial scale and *ex situ* conservation of this important medicinal plant species. Leaf derived callus cultures can be exploited further for isolation of totipotent protoplast and genetic transformation. These formulations may also be employed for root culture for deriving cell suspension cultures for producing and enhancing many important secondary metabolites of sarpgandha by elicitation and immobilization procedures.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 7
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Massive *In vitro* Propagation from Cultured Nodal Segment of Three *Citrus species*

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ABSTRACT

Nodal sections excised from *in vitro* grown saplings of three citrus *species viz*., *Citrus aurantiafolia*, *Citrus reticulata* and *Citrus sinensis* were inoculated on diverse modifications of basal MS medium for manifold shootlet production. *In vitro* morphogenesis followed by plant regeneration speckled significantly among species and culture medium fortification. In this investigation, shootlets were developed directly *via* auxiliary bud proliferation along with from callus tissue. Culture medium MS5N.5B/MS5N.Kn (MS + 5.0 mgl^{-1} NAA + 0.5 mgl^{-1}BA/Kn) convinced callusing in higher rates. Nutrient medium MS.1Td.5N/MS.5B.5N (MS + 0.1 mgl^{-1} TDZ/0.5 BA + 0.5 mgl^{-1} NAA) boosted plantlet multiplying competence. Whereas, plantlets per explant in higher frequencies (s) of bigger length were recognized on nutrient medium MS.1Td or MS.2Td (MS + 0.1/0.2 mgl^{-1} TDZ). In respect to *in vitro* rooting, root initiating efficacy in higher frequencies was verified on medium MS.5IB (MS + 0.5 mgl^{-1} IBA), while roots in greater numbers were documented on rooting medium MS2IB.5Kn (MS + 2.0 mg l^{-1} IBA + 0.5 mg l^{-1} Kn), whereas nutrient medium MS.5IB.5B (MS + 0.5 mg l^{-1} IBA + 0. 5 mg l^{-1} BAP) improved mean root length. In respect to interspecific *in vitro* response, usually, Acid lime tracked by Mandarin and Sweet orange performed authoritatively for the almost culture stages. The *in vitro* developed shootlets were efficaciously adapted and shifted under field conditions.

Keywords: Citrus aurantiafolia; citrus reticulata; citrus sinensis; nodal segment culture; direct and indirect organogenesis and plantlet regeneration.

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1. INTRODUCTION

Citrus species usual a woody subtropical fruit crop plant belongs Rutaceae family. Its fruits deliver vitamins, minerals and several other cruxes. Fruits of this family are identically venerated all over the world owing to the sense of taste, fragrant savor and medicinal possessions [1-6]. They are broadly employed to prevent flu and coldness and sustain the resistant arrangement [7]. Citrus fruits are also proved beneficial for patients suffering to health glitches for instance gastritis, fever and arterial induration. The juice of this species is employed in the pharmacological business as it encompasses a higher extent of citric acid which having rich source of ascorbic citric acids along with essential oils [8]. There are also verdicts about positive belongings of citrus fruits against cancer of gastrointestinal and upper respiratory tracts [9]. The genus is grown for its fruit and scent, impartially because of flavonoids and limonoids [10-11].

Citrus is being propagated by means of seed and “shield” / “T” budding, but from seed it does not guarantee true to type plants owing to cross-pollinating fauna. In addition, these plants take more time to bear fruits. The less-obtainability of reliable and disease-free propagating stock and shootlets for grafting and budding makes field multiplication problematic to citrus species. Prearranged the enormous status of the *Rutaceae* family in innumerable branches of business, the micropropagation of *Citrus* has constantly enthused countless apprehension among researchers. There is a cultivating mandate to advance new cultivars of plants resistant to different pathogens and unfriendly environmental conditions and eminent by high quality of fruits [12]. Traditional practices for generating new species are not effective in the instance of citrus species owing to the physiological impediments linked with sexual reproduction for instance heterozygosity and polyembryony [13-17]. Citrus plantations face an array of snags such as pests, slow growth, susceptibility to disease, sensitivity to low temperatures and substantial fatalities throughout storage [18,1,3,11,17]. Plant tissue culture procedure can resolve these complications [19-22]. Moreover, this technique can also form plants comparatively in huge numbers as compared to conventional methods [23-44]. Furthermore, *in vitro* cultures eradicate infections and can be faster than traditional plant cultures systems [45,17].

Formation of callus followed by an efficient plantlet regeneration are the chief phases in the biotechnological exploitation *in vitro*. In citrus, mature cotyledons [46-49,1], hypocotyls [50], shoot tip [51-56], nodal segment [57-64,18,2], root segment [44], ovule [65,66,47], nucleus tissues [11,66] and style and stigma [14] have been employed by different investigators throughout the world with varying notch of accomplishment.

The utilization of biotechnological interventions particularly micropropagation facilitate a better substitute for massive multiplication of true-to-type regenerants. Plant regeneration through nodal section cultures comprises bud multiplication *via* auxiliary buds proliferations, owns less possibility of somaclonal variation among regenerants as compared to callus mediated regeneration alleyway. The kinds and concentrations of plant growth regulators added into the culture medium and culture conditions are the most deciding factors that regulate the

route of in *vitro* morphogenesis [67,48,49,62,11]. Consequently, current investigation was commenced with intention to set a protocol for establishment, regeneration, rooting and hardening of locally acclimatized and popular cultivars of three different species of citrus *namely*., *Citrus aurantiafolia*, *Citrus reticulata* and *Citrus sinensis* by culturing nodal segment explants to evade further problems of adaptation and tailoring transgenic plants resistant against different biotic and abiotic factors.

2. MATERIALS AND METHODS

2.1 Explant Material

Three diverse species of citrus *viz: Citrus aurantiafolia*, *Citrus reticulata* and *Citrus sinensis* were preferred for the *in vitro* studies.

2.2 Culture Media

To instigate with an initial experimentation, two diverse basal media *viz*., MS [68] and WP [69] were adored to search out better *in vitro* response. All through the initial study, MS basal medium was proved more amenable than WP medium (data not presented).

Henceforth, for later investigation basal MS medium was employed. All basal media were made using readymade basal MS medium (HiMidiaTM) and fortified with three diverse sets of plant growth regulators to fortify medium. In first set: three different auxins, *viz.,* 2, 4-D, NAA and 2, 4, 5-T (alone), in second situate: three diverse cytokinins *i e.,* BAP, Kn and TDZ (solely) and in third arrangement: diverse cytokinins (BAP, Kn and TDZ) in combinations with an auxin NAA in variable concentrations to accomplish the best *in vitro* response. In addition to MS basal macro and micro salts, vitamins, all initial media was supplied with 30.0 gl-1 sucrose and the final volume was made to 1000 ml and pH was adjusted to 5.8±0.1 with 1N KOH solution. After correcting the pH, agar powder @ 7.5 gl-1 was incorporated to the media for semi-solidification. Warm culture media, still in liquid state was dispensed into baby food bottles (50-60 ml / bottle) and culture tubes (15-20 ml / tube) tracked by autoclaving at 121^{o}C under 15 psi pressure for 25 minutes. Readymade MS basal medium, plant growth regulators and other components were acquired from Hi-media Laboratories, Mumbai, India.

2.3 Surface Sterilization of Seed

Seeds were acquired from mature fruits of various *Citrus* species and were dried and subsequently splashed with 2% Tween 20 (v/v) (a commercial detergent) for 15- 20 minutes trailed by washing systematically with running tap water for 30 minutes to eradicate dirt and residues tracked by a treatment with 70% (v/v) ethanol for 2 minutes. Then seed were treated with 2% Bavistin® (BASF, Germany) for five minutes tracked by a treatment of 0.2% $HgCl_2$ for seven minutes. Lastly, seed were cleaned 4-5 times with sterile double distilled water and soaked for 1- 2 minutes and were cultured on culture tubes comprising agar gelled water (7.5 gl^{-1} agar) under diffused luminance of 16 m mol m^{-2} s^{-1} provided with white fluorescent lights.

2.4 Nodal Segment Excision and Plating Technique

Nodal sections was acquired from one month old *in vitro* germinated seedlings. All the species employed in investigation were polyembryonic in nature and having three shootlets. Two of them are gameting seedlings and one nucellar. Nucellar seedling was employed for *In vitro* culture establishment with the purpose of obtain true-to-type plantlets. Seedling was divided in to 2-3 parts having at least one node and 1-2 pieces of nodal segments/sections were cultured in baby food bottles comprising culture medium.

2.5 Culture Conditions

Cultures wrapped with Lab film (Parafilm®) were incubated under complete darkness at 25±2°C for one week. Later *In vitro* cultured explants were imperiled to photoperiod command of 16 hours light / 8 hours dark cycle at an intensity of 2000-lux luminance facilitated by PAR lamps.

2.6 Regeneration of Plantlets

Afterward 4-5 weeks of initial inoculation, cultured nodal sections followed either direct organogenesis (without callus formation) alleyway or indirect mode (*via* callus formation) plantlet regeneration. Multiple shoots acquired from direct organogenesis (auxiliary bud proliferation) were transferred to elongation medium which was MS basal medium amended with 1.0 mgl^{-1} GA3, 15.0 gl^{-1} sucrose and 7.5 gl^{-1} agar powder and calli obtained from indirect organogenesis were subsequently subcultured on regeneration medium which has similar ingredients as initial medium for regeneration of shootlets. Cultured baby food bottles /culture tubes were exposed to 25±2^{o}C temperature and photoperiod regimes of 60 mmol m^{-2} s^{-1} luminance provided by cool fluorescent tubes for 16 hr.

2.7 *In vitro* Rooting of Regenerants

Regenerants were then placed to MS rooting medium fortified with diverse concentrations of IBA, NAA and Kn (alone) as well as IBA in combination with BA and Kn, 15.0 gl^{-1} sucrose and 7.5 gl^{-1} agar. For rooting, reduced concentration of sucrose was employed based on previous work steered by diverse researchers and initial experiences of our laboratory.

2.8 Acclimatization of Regenerants

The shootlets acquired after root initiation were prudently detached from the medium of the culture vessels employing forceps to avoid damaging them and were thoroughly washed with running tap water to separate the sticked agar and were planted in 2.5 cm root trainers filled with 1:1:1 sand, soil and FYM sterilized mixture. Root trainers with transplanted plants were placed under 28±2^{o}C and 70±5% RH for 15-20 days in an Environmental Growth Cabinet tracked by 2-3 weeks in Net House under 30±5^{o}C and 60±5% RH for adaptation. Lastly, acclimatized plants were subjected under field conditions.

2.9 Experimental Design and Analysis of Data

The experiments were conducted in factorial Completely Randomized Design to search out the significance of species, culture medium combination and their interaction. Each treatment comprised of two replications. Per replication around 80-100 nodal sections were inoculated on each media combination. The arc-sine transformation was adopted before the analysis of data, where data were in percentage. The data were analyzed as per method recommended by Snedecor and Cochran [70].

3. RESULTS AND DISCUSSION

Contingent to the nature of species and diverse plant growth regulators, inoculated nodal sections tracked either direct or indirect route of plantlet regeneration. In direct slant, shootlets were developed from explant surface directly without intervening callus formation (*via* auxiliary bud proliferation) and in indirect route; plantlets were regenerated *via* callus formation (indirect organogenesis). In most circumstances, shootlets were developed directly from the meristematic zones from cultured nodal sections (Fig.1 A-I). Inoculated nodal sections proliferated two (Fig.1A-C) and multiple shootlets (Fig.1D-I) that afterward elongated. Shootlet initiation started around 3-7 days from initial inoculation which proliferated in 7 to 20 days. Nevertheless, the period speckled from culture to culture and in a few cases shootlets formed after 20 days and were elongated in 25-40 days. In indirect pathway, plantlet regenerated *via* indirect organogenesis (Fig.2 A-E). The earlier response of inoculated explants was same after 7 -10 days and mostly free from explant and culture medium combination. All explants became swollen and no callus proliferation was apparent in first few days. Callus proliferation generally started from the portion that was in contact with the medium and spread upward after 7 days of inoculation (Fig. 2A). After 4-5 weeks of inoculation, callus forming explants were documented. Initiated callus tissue established different phenotypes. These pheno-variants were wet, rough, hard, dense and glossy, imitated diverse developmental abilities. Shootlet formation ensued habitually (Fig.2 C-E) after sub culturing of these pheno-variant calli. Nodal sections derived callus were proliferated shootlet *via* indirect somatic embryogenesis (Fig.2 B-C) along with indirect organogenesis (Fig.2 D-E). Plantlet regeneration started after 15-20 days from initial culturing. Nevertheless, the period speckled from culture to culture and in some instances, shootlets formed even after 35 days. Complete plantlets regenerated *via* direct as well as indirect organogenesis were counted as regenerated plantlets. Regenerated shoots alone were also considered as plantlets as they gave rise to complete plants after rhizogenesis after transferring on rooting medium (Fig.1 J-L; Fig.2F). Root formation initiated after 10-15 days of transferring of shootlets in rooting medium. Root trainers with transplanted plants were subjected in an Environmental Growth Chamber for hardening for 10-25 days (Fig.2 G). The plantlets, after adaptation in the Green House circumstances (Fig.2H) transferred in the field (Fig.2I). Regenerants were assessed visually on the basis of appearance. Even if the traits were not recorded quantitatively, regenerated plantlets were phenotypically usual and similar to its mother plants.

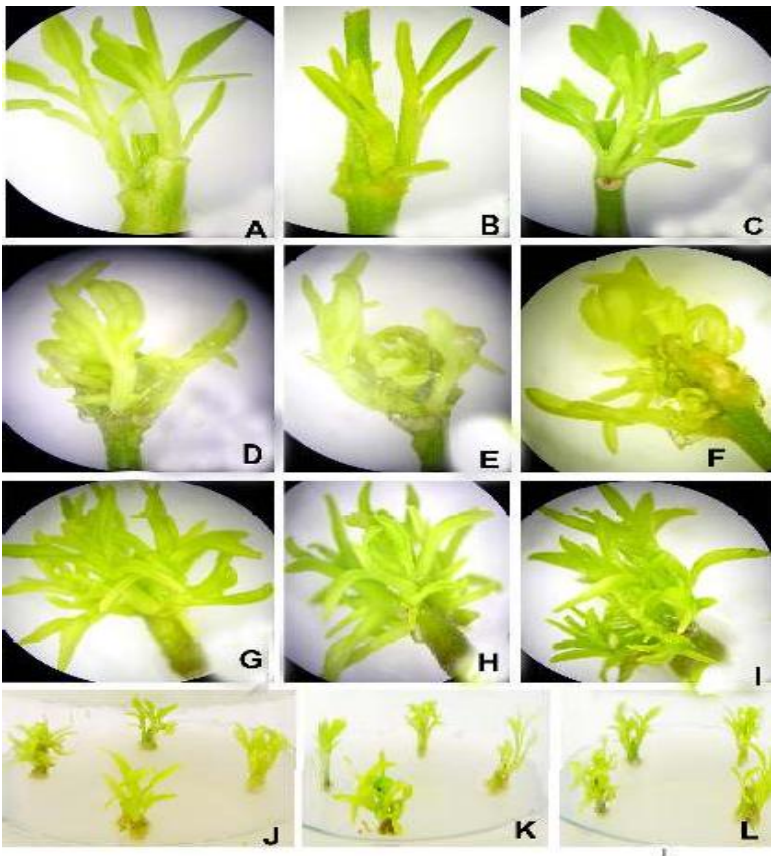


Fig. 1. Plant regeneration *via* direct organogenesis from nodal segment culture in *Citrus species*: A. Direct auxiliary bud proliferation from cultured nodal segment of *Citrus aurantiafolia*; B. Direct auxiliary bud proliferation from cultured nodal segment of *Citrus reticulata*;C. Direct auxiliary bud proliferation from cultured nodal segment of *Citrus sinensis*; D. Multiple shoot initiation from cultured nodal segment of *Citrus aurantiafolia*; E. Multiple shoot initiation from cultured nodal segment of *Citrus reticulata*. Multiple shoot initiation from cultured nodal segment of *Citrus sinensis*; G Multiple shoot formation and elongation from cultured nodal segment of *Citrus aurantiafolia*; H. Multiple shoot formation and elongation from cultured nodal segment of *Citrus reticulata*; I. Multiple shoot formation and elongation from cultured nodal segment of *Citrus sinensis*; J. Elongated shoot transferred in rooting medium of *Citrus aurantiafolia*; K. Elongated shoot transferred in rooting medium of *Citrus aurantiafolia;* and L. Elongated shoot transferred in rooting medium of *Citrus aurantiafolia*

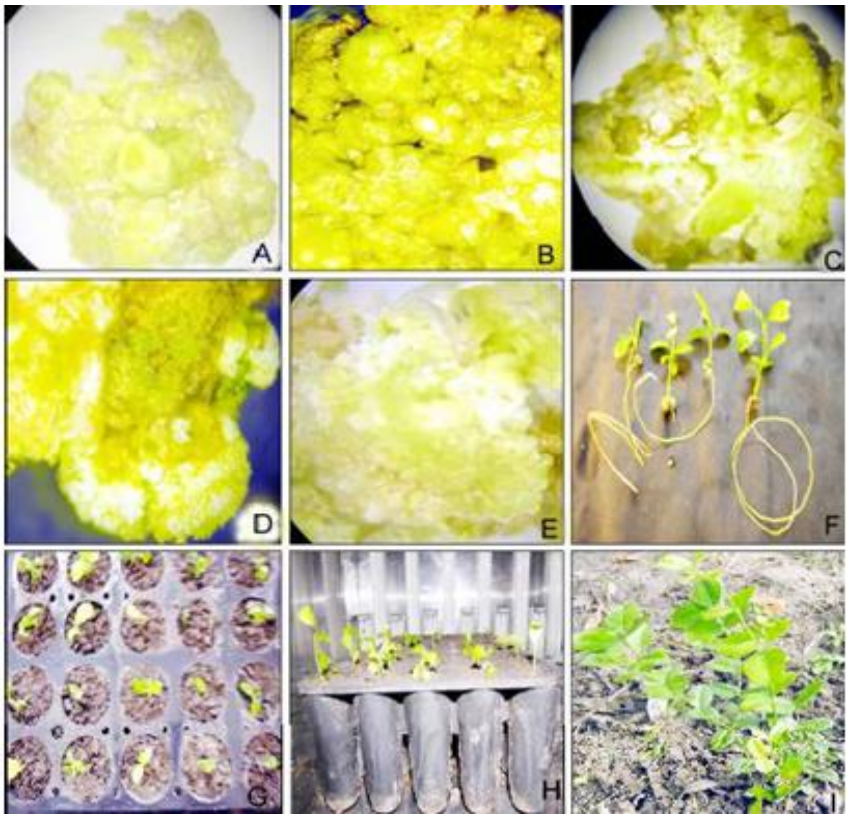


Fig. 2. Plant regeneration *via* indirect organogenesis from nodal segment culture in *Citrus species*: A. Callus formation from cultured nodal segment; B. Initiation of globular somatic embryos; C. Germination of somatic embryo; D. Initiation of protuberances on surface of callus mass; E. Shoot initiation *via* indirect organogenesis; F. *In vitro* rooting of regenerants; G. Hardening of regenerants in Environmental Growth Cabinet; H. Hardening of regenerants in greenhouse; and K. Plant transferred in Field

The analysis of variance offered in Tables 1-4 indicated that there were highly significant ($p<0.01$) differences present amongst the response of species, nutrient media combinations along with their interactions in respect to overall callus induction, shoot proliferating efficiency, number of shoot (s) per explant and mean shoot length, root proliferating efficiency, number of root (s) and mean root length. It designates the incidence of substantial magnitude of variation amongst the diverse culture media combinations, species in addition to their interactions for the most of parameters inspected.

Table 1. Effect of different auxins (alone) in varying concentrations on *In vitro* response of cultured nodal segments

Culture media x Species	Callus induction (%)				Shoot proliferating explants (%)				No. of shoot (s) per responding explant				Mean shoot length (in cm)			
	Acid lime	Mandarin	Sweet orange	Mean	Acid lime	Mandarin	Sweet orange	Mean	Acid lime	Mandarin	Sweet orange	Mean	Acid lime	Mandarin	Sweet orange	Mean
MS.5N	60.58	62.33	61.74	**61.55**^{b}	17.16	26.50	29.45	**24.37**^{c}	2.01	1.98	2.02	**1.98**^{a}	0.62	0.58	0.59	**0.59**^{b}
MSN	61.53	60.66	64.97	**62.38**^{ab}	26.90	23.09	22.31	**24.10**^{c}	1.91	1.89	1.82	**1.87**^{a}	0.53	0.58	0.54	**0.55**^{b}
MS2N	60.11	62.53	63.71	**62.11**^{b}	46.19	27.30	24.96	**32.81**^{a}	2.01	2.12	2.20	**2.11**^{a}	0.57	0.54	0.53	**0.54**^{b}
MS3N	45.01	42.22	44.49	**43.90**^{e}	29.21	27.59	28.83	**28.54**^{b}	1.86	1.71	1.73	**1.97**^{a}	1.06	0.43	1.01	**0.83**^{a}
MS4N	42.20	44.55	43.01	**43.25**^{e}	19.53	19.09	21.32	**19.98**^{de}	1.39	1.29	1.19	**1.29**^{a}	0.49	0.22	0.35	**0.35**^{c}
MS5N	40.99	41.53	41.33	**41.28**^{e}	17.22	15.01	14.93	**15.72**^{g}	1.11	1.09	1.05	**1.08**^{a}	0.33	0.10	0.41	**0.28**^{c}
MS.5D	62.23	64.52	64.98	**63.91**^{a}	15.99	16.87	13.12	**15.30**^{gh}	1.42	1.32	1.22	**1.32**^{a}	0.26	0.69	0.63	**0.52**^{b}
MSD	64.09	63.36	64.97	**64.61**^{a}	28.23	25.66	23.65	**25.84**^{c}	1.91	1.95	1.82	**1.89**^{a}	0.53	0.71	0.64	**0.62**^{ab}
MS2D	67.59	66.31	65.93	**66.61**^{a}	44.20	20.30	21.65	**28.23**^{b}	1.87	1.83	1.71	**1.80**^{a}	0.58	0.41	0.53	**0.50**^{b}
MS3D	57.77	58.28	58.90	**58.31**^{c}	22.19	20.19	20.30	**21.38**^{d}	1.83	1.73	1.63	**1.73**^{a}	0.58	0.29	0.32	**0.29**^{c}
MS4D	55.55	53.93	54.77	**54.75**^{d}	29.03	18.53	17.01	**21.52**^{d}	1.78	1.29	1.41	**1.49**^{a}	0.19	0.33	0.29	**0.27**^{c}
MS5D	51.79	49.99	48.64	**50.14**^{d}	13.33	17.75	18.30	**16.46**^{g}	1.12	1.17	1.11	**1.13**^{a}	0.12	0.11	0.23	**0.15**^{c}
MS.5T	59.53	57.52	58.50	**58.51**^{bc}	18.93	16.35	19.83	**18.37**^{er}	1.83	1.06	1.33	**1.40**^{a}	0.49	0.52	0.53	**0.51**^{b}
MST	57.55	58.93	57.53	**58.00**^{c}	17.35	18.35	17.05	**17.58**^{f}	1.89	1.14	1.50	**1.50**^{a}	0.41	0.32	0.37	**0.36**^{bc}
MS2T	59.01	58.32	48.53	**55.28**^{cd}	16.90	17.85	16.30	**17.01**^{fg}	1.33	0.86	1.16	**1.11**^{a}	0.39	0.35	0.30	**0.34**^{c}
MS3T	39.93	39.99	40.23	**40.05**^{e}	15.9	16.88	16.87	**16.55**^{g}	1.77	1.79	1.84	**1.60**^{a}	0.41	0.23	0.30	**0.31**^{c}
MS4T	39.56	38.53	38.88	**38.99**^{e}	14.39	15.05	15.53	**14.99**^{h}	1.52	1.08	1.55	**1.38**^{a}	0.39	0.19	0.29	**0.29**^{c}
MS5T	35.50	32.19	30.16	**32.61**^{f}	13.03	14.33	13.99	**13.78**^{h}	1.11	1.09	1.05	**1.08**^{a}	0.12	0.11	0.25	**0.16**^{c}
Mean	**53.36**	**53.09**	**52.84**		**22.53**^{a}	**19.81**^{b}	**19.74**^{b}		**1.64**	**1.46**	**1.58**		**0.43**^{a}	**0.37**^{a}	**0.45**^{a}	
CD 0.05				**NS**				**0.72**				**NS**				**0.12**
Species				**5.27**				**1.77**				**1.58**				**0.29**
Media				**9.13**				**3.07**				**NS**				**0.50**
S x M																

- *Values within column followed by different letters are significantly different at 5% probability level.*
- *NS:Non-significant*

Table 2. Effect of different cytokinins (alone) in varying concentrations on *In vitro* response of cultured nodal segments

Culture media x Species	Callus induction (%)				Shoot proliferating explants (%)				No. of shoot (s) per responding explant				Mean shoot length (in cm)			
	Acid lime	Mandarin	Sweet orange	Mean	Acid lime	Mandarin	Sweet orange	Mean	Acid lime	Mandarin	Sweet orange	Mean	Acid lime	Mandarin	Sweet orange	Mean
MS.5B	15.20	14.93	14.69	**14.94**^{a}	70.19	71.04	75.17	**72.81**^{a}	2.91	2.83	2.36	**2.70**^{d}	1.19	1.12	1.20	**1.17**^{b}
MSB	14.36	15.92	14.02	**14.76**^{ab}	69.10	65.55	65.42	**66.69**^{b}	2.52	2.66	1.35	**2.17**^{de}	1.03	1.12	1.19	**1.11**^{b}
MS2B	13.22	13.63	14.55	**13.80**^{b}	55.11	56.19	63.07	**58.12**^{d}	2.31	2.21	1.19	**1.90**^{e}	1.07	1.13	0.99	**1.06**^{b}
MS3B	12.36	10.86	11.02	**11.41**^{b}	60.31	50.09	60.32	**56.90**^{de}	1.90	1.91	1.17	**1.66**^{e}	1.19	1.12	0.83	**1.04**^{b}
MS4B	8.66	9.01	9.53	**9.06**^{c}	55.01	55.73	51.20	**54.91**^{e}	1.84	1.20	1.11	**1.38**^{e}	1.01	1.03	0.99	**1.01**^{b}
MS5B	6.53	6.93	5.01	**6.15**^{c}	45.22	40.17	45.31	**43.96**^{fg}	1.19	1.12	1.02	**1.11**^{e}	0.99	0.81	0.83	**0.87**^{b}
MS.5Kn	13.22	10.52	12.20	**11.98**^{b}	65.15	62.09	65.33	**64.19**^{b}	2.71	2.80	2.53	**2.68**^{d}	1.19	1.15	1.21	**1.18**^{b}
MSKn	10.90	11.01	9.98	**10.63**^{b}	65.09	62.02	65.05	**64.05**^{b}	2.63	2.72	2.50	**2.61**^{d}	1.13	1.11	1.13	**1.12**^{b}
MS2Kn	9.53	8.03	8.26	**8.60**^{c}	60.12	60.15	55.09	**58.45**^{cd}	1.22	1.37	1.52	**1.37**^{e}	1.10	0.98	1.01	**1.03**^{b}
MS3Kn	9.93	6.71	6.15	**7.87**^{c}	55.12	57.12	53.05	**55.09**^{e}	2.09	1.77	2. 01	**1.97**^{e}	1.02	1.10	0.95	**1.02**^{b}
MS4Kn	6.03	7.75	7.25	**7.01**^{c}	55.01	55.73	51.31	**45.95**^{f}	1.27	1.02	1.12	**1.13**^{e}	0.93	1.07	1.03	**1.01**^{b}
MS5Kn	5.53	6.97	5.20	**5.90**^{c}	40.18	40.29	38.02	**39.49**^{g}	1.07	1.83	1.08	**1.32**^{e}	0.99	0.93	0.89	**0.93**^{b}
MS.1Td	11.92	10.55	11.03	**11.16**^{b}	70.86	80.29	80.12	**77.09**^{a}	11.42	13.20	15.29	**13.30**^{a}	4.23	3.93	4.01	**4.01**^{a}
MS.2Td	10.99	9.22	9.93	**10.04**^{bc}	75.17	65.12	60.12	**66.80**^{b}	12.32	11.52	14.23	**12.69**^{a}	4.11	3.99	4.53	**4.31**^{a}
MS.3Td	9.02	9.82	8.58	**9.14**^{c}	70.05	60.33	51.31	**63.49**^{bc}	8.32	10.39	11.92	**10.21**^{b}	4.42	4.33	4.49	**4.35**^{a}
MS.4Td	8.23	8.66	8.01	**8.30**^{c}	55.12	50.17	50.33	**51.87**^{e}	8.93	11.52	9.11	**9.85**^{b}	2.93	2.82	2.73	**2.82**^{ab}
MS.5Td	8.08	7.88	7.65	**7.87**^{c}	40.18	40.29	38.02	**39.49**^{g}	4.92	3.02	5.29	**4.41**^{c}	2.81	2.70	2.69	**2.73**^{b}
MSTd	5.90	5.85	5.81	**5.85**^{c}	65.08	55.12	55.07	**58.42**^{d}	4.52	4.59	5.12	**4.74**^{c}	2.63	2.53	2.59	**2.58**^{b}
MS2Td	5.01	5.72	5.44	**5.39**^{c}	60.32	50.32	55.12	**55.26**^{e}	4.02	4.11	4.39	**4.17**^{c}	2.03	2.19	2.22	**2.14**^{b}
Mean	**9.71a**	**9.44a**	**9.21a**		**59.4a**	**56.68b**	**57.23b**		**3.80**	**3.91**	**4.07**		**1.81**	**1.77**	**2.13**	
CD 0.05																
Species				**1.93**				**2.19**				**NS**				**NS**
Media				**4.86**				**5.51**				**1.23**				**1.95**
S x M				**8.42**				**9.55**				**2.14**				**NS**

- *Values within column followed by different letters are significantly different at 5% probability level.*
- *NS: Non-significant*

Table 3. Combined effect of different added auxins and cytokinins in varying concentrations and combinations on *in vitro* response of cultured nodal segments

Culture media x Species	Callus induction (%)				Shoot proliferating explants (%)				No. of shoot (s) per responding explant				Mean shoot length (in cm)			
	Acid lime	Mandarin	Sweet orange	Mean	Acid lime	Mandarin	Sweet orange	Mean	Acid lime	Mandarin	Sweet orange	Mean	Acid lime	Mandarin	Sweet orange	Mean
MS.5B.5 N	68.19	60.27	66.01	**64.82**^{f}	96.23	72.80	95.07	**88.03**^{a}	6.12	4.27	3.27	**4.54**^{ab}	2.12	1.40	1.90	**1.80**^{b}
MSB.5 N	62.53	61.95	61.27	**61.91**^{g}	90.01	80.05	70.33	**80.13**^{b}	4.09	3.33	2.11	**3.17**^{b}	2.19	2.19	1.39	**1.92**^{a}
MS2B .5N	65.23	60.56	61.95	**62.58**^{fg}	70.19	71.04	75.17	**72.81**^{c}	3.98	3.12	2.03	**3.04**^{bc}	1.81	2.11	1.12	**1.68**^{b}
MS3B.5N	58.59	55.94	58.56	**57.69**^{hi}	64.52	60.03	66.04	**63.53**^{e}	6.02	3.29	2.26	**3.85**^{b}	1.79	1.67	1.90	**1.78**^{b}
MS4B.5N	56.38	57.76	57.94	**57.36**^{i}	60.32	70.05	55.05	**62.47**^{e}	2.23	2.10	2.03	**2.12**^{c}	1.65	1.26	1.03	**1.31**^{b}
MS5B.5N	56.13	54.94)	55.88	**55.65**^{i}	60.31	50.09	60.32	**56.90**^{g}	2.13	2.09	1.17	**1.79**^{c}	1.55	1.19	0.99	**1.24**^{b}
MS.5Kn.5N	65.50	64.99	64.78	**65.09**^{ef}	81.11	90.12	80.66	**83.63**^{b}	4.16	4.12	3.76	**4.01**^{ab}	2.02	0.83	1.21	**1.35**^{b}
MSKn.5N	62.91	62.52	61.59	**62.34**^{g}	70.55	73.09	72.12	**71.92**^{c}	2.85	3.11	2.19	**2.71**^{c}	2.13	2.15	1.03	**1.77**^{b}
MS2K.5N	60.51	60.83	60.01	**60.45**^{gh}	55.12	60.04	55.59	**56.91**^{g}	4.12	4.01	3.15	**3.76**^{b}	2.04	2.11	1.14	**1.76**^{b}
MS3Kn.5N	57.83	59.90	59.13	**58.95**^{h}	60.12	60.15	55.09	**58.45**^{g}	3.11	2.88	2.18	**2.72** ^{c}	1.99	2.13	1.13	**1.75**^{b}
MS4Kn.5N	60.21	58.28	53.61	**57.36**^{i}	65.09	62.02	65.05	**64.05**^{de}	3.66	2.13	2.90	**2.89**^{c}	1.44	2.01	2.43	**1.86**^{ab}
MS5Kn.5N	65.52	58.13	58.77	**60.80**^{g}	61.91	61.19	60.23	**61.18**^{ef}	1.05	1.83	1.08	**1.32**^{c}	1.62	1.39	2.33	**1.78**^{b}
MSN.5B	62.26	69.72	68.49	**66.82**^{e}	60.07	75.12	55.12	**63.64**^{e}	3.11	3.03	2.97	**3.03**^{c}	2.38	1.01	1.91	**1.76**^{b}
MS2N.5B	58.13	83.93	84.59	**75.55**^{b}	71.29	69.12	72.09	**70.85**^{c}	3.09	2.99	2.72	**2.93**^{c}	2.03	1.29	1.90	**1.74**^{b}
MS3N.5B	58.76	79.33	79.94	**72.67**^{c}	63.12	68.13	65.79	**65.68**^{cd}	2.92	2.81	2.83	**2.85**^{c}	1.70	1.11	1.70	**1.50**^{b}
MS4N.5B	54.94	83.71	82.19	**73.61**^{bc}	62.31	68.03	65.32	**65.22**^{d}	1.87	1.89	1.95	**1.90**^{c}	1.3	1.09	1.13)	**1.28**^{b}
MS5N.5B	60.20	84.55	88.77	**77.17**^{a}	55.07	50.01	45.09	**50.05**^{h}	1.73	1.74	1.53	**1.66**^{c}	1.49	1.17	1.12	**1.26**^{b}
MSN.5Kn	63.01	80.11	80.03	**74.38**^{b}	72.29	71.82	72.09	**72.04**^{c}	3.23	3.21	3.11	**3.18**^{b}	1.43	1.19	1.03	**1.21**^{b}
MS2N.5Kn	61.52	75.20	75.01	**70.57**^{cd}	70.04	70.12	72.19	**70.78**^{c}	1.87	1.11	1.91	**1.63**^{c}	1.01	1.12	1.11	**1.08**^{b}
MS3N.5Kn	60.83	79.52	73.10	**71.15**^{c}	62.19	65.23	60.53	**62.65**^{e}	2.81	2.89	2.95	**2.88**^{c}	2.24	1.18	1.09	**1.50**^{b}
MS4N.5Kn	59.90	83.30	81.69	**74.96**^{b}	62.11	59.23	60.33	**60.57**^{f}	2.79	2.75	2.71	**2.70**^{c}	2.07	1.12	1.11	**1.43**^{b}
MS5N.5Kn	60.28	82.15	84.61	**75.68**^{ab}	59.20	57.19	57.93	**58.55**^{fg}	1.91	1.43	1.33	**1.55**^{c}	2.21	1.03	0.93	**1.39**^{b}
MS.1Td.5N	56.05	76.13	76.20	**69.46**^{d}	88.50	89.09	88.95	**88.84**^{a}	5.33	4.98	4.60	**4.97**^{a}	1.73	1.01	2.20	**1.64**^{b}
MSTd.5N	65.99	68.56	66.72	**67.75**^{de}	82.01	81.35	80.63	**81.33**^{b}	4.23	3.72	3.01	**3.65**^{b}	0.40	2.13	2.01	**1.51**^{b}
MS2Td.5N	42.10	40.20	40.29	**40.86**^{J}	45.32	30.06	50.51	**41.85**^{i}	1.98	1.08	1.10	**1.38**^{c}	0.98	1.11	1.15	**1.08**^{b}
Mean	**59.30**^{b}	**68.09**^{a}	**68.04**^{a}		**69.59**^{a}	**69.21**^{a}	**68.47**^{b}		**3.30**^{a}	**2.97**^{a}	**2.62**^{a}		**1.73**^{a}	**1.44**^{b}	**1.43**^{b}	
CD 0.05																
Species				**1.04**				**1.23**				**0.61**				**0.28**
Media				**2.99**				**3.55**				**1.75**				**0.81**
S x M				**5.18**				**6.15**				**3.04**				**1.40**

Table 4. Effects of different plant growth regulators on *in vitro* rooting of shootlets

Culture media ▼ Species	Root proliferating shootlets (%)				Numbers of roots				Root length (in cm)			
	Acid lime	**Mandarin**	**Sweet orange**	**Mean**	**Acid lime**	**Mandarin**	**Sweet orange**	**Mean**	**Acid lime**	**Mandarin**	**Sweet orange**	**Mean**
MS.5IB	82.12	77.15	73.66	**77.64^{a}**	2.99	2. 07	2.37	**2.47^{b}**	3.33	3.30	3.34	**3.32^{a}**
MS IB	76.09	71.32	72.09	**73.16^{c}**	2.18	3.10	3.33	**2.87^{b}**	3.19	3.15	3.19	**3.17^{b}**
MS2 IB	71.22	69.12	66.33	**68.89^{e}**	3.19	2.29	2.93	**2.80^{b}**	2.99	2.31	2.25	**2.28^{bc}**
MS3 IB	61.32	62.05	60.12	**61.16^{g}**	1.12	1. 08	1.29	**1.16^{b}**	2.12	2.30	2.02	**2.14^{c}**
MS.5N	75.12	71.19	74.04	**73.45^{c}**	2.20	3.12	3.98	**3.10^{b}**	3.20	3.39	3.12	**3.23^{ab}**
MSN	70.66	70.60	71.10	**70.45^{de}**	1.98	1.58	2.22	**1.92^{b}**	2.12	2.30	2.02	**2.14**
MS2N	69.52	68.12	69.16	**68.93^{e}**	1.10	1. 09	1.87	**1.35^{b}**	3.09	3.19	3.03	**3.10^{b}**
MS3N	61.33	59.72	58.79	**59.94^{gh}**	1.20	1. 07	1.10	**1.12^{b}**	1.78	1.70	1.01	**1.52^{c}**
MS.5Kn	79.12	75.10	76.66	**76.96^{b}**	3.19	2.29	2.93	**2.80^{b}**	3.59	3.23	3.08	**3.30^{a}**
MSKn	67.03	61.01	65.10	**64.38^{f}**	2.19	2. 09	2.10	**2.12^{b}**	2.97	2.78	2.12	**2.62^{b}**
MS2Kn	75.42	77.03	72.33	**74.92^{bc}**	3.33	3.13	3.27	**3.24^{ab}**	2.11	2.61	2.03	**2.25^{c}**
MS3Kn	58.17	60.66	56.10	**58.31^{h}**	1.20	1.97	1.33	**1.50^{b}**	1.99	1.60	1.49	**1.69^{c}**
MS.5IB.5B	79.10	77.32	75.12	**77.18^{ab}**	2.91	3.22	3. 06	**3. 06^{b}**	3.60	3.78	3.29	**3.61^{a}**
MS IB.5B	75.22	75.10	71. 09	**74.13^{bc}**	3.71	2.97	2.77	**2.97^{b}**	3.53	3.33	3.45	**3.38^{a}**
MS2 IB.5B	72.10	75.11	70.33	**72.51^{cd}**	2.11	1.81	2. 03	**1.98^{b}**	3.12	3.03	2.99	**3.04^{b}**
MS.5IB.5Kn	70.11	71.92	73.10	**71.71^{d}**	2.91	2.92	2.98	**2.98^{b}**	3.47	3.52	3.42	**3.47^{a}**
MS IB.5Kn	73.01	71.34	75.91	**73.42^{c}**	2.98	2.22	2.87	**2.69^{b}**	3.42	3.49	3.39	**3.43^{a}**
MS2 IB.5Kn	79.12	75.10	76.66	**76.96^{b}**	3.97	3.12	3.50	**3.53^{a}**	3.59	3.23	3.08	**3.30^{a}**
MS IB.5Kn	67.03	61.01	65.10	**64.38^{f}**	1.13	1.19	1.93	**1.44^{b}**	2.97	2.78	2.12	**2.62^{b}**
Mean	**71.90^{a}**	**70.35^{b}**	**69.69^{b}**		**2.38**	**2.24**	**2.52**		**2.97^{a}**	**2.91^{a}**	**2.68^{a}**	
CD (0.05)												
Species				**1. 07**				**NS**				**0.40**
Media				**2.69**				**2.32**				**1.00**
S x M				**NS**				**NS**				**1.74**

- *Values within column followed by different letters are significantly different at 5% probability level.*
- *NS: Non-significant*

Higher concentration of an auxin NAA in amalgamation with a lower concentration of BAP/Kn cytokinin enabled higher grade of callus initiation. Higher callus initiation occurrence was experimented on culture media MS5N.5B/ MS5N.5Kn as compared to MS5Kn.5N/MS5B.5N that suggested that higher concentration of an auxin in association with a lower concentration of a cytokinin is needed for this aim. Much lower outcomes with 2, 4-D, NAA or 2, 4, 5-T auxin alone with media MS5D, MS5N or MS5T as well as BAP, kinetin or TDZ cytokinin alone with media MS5B, MS5Kn or MS2Td even in higher concentrations as compared to combination of NAA with BAP/Kn revealed that a cytokinin as well as an auxin (alone) were inadequate for higher degree of callus initiation. However, culture medium fortified with auxins (alone) initiated calli in higher frequencies as compared to culture medium containing cytokinins (alone). Similar findings have also been reported by Vibhute et al. [49] for hypocotyl, Vibhute et al. [50] for mature cotyledon and Vibhute et al. [62] for nodal segment cultures in *Citrus species,* for different explants cultures [71-78] in soybean in onion [79-80], muskmelon [81-82] and Indian mustard [83-85].

In respect to shoot proliferating aptitude, culture media containing auxin alone in varying concentrations proliferated shootlets in lower frequency, *i.e.,* lesser number of explants proliferated shoots. Culture media MS.1Td and MS.5B containing lower concentration of cytokinin as sole proliferated shoots in higher percentage (72%) suggested that a lower concentration of cytokinin is necessitated for this purpose. However, shoot proliferating efficiency was enhanced with addition of an auxin in combination with a cytokinin. Nutrient media MS.1Td.5N and MS.5B.5N containing lower concentrations of both cytokinin BAP/TDZ as well as auxin NAA, showed higher shoot proliferating ability (more than 88% explants proliferated shoots) suggested that nodal segment cultures of citrus performed well in combinations. Similar results have been addressed by various other scientists for ovule culture [47], nodal segment culture [86,56,61,62], shoot tip culture [86], hypocotyl culture [50] and mature cotyledon culture [49] in *Citrus species*. In terms of number (s) of shoots per responding explant, culture media amended with auxins (alone) in variable concentrations proliferated shootlets in lesser numbers. Nutrient media MS.1Td and MS.2Td (encompassing very lower concentration of a cytokinin alone was responded well (~13 shoots per responding explant) proposed that a lower concentration of cytokinin is needed for this target. Lower level of cytokinin persuaded early bud sprouting. Multiple shoot induction needed the incidence of cytokinin in the culture medium, which is in closely related to results of other investigations [87,2, 11]. Nevertheless, lesser number of shootlets was formed on nutrient media fortified with an auxin alone along with an auxin combined with a cytokinin. Application of TDZ, a cytokinin as sole at the concentration of 1.0 mgl^{-1} was proved suitable for forming of shootlets of higher length. Shootlets of higher length was recovered on culture media MS.3Td, MS.1Td and MS.4Td (fortified with TDZ in variable concentrations) as compared to regeneration media MSB.5N/MS3N that advised that higher concentration of a cytokinin in combination with a lower concentration of an auxin as well as higher concentration of an auxin (alone) is not adequate for formation of shootlets of

higher length. Furthermore, other cytokinins (BAP/ Kn) were not proved passable for this parameter.

In this research, *in vitro* rooting was investigated to be increasing subsequently shifting shootlets into root inducing medium. In citrus, auxins like IBA [88-104, 56, 86], IAA [61] and NAA [105] were proved an effective in inducing *in vitro* rooting. In current investigation, MS basal medium fortified with either IBA alone in range of 0.5-2.0 mgl^{-1} or in combinations with cytokinins (BAP/Kn) at the level of 0.5 mgl^{-1} was proved to be optimal for *in vitro* root proliferation. Root proliferation capability in higher magnitude was displayed by rooting media MS.5I and MS.5I.5B as compared to MS3I, MS3Kn or MS3N advised that IBA needed in lower concentration and appropriate for this purpose. The number (s) of roots increased with application of higher concentration of IBA (2.0 mgl^{-1}) in combinations with lower concentration (0.5 mgl-1) of a cytokinin (Kn/BAP). The root of higher length was recovered from medium fortified with equal concentrations of IBA (0.5 mgl^{-1}) and 0.5 mgl-1 cytokinin (BAP/Kn). Comparable findings were also documented by Gill et al. [46] and Syamal et al. [56] in citrus. Rooting medium amended with IBA alone resulted better in comparison to nutrient media supplemented with other auxin (NAA) even in higher proportions advised that IBA encourages *in vitro* rooting of plantlets acquired from nodal segment cultures in *Citrus*. Auxins encouraged adventitious root formation on intact plants in addition to excised stems. Among these, IBA was the most active than any other plant growth regulators in the most of the cases, deceptively since it is not devastated by IAA oxidase or other enzymes and hence continues longer.

In this study, substantial variation for *in vitro* response was documented amongst three species. Overall, Acid lime species tracked by Mandarin and Sweet orange performed better for the all of the culture stages. In *Citrus*, interspecific differences have also been testified for different explants cultures by Parthasarathy et al. [106]. Moreover, genotypic differences have also been reported by Parthasarathy et al. [106].

Besides interspecific and nutrient medium effect on all stages of various explants cultures, strong species x culture medium interactions for all the culture phases were also evident. Either nutrient medium MS5N.5B or MS5N.5Kn or both induced more than 80% calli. Either nutrient media MS.1Td.5N or MS.5B.5N or both proliferated shootlets more than 80%. While, for numbers of shoots per explant, either culture medium MS.1Td or MS.21Td or both performed well for the all species. This divulges that besides diverse responses of species for nutrient medium, specific species does not essentially respond in the same way to each of different nutrient media investigated. This recommends that a certain species designated for advance work could be inoculated on the most appropriate medium to acquire maximum response. Likewise, the probability occurs for enhancement of *in vitro* competence of a specific species by further culturing in modified nutrient medium.

In this research, generally nodal segment cultures displaying higher number of morphogenic calli regenerated more plantlets. Nevertheless, the procedure of morphogenesis was not an assumption of higher regeneration occurrences. For different explants cultures, single species or nutrient medium demonstrating higher morphogenic callus formation may regenerate plantlets in lesser numbers compared to lower morphogenic calli. Such aberrations happened as a single morphogenic calli formed none or several plants (up to 13 in clusters). Moreover, not all the regenerants converted into whole plants *i.e.,* shoots with roots. From time-to-time shootlet could not develop after initiation, in a few cases they were distorted and not all the shootlets developed without roots that were able to yield roots even on rooting medium.

In this research, plantlets generally initiated through direct organogenesis without intervening callus stage (*via* auxiliary bud proliferation). More prospect occurs for the production of the identical clones of the donor plants of such plantlets, since they are not exposed to the factors daunting somaclonal variations owing to evading long callus phase. Saini et al. [107], Savita et al. [108], Vijayakumari et al. [109]. Starrantino and Caruso [57] and Marin and Duranvila [58] for nodal segment culture of citrus and Bele et al. [25] for nodal segment culture of sandalwood documented analogous findings.

4. CONCLUSION

In this investigation, plant regeneration in higher frequencies was attained under optimized conditions. Nodal segment explant cultures of *Citrus species* contrary to other fruit crops give rise to higher numbers of shootlets. Higher plantlet regeneration was attained *via* direct organogenesis which offers a reliable means for producing identical propagules from mature trees selected for desired horticultural characteristics. Higher frequency of plantlets regeneration during current study was higher than the previous reports, The established protocol would help for genetic transformation (direct and / or *via* vector) purposes as well. A system for plant regeneration in *Citrus* is therefore obtainable, which could facilitate its application for *Citrus* improvement through conventional breeding and/or biotechnological strategies. Investigation involving other plant growth regulators may be prolific in improving shoot proliferation in forthcoming days.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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selection and characterized whitefly and YMV with molecular markers for soybean disease control in MP, isolated several plants growth-promoting rhizobacteria (PGPR) from the rhizosphere displaying various direct plant growth promoting attributes and generated more than 600 sequences for different genes generated from PGPRs have been published in the NCBI domain, performed DNA fingerprinting of major crops, including soybean, minor millets and different medicinal plant species. Filed a patent on newly developed methods for genotype identification based on simple sequence repeats marker data in 2017, which is under review. He also revealed DNA barcode in various medicinal plants with universal markers A patent "DNA barcode for species identification of sedge plants and methods thereof" was granted earlier this year. Another patent on DNA barcoding coupled high resolution melting analysis is under review. As a breeder he developed 2 varieties of rice and collaborator in 2 varieties of soybean (JS 20-94 and JS 20-116) and one variety of chickpea. He has teaching experience of more than 40 years and guided 59 post-graduate and 14 doctoral students in Agriculture Biotechnology and Plant Breeding & Genetics. He is a Supervisor/Mentor of five National Post-doctoral Fellow from DBT, DST and CSIR. He has published more than 110 Scientific Papers (out of which 29 are on soybean) in refereed journals, more than 60 papers presented in conferences, 01 book and 12 Book chapters.

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Chapter 11
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Assessment of Groundnut Germplasm Lines for Foliar Fungal Diseases and Population Structure Analysis using Gene Based SSR Markers

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ABSTRACT

Introduction: Peanut (*Arachis hypogaea* L.) is an exceptional cash crop having versatile applications of each plant part. Rust and late leaf spot (LLS) are two main foliar fungal diseases of groundnut and can cause yield damage up to 70%. Employment of fungicide is expensive approach and it is not environment-friendly also, consequently breeding new cultivars with genetic resistance is sustainable, eco-friendly and cost-effective method.
Aim: The present investigation aimed to screen groundnut germplasm lines employing allele specific molecular markers against foliar fungal diseases (LLS and rust).
Study Material: The present investigation contained 30 uncharacterized germplasm lines along with 4 check varieties of peanut. A set of 4 gene based SSR molecular markers were designated for screening of groundnut germplasm lines against foliar fungal diseases *i.e*., LLS and rust.
Results: For polymorphic SSR markers, a total of 14 alleles were identified, with an average of 3.5 alleles per locus. With an average of 0.47, the gene diversity and Polymorphic Information Content (PIC) values ranged between 0.3972-0.5778. In a UPGMA tree based on SSRs, the genetic links between peanut genotypes are shown. Four major demographic groups were formed using principle coordinate analysis (PCA) depending on origin. The population structure of the 34 genotypes was assessed employing STRUCTURE v2.3.3 software based on SSR markers. The optimum K value was determined by employing Structure Harvester, where the highest peak was observed at delta K = 2. The number of sub populations (K) was identified based on maximum likelihood and delta K (dK) values, with two core and pure groups and an admixture group. Present investigation identified four germplasm lines *viz*., AH8054, CS21181, CS708 and Akola White that may be used as foliar disease resistant cultivar for groundnut hybridization and improvement programme.

Keywords: Groundnut germplasm; SSR markers; population structure; PCoA.

1. INTRODUCTION

Peanut (*Arachis hypogea* L.) is one of the most important oilseed crops grown in the semi-arid tropics. It is an imperative self-pollinated oilseed crop cultivated in more than 100 nations with diverse agro-climatic situations on around on ~27.9 Mha land with worldwide yield of 47 metric tons in 2017 [1-5]. India positions leading with occupying cultivated area of 5.31 Mha (FAOSTAT 2017). Groundnut is one of the most important crops grown during the Kharif season, with Gujarat leading the way, followed by Andhra Pradesh, Tamil Nadu, Rajasthan, and Karnataka. The cultivated peanut (*Arachis hypogaea* L.) is an allotetraploid (2n = 4x = 40) with a genomic size of 2891 Mbp that evolved from a single hybridization and polyploidization event in South America. Peanut sub-genomes contain about

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64% repetitive sequence [6], thus less probabilities of polymorphism have been informed. In India, it is primarily an important oilseed crop but growing realization of confectionary qualities traits in kernel, it is now being realized as a food and fodder crop. It is esteemed as a rich source of energy in form of oil (48-50%) and protein (25- 28%) in the kernels. It delivers 564 kcal of energy from 100 g of kernels [7]. In addition, this, it encompasses several health advantageous nutrients for instance minerals, vitamins and antioxidants. Its haulms offer nutritious fodder for livestock. It comprises protein (8-15%), lipids (13%), minerals (9-17%) and carbohydrate (38-45%) higher than cereal fodder. The digestibleness of nutrients in peanut haulm is around 53% and that of crude protein 88% when fed to animals [8-12].

Cultivated groundnut is mainly grown in the semi-arid tropics region by poor farmers. Consequently, crop productivity has been adversely hampered by an array of biotic and abiotic factors. The main biotic stresses are early leaf spot (*Cercospora* spp.), late leaf spot (*Phaeoisariopsis personata*), rust (*Puccinia arachidis*), mottle virus *etc*. Moreover, aflatoxin adulteration severely distresses product quality and significantly diminishes worth of the crop plant along with grains. Among these, foliar fungal diseases *viz*., early leaf spot (*Cercospora arachidicola* Hori), late leaf spot and rust (*Puccinia arachidis* Speg.) damaged this crop significantly. Foliar fungal diseases are the major production constraints of groundnut worldwide wherever the crop is grown. These diseases can cause more than 70% loss in yield besides harmfully influencing the quality of the produce (pods, seeds and haulms) [13]. Late leaf spot is a major and widely distributed disease. It can cause defoliation and reduce pod and fodder yields about 50% and adversely affect quality of its produce [14]. Rust is also economic important disease-causing yield losses in range of 10 - 52%, in addition to a decline in seed quality [14]. Foliar diseases can be control by chemical measures but they increase costs of production thus beyond the reach of small and marginal farmers and also pollute the environment. Chemical control measures are accessible but they increase production costs by 10% [15] and are beyond the scope of small and marginal farmers. In view of above actualities, development and growing of resistant cultivars is the best feasible opportunity to abate economic losses of farmer and sustains good quality of the product. Conventional breeding has been the major path for providing modern groundnut cultivars to farmers and has been efficacious in some crops but groundnut has lagged behind owing to lack of information about molecular markers linked to traits of interest. Significant progress in the area of molecular breeding increased the output of breeding tactics, especially where phenotypic selection is problematic, expensive and more importantly lack accuracy. Modern breeding methods *i.e.,* molecular breeding may be supportive for solving these difficulties.

Marker-aided selection is an imperative device to select enhanced tolerance/resistance lines against these stresses and has the potential to allow faster and larger gains through genetic improvement of popular varieties [16-36]. The transfer of targeted traits has been accomplished in 2-3 years through marker- aided backcrossing (MABC) as contrast to 6- 8 years required with conventional breeding methods [37]. Over the last few years, about 5000 SSR markers have been developed for peanut [38,39,40]. Zhao et al. [41] has recognized a total of 135,529 and 199,957 SSRs from the 1,084.3 and 1,353.8 Mb genomic sequences of *A. duranensis and A. ipaensis*, correspondingly. Identification of resistant sources and knowledge of components and mechanism of resistance are the pre-requisite for the accomplishment of disease resistance breeding programmes. Inadequate disease occurrence also confuses the selection of resistant plants under field conditions [42]. Mondal et al. [38] and Chaudhari et al. [43] employed a set of 340 diverse groundnut genotypes and screened for LLS and rust resistance along with yield attributing traits across three locations in India under natural and artificial disease epiphytotic circumstances. The investigation exposed significant variation among the genotypes for LLS and rust resistance at different environments. Recently Chu et al. [44] established an RIL population from crossing Florida-07 × GP- NC WS16 and applied this population to map QTLs associated with ELS and LLS resistance. Screening and identification of germplasm and advance breeding lines against foliar fungal diseases is one of the primary objectives for resistant breeding. For late leaf spot and rust diseases, the investigation of Pandey et al. [45] has provided allele-specific PCR-based markers using QTL-seq approach. These newly developed markers are cost-effective and very calm to genotype for developing improved groundnut lines with enhanced resistance to LLS and rust. The current study was conducted to screen peanut germplasm line(s) employing gene-linked SSR molecular markers and identification of superior germplasm line(s) for higher yield along with foliar fungal disease resistance.

2. MATERIALS AND METHODS

The genetic materials comprised 30 uncharacterized germplasm lines and 4 varieties of peanut were acquired from DGR, Junagadh (Table 1). Check varieties for the experiment embraces GPBD4, foliar disease resistance and a high yielding variety; KDG128, foliar disease resistance and a high yielding variety; JGN3, variety released from Madhya Pradesh and sensitive to foliar diseases and Gangapuri, old local variety sensitive for foliar disease.

2.1 Genomic DNA Isolation and Genotyping with Gene Based Markers

About 3 to 5 young leaves from all the 34-peanut genotype were sampled from the 20 days old seedling grown in the field. Leaf samples were collected from first three plants to maintain homogeneity and disinfected by ethanol wiping to eradicate infections of diseases. The genomic DNA was extracted employing CTAB method [46] with minor modification [47-48]. The quality of the DNA was examined on 1% agarose gel and the DNA concentration was appraised with the micro volume spectrophotometer (Helix Biosciences, New Delhi, India). The DNA concentration for use in polymerase chain reaction (PCR) was diluted to 20-30 ng/µl.

A set of 4 gene-specific markers were designated for screening of peanut germplasm lines against foliar fungal diseases *i.e*., LLS and rust [17] (Table 2). The primers were synthesized by Eurofins Genomics India Pvt. Ltd. Polymerase chain reaction was accomplished in 10 µl reaction mixture encompassing of 1X PCR buffer, 0.1 U Taq DNA polymerase, 1 µl dNTP (1 mM), 0.5 µl of forward and reverse primers each (10 pM) and 2 µl (20 ng/µl) of genomic DNA in a thermocycler (Bio- Rad, USA). The PCR protocol encompassed of initial denaturation step of 94ºC for 3 min tracked by 35 cycles of 94ºC for 1 min, annealing at 55ºC for 30 sec, elongation at 72ºC for 1 min with final extension at 72ºC for 10 min. The PCR products were resolved on 3% agarose gel at 120V for 2-3 hrs and documented using UVP, Gel Documentation (Thermo Fisher Scientific). Gel scoring was done by base pair analysis using ladder based on banding pattern. Data sheet was prepared to run in population structure and allele pattern A/A was employed if band was in the upper side and pattern B/B was used if the band was in the lower side, in heterozygous condition banding pattern was A/B and in case of no amplification -/- was assigned.

2.2 Molecular Screening and Population Structure Analysis

Owing to the allele size the genetic profile of 34 peanut genotypes was scored employing SSR markers. The observations were chronicled for major allele frequency, polymorphism information content (PIC) and genetic distance by using Unweighted Pair Group Method for Arithmetic average (UPGMA) tree employing Power Marker v3.25 software [49] and the dendrogram was constructed using MEGA 6.0 software [50]. SSR data was again subjected to cluster analysis tracked by bootstrap analysis with 1000 permutations for all the genotypes using Mega 6.0 software. The population structure for 34 peanut genotypes encompassing both germplasm lines and released check varieties was inferred employing Structure 2.3.4 software [51]. The structure harvests were envisaged using Structure Harvester from which Evanno plots [52] were constructed. Principle Co-ordinate Analysis (PCoA) based on origin was documented employing GenAlex software.

3. RESULTS AND DISCUSSION

3.1 Phylogenetic Analysis and PIC Information

A set of four gene-specific primers were amplified across 30 groundnut germplasm lines along with 4 check cultivars. A total of 14 alleles were detected with an average of 3.5 alleles per locus for polymorphic SSR markers. The gene diversity and Polymorphic Information Content (PIC) values ranged between 0.3972-0.5778 for the markers GMRQ975 to GMRQ517 respectively with an average of 0.47 (Table 3). The primer which displayed highest gene diversity and PIC values was GMRQ517 while the lowest gene diversity and PIC values was evidenced for the marker GMRQ975. The major allele frequency varied between 0.53 (GMRQ517) to 0.67 (GMRQ786) with a mean worth of 0.61. The genetic relationships among peanut genotypes are presented in SSR based UPGMA tree (Fig. 1). All

the genotypes are congregated into 7 clusters. The genotypes in Cluster 1: TIPO, AH7608, CS23, JGN3, 51-44; Cluster 2:EC21010, AH6644, U2246, CS60, AH6312, AH7999; Cluster 3: KDG28, CS8180, TINAN WOR, TE STU KB, Gangapuri; Cluster 4: YOU KAICH, SUTHERN CROSS; Cluster 5: GPBD4, AH8054, CS21181, CS708, Akola White; cluster 6: Colorado Manfre; Cluster 7: AH7618, CS2377, A6, USA53, CS8501, ERECT PEANUT, 101U4717 and CS888 NC1 were forming group other than check varieties. Cluster 1 is forming group with JGN3 variety that is very sensitive to foliar fungal diseases, whereas cluster 2 is forming separate group having no check variety. Cluster 3 is grouped with KDG128 and Gangapuri hence showing moderately sensitive genotypes whereas clusters 4, 6 and 7 are forming separate groups having no check variety. Cluster 5 is grouping with GPBD4 hence may be resistant to foliar fungal diseases. The clusters based on SSR markers have been found to have relationship with sensitivity to foliar disease resistance. Most of the genotypes with the similar degree of foliar fungal disease resistance were clustered into same group.

3.2 Principle Co-ordinate Analysis for SSR

Principle Co-ordinate analysis (PCA) based on origin formed 4 major population groups. Group 1 included accessions from India, Argentina, USA and Taiwan, Group 2 included accessions mostly from India, Gambia, USA, Argentina and United Kingdom. Third group consisted of accessions from India, USA, United Kingdom, Argentina and the fourth group included accessions were largely from India and USA. The accessions in all the 4 groups included India. It means all the varieties of India are having highly diversified characteristics (Fig. 2).

Table 1. List of groundnut germplasm lines and their country of origin

S. No.	Country of origin	Germplasm line
1.	India	CS23, CS50, CS709, CS818-2, CS850-1, CS888, CS2118-1, CS2377, AS7808, AH7999, AH8054, AH7516, Akola White,51-44 Erect Peanut, GPBD4, KDG128, Gangapuri, JGN3
2.	USA	Southern Cross, USA53, Wo Rte Stu Kei, You Kalch
3.	SUDAN	A6, AH8312, Colorado Manfre
4.	Taiwan	Tainan#1
5.	Argentina	TIPO, U2-24-6, U4-7-9, U4-7-17
6.	Ghana	EC21010
7.	UNK	10-1
8.	UN	AH6644

Table 2. Allele specific primers used for screening of foliar fungal diseases of groundnut [45]

Trait	Marker name	Forward sequence	Reverse sequence	Annealing temp (ºC)	Size (in bp)
Rust	GMRQ517	TGTACCTGAAAT GCAAGTTGAGAC	AATGTATGTGT GTTGGGCCC	59	150
Rust	GMRQ786	AACATTGTAACA CTCACCTGGCTA	TCATGCTTGAA C TGTGCCTC	59	200
Rust	GMRQ843	AGCCTTGCGACT AGGTTCAT	CATGGTGAGA GACGCGTAAG	59	200
LLS	GMRQ975	GGTATCATGATG AATTTTTAGAAGA CTAGG	GAAATTTGGC TT TGGGTTCA	59	150

Table 3. Allele specific SSR markers presenting Major Allele Frequency (MAF), number of alleles, gene diversity and Polymorphic Information Content (PIC) in groundnut using power marker v3.25 software

Marker	MAF	Genotype number	Allele number	Gene diversity	PIC
GMRQ517	0.5294	4.0000	4.0000	0.6315	0.5778
GMRQ786	0.6765	3.0000	3.0000	0.4896	0.4400
GMRQ843	0.6176	4.0000	4.0000	0.5536	0.5022
GMRQ975	0.6176	3.0000	3.0000	0.4931	0.3972
Mean	**0.6103**	**3.5000**	**3.5000**	**0.5420**	**0.4793**

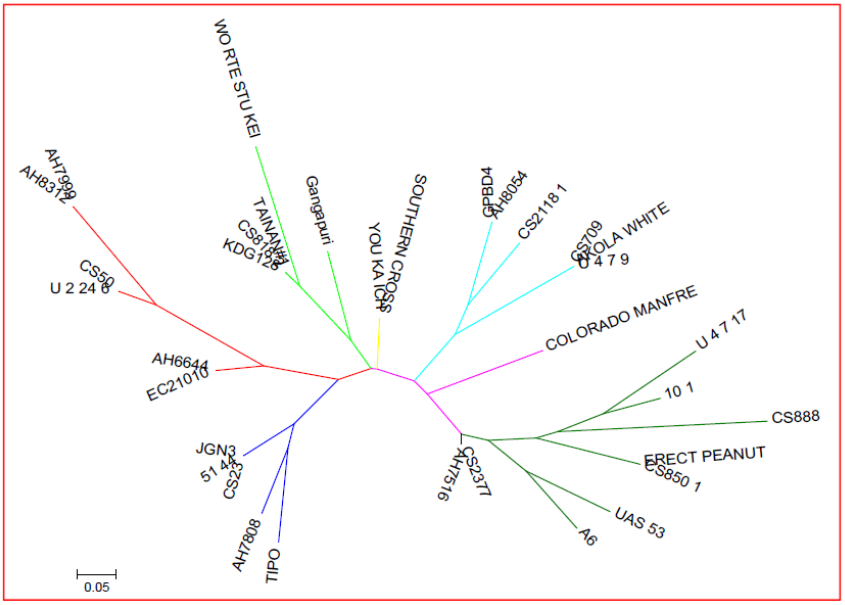


Fig. 1. Dendrogram of 34 groundnut germplasm lines based on banding pattern analysis of gene based SSR markers using MEGA 6.0 software

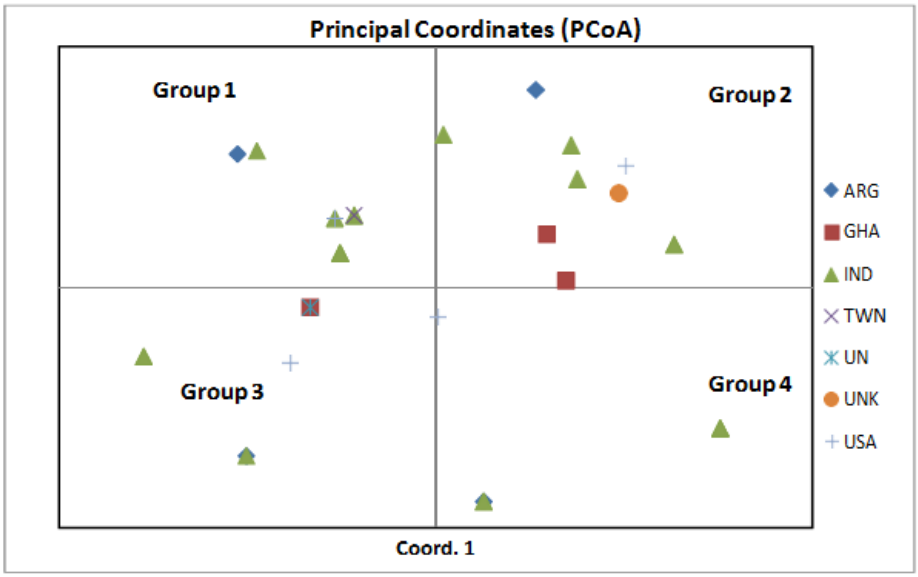


Fig. 2. Principal Coordinates Analysis (PCoA) based on allelic variation using SSR markers representing origin of groundnut germplasms line using GenAlex software

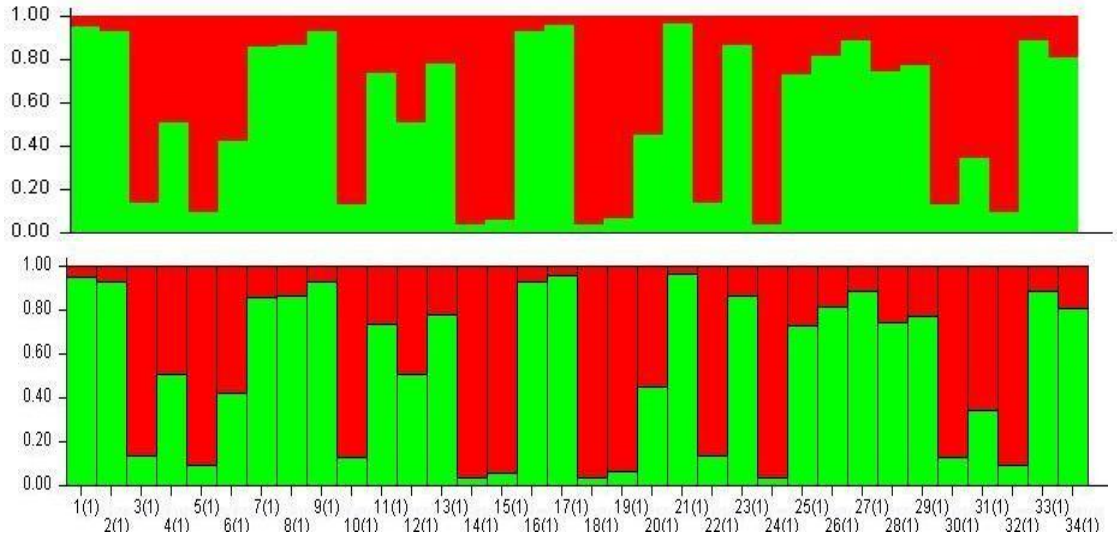


Fig. 3. Single line analysis and multiple line analysis representing two pure and an admixture group of groundnut germplasm lines by Structure 2.3.4 software

3.3 Population Structure Analysis

The population structure of the 34 peanut genotypes was assessed employing STRUCTURE v2.3.3 software based on SSR molecular markers. The optimum K value was determined by using Structure Harvester, where the highest peak was documented at delta K = 2. The number of sub populations (K) was detected owing to maximum likelihood and delta K (dK) values, with two core and pure groups and an admixture group (Figs. 3 and 4). Employing a membership probability threshold of 0.80, 18 genotypes were assigned to group1 (G_1), 11 genotypes to G_2 and 5 genotypes were allocated to admixture group G_3. The relationship between groups derived from STRUCTURE explained that G_1 and G_2 encompassed of distinct types. This indicated that the population structure was in accordance with clustering of peanut genotypes formed employing UPGMA tree based on SSR molecular data.

Molecular marker analysis on peanut germplasm lines employing an array of molecular markers for instance microsatellites or simple sequence repeats (SSRs), randomly amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) in overall has shown very low variation in cultivated gene pool owing to the evolutionary genetic bottleneck in the form of polyploidy and self-pollination [53,54,4,10]. Conversely, wild diploid *Arachis* species displayed comparatively higher variation, providing a rich source of genetic variation for genetic and genomic investigations. Among diverse marker systems examined in the groundnut, like other plant species, SSR markers have been found more informative and beneficial for genetic analysis and breeding applications [55,56,57,45] rather than morpho-physiological [58-59] and biochemical [60-66] characterization. There are numerous reports on in peanut gene-linked markers has been investigated against foliar fungal diseases [67,45,38]. Biotic stresses are one of the major yield restraining factors in peanut. Foliar fungal diseases of groundnut cause significance yield loss. Chemical control measures are obtainable but they upsurge production costs by 10% and are beyond the reach of small and marginal farmers as well as they are not eco-friendly tactics. Development of resistant variety is eco-friendly and cost-effective method. Marker-aided breeding spring exact result in less time for resistant variety development programme. In last decade's molecular breeding approaches has significantly augmented genome trait transfer in peanut improvement against foliar fungal diseases with higher yield [41]. In groundnut, the DNA polymorphism is very low so using genomics assisted breeding can be helpful to increase peanut productivity. Pandey et al. [45], have detected a highly informative set of SSR markers employing a preliminary set of 4400 SSR molecular markers across 20 genotypes representing parents of 15 mapping populations. Mapping of quantitative trait loci (QTL) is one of the auspicious ways to detect gene-based markers as well as resistant lines of groundnut and has been

applied widely for disease resistance [68, 69,70,71, 72]. Clevenger et al. [73] employed recombinant inbred line population segregating for quantitative field resistance was used to identify quantitative trait loci (QTL) using QTL-seq. This research establishes that QTL-seq can be employed to rapidly detect QTLs controlling highly quantitative traits in polyploid crops with complex genomes. Pandey et al. [45] recommended expediency of QTL‡seq approach in precise and rapid identification of candidate genomic regions and development of diagnostic markers for breeding applications. They employed that approach to locate the genomic region and candidate genes associated with resistance to rust and LLS in peanut. Freshly Chu et al. [44] developed an RIL population from crossing Florida-07×GP- NC WS 16 and applied this population to map QTLs associated with ELS and LLS resistance. For QTL mapping and gene identification, screening of germplasm line (s) and identification of donors to make crosses is one of the elementary necessities for resistant breeding. Gene-specific markers are cost effective and easy tactic for screening of peanut germplasm lines. In our investigation. we employed these markers and detected superior germplasm line(s) which may be used in hybridization programme for mapping and varietal improvement programme in groundnut.

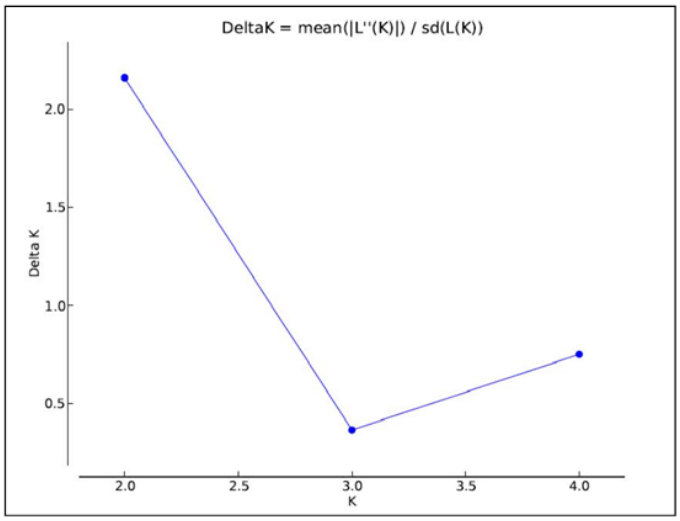


Fig. 4. Delta K value at maximum peak representing number of populations obtained by structure software analysis

4. CONCLUSION

Foliar fungal diseases *viz*., LLS and rust are the chief limiting reasons for peanut productivity and haulm benefits that deliver nutritious fodder for livestock. Development of foliar fungal disease resistant cultivar is cost effective and eco-friendly tactic. Resistant variety needs efficient donor for inclusion in hybridization programme, consequently germplasm screening is promising approach. Present investigation identified four germplasm lines including AH8054, CS21181, CS708 and Akola White to be employed as donor in peanut hybridization programme. The population structure of the 34 peanut genotypes was assessed using STRUCTURE v2.3.3 software based on SSR molecular markers. Using a membership probability threshold of 0.80, 18 genotypes were allotted to group1 (G_1), 11 genotypes to G_2 and 5 genotypes were allotted to admixture group G_3. The relationship between groups resulting from STRUCTURE elucidated that G_1 and G_2 embraced of distinct types. This designated that the population structure was in agreement with clustering of peanut genotypes formed employing UPGMA tree based on SSR molecular data.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Standardization of *In-vitro* Regeneration Protocol in *Gerbera jamesonii Bolus* Ex Hooker F.

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ABSTRACT

Introduction: The gerbera belong to the family Asteraceae is the chief cut flowers and ranks among the top ten cut flowers in the universe. For commercial propgation of this plant species, planting material is required on large-scale which requires the employment of plant tissue culture techniques for massive *in vitro* propagation.
Study Objectives: In this investigation**,** an effort was made to compute optimal concentration of plant growth regulators added in culture medium and optimize other physical factors exhibiting higher *in vitro* response by culturing mature embryo *in vitro*.
Results: Nutrient media MS3D.5B (MS + 3.0 mgl^{-1} 2, 4 D + 0.5 mgl^{-1}BA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar powder) evidenced more appropriate for callus initiation. Inoculation media MS2N.5iP/MS3N.5ip (MS + 2.0/3.0mg l^{-1} NAA + 0.5 mgl^{-1}2-ip + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) displayed higher *in vitro* response *i.e.,* numbers of shoot proliferating explants and numbers of shoot (s) per explant. While, shoot of higher length was recovered on culture medium MSB/MS2B (MS + 2.0/3.0 mgl^{-1}BA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar). Enhanced *in vitro* rooting response (root proliferating efficiency, numbers of roots and root of higher length) were documented on rooting medium MS.1IB (MS + 0.1 mgl^{-1}IBA + 15.0gl^{-1} sucrose + 7.5 g l^{-1} agar). The regenerants were transferred to pots and hardened in Environmental Growth Cabinet and net House and subsequently shifted to field conditions efficaciously. Phenotypic normal plants were acquired.

Keywords: Gerbera jamesonii; mature embryo culture; direct and indirect organogenesis; direct and indirect somatic embryogenesis and plantlet regeneration.

ABBREVIATIONS

MS : Murashige and Skoog medium;
NAA : α-Naphthalene acetic acid;
2, 4-D : 2, 4-dichlorophenoxyacetic acid;
2, 4, 5-T : 2, 4, 5 - trichlorophenoxyacetic acid;
BA : 6-benzylaminopurine;
Kn : Kinetin;
TDZ : Thidiazuron;
2-ip : N-isopentenyl amino purine;
IBA : Indole-3-butyric acid.

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1. INTRODUCTION

The gerbera belonging to the family Asteraceae is one of the chief cut flowers and grades among the top ten cut flowers in the world [1-2]. The species, a perennial herb and inborn in South Africa and Asia is cultivated throughout world in an extensive array of climatic circumstances. The genus *Gerbera* was baptized in honors of a German naturalist Traugott Gerber, who toured Russia in 1743. The genus comprises of around forty species. Out of the chronicled species, only one species *Gerbera jamesonii* is under farming. The flower makes an exceptional choice for any gift basket envisioned to brighten someone's day, or revel joyous events. The gerbera daisy has long been a sign of loveliness, purity and blamelessness.

Gerbera is being propagated by both sexual and asexual approaches. Most of the commercially cultivated varieties are propagated by vegetative methods to uphold homogeneousness and genetic limpidness. Among the vegetative methods, multiplication by division of clumps is the most communal technique employed for many decades. It can also be propagated by means of cuttings. The plants multiplication by these techniques is too slow to be commercially feasible. For commercialization of this crop, planting material is needed on huge scale which necessitates the advancement of an easier, faster and frugally feasible technique of propagation. The usage of biotechnological interventions particularly micropropagation facilitate an exclusive choice for mass multiplication of identical planting material. Plant Tissue Culture is an influential device for cloning and propagation of horticultural crop plants [3-31], particularly ornamentals [32-41]. Micropropagation methods upsurge the gauge and rapidity of production and yield an enhanced product [42-48]. Clonal propagation of gerbera has efficiently used to produce genetically pure plants certifying an exclusive colour shape for each variety. This technique allows a million-fold developments per year of a wanted crop species [49-50].

In gerbera, different explants have been employed proficiently to produce regenerable cultures *via* somatic embryogenesis and/or organogenesis are shoot tips [51-52,49], leaf discs [54-61,40,41,50,53], mature embryo [38], capitulum [62-66,52,56,57], petiole [67,58], flower stack [68], floral buds [69-71], floral bracts [72], torus [65], inflorescence [73] and cell suspension cultures [74,76,59,38] with varying degree of accomplishment.

Even though, some investigations have been carried out in some laboratories around the world to attain productive *in vitro* culture system of *Gerbera jamesonii* with differing regeneration competence, diminutive research has been done so for in M.P. on these facets. Moreover, *in vitro* response of plant tissues hinges on nature of genotype, the physiological status of the donor plant, the type of explant, constituents of nutrient medium and their interactions [77]. In light of these aspects, an effort was made to calculate optimal amount of plant growth regulators supplemented in nutrient medium and optimize other physical factors displaying higher *in vitro* morphogenesis by culturing of mature embryo *in vitro*.

2. MATERIALS AND METHODS

Mature embryo of Pink cultivar of *Gerbera jamesonii* were inoculated on diverse modifications of two basal media *viz*: Murashige & Skoog [78] and Gerbera multiplication medium. Seeds were acquired from Balaji Nursery, Indore (M.P.). In initial experimentations, MS medium was responded better than Gerbera multiplication medium (data not presented), henceforth, for advanced investigations basal MS medium was employed. All culture media were prepared from readymade basal medium (HiMidia^{TM}) and fortified with three different sets of plant growth regulators to fortify MS basal media. In first set: three diverse auxins, *viz.,* 2, 4-D, NAA and 2, 4, 5-T (alone), in second set: three different cytokinins *viz*: BAP, Kn and 2-ip (sole) and in third set: diverse auxins NAA and 2,4-D in association with cytokinins BAP, Kn and 2-ip in changing concentrations. In addition to MS basal macro and micro salts, vitamins, all culture media was added with 30.0 gl^{-1} sucrose and the final volume was made to 1000 ml and pH was adjusted to 5.8 ± 0.1 with 1N KOH solution before autoclaving. After adjusting the pH, agar powder @ 7.5 gl^{-1} was incorporated to the media as a semi-solidifying agent. Warm culture media, still in liquid state was dispensed into baby food bottles (50-60 ml / bottle) and culture tubes (15-20 ml/ tube) tracked by autoclaving at 121^{o}C under 15 psi pressures for 25 minutes.

Nevertheless, in case of pouring in Petri dishes, autoclaved warm culture media was poured into pre-sterilized 100 x17 mm glass Petri dishes (25-30 ml / dish) under aseptic conditions of Laminar Air Flow Clean Air Cabinet. Readymade basal media, plant growth regulators and other ingredients were procured from Hi-media Laboratories, Mumbai, India.

Explant mature embryos were excised from mature seeds and surface sterilized by washing with 2% Tween 20 (v/v) (a commercial detergent) for 15-20 minutes to eradicate dirt and residues followed by treating with 70% (v/v) ethanol for 30 seconds. Then seeds were subjected to different concentrations of Bavistin® (BASF, Germany) tracked by tumbling in two surface sterilant *i.e*., Ca $(OCl)_2$ and $HgCl_2$ in different concentrations and combinations for various periods (Table 1). Lastly, seeds were washed 4-5 times with sterile double distilled water and drenched for 12 hours in sterilized double distilled water. Mature embryos from pre-soaked seeds were excised and inoculated on the inoculation medium in 100 x 17 mm glass petri dishes and wrapped with Lab film (Parafilm®) and incubated under complete darkness at 25±2°C for one week. Afterward *in vitro* inoculated explants were exposed to photoperiod regime of 16 hours light / 8 hours dark cycle at an intensity of 2000-lux luminance provided by Photo-synthetically Active Radiation lamps at 25±2 ^{0}C and 60% RH regimes. After 4-5 weeks of initial inoculation, callus cultures were sub cultured on initial medium for regeneration of plantlets. Multiple shoots, acquired from direct organogenesis, (auxiliary bud proliferation) were transferred to elongation medium which was MS basal medium amended with 1.0 mgl^{-1} GA_3, 20.0 gl^{-1} sucrose and 7.5 gl^{-1} agar. Cultured baby food bottles /culture tubes were subjected to 25 ± 2^{o}C temperature and photoperiod regimes of 60 μ mol m^{-2} s^{-1} luminance facilitated by cool fluorescent tubes for 16 hr., For root induction, regenerants were later transferred to MS rooting medium fortified with diverse concentrations of IBA, NAA, Kn and combination of IBA with BA and Kn, 15.0 gl^{-1} sucrose and 7.5 gl^{-1} agar. For regeneration, elongation and rooting, reduced level of sucrose was applied based on previous work carried out by different researchers and preliminary experiences of our laboratory.

Regenerants were cautiously cleaned with running tap water to eradicate the sticked agar and were planted in 2.5 cm root trainers occupied with 1:1:1 sand, soil and FYM sterilized mixture. Root trainers with transplanted plantlets were relocated under 30±2^{o}C and 60 ± 5% Relative Humidity for 20-25 days to an Environmental Growth Cabinet for hardening. Afterward these regenerants were subjected to the green and net houses for 30-35 days for adaptation. Adapted plants lastly were transferred under field conditions.

The observations were documented for callus induction, numbers of shoot proliferating explants, average number of shoots per explant and mean shoot length for mature embryo culture and for *in vitro* rooting, for root proliferation, numbers of root (s) and an average root length. The experiment was conducted in Completely Randomized Design to realize the significance of diverse nutrient medium combination. Each treatment was containing of two replications. Per replication about 100-120 explants were inoculated on each media. The arc-sine transformations were adopted before data analysis, as all data were in percentage. The data were evaluated as per method prosed by Snedecor and Cochran [79].

3. RESULT AND DISCUSSION

Mature embryo was inoculated on diverse modifications of MS medium. The significance of governing contamination for accomplishing the targets of tissue culture. Diverse techniques with its individual recompences and drawbacks have been advised till now in various flowering plants. Employing sodium hypochlorite, mercuric chloride, alcoholic and antibiotic solutions are the mostly used. The sterilization process for capitulum explants of gerbera former was adopted by Shabanpour [7]. He pre-treated explants with 70% ethanol for 90 seconds and for 15 minutes with 1% aqueous solution of sodium hypochlorite and recovered only 34% disinfected explants. In our research, application of 0.5% bavistin in combination with 0.1% $HgCl_2$ exposed for 30 minutes braced higher proportion of aseptic culture (77.18%) establishment along with survival percentage (74.38%) (Table 1).

In present study, cultured mature embryos followed either direct or indirect pathway of plant regeneration depending upon the nature of different plant growth regulators supplemented in basal culture media. In direct approach, plantlets were regenerated on explant surface directly without callus

formation (*via* direct organogenesis) and in indirect mode; plantlets were originated from callus mass (either *via* indirect somatic embryogenesis or organogenesis).

Table 1. Effect of different surface sterilizing and antifungal agents on recovery of aseptic cultures

Treatments	Concentration (%)	Exposure time (in minutes)	Aseptic culture (%)	Survival of explants (%)
$Ca(OCl)_2$	5	10	12.37^{p}(20.56)	23.64^{k} (29.08)
$Ca(OCl)_2$	5	15	15.42^{o}(20.56)	28.01^{j} (31.94)
$Ca(OCl)_2$	5	20	36.49^{l} (23.10)	43.97^{f} (41.52)
$Ca(OCl)_2$	10	10	24.91^{n} (37.15)	45.98^{ef} (42.68)
$Ca(OCl)_2$	10	15	28.92^{m} (29.92)	54.54^{d} (47.59)
$Ca(OCl)_2$	10	20	38.05^{kl} (35.52)	62.57^{c} (52.26)
$Ca(OCl)_2$	15	10	37.25^{l} (38.07)	30.42^{l} (33.46)
$Ca(OCl)_2$	15	15	44.99^{j} (37.60)	44.15^{f} (41.62)
$Ca(OCl)_2$	15	20	46.01^{ij} (42.11)	24.37^{k} (29.56)
$Ca(OCl)_2$	20	10	64.59^{e} (53.46)	65.46^{b} (53.99)
$Ca(OCl)_2$	20	15	47.03^{i} (43.28)	64.17^{c} (53.21)
$Ca(OCl)_2$	20	20	48.75^{hi} (44.27)	62.71^{a} (52.34)
$HgCl_2$	0.1	2	39.92^{k} (39.17)	59.15^{d} (50.25)
$HgCl_2$	0.1	5	43.02^{j} (40.97)	25.62^{jk} (30.39)
$HgCl_2$	0.2	2	50.23^{gh} (45.11)	74.13^{a} (59.41)
$HgCl_2$	0.2	5	53.02^{g} (46.71)	34.51^{h} (35.96)
$HgCl_2$	0.2	10	76.86^{b} (61.23)	62.63^{c} (52.30)
Bavistin + $Ca(OCl)_2$	0.5 + 10	30	71.29^{cd} (57.58)	29.91^{i} (33.14)
Bavistin + $Ca(OCl)_2$	0.5 + 15	30	73.68^{c} (59.11)	37.64^{g} (37.83)
Bavistin + $Ca(OCl)_2$	0.5 + 20	30	64.72^{e} (53.54)	47.20^{e} (43.38)
Bavistin + $HgCl_2$	0.5 + 0.1	20	56.32^{f} (48.61)	67.35^{b} (55.13)
Bavistin + $HgCl_2$	0.5 + 0.2	20	69.09^{d} (56.20)	63.61^{c} (52.88)
Bavistin + $HgCl_2$	0.5 + 0.1	30	78.76^{b} (62.54)	74.32^{a} (59.53)
Bavistin + $HgCl_2$	0.5 + 0.2	30	83.54^{a} (66.05)	64.61^{bc} (53.48)
Mean			**50.22 (45.06)**	**49.61 (44.70)**
$CD_{0.05}$			**2.99**	**2.85**

• Ca (OCl)2: Calcium hypochlorite, HgCl2: Mercuric chloride
• Figures in parenthesis are transformed values (Arc-sine transformation)
• Values with in column followed by different letters are significantly different at 5% probability level

An initial response of inoculated mature embryo was identical after 4-7 days and mostly sovereign from inoculation media. All explants became engorged and no callus proliferation was evident in initial few days. Callus proliferations generally happening from embryonic axis after 4-7 days of culture (Fig.1A&B). In indirect organogenesis mode, plantlets shaped from the nodules arising on the surface of the callus (Fig. 1C). Shoot initiation happening around 10 days from initial inoculating (Fig. 1D), nevertheless, the duration differed from culture to culture and in a few cases shootlets formed even after 35 days of initial culture. Plantlets also produced *via* direct organogenesis (Fig. 1E-F). In direct organogenesis, adventitious constructions were shaped on explant surface and happening around 10-14 days from initial culturing. However, the duration varied from culture to culture and in a few cases adventitious structures formed after 28-35 days as well. After some time, these adventitious structures converted in to shootlets (Fig. 1G-I). When root formation was not obtained, shoots were then subjected to the rooting medium (Fig. 1J). Most of the calli, after prolonged culturing on the induction media gave rise to plants. Nevertheless, transfer into elongation medium facilitated higher plant regeneration with elevated growth frequency. In this research, plantlets were regenerated in enormous numbers, that may be employed for mass *in vitro* propagation. Rooted plants were transferred under 28±2^{o}C and 65±5% relative humidity for 20-25 days to an Environmental Growth Cabinet for hardening (Fig. 1K). Afterward these plantlets were subjected to the net house/ poly house (Fig. 1L) for 25-30 days before transferring under field conditions.

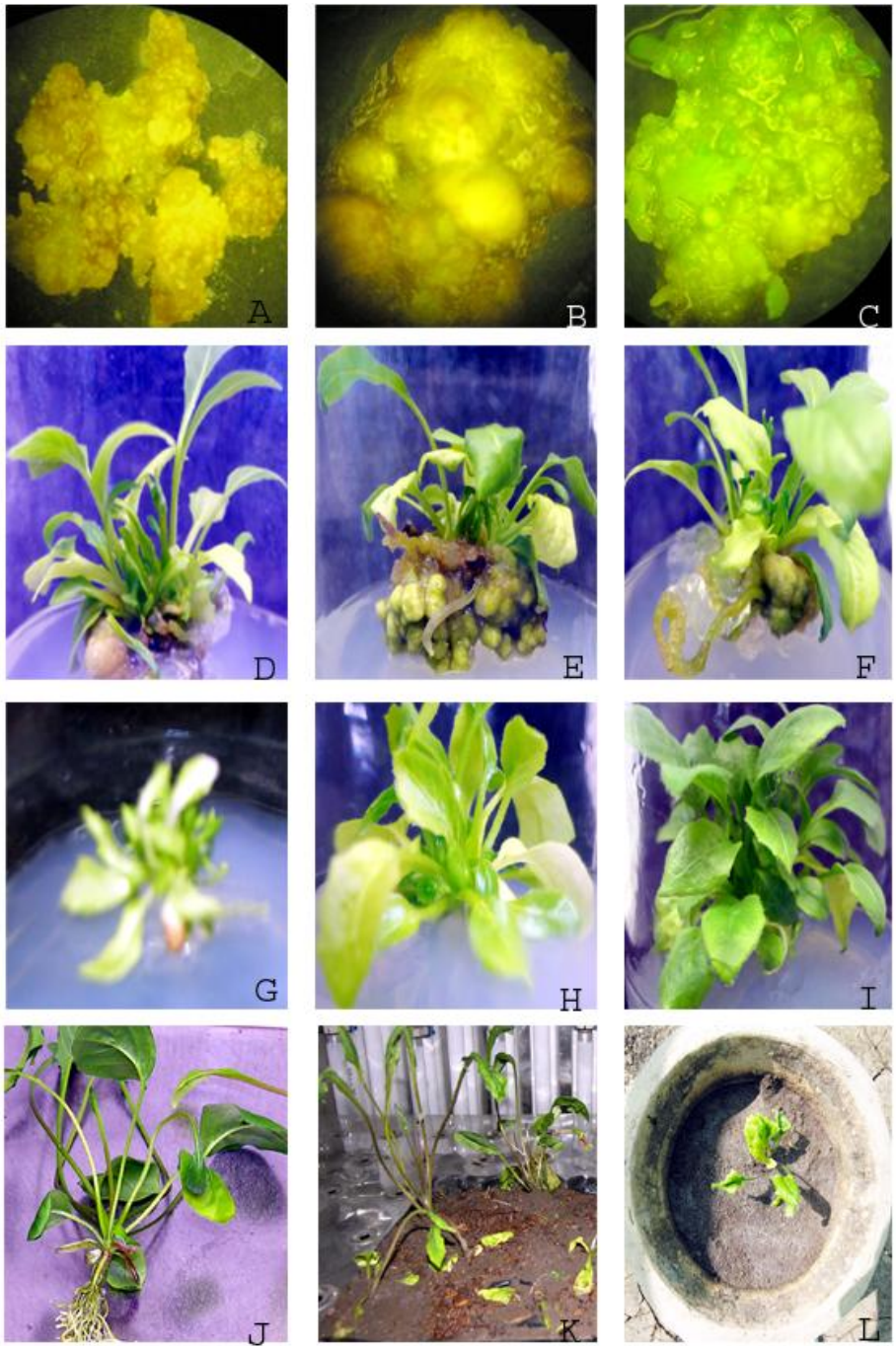


Fig. 1. Plant regeneration in Gerbera jamesonii from cultured mature embryo: A. Formation of callus after 15-20 days; B. Initiation of somatic embryo of globular stage; C. Shoot proliferation after 20-25 days; D. Multiple shoots formation via indirect organogenesis after 30-35 days; E. Multiple shoots formation via indirect somatic embryogenesis after 30-35 days; F. Multiple shoots and root formation via indirect somatic embryogenesis; G-I. Formation of multiple shoots via direct organogenesis; J. *In vitro* rooting; K. Regenerants transferred in Environmental Growth Chamber after 40-45 days and L. Plantlet transferred in Net House after 45-50 days for hardening

The ANOVA given in Tables 2-5 indicated that there were exceedingly substantial ($p<0.01$) differences exist among the response of diverse nutrient media combinations in respect to all culture phases *viz*., callus induction, shoot proliferating efficiency, numbers of shoot (s) per explant, mean

shoot length for mature embryo explant (Tables 2-4) and root proliferating efficiency, numbers of root (s) and mean root length (Table 5) for *in vitro* rhizogenic response. It designates the incidence of substantial magnitude of erraticism amongst diverse nutrient media amalgamations.

Table 2. Effect of different auxins alone in varying concentrations on *in vitro* response of mature embryo cultures

Culture Media	Plant growth regulators mgl^{-1}			*In vitro* response			
	2,4-D	**NAA**	**2,4,5-T**	**Callus induction (%)**	**Shoot proliferating explant (%)**	**No. of shoot (s)/explant**	**Mean shoot length (in cm)**
MS.5D	0.5	-	-	24.67^{e} (29.76)	11.01^{g} (19.35)	2.30^{a} (8.68)	1.41^{ab} (6.78)
MSD	1.0	-	-	29.52^{c} (32.89)	9.80^{h} (18.20)	2.01^{b} (8.12)	1.62^{a} (7.27)
MS2D	2.0	-	-	33.02^{ab} (35.06)	7.12^{hi} (15.43)	1.87^{b} (7.84)	1.95^{a} (7.96)
MS3D	3.0	--	-	35.09^{a} (36.31)	5.21^{ij} (13.13)	1.95^{b} (7.97)	1.18^{b} (6.21)
MS4D	4.0	-	-	34.32^{a} (35.84)	3.89^{j} (11.28)	1.79^{b} (7.62)	0.88^{b} (5.35)
MS5D	5.0	-	-	28.31^{d} (32.13)	2.20^{j} (8.28)	0.55^{b} (3.77)	0.61^{b} (4.42)
MS.5N	-	0.5	-	24.00^{e} (29.32)	40.31^{a} (39.40)	2.53^{a} (9.09)	1.73^{a} (7.50)
MSN	-	1.0	-	28.44^{cd} (32.21)	34.28^{b} (35.82)	2.52^{a} (9.10)	2.03^{a} (8.07)
MS2N	-	2.0	-	30.30^{bc} (33.38)	33.96^{bc} (35.63)	2.15^{a} (8.38)	2.14^{a} (8.29)
MS3N	-	3.0	-	33.82^{a} (35.54)	31.00^{c} (33.82)	2.08^{a} (8.21)	0.87^{b} (5.33)
MS4N	-	4.0	-	28.38^{d} (32.17)	25.98^{d} (30.62)	1.80^{b} (7.69)	0.75^{b} (4.90)
MS5N	-	5.0	-	24.96^{de} (29.95)	9.67^{h} (18.09)	1.81^{b} (7.71)	0.48^{b} (3.90)
MS.5T	-	-	0.5	16.44^{g} (23.90)	24.70^{d} (29.78)	1.85^{b} (7.79)	1.37^{b} (6.66)
MST	-	-	1.0	19.68^{fg} (26.32)	17.56^{e} (24.75)	2.07^{ab} (8.25)	1.78^{a} (7.63)
MS2T	-	-	2.0	22.01^{f} (27.96)	16.11^{e} (23.64)	1.86^{b} (7.83)	1.72^{a} (7.49)
MS3T	-	-	3.0	22.88^{ef} (28.56)	14.59^{ef} (22.43)	1.62^{b} (7.30)	1.13^{b} (6.08)
MS4T	-	-	4.0	23.39^{e} (28.91)	11.99^{fg} (20.23)	1.05^{bc} (5.86)	1.07^{b} (5.91)
MS5T	-	-	5.0	18.87^{g} (25.72)	9.97^{gh} (18.37)	0.95^{c} (5.57)	1.02^{b} (5.79)
Mean				**26.56 (23.24)**	**17.19 (23.24)**	**1.82 (7.60)**	**1.32 (6.42)**
$CD_{0.05}$				**3.49**	**3.21**	**1.02**	**1.00**

• Figures in parenthesis are transformed values (Arc-sine transformation)
• Values within column followed by different letters are significantly different at 5% probability level

Nutrient media MS3D.5B/ MS2D.5B/ MS3N.5ip (supplemented with comparatively higher magnitude of an auxin in association with a lower concentration of a cytokinin) enable callus induction in higher frequencies. Inoculation media MS3D/MS4D/ MS3N/ MS2D (comprehending a modest to higher concentration of an auxin 2,4-D and NAA) as well as MS3ip/ MS2ip/ MSip/ MS.5ip (comprising a reasonable to higher concentration of 2-ip) also initiated callus, nevertheless, proportions were investigated modest. While, nutrient medium amended with other auxin 2,4,5-T and cytokinins: BA and Kn in comparable concentrations persuaded callus induction in lower frequencies that suggested that 2,4-D either sole or in combination with cytokinin is better for callus proliferation from cultured mature embryos. Aswath and Choudhary [68], Kumar and Kanwar [58], Patidar et al. [38] and Bhatt et al. [39-41] also testified analogous results for various explants cultures in gerbera.

In respect to shoot proliferating aptitude, numbers of shootlets and an average shoot length, inoculation media comprising auxin alone in diverse concentrations demonstrated lower shoot proliferation ability, *i.e.,* lesser number of explants proliferated shootlets, lesser numbers of shoot (s) per explant recovered along with shootlets of minimum length. Shoot proliferation in very lower frequency has been evinced on medium fortified with 2,4-D as sole, whereas nutrient medium amended with NAA performed much better. Previous investigations also testified that cytokinin is needed for shoot organogenesis [80]. So as to attain the best *in vitro* response, basal medium was also amended with various types of cytokinins in differing concentrations. In this research, nutrient media supplemented with cytokinin as sole in modest concentrations (BAP in concentration of 1.0-2.0

mgl^{-1} and 2-ip in magnitude of 0.1-0.5 mgl^{-1}) were responded better (more than 40% explants proliferated shootlets) advised that a moderate concentration of cytokinin is necessitated for this target. Former investigations also confirmed that a lower level of BAP persuaded early bud sprouting in many species. Multiple shoot induction needed the attendance of a cytokinin in the nutrient medium, which is in faithfully pertinent to result in other investigations in gerbera [53,55,60,37]. Nevertheless, shoot proliferating aptitude and numbers of shoot (s) were boosted when an auxin and a cytokinin were added into inoculation medium in combinations. Nutrient media MS2N.5ip/MS3N.5ip encompassing comparative higher proportions of an auxin (NAA) in association with a lower concentration of a cytokinin (BA), displayed higher *in vitro* retort (more than 53% explants proliferated shootlets with 7 shoots/explant) than nutrient medium comprehending a higher concentration of cytokinins as well as an auxin as alone. This outcome is an agreement with findings of Hasbullah et al. [67] and Patidar et al. [38] as they also advised that many commercial ornamental plants are being propagated by *in vitro* culture on the nutrient medium comprising an auxin and a cytokinin in combinations. Additionally, addition of strong auxin (NAA) with BAP encouraged better shoot formation compared to a weak auxin like IBA [62]. Nevertheless, supplementation of an auxin in higher concentration as compared to a cytokinin in the nutrient medium caused in the embarrassment of shoot formation. Similar trend has also been addressed in soybean [71-87] and in Indian mustard [88-89]. In respect to mean shoot length, induction medium amended with BAP in an array of 0.5-3.0 mgl^{-1} supported production of shootlets of comparatively higher length.

Table 3. Effect of different cytokinins alone in varying concentrations on *in vitro* response of mature embryo cultures

Culture Media	Plant growth regulators mgl^{-1}			*In vitro* response			
	BAP	Kn	2-ip	Callus induction (%)	Shoot proliferating explant (%)	Numbers of shoot(s)/explant	Mean shoot length (in cm)
MS.5B	0.5	-	-	13.05^{f} (21.16)	35.80^{d} (36.73)	4.32^{b} (11.55)	2.63^{a} (9.27)
MSB	1.0	-	-	15.34^{f} (23.04)	45.92^{a} (42.64)	6.25^{a} (14.33)	2.88^{a} (9.70)
MS2B	2.0	-	-	19.86^{d} (26.44)	44.53^{a} (41.84)	5.65^{a} (13.59)	2.83^{a} (9.61)
MS3B	3.0	-	-	18.20^{e} (25.24)	38.28^{c} (38.20)	4.63^{ab} (12.37)	2.65^{a} (9.31)
MS4B	4.0	-	-	15.90^{ef} (23.48)	36.71^{cd} (37.28)	2.41^{b} (8.77)	2.58^{a} (9.18)
MS5B	5.0	-	-	14.52^{f} (22.38)	35.44^{d} (36.52)	2.32^{b} (8.67)	2.52^{a} (9.11)
MS.5Kn	-	0.5	-	19.18^{de} (25.93)	34.72^{d} (36.09)	2.41^{b} (8.84)	2.53^{a} (9.13)
MSKn	-	1.0	-	14.62^{f} (22.43)	34.35^{d} (35.86)	3.46^{b} (10.72)	2.55^{a} (9.15)
MS2Kn	-	2.0	-	20.13^{d} (26.65)	39.90^{bc} (39.16)	3.95^{b} (11.13)	2.65^{a} (9.33)
MS3Kn	-	3.0	-	24.22^{c} (29.46)	37.54^{c} (37.77)	3.49^{b} (10.76)	2.59^{a} (9.23)
MS4Kn	-	4.0	-	13.80^{f} (21.79)	35.62^{d} (36.63)	2.35^{b} (8.74)	2.55^{a} (9.16)
MS5Kn	-	5.0	-	8.79^{g} (17.21)	34.92^{d} (36.21)	1.97^{b} (7.91)	2.52^{a} (9.09)
MS.1ip	-	-	0.1	25.15^{c} (30.08)	34.92^{d} (36.20)	3.59^{b} (10.66)	1.89^{ab} (7.90)
MS2ip	-	-	0.2	29.12^{b} (32.64)	40.42^{b} (39.46)	4.20^{b} (11.73)	2.25^{a} (8.62)
MS.5ip	-	-	0.5	29.13^{b} (32.65)	44.90^{a} (42.06)	8.40^{a} (16.77)	2.20^{a} (8.52)
MSip	-	-	1.0	29.75^{b} (33.03)	39.12^{c} (38.70)	1.79^{b} (7.68)	0.95^{b} (5.59)
MS2ip	-	-	2.0	29.95^{b} (33.15)	35.65^{d} (36.64)	1.73^{b} (7.55)	1.30^{b} (6.50)
MS3ip	-	-	3.0	35.06^{a} (36.29)	33.70^{d} (35.47)	1.59^{b} (7.24)	1.00^{b} (5.73)
Mean				**20.88**	**37.91**	**3.58**	**2.28**
$CD_{0.05}$				**3.86**	**3.41**	**3.17**	**1.18**

• Figures in parenthesis are transformed values (Arc-sine transformation)
• Values within column followed by different letters are significantly different at 5% probability level

Table 4. Combined effects of added different auxins and cytokinins *on in vitro* response for cultured mature embryo

Culture Media	Plant growth regulators Mgl^{-1}					*In vitro* response			
	2,4-D	NAA	BAP	Kn	2-ip	Callus induction (%)	Shoot proliferating explants (%)	No. of shoot(s) /explant	Mean shoot length (in cm)
MS.5D.5B	0.5	-	0.5	-	-	27.01^{a} (31.30)	19.25^{ij} (26.01)	2.54^{d} (8.98)	1.79^{a} (7.39)
MSD.5B	1.0	-	0.5	-	-	30.36^{d} (33.42)	22.64^{h} (28.40)	2.61^{d} (9.11)	1.87^{a} (7.56)
MS2D.5B	2.0	-	0.5	-	-	41.12^{a} (39.87)	24.73^{g} ()29.80	2.32^{d} (8.62)	1.95^{a} (7.76)
MS3D.5B	3.0	-	0.5	-	-	43.59^{a} (41.30)	19.12^{j} (25.91)	2.36^{d} (8.70)	1.12^{b} (5.96)
MS4D.5B	4.0	-	0.5	-	-	35.22^{c} (36.38)	17.11^{jk} (24.42)	2.31^{d} (8.58)	0.89^{b} (5.41)
MS5D.5B	5.0	-	0.5	-	-	31.02^{d} (33.83)	13.44^{lm} (21.48)	1.23^{d} (6.36)	0.86^{b} (5.32)
MS.5D.5Kn	0.5	-	-	0.5	-	25.03^{e} (30.00)	14.70^{kl} (22.52)	2.14^{d} (8.36)	2.02^{a} (8.17)
MSD.5Kn	1.0	-	-	0.5	-	30.00^{d} (33.19)	11.97^{m} (20.22)	2.44^{d} (8.85)	2.08^{a} (8.29)
MS2D.5Kn	2.0	-	-	0.5	-	33.62^{c} (35.41)	9.20^{n} (17.63)	2.02^{d} (8.100	1.69^{ab} (7.47)
MS3D.5Kn	3.0	-	-	0.5	-	34.92^{c} (36.19)	6.82^{no} (15.09)	1.89^{d} (7.90)	1.72^{a} (7.53)
MS4D.5Kn	4.0	-	-	0.5	-	33.62^{c} (35.41)	4.81^{op} (12.61)	1.83^{d} (7.77)	0.86^{b} (5.32)
MS5D.5Kn	5.0	-	-	0.5	-	34.92^{c} (36.19)	3.05^{p} (10.05)	1.57^{d} (7.19)	0.67^{b} (4.69)
MS.5N.5B	-	0.5	0.5	-	-	32.19^{c} (34.98)	36.51^{d} (37.16)	4.82^{bc} (12.47)	1.72^{a} (7.53)
MSN.5B	-	1.0	0.5	-	-	27.97^{de} (31.90)	41.67^{c} (40.19)	6.80^{ab} (14.97)	1.85^{a} (7.81)
MS2N.5B	-	2.0	0.5	-	-	24.69^{e} (29.77)	44.08^{bc} (41.58)	6.72^{b} (14.87)	1.59^{b} (7.24)
MS3N.5B	-	3.0	0.5	-	-	31.12^{d} (33.89)	33.35^{e} (35.26)	2.65^{d} (9.36)	1.54^{b} (7.13)
MS4N.5B	-	4.0	0.5	-	-	31.56^{cd} (34.16)	30.52^{f} (33.52)	2.57^{d} (9.22)	1.19^{b} (6.26)
MS5N.5B	-	5.0	0.5	-	-	26.80^{e} (31.14)	21.54^{hi} (27.64)	1.54^{d} (7.13)	1.02^{b} (5.79)
MS.5N.5ip	-	0.5	-	-	0.5	25.02^{e} (29.98)	38.20^{d} (38.16)	2.72^{cd} (9.49)	1.82^{s} (7.75)
MSN.5ip	-	1.0	-	-	0.5	23.79^{e} (29.17)	45.01^{a} (42.12)	7.98^{a} (16.28)	2.25^{a} (8.62)
MS2N.5ip	-	2.0	-	-	0.5	26.43 (30.91)	55.22^{a} (47.98)	9.30^{a} (17.69)	2.50^{a} (9.09)
MS3N.5ip	-	3.0	-	-	0.5	33.21^{c} (35.17)	53.17^{a} (46.80)	7.82^{a} (16.23)	2.12^{a} (8.37)
MS4N.5ip	-	4.0	-	-	0.5	36.12^{bc} (36.93)	36.12^{d} (36.92)	2.03^{d} (8.19)	0.97^{b} (5.65)
MS5N.5ip	-	5.0	-	-	0.5	39.00^{ab} (38.63)	21.20^{i} (27.40)	1.70^{d} (7.49)	0.87^{b} (5.35)
Mean						**31.18 (33.85)**	**25.98^{i} (29.54)**	**3.41 (10.08)**	**1.54 (6.98)**
$CD_{00.5}$						**4.73**	**2.71**	**2.77**	**1.03**

• Figures in parenthesis are transformed values (Arc-sine transformation)
• Values within column followed by different letters are significantly different at 5% probability level

Table 5. Combined effect of different plant growth regulators added in MS basal media for induction of *in vitro* rooting

Culture Media combinations ▼	Plant growth regulators mg l^{-1}				*In vitro* rooting response		
	IBA	NAA	BAP	Kn	Root proliferating shootlets (%)	No. of root (s) per shootlet	Root length (in cm)
MS.1IB	0.1	-	-	-	98.74^{a} (83.60)	10.71^{a} (19.08)	6.15^{a} (14.34)
MS.5IB	0.5	-	-	-	96.17^{a} (78.84)	10.33^{b} (18.73)	4.32^{b} (11.98)
MSIB	1.0	-	-	-	93.94^{b} (75.74)	8.54b^{c} (16.97)	5.07^{b} (13.00)
MS2IB	2.0	-	-	-	89.61^{c} (71.19)	6.81^{d} (15.08)	4.02^{b} (11.55)
MS3IB	3.0	-	-	-	86.50^{d} (68.43)	6.06^{e} (14.22)	2.56^{c} (9.19)
MS.1N	-	0.1	-	-	92.81^{b} (74.44)	7.42^{cd} (15.79)	2.37^{c} (8.82)
MS.5N	-	0.5	-	-	89.55^{c} (71.12)	8.32^{c} (16.74)	2.27^{c} (8.61)
MSN	-	1.0	-	-	86.21^{d} (68.24)	6.09^{e} (14.28)	1.82^{c} (7.68)
MS2N	-	2.0	-	-	84.88^{d} (67.10)	6.16^{de} (14.36)	2.21^{c} (8.50)
MS3N	-	3.0	-	-	79.49^{ef} (63.04)	5.51^{e} (13.56)	2.76^{c} (9.55)
MS.1Kn	-	-	-	0.1	84.81^{d} (67.04)	4.36^{f} (12.04)	2.04^{c} (8.12)
MS.5Kn	-	-	-	0.5	79.98^{e} (63.41)	4.69^{ef} (12.47)	2.33^{c} (8.74)
MSKn	-	-	-	1.0	78.76^{f} (62.55)	3.91^{f} (11.39)	3.54^{bc} (10.82)
MS2Kn	-	-	-	2.0	76.83^{f} (61.21)	3.72^{f} (11.10)	2.16^{c} (8.43)
MS3Kn	-	-	-	3.0	71.79^{g} (57.90)	3.96^{f} (11.46)	2.53^{c} (9.11)
MS.5IB.5B	0.5	-	0.5	-	79.63^{e} (63.17)	3.94^{f} (11.40)	2.56^{c} (9.20)
MSIB.5B	1.0	-	0.5	-	78.35^{f} (62.25)	3.66^{f} (11.02)	2.06^{c} (8.25)
MS2IB.5B	2.0	-	0.5	-	70.20^{g} (56.89)	3.16^{f} (10.23)	1.83^{c} (7.75)
MS.5IB.5Kn	0.5	-	-	0.5	84.82^{d} (67.07)	3.89^{f} (11.36)	3.44^{c} (10.66)
MSIB.5Kn	1.0	-	-	0.5	80.40^{e} (63.70)	4.21^{f} (11.82)	2.33^{c} (8.77)
MS2IB.5Kn	2.0	-	-	0.5	76.48^{f} (60.97)	3.05^{f} (10.04)	3.01^{c} (9.98)
Mean					**83.80 (67.04)**	**5.64 (13.48)**	**2.92 (9.67)**
$CD_{0.05}$					**3.01**	**1.79**	**1.77**

• Figures in parenthesis are transformed values (Arc-sine transformation)

• Values within column followed by different letters are significantly different at 1% probability level

In this study, rhizogenesis incidence was inspected to be higher after transferring shootlets into rooting medium. Generally, auxins like IBA [90-91,75,54,62,52,61,38-41], IAA [56,72] as well as NAA [54,38] were investigated effective in inducing *in vitro* rooting. In current investigation, MS medium fortified with 0.1-0.5 mgl^{-1}IBA was evidenced to be optimal for demonstrating higher *in vitro* rooting response. Maximum *in vitro* root proliferation, numbers of roots and root of higher length were documented on rooting media MS.1IB/ MS.5IB. The findings undoubtedly designated that rooting of *in vitro* shootlets of gerbera needed a lower concentration of IBA with higher nutrient level. As reported earlier that auxin encouraged adventitious root development on intact plants in addition to excised stems. Of these, IBA was the most operative than any other plant growth regulators in the most of the cases deceptively perhaps it is not demolished by IAA oxidase or other enzymes and hence continues for longer time. The above findings are in conformity with the previous results of Shabanpour et al. [52], Patidar et al. [38], Bhatt et al. [39-41] and Nhut et al. [75].

4. CONCLUSION

The outcomes of current investigation established that under circumstances of these experimentations, *in vitro* response of mature embryo cultures was under genetic control. As explants inoculated on diverse nutrient media combinations, significant differences were evident for all the culture stages. Trend revealed in the present research recommended that explant (s) with higher regeneration competence may be employed for mass *in vitro* propagation and advance biotechnological work in gerbera.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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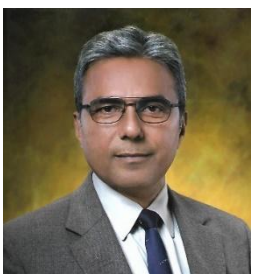
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Chapter 1
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Plantlet Regeneration from Cultured Nodal Segments in Sandalwood (*Santalum album* Linn.)

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ABSTRACT

Study Objectives: An effort has been made to compute the optimal quantity of plant growth regulators to be added in culture medium and other physical factors exhibiting higher *in vitro* morphogenesis with 'elite' lines of sandalwood by culturing nodal segment.
Results: Higher percentage of direct somatic embryogenesis, number(s) of somatic embryo per explant and plantlet regeneration *via* direct organogenesis were evidenced on MS medium augmented with a moderate concentration of TDZ (1.0 mgl^{-1}) in combination with comparatively a lower concentration of NAA (0.5 mgl^{-1}). A comparative higher concentration of BAP (1.0-2.0 mgl^{-1}) in amalgamation with a lower concentration of NAA (0.5 mgl^{-1}) encouraged frequency of indirect somatic embryogenesis. From culture media fortified with a greater concentration of BA at 4.0 mgl-^{1} in combination with a lower concentration of NAA, the proportion of organ development directly from the surface of cultured explants was recovered (0.5 mgl-^{1}). Maximum plantlets regenerated *via* somatic embryogenesis (direct and/or indirect) on regeneration medium fortified with 2.0 mgl^{-1}TDZ in combination with 1.0 mg $l^{-1}GA_3$, while plantlets in higher frequencies *via* indirect organogenesis was achieved with regeneration medium modified with relatively lower concentration of TDZ (1.0 mg l^{-1}) in amalgamation with 0.5 mgl^{-1} GA_3 and 0.5 mg^{l-1} NAA. The plantlets were transferred to pots and hardened in Environmental Growth Cabinet and Net House during initial weaning period and shifted to field magnificently. Morphologically usual plants were obtained.

Keywords: Santalum album; nodal segment culture; somatic embryogenesis; organogenesis; plantlet regeneration.

ABBREVIATIONS

MS-Murashige and Skoog Medium; BA-6- Benzyl aminopurine; TDZ-Thidiazuron; KN-Kinetin; NAA-Naphthalene acetic acid; 2,4-D-2,4-Dichlorophenoxyacetic acid; 2, 4, 5-T - 2, 4, 5-Trichlorophenoxyacetic acid; IBA-Indole-3- Butyric acid; GA_3: Gibberellic acid.

1. INTRODUCTION

Sandalwood is medium-sized hemi parasitic trees and distinguished members are Indian sandalwood (*Santalum album* L.) and Australian sandalwood (*Santalums picatum*). Indian sandalwood belonging to the family santalaceae which is one of the imperative tree species of tropical forests since it crops essential oil in the heartwood which is employed widely in the enrage and perfumery industry [1,2,3].

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Its oil is one of the eldest recognized perfume materials with 4000 years of incessant application. Chief ingredients of sandalwood oil are α- santalol (60 %), ß- santalol (30%) and α- and ß - santalene. The world renowned East Indian sandalwood oil is extracted from the sturdily scented heartwood [4] of this tree. Sandalwood oil is used mainly in perfumery due to its outstanding fixative possessions. It is also applied in preparing all kinds of perfume compositions particularly Indian attars like Hina, Gulab, Kewda and Jasmine in which the natural essential oils from distillate of floral distillation are fascinated in sandalwood oil. With neem oil, it is employed as preventive. It is used for healing wounds and sores instigated by the smallpox vaccination. In addition, it is employed as cardiotonic, diuretic, moisturizer, astringent, antifungal and antimicrobial. It is also one of the premium woods for statuette owing to smooth and uniform fibers. Saw dust from heartwood is mostly used in incense for scenting cloths and storerooms.

Production of sandalwood has tumbled abruptly over the past decades owing to the mounting of unlawful sandalwood dispensation units. Traditional breeding of sandalwood for introgression of new genetic information can be an affluent and tough task due to its long generation time, sexual incompatibility and heterozygous nature [5,6]. Furthermore, a main danger to sandalwood trees is the spike disease which has a distressing consequence and very often completely eliminates the plantation [7]. Efforts to control and eliminate the disease have been ineffective. Per se, it is, imperious to advance substitute procedures for swift and large-scale multiplication of the species. Studies were conducted on sandalwood with the chief target of evolving techniques for clonal multiplication of the elite species which are also disease free as such trees are identified to exist in sandalwood plantations. *In vitro* and mostly somatic embryogenesis, techniques has been employed for pretty some time in different crop species including sandalwood for the regeneration of plantlets Tissue culture technology can be employed to encounter complications of traditional propagation methods by microcloning of elite lines [8-11].

Extensive effort has been conducted in sandalwood by several investigators all over the world employing different explants for instance embryo [12,13], hypocotyls [3,13], shoot tip [14,15], nodal segment [5,16-21], leaf disc [6,22,23], seedling [24], endosperm [4], cotyledons [13], protoplast [25,26,27] and cell suspension cultures [28,29,30] with varying notch of accomplishment. Somatic embryogenesis in higher frequencies have been conveyed by Herawan et al. [31], Ilah et al. [32] and Isah [33]. Considering all truths in view for the present situation, an effort has been made to calculate the optimum quantity of plant growth regulators to be supplemented in culture medium and other physical factors displaying higher *in vitro* morphogenesis with 'elite' lines of M.P. and contiguous zones by culturing nodal segment since it is obtainable throughout the year and could be used as explant spring.

2. MATERIALS AND METHODS

The 'elite' cultivar of sandalwood has been chosen for the current investigation by means of nodal segment as an "explant." The experimental materials were collected from 5-10 years old plants, planted at Bahadri Farm, KNK, College of Horticulture, Mandsaur, M. P. India.

2.1 Culture Media

To instigate with an initial experimentation, two diverse fortifications of basal media *viz*: MS [34] and WP [35] were investigated to distinguish better *in vitro* response. In preliminary experiments, MS basal medium was evident more responsive than WP medium (data not presented), consequently, for the succeeding investigations basal MS medium was employed. In initial experiment with respect to plant growth regulators, nodal segments were cultured on MS media amended with two different auxins, *namely*: 2,4-D and NAA alone as well as three diverse cytokinins *viz*: BAP, kinetin and TDZ as sole in varying concentrations to find out better *in vitro* response. In initial investigations, it was inspected that an auxin or a cytokinin alone is not passable for inducing morphogenesis in higher frequencies (data not presented). Subsequently, for final experimentation, basal MS medium was supplemented with diverse concentrations of BAP, TDZ and Kn in combination with NAA and 2,4-D in variable concentrations. In addition to MS basal macro and micro salts, vitamins, all culture media were fortified with 30.0 gl^{-1} sucrose and the final volume was kept to 1000 ml and pH was adjusted to 5.8 ± 0.1 with 1N KOH solution. After adjusting the pH, agar powder @ 7.5 g l^{-1} was incorporated to

the media as a semi-solidifying agent. Warm culture media, still in liquid state were dispensed into baby food bottles (50-60 ml / bottle) tracked by autoclaving at 121°C under 15 psi pressure for 20-25 min. Readymade basal media, plant growth regulators and other ingredients were purchased from Hi-media Laboratories, Mumbai, India.

2.2 Explant Collection and Surface Sterilization

The indeterminate shoots were expunged and collected in distilled water from plants planted in orchard. Top 10 to 20 nodal segments were clipped to 0.5-1.0-centimeter length and washed under running tap water for 30 min to eradicate adhering dirt particles. Nodal segments were then sited into double distilled water comprehending 2% Tween 20 (v/v) for 30 min to eliminate the adhering fine particles. The cleaned segments were then treated with 70% (v/v) ethanol for 2 min tracked by treatment with diverse concentrations of Bavistin® (BASF, Germany) and aqueous solution of two different surface sterilizing agents *i.e.,* $HgCl_2$ and $Ca(OCl)_2$ in various concentrations and combinations for diverse durations with initial vacuum of 100 psi (Table 1). Lastly, the segments were exposed to treatment with 4-5 rinsing with sterile double distilled water.

Table 1. Effects of different surface sterilizing and antifungal agents on recovery of aseptic culture in sandalwood

Treatments	Concentration (%)	Exposure time (in min)	Aseptic culture (%)	Survival of explants (%)
Ca (OCl) $_2$	5	10	12.96^{p} (21.08)	26.54^{m} (30.99)
Ca (OCl) $_2$	5	15	15.07^{o} (22.82)	26.62^{m} (31.04)
Ca (OCl) $_2$	5	20	26.34^{n} (30.86)	26.67^{m} (31.08)
Ca (OCl) $_2$	10	10	36.14^{m} (36.94)	33.72^{l} (35.48)
Ca (OCl) $_2$	10	15	37.96^{lm} (38.02)	46.41^{j} (42.92)
Ca (OCl) $_2$	10	20	44.23^{i} (41.67)	58.10^{g} (49.64)
Ca (OCl) $_2$	15	10	40.07j^{k} (39.26)	34.88^{l} (36.18)
Ca (OCl) $_2$	15	15	43.17^{i} (41.06)	46.59^{j} (43.03)
Ca (OCl) $_2$	15	20	46.49^{h} (42.97)	49.14^{i} (44.49)
Ca (OCl) $_2$	20	10	48.36^{g} (44.04)	52.11^{h} (46.19)
Ca (OCl) $_2$	20	15	49.12^{g} (44.48)	57.34^{g} (49.20)
Ca (OCl) $_2$	20	20	52.36^{f} (46.33)	61.35^{f} (51.54)
$HgCl_2$	0.1	2	38.30^{kl} (38.22)	68.50^{b} (55.84)
$HgCl_2$	0.1	5	44.23^{i} (41.67)	68.43^{b} (55.79)
$HgCl_2$	0.2	2	48.06^{gh} (43.87)	67.39^{b} (55.16)
$HgCl_2$	0.2	5	49.64^{g} (44.78)	62.22^{ef} (52.05)
$HgCl_2$	0.2	10	49.64^{g} (44.78)	27.55m (31.64)
Bavistin+Ca (OCl) $_2$	0.5+10	30	40.25^{j} (39.36)	38.16^{k} (38.13)
Bavistin + Ca (OCl) $_2$	0.5+15	30	57.01^{e} (49.01)	49.45^{i} (44.67)
Bavistin + Ca (OCl) $_2$	0.5+20	30	75.95^{c} (60.61)	68.43^{b} (55.79)
Bavistin+ $HgCl_2$	0.5+0.1	20	77.95^{b} (61.97)	75.93^{a} (60.60)
Bavistin+ $HgCl_2$	0.5+0.2	20	82.23^{a} (65.05)	63.13^{de} (52.59)
Bavistin + $HgCl_2$	0.5+0.1	30	68.46^{d} (55.81)	64.15^{c} (53.20)
Bavistin+ $HgCl_2$	0.5+ 0.2	30	69.80^{d} (56.64)	61.04^{f} (51.36)
Mean			]48.07(43.80)	51.41 (45.78)
CD (0.05%)			1.830	1.791

Ca (OCl)$_2$: Calcium hypochlorite, $HgCl_2$: Mercuric chloride;
Figures in parenthesis are transformed values (Arc-sine transformation).
Values within column followed by different letters are significantly differed at 5% probability level.

2.3 Plating Technique and Culture Conditions

Baby food bottles were cultured with one piece of nodal segment and wrapped with Parafilm® and incubated under complete darkness at 25±2°C for one week. Later *in vitro* cultured explants were imperiled to photoperiod regime of 16 h light and 8 h dark at an intensity of 2000-lux luminance providing by PAR lamps.

Table 2. In vitro morphogenesis in cultured nodal segments on different fortification of MS media

Culture Media ▼	Plant growth 2,4-D	Regulators mg l^{-1} NAA	BA	TDZ	Kn	Direct somatic embryogenesis	Indirect somatic embryogenesis	*Average no. of somatic embryos	Direct organogenesis	Indirect organogenesis	*Plantlet regeneration *via* direct organogenesis
MS.5D.5Td	0.5	-	-	0.5	-	22.43^{ij} (28.25)	7.50^{e} (15.89)	251.14^{l}(2.40)	20.35^{l} (26.80)	6.56^{f} (14.83)	80.96^{u} (1.91)
MSD5Td	1.0	-	-	0.5	-	25.08^{gh} (30.04)	8.34^{e} (16.78)	266.38^{k}(2.43)	28.12^{e} (32.01)	9.78^{d} (18.22)	112.13^{h} (2.05)
MS2D.5Td	2.0	-	-	0.5	-	20.97^{jk} (27.24)	10.16^{d} (18.56)	295.94^{i}(2.47)	26.15^{f} (30.740	9.65^{d} (18.09)	104.22^{k} (2.02)
MS3D.5Td	3.0	-	-	0.5	-	17.53^{lm} (24.73)	11.17^{c} (19.50)	326.12^{f}(2.52)	28.24^{e} (32.08)	9.63^{d} (18.07)	112.92^{g} (2.05)
MS4D.5Td	4.0	-	-	0.5	-	15.96^{m} (23.53)	8.94^{e} (17.39)	335.06^{e}(2.53)	21.32^{ij} (27.480	9.19^{e} (17.64)	84.35^{t} (1.93)
MS5D.5Td	5.0	-	-	0.5	-	6.74^{n} (15.04)	5.51^{f} (13.57)	270.65^{j}(2.44)	17.55^{kl} (24.75)	7.17^{f} (15.53)	68.41^{w} (1.84)
MS.5B.5N	-	0.5	0.5	-	-	26.44^{g} (30.93)	18.25^{a} (25.27)	116.58^{u}(2.07)	24.48^{g} (29.64)	13.04^{b} (21.15)	96.18^{o} (1.98)
MSB.5N	-	0.5	1.0	-	-	31.55^{de} (34.16)	19.18^{a} (25.56)	135.80^{o}(2.13)	27.35^{e} (31.52)	17.56^{a} (24.76)	108.04^{j} (2.04)
MS2B.5N	-	0.5	2.0	-	-	33.08^{d} (35.09)	17.56^{a} (24.76)	345.83^{d}(2.54)	19.44^{jk} (26.14)	18.56^{a} (25.50)	76.23^{v} (1.88)
MS3B.5N	-	0.5	3.0	-	-	35.55^{c} (36.58)	14.09^{b} (22.03)	317.29^{g}(2.51)	22.35^{hi} (28.20)	12.14^{bc}(20.37)	88.94^{q} (1.95)
MS4B.5N	-	0.5	4.0	-	-	32.62^{d} (34.81)	10.23^{d} (18.62)	78.24^{w} (1.90)	36.49^{c}(37.150)	11.79^{c} (20.06)	144.16^{d} (2.16)
MS5B.5N	-	0.5	5.0	-	-	29.23^{f} (32.71)	14.54^{b} (22.41)	28.38^{x} (1.45)	16.07^{l} (23.62)	9.63^{d} (18.05)	64.50^{x} (1.81)
MS.5Td.5N	-	0.5	-	0.5	-	33.55^{cd} (35.38)	7.43e^{f} (15.81)	315.74^{h}(2.50)	26.37^{ef} (30.88)	10.12^{d} (18.52)	104.15^{l} (2.02)
MSTd.5N	-	0.5	-	1.0	-	44.12^{a} (41.61)	9.11^{e} (17.53)	368.68^{a}(2.57)	27.11^{e} (31.36)	13.54^{b} (21.57)	212.10^{a} (2.33)
MS2Td.5N	-	0.5	-	2.0	-	39.63^{b} (39.00)	10.15^{d} (18.55)	354.35^{b}(2.55)	21.63^{i} (27.700	12.55^{b} (20.73)	84.56^{s} (1.93)
MS3Td.5N	-	0.5	-	3.0	-	30.18^{ef} (33.31)	11.18^{c} (19.51)	348.56^{c}(2.54)	22.08^{i} (28.01)	11.78^{c} (20.05)	88.28^{r} (1.95)
MS4Td.5N	-	0.5	-	4.0	-	28.59^{f} (32.31)	8.25^{e} (16.660	128.32^{q}(2.11)	26.59^{e} (31.02)	9.18^{e} (17.63)	104.02^{m} (2.02)
MS5Td.5N	-	0.5	-	5.0	-	25.14^{g} (30.07)	7.08^{f} (15.43)	118.38^{t}(2.08)	30.33^{d} (33.40)	7.72^{f} (16.10)	120.26^{f} (2.08)
MS.5Kn.5N	-	0.5	-	-	0.5	18.65^{l} (25.55)	6.33^{f} (14.57)	116.38^{v}(2.07)	48.53^{b} (44.14)	6.56^{f} (14.83)	192.40^{c} (2.29)
MSKn.5N	-	0.5	-	-	1.0	21.41^{j} (27.54)	8.29^{e} (16.73)	125.76^{r}(2.10)	23.72^{gh} (29.13)	9.12^{e} (17.57)	92.93^{p} (1.97)
MS2Kn.5N	-	0.5	-	-	2.0	23.06^{hi} (28.68)	9.15^{de} (17.58)	131.06^{p}(2.12)	24.56^{fg} (29.69)	10.04^{d} (18.45)	108.94^{i} (2.04)
MS3Kn.5N	-	0.5	-	-	3.0	20.23^{k} (26.71)	10.48^{cd} (18.86)	150.83^{m}(2.18)	35.54^{c} (36.58)	10.52^{cd} (18.90)	140.22^{c} (2.15)
MS4Kn.5N	-	0.5	-	-	4.0	19.16^{kl} (25.94)	7.53^{e} (15.920	142.52^{n}(2.15)	53.63^{a} (47.06)	9.27^{de} (17.72)	96.28^{n} (1.98)
MS5Kn.5N	-	0.5	-	-	5.0	15.69^{m} (23.31)	5.54^{f} (13.61)	118.68^{s} (2.08)	49.44^{b} (44.66)	7.93^{ef} (16.35)	196.23^{b} (2.30)
Mean						25.69 (30.11)	10.25 (18.39)	216.11 (2.27)	28.23 (31.82)	10.54 (18.78)	111.73 (2.03)
CD (0.5%)						2.0591	1.960	0.027	1.899	1.920	0.0271

- *Figures in parenthesis are transformed values (Arc-sine transformation).*
- **Figures in parenthesis are transformed values (Log transformation).*
- *Values within column followed by different letters are significantly differed at 5% probability level.*

Table 3. Effect of different plant growth regulators on plantlet regeneration from cultured nodal segments *via* indirect somatic embryogenesis and organogenesis

Culture media▼	**Plant growth regulator mgl^{-1}**					**Plant regeneration *via* somatic embryogenesis**	**Plant regeneration *via* indirect organogenesis**
	BA	TDZ	Kn	GA_3	NAA		
MS.5B.5GA	0.5	-	-	0.5	-	241.58^{r} (2.39)	146.50^{o} (2.17)
MSB.5GA	1.0	-	-	0.5	-	246.30^{q} (2.40)	153.64^{m} (2.19)
MS2B.5GA	2.0	-	-	0.5	-	289.38^{j} (2.47)	167.04^{k} (2.23)
MS.5BGA	0.5	-	-	1.0	-	250.86^{p} (2.40)	82.68^{v} (1.92)
MSBGA	1.0	-	-	1.0	-	314.74^{g} (2.50)	120.68^{t} (2.08)
MS2BGA	2.0		-	1.0	-	327.54^{d} (2.52)	179.76^{i} (2.26)
MS.5Td.5GA	-	0.5	-	0.5	-	267.50^{n} (2.43)	133.53^{p} (2.13)
MSTd.5GA	-	1.0	-	0.5	-	281.58^{l} (2.45)	189.58^{g} (2.28)
MS2Td.5GA	-	2.0	-	0.5	-	331.10^{c} (2.52)	198.92^{d} (2.30)
MS.5TdGA	-	0.5	-	1.0	-	313.56^{h} (2.50)	127.50^{q} (2.11)
MSTdGA	-	1.0	-	1.0	-	324.24^{f} (2.52)	157.58^{l} (2.20)
MS2TdGA	-	2.0	-	1.0	-	348.24^{a} (2.54)	198.82^{e} (2.30)
MS.5Kn.5GA	-	-	0.5	0.5	-	102.88^{x} (2.01)	125.63^{r} (2.10)
MSKn.5GA	-	-	1.0	0.5	-	129.54^{v} (2.12)	148.56^{n} (2.18)
MS2Kn.5GA	-	-	2.0	0.5	-	170.54^{t} (2.23)	120.88^{s} (2.09)
MS.5KnGA	-	-	0.5	1.0	-	122.52^{w} (2.09)	90.78^{u} (1.96)
MSKnGA	-	-	1.0	1.0	-	167.30^{u} (2.23)	35.84^{x} (1.56)
MS2KnGA	-	-	2.0	1.0	-	192.58^{s} (2.29)	58.66^{w} (1.77)
MS.5B.5GA.5N	0.5	-	-	0.5	0.5	262.08^{o} (2.42)	183.12^{h} (2.27)
MS.5Td.5GA.5N	-	0.5	-	0.5	0.5	301.14^{i} (2.48)	284.07^{b} (2.46)
MS.5Kn.5GA.5N	-	-	0.5	0.5	0.5	278.58^{m} (2.45)	174.30^{j} (2.24)
MSB.5GA.5N	1.0	-	-	0.5	0.5	283.52^{k} (2.46)	203.26^{c} (2.31)
MSTd.5GA.5N	-	1.0	-	0.5	0.5	325.04^{e} (2.52)	289.44^{a} (2.47)
MSKn.5GA.5N	-	-	1.0	0.5	0.5	340.64^{b} (2.54)	198.16^{f} (2.30)
Mean						258.87 (2.39)	157.04 (2.16)
CD (0.5%)						0.019	0.026

- *Figures in parenthesis are transformed values (Log transformation).*
- *Values within column followed by different letters are significantly differed at 5% probability level*

Table 4. Response of regenerated shootlets on MS basal medium with different concentrations of plant growth regulators tried for induction of *in vitro*

Culture media combinations▼	Plant growth regulators mgl^{-1}					*In vitro* rooting response
	NAA	**IBA**	**Kn**	**BA**	**GA_3**	
MS.1N	0.1	-	-	-	-	Healthy for 1 month, no rooting, dried.
MS.5N	0.5	-	-	-	-	As above
MSN	1.0	-	-	-	-	Growth + healthy for 2 months, no rooting
MS5N	5.0	-	-	-	-	As above
MS10N	10.0	-	-	-	-	Dried within one month, no rooting
MS.1IB	-	0.1	-	-	-	Healthy for 1 month, no rooting, dried
MS.5IB	-	0.5	-	-	-	As above
MSIB	-	1.0	-	-	-	Healthy for 3 months, no rooting
MS5IB	-	5.0	-	-	-	Healthy for 4 months, no rooting
MS10IB	-	10.0	-	-	-	Growth + healthy for 6 months, no rooting
MS.1Kn	-	-	0.1	-	-	Growth + callusing at the cut end, no root formation
MS.5Kn	-	-	0.5		-	Adventitious shoots formed, no rooting
MSKn	-	-	1.0		-	Hairy roots
MS5Kn		-	- 5.0		-	Hairy roots
MS10Kn		-	- 10.0		-	Hairy roots
MS.1IB.5B		-	0.1	-	0.5	- Growth + healthy for 4 months, adventitious shoots formed, no rooting
MS.5IB.5B		-	0.5	-	0.5	- As above
MSIB.5B		-	1.0	-	0.5	- As above
MS5IB.5B		-	5.0	-	0.5	- Healthy for 4 months, no rooting
MS10IB.5B		-	10.0	-	0.5	- Growth + healthy for 6 months, no rooting
MS.1IB.5Kn		-	0.1	0.5	-	- No growth, healthy for 1 month, no rooting
MS.5IB.5Kn		-	0.5	0.5	-	- As above
MSIB.5Kn		-	1.0	0.5	-	- As above
MS5IB.5Kn		-	5.0	0.5	-	- Healthy for 4 months, no rooting
MS10IB.5Kn		-	10.0	0.5	-	- Growth + healthy for 6 months, no rooting
MS.1IB.5Kn.5G		-	0.1	0.5	-	0.5 Healthy for 1 month, no rooting
MS.5IB.5Kn.5G		-	0.5	0.5	-	0.5 As above
MSIB.5Kn.5G		-	1.0	0.5	-	0.5 Healthy for 3 months, no rooting
MS5IB.5Kn.5G		-	5.0	0.5	-	0.5 Healthy for 4 months, no rooting
MS10IB.5Kn.5G		-	10.0	0.5	-	0.5 Growth + healthy for 6 months, no rooting

2.4 Histological Analysis

Somatic embryos of diverse developmental phases and periods of culture displaying morphogenesis were fixed in FAA and processed in alcohol-xylol series for histological investigations. Sections were cut at 10 μm and stained with safranin-fast green.

2.5 Regeneration of Plantlets

Afterward 4-5 weeks of primary culturing, somatic embryoids and calli were shifted to MS regeneration medium supplemented with various concentrations and combinations of BAP, TDZ, Kn, NAA and GA_3, 20.0gl^{-1} sucrose and 7.5gl^{-1} agar. Nevertheless, in cases where organ shaped directly on explants surface was sub-cultured on initial medium for regeneration. Cultures were subjected at 25±2^{o}C for 12 hr photoperiod regimes.

2.6 In *vitro* Rooting of Regenerants

Regenrants were subsequently transferred to MS rooting medium fortified with different concentrations of IBA, NAA, BA, Kn and GA_3 alone as well as in combinations, 15.0 gl^{-1} sucrose and 7.5 gl^{-1} agar for root formation. For regeneration and rooting, reduced level of sucrose was employed based on the work carried out by various scientists of the word in addition to preliminary experiences of our laboratory.

2.7 Hardening of Regenerants

The regenerants were de-flasked from cultures and were systematically splashed with running tap water to eradicate the adhering agar and they were planted in 2.5 cm root trainers filled with 1:1:1 sand, soil and FYM sterilized mixture. Root trainers with transplanted plants were subjected to Environmental Growth Cabinet under 30±2°C temperature and 65±5% relative humidity regimes for 15-20d for acclimatization. Adapted plants were then shifted to Net House for 30 d for hardening before transplanting to the field.

2.8 Observations

Observations were recorded for number (s) of direct and indirect somatic embryo inducing explants, average number of somatic embryos, number of shoots/ embryoid, number of direct and indirect organogenic calli forming explants and number (s) of shoots *via* direct and indirect organogenic calli and somatic embryo.

2.9 Experimental Design and Analysis of Data

The experiment was arranged in completely randomized design to realize the significance of different culture medium with two parallel experiments (replications). About 100- 120 explants were cultured on each media. Arc-sine transformation (where values lie below100%) and log transformation (wherever values surpassed more than 100%) was made as per necessity before analysis of data. The data were analyzed as per method recommended by Snedecor and Cochran [36].

3. RESULTS AND DISCUSSION

3.1 Establishment of Aseptic Culture

During culture establishment, the nodal segment excised from top 1-10 nodes could not persuade higher bud break, which may be as a result of tender nature of buds. The acquaintance of explants with sterilant may kill these soft buds. The maximum bud break was detected from 10-15 nodes, perhaps owing to juvenile state and capacity to endure the toxic effect of sterilant [37-39]. These outcomes are in agreement to the results of Tran Than Van [40], Mishra et al. [41], Sharma et al. [42], Kurmi et al. [43] and Vibhute et al. [44] who had also found higher morphogenic response followed by

plantlet regeneration that appeared to be highly dependent on position of explant. The studies done earlier have revealed that lower or mid portion of the branch are easier to establish *in vitro* than upper part of the branch [45, 42-45]. The explants from young shoot tips were used as inoculums to regenerate shoots from the meristematic tissue through direct organogenesis. Internodal stem segment measuring 1 cm and retaining one node without leaves were found to be the most potential explant for shoot organogenesis, in comparison to the apical meristem (2-3 mm from the shoot tip) and shoot tip of 1-2 cm length along with shoot apex.

3.2 In vitro Morphogenesis

Nodal segment explants of were inoculated on MS medium with diverse PGRS regimes. The plantlets were regenerated *via* two dissimilar paths: direct (somatic embryogenesis/organogenesis) and indirect (somatic embryogenesis/organogenesis). In direct mode, shootlets developed from somatic embryoids shaped on explants surface without an intervening callus phase, shoot bud meristemoids looked on surface of callus mass in indirect mode. The results described here showed that most of the cells in sandalwood, which were still actively dividing, were embryogenically capable which tracked two general patterns of embryogenesis. It has been well reported that direct embryogenesis occurs from pre-embryogenic determined cells, while indirect somatic embryogenesis requires the induction of embryogenically determined cells [46,47].

Early response of cultured nodal segments was comparable after 4-7 d on most of the media combinations tried. All explants became swollen and no callus seemed on swollen explants. Callus proliferation generally initiated from the portion in contact with the medium after 10-14 d of culture (Fig.1F; Fig.2 D-E). In direct embryogenesis, embryo like structures originated directly on the entire surface of explants. Embryoid formation started appearing after 7 d from primary culturing (Fig. 1A-F). Whereas in indirect embryogenesis, embryos started appearing after 14 d (Fig. 1G) on the surface of callus mass. Embryoid like structures were round with irregular out lines that frequently appeared as shining structures in clusters (Fig. 1A-B). The combination of various media treatments greatly affected embryoid differentiation and in few cases embryoids developed after 35d of first sign of callus formation. During present investigations, somatic embryogenesis followed four developmental stages *i.e*., globular (Fig. 1A-B), heart (Fig. 1C), torpedo (Fig. 1D) and cotyledonary (Fig. 1 E) followed by germination (Fig. 1F). These developmental stages also documented during different explants cultures by various scientists including Tiwari et al. [48] for onion, Jhankare et al. [49] for Withania, Bairwa et al. [50] for muskmelon, Uikey et al. [51] for sarpgandha, Patidar et al. [52] for chitrak, Sharma et al. [53] for grape, Upadhyay et al. [54] and Mishra et al. [55] for soybean and Shyam et al. [56,57,58] for Indian mustard. Secondary embryogenesis could be seen on the entire surface of primary embryos (Fig. 1B) and formed plantlets in huge numbers (Fig. 1F). Histological analysis revealed that the initial green translucent globular structure initiated from epidermal and sub-epidermal layers of the explant. Clear bipolar, it's with shoot and root poles as well as continuous procambial strands and distinct epidermis was observed without vascular connections with the explant (Fig. 1I). A pre-embryo was found which looked to develop from a single cell of sub epidermal cells. The section also exposed a secondary globular structure which grew on the surface layer of primary somatic embryos (Fig. 1 J-L). As division in superficial cells progressed, the cortical parenchyma cells actively divided in periclinal and anticlinal directions, which partly led to the nodular appearance; they later became the multicellular suspensor of the somatic embryos (Fig. 1J). It is likely that a single epidermal cell had undergone anticlinal division before the uppermost cell differentiated into the embryogenic mass. It was not clear if secondary somatic embryos originated from single cell. As superficial cells formed somatic embryos, new pre-embryo developed at adjacent deeper zones of the explant and subsequently, parenchymatous cells of cortex also formed pre- embryo (Fig. 1K-L).

In direct organogenesis, adventitious shoot primordia appeared after 7-10 d on explant surface. Green adventitious primordial formation started seeming in some instances. However, the advent of adventitious structure could be attained after 28 d (Fig.2 A-C). In indirect organogenic mode, shootlets developed from the surface of the callus (Fig.2 F-H). Shoot differentiation generally started meristemoids 10 d from initial culturing (Fig.1E). However, the duration varied from culture to culture and in a few cases the shoots started appearing after 45d. Most of the calli, after prolonged culturing on the induction media gave rise to plants. However, transfer into regeneration medium allowed

higher plant formation and growth rate (Fig.2 H). In cases, where there was no root formation attained, shootlets were subsequently transferred into the rooting medium (Fig.2 I). The rooted plantlets were elongated after transferring into elongation medium. Well-developed plantlets were transferred under 28±2^{o}C and 60±5% relative humidity for 20-25 d in an Environmental Growth Cabinet and subsequently (Fig.2 J). to the Net House/ Poly House (Fig.2 K) for 25-30 d for hardening before actual transfer in the field conditions (Fig.2 L).

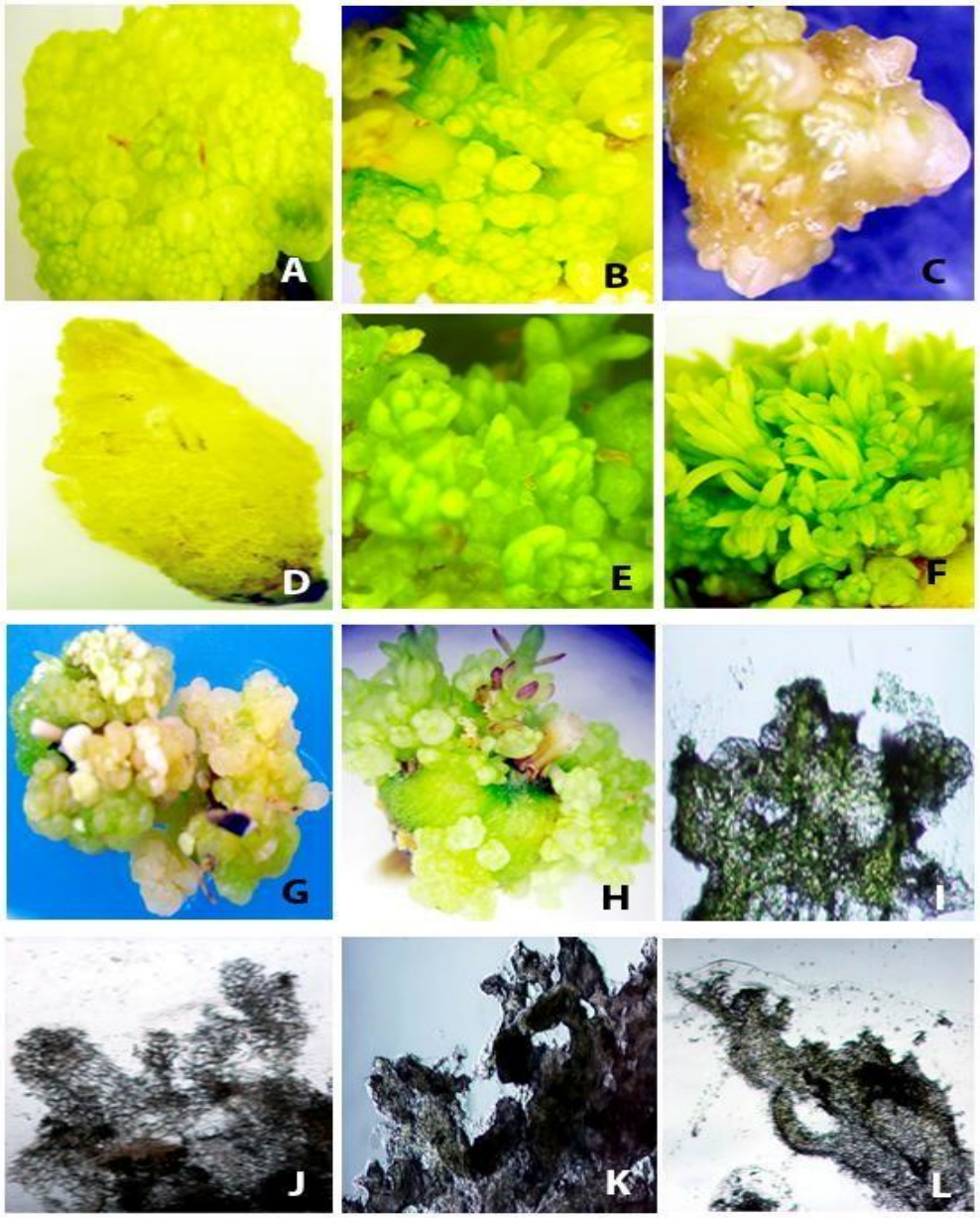


Fig. 1. Plant regeneration in *Santalum album via* somatic embryogenesis

A. Somatic embryo of globular stage; B. Formation of secondary embryos; C. Somatic embryo of heart stage; D. Somatic embryo of torpedo stage; E. Somatic embryo of cotyledonary stage; F. Germination of somatic embryo; G. Formation of globular stage somatic embryo via indirect mode; H. Plant regeneration from indirect somatic embryogenesis; I. Longitudinal section through green globular somatic embryo showing bipolarity by developing procambial strand with a close radicular and without vascular connections with the mother tissue; J. Somatic embryos differentiated from cortical parenchyma; and K-L. Development of leaf primordia with initiation of secondary somatic embryos

3.3 Effect of Culture Media and Plant Growth Regulators on *in vitro* Morphogenesis

A wide range of basal medium have been employed such as MS medium [6,13,16,17,18,22,24,59,60,61,62,63,64.65], WP medium [22,24] and White medium [17,66] for culture establishment of different plant species. The present outcomes disclosed that, basal MS medium used throughout the experiment was more responsive as compared to WP medium in course of preliminary experiments. Moreover, as observed during present investigations it is clear from obtained data that the composition of culture media does not seem to play major role in *in vitro* response as much as the type and concentration of plant growth regulators, henceforward for nodal segment culture, three different sets of culture media were formulated by supplementing different auxins as alone, diverse cytokinins as sole as well as auxins 2,4-D and NAA in combinations with cytokinins: BA, TDZ and Kn to basal MS media to accomplish the best *in vitro* performance.

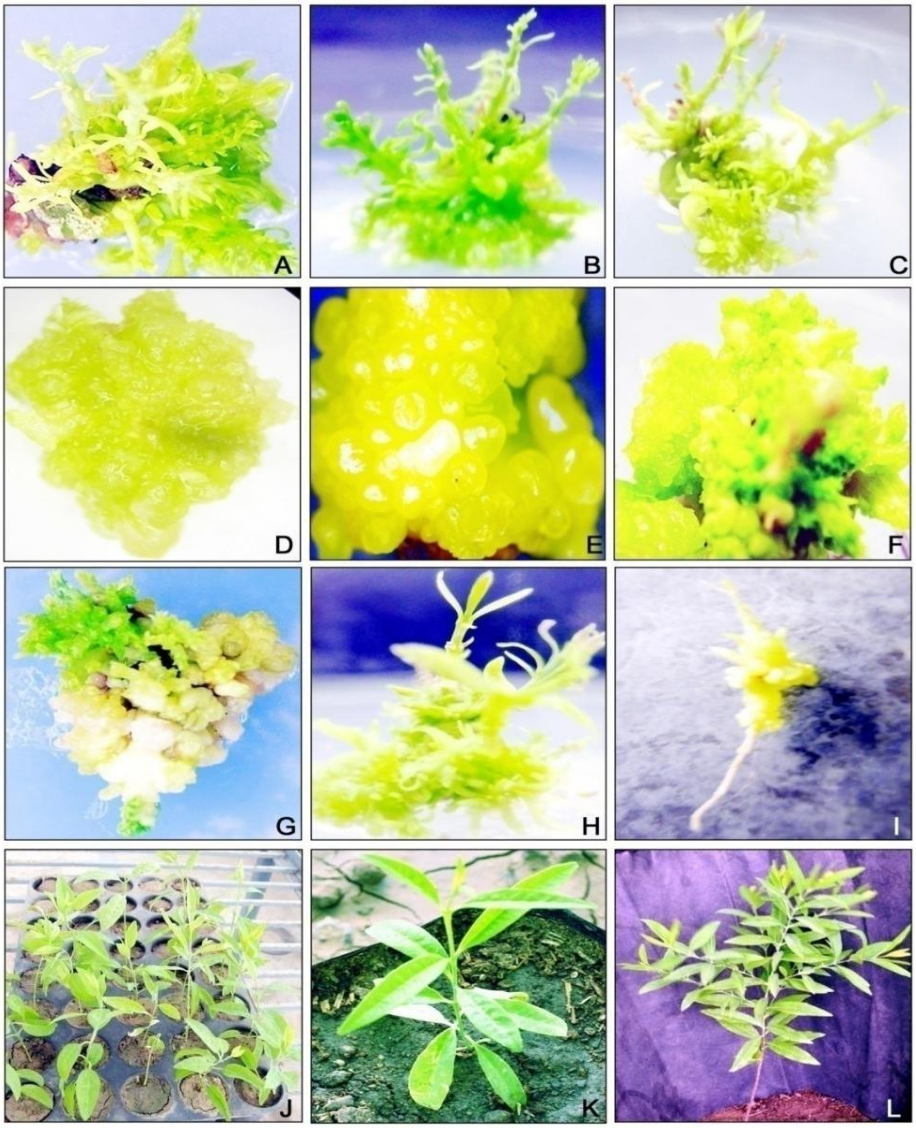


Fig. 2. Plant regeneration in *Santalum album via* organogenesis

A-C. Formation of multiple shoots via direct organogenesis; D-E. Callus formation; F-G. Initiation of multiple shoots; H. Formation of multiple shoots; I. In vitro rooting; J. Regenerants transferred in Environmental GrowthCabinet; K. Regenerants transferred in Net House for hardening; and L Plantlets transferred in field

Application of auxin 2, 4-D preferred the callus formation in higher frequencies at a concentration ranging from 1.0-7.0 mg l^{-1}. The callus was found to be enlarged with an increase level of 2, 4-D up to 7.0 mg l^{-1}. Beyond this concentration the calli proliferation twisted into dark black colour necrosed subseal and the best embryogenic response in terms of percentage of cultures forming somatic embryos and average number of somatic embryos per responding explant, was attained with addition of 0.5-5.0 mgl^{-1} 2, 4-D. Increasing the 2,4-D concentration up to 5.0 mg l^{-1} 2,4-D did not improve the embryogenic response (data not presented). Auxin 2,4-D initiated direct somatic embryogenesis in higher frequencies at concentration ranging from 0.5-3.0 mgl^{-1}. Indirect somatic embryogenesis and organogenesis were higher with culture media supplemented with 2, 4-D in the range of 1.0- 4.0 mgl^{-1}. However, direct organogenesis and plantlet regeneration efficiency was found to be quite low with application of 2, 4-D alone irrespective of all tested concentrations. Earlier similar results also reported by other researchers in different plant species [67-72].

Auxin NAA was able to induce somatic embryo formation at moderate frequency. However, organogenic response was found to be higher with application of NAA in range of 0.5-5.0 mgl^{-1}. Culture medium supplemented with NAA alone, produced calli in lower to higher frequencies dependent upon the concentrations (1.0-5.0 mgl^{-1}). Maximum calli initiated on culture medium supplemented with NAA at the concentration of 4.0 mgl^{-1}. Beyond this concentration ratio of non-morphogenic calli increased subsequently. NAA promoted direct organogenesis at concentration ranging from 1.0-4.0 mgl^{-1}. Plantlet regeneration efficiency was also found to be higher with application of NAA as compared to other auxins tested. Supplementation of NAA facilitated organogenesis in other crop species as reported previously by various investigators [73-76]

Among tested cytokinins, a higher proportion of explants responded well on media containing TDZ than with BA or kinetin. The supplementation of diverse concentrations of TDZ, as alone in the medium, displayed poor callus induction and organogenic calli formation. Nevertheless, culture medium amended with TDZ improved direct somatic embryo induction and plantlet regeneration efficiency as compared to media supplemented with NAA and/or 2, 4-D. BAP and kinetin performed poorly. Cultures produced in media supplemented with kinetin resulted in the formation of shoots of higher length with lesser numbers and low morphogenic frequency in advance phase of cultures.

Preliminary investigations to launch plant growth regulator type, combinations and concentrations revealed that auxins or cytokinins alone were not effective for accomplishing higher *in vitro* response for nodal explant culture. Either formation of morphogenic calli or plantlet regeneration efficiency was inspected low to moderate. Consequently, while determining final experimentation auxins NAA and 2,4-D in combinations with cytokinins: BA, Kn and TDZ in varying concentrations was applied for realizing the best *in vitro* response. The analysis of variance presented in Tables 2-3 revealed highly significant ($p<0.05$) differences among the response of different culture media combinations in terms of number of direct and indirect somatic embryo inducing explants, average number of somatic embryos, number of shoots/ embryoid, number of direct and indirect organogenic calli forming explants and number of shoots/ direct and indirect organogenic calli. It designates the incidence of substantial magnitude of variability amongst different culture media amalgamation. The outcome of plant growth regulators combinations is presented in the Tables 2 and 3. Higher proportion of direct somatic embryogenesis, average number of somatic embryos per explant and plantlet regeneration *via* direct organogenesis were documented on culture medium comprehending a moderate concentration of TDZ (1.0 mgl^{-1}) in combination with a lower concentration of NAA (0.5 mgl^{-1}). Previous investigations on somatic embryogenesis of white ash [77] walnut, watermelon, muskmelon, geranium, grape [78,79], peanut [80], gladiolus [81,82] and sandalwood [6,17,20] have also been investigated for the significant role of TDZ. In, the optimum concentration of TDZ for somatic embryo induction was quite low in sandalwood as compared to other species. TDZ at low concentrations (0.5-1.0 mgl^{-1}) has been reported to be more efficient in inducing organogenesis or somatic embryogenesis than other cytokinins, particularly in recalcitrant woody species [79]. The explanations for the high bustle of TDZ in woody species have not been examined at the physiological or molecular level. A carbon isotope study showed that TDZ was very stable in the culture media and persistent in plant tissue [82]. It has been suggested that TDZ helps to establish the internal optimum balance of cytokinin and auxin required for induction and expression of somatic embryogenesis [78,83,84].

A comparative higher concentration of BAP (1.0-2.0 mgl^{-1}) in combination with a lower concentration of NAA (0.5 mgl^{-1}) encouraged indirect somatic embryogenesis and organogenesis. In preceding studies steered by innumerable scientists [85-96] in different crop plants reinforced current outcomes. Ratio of organ formation directly from surface of cultured explants was recovered from culture medium amended with a higher concentration of BA at the concentration of 4.0 mgl^{-1} combined with a lower concentration of NAA (0.5 mgl^{-1}). Maximum plantlets regenerated *via* somatic embryogenesis (direct and/or indirect) on regeneration medium fortified with 2.0 mgl^{-1}TDZ in combination with 1.0 mg $l^{-1}GA_3$, while plantlets in higher frequencies *via* indirect organogenesis was acquired with regeneration medium amended with comparative lower concentration of TDZ (1.0 mg l^{-1}) in combination with 0.5 mgl^{-1} GA_3 and 0.5 mg^{l-1} NAA. The system described here required different hormonal combinations and concentrations from those formerly testified by Bapat et al. [27] who employed media comprising IAA and BAP to form the most normal somatic embryos, multiplication, germination and conversion into plantlets and distinguished the problem of low conversion frequency. In the current investigation, TDZ alone or with NAA can spontaneously induced embryogenesis at a higher frequency and with greater reproducibility. This conclusion is a harmony to the outcome of Rugkhla and Jones [15] who also evidenced 100% somatic embryo induction with application of TDZ alone as well in combination with 2, 4-D. Nevertheless, in this research, NAA performed better than 2,4-D. Mature somatic embryos needed supplementation of GA_3 for germination, conversion and elongation of plantlets, otherwise several abnormal somatic embryos were gotten and this occasioned in a low conversion into shootlets.

3.4 Histological Confirmation

Validation of somatic embryogenesis is grounded on histological evidence that the structure is bipolar and has no vascular connection to the explant [97]. The most appropriate regeneration systems for transformation are direct or repetitive production of somatic embryos or *de novo* shoot organogenesis, which instigated from single cells of the epidermal layer [98,99]. Histological annotations designated that differentiation of maternal tissue led to a suspensor-like structure of primary somatic embryos. The occurrence of a narrow suspensor directed a single cell origin and a broad suspensor area specified a multicellular source of somatic embryos [46]. Both narrow and broad suspensors were found in the current investigation. Comparable results have also been documented by Rugkhla and Jones [16] in sandalwood. Somatic embryos of single or multiple cell origin resulting from the epidermal layer were found in *Medicago sativa* [100] and from epidermal and cortical cells in *Trifolium repen* [46]. The single cell origin of secondary somatic embryos was recorded in walnut [101] and employed efficaciously in evolving solid transgenic plants [102].

3.5 In vitro Rooting Response

Regenerated shootlets of 3-4 months old and 1–2-inch height was isolated from the mother tissue and transferred on diverse fortification of MS medium for rooting (Table 4). Different hormonal treatments employing to MS basal agar gelled and liquid media, disastrous to form root even affects prolonged /incubation under diverse culture conditions. It is exciting to note that in all media combinations, shootlets were implanted on MS /agar gelled medium tried to induce roots, preferred healthy growth of shootlets with low frequency (1-3%), of rooting. Combination of IBA with kinetin induced formation of white friable calli under at the cut end; without root. Different combinations of IBA with BA rather induced adventitious shoot formation instead of rooting. Agar gelled medium with different concentrations of IBA in combination of BA/ kn and GA_3 also did not induce root formation from the excised shoots. These results are in close promise with previous research of Sarangi et al. [17] who also failed to induce *in vitro* rooting in sandalwood disdain of a long and repetitive exertion.

Plantlets regenerated *via* direct somatic embryogenesis have more prospect to be true to the type. Whereas with plants regenerated *via* callus cultures, option to get variation may not be ruled out. Plantlets also originated through direct organogenesis (*via* auxiliary bud proliferation). More probability occurs for the formation of the identical clones of the donor plants of such plantlets, since they are not exposed to the factors imposing somaclonal variations owing to bypassing long callus phase. Sarangi et al. [17], Bele et al. [5] and Tripathi et al. [13,30] attained parallel outcomes for diverse explants cultures in sandalwood. During current investigation, organogenesis in form of root

never lead to viable plants, whereas shoot forming cultures were able to crop complete plants by succeeding transferring into rooting media. Regeneration of plants *via* somatic embryogenesis is favored to organogenesis as embryoids generally arise from single cell and genetic manipulations could be carried through succeeding generations.

4. CONCLUSION

The current study points that under suitable circumstances nodal segment culture of sandalwood is more capable in contrast to other woody species. Higher number of shoots, embryogenic frequency and the conversion rate of embryoids into plantlets attained throughout present experimentation were higher as compared to the earlier discoveries. The events designated currently deliver operative approach for genetic transformation (direct and/ or *via* vector). Per se present plant regeneration technique in sandalwood is obtainable, which has instant potential for breeding and biotechnological investigations. Rooting is sporadic, and additional investigations are in advancement to get supportable *in vitro* rooting.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 11
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Production of Synthetic Seed from Encapsulated Nodal Segments of Giloe (*Tinospora cordifolia* Willd.)

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ABSTRACT

Tinospora cordifolia (Willd.) Miers is generally acknowledged as giloe belong to the family Menispermaceae employed for medicinal purpose, particularly in Ayurveda for curing different diseases.

Standardization of the concentration of encapsulation matrix and hardening solution affecting the frequency of germination to plant idyllic synthetic seeds of *Tinospora cordifolia* tracked by an efficient plantlet regeneration.

The current investigation was carried out at Plant Tissue Culture & Genetic Transformation Laboratory, Department of Plant Molecular Biology & Biotechnology, College of Agriculture, Gwalior, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, M.P., India during 2017 to 2020.

A procedure was recognized for encapsulation of nodal segments of *Tinospora cordifolia* excised from 18-month-old *in vitro* derived plants for short-term conservation and further propagation. Conservation strategies prerequisite to be implemented for constant supply to encounter the ever-increasing demands and sustainable utilization of resources. Diverse concentrations and combinations of gelling matrix *viz*., sodium alginate and complexing agent *i.e*., Calcium chloride were experienced to optimize amalgamation to achieve uniform beads.

The best gelling composition was established using 2.5% sodium alginate and 100 mM calcium chloride. The maximum conversion rate of encapsulated beads was attained on Murashiage and Skoog's medium amended with 2.0 mgl^{-1} BAP in association with 0.2mgl^{-1} NAA. Well, established regenerants were hardened successfully, acclimatized and established first under net house circumstances tracked under field conditions.

In this research, syn seed production procedure in giloe (*Tinospora cordifolia*) has been standardized. Current method warrants feasible tactic for multiplication, conservation and germplasm exchange through artificial, seed development.

Keywords: Giloe; synthetic seed; micropropagation; encapsulation; germplasm conservation; plantlet regeneration.

ABBREVIATIONS

$CaCl_2.2H_2O$: Calcium chloride; BA: Benzyl adenine; IBA: Indole-3-butyric acid; NAA: α-naphthalene acetic acid; MS: Murashiage and Skoog medium; PGR: Plant growth regulators.

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1. INTRODUCTION

Tinospora cordifolia (Willd.) Miers is usually acknowledged as giloe widely spreading, glabrous, succulent, climbing shrub belonging to the family Menispermaceae [1,2]. It is dispersed all over the tropical region of Nepal, India, Sri Lanka and China mounting to an altitude of 1,200 m asl. It prospers in the tropical region in forests and other habitat. Stems, roots, leaves and starch obtained from the roots and stems are employed for medicinal purpose, particularly in Ayurveda [3-6,2]. In current Covid pandemic situation, this was the most wanted plant species as immune modulator to increase immunity. Owing to the incidence of immense medicinal properties [2] the plant has been over subjugated by pharmaceutical industries and traditional peoples for outmoded medicines and nonexistence of prearranged cultivation led to the critical shortage of this plant to encounter the current claim. Furthermore, viability of seeds is very less, poor seed set and germination of seeds are the chief complications allied with its traditional propagation. Conservation strategies prerequisite to be implemented for constant supply to encounter the ever-increasing demands and sustainable utilization of resources. Plant cell, tissue and organ culture techniques is proving very convenient to solve these problems [7, 8]. It could be employed for massive *in vitro* propagation [9-40], synthetic seed production [41-43], raising embryogenic cell suspension cultures [44-52], production of useful secondary metabolites [53-56] *in vitro* selection in conjunction with somaclonal variation against different biotic [57-59] and abiotic [60] stresses as well as quality improvement [61].

A modified synthetic seed production process is considered a precious supernumerary acquaintance of propagation in many commercially domineering crop plants including giloe and a remarkable technique for mass propagation of elite plant genotypes. The production of plant clones multiplied by tissue culture and dispersed as synthetic seeds could be an appropriate substitute to the pricey crop plants. The conveyance of artificial seeds also allows difficulties like inventiveness abundant traditions for ascending up *in vitro* cultures and acclimatization to *ex vitro* conditions [62-63]. Synthetic seeds/artificial seeds or syn-seeds have been developed by several investigators in diverse crop plants by encapsulating an array of explants for instance, somatic embryos [64], shoot buds/tips [65-69], nodal segments [70-75,43], cell aggregates [76], auxiliary buds [77,76] and micro shoots/cutting [78-80,41] that may be further propagated as a seed and transformed into a plant under *in vitro* or *in vivo* conditions.

This encouraged to advance synseed technique for propagation, short term conservation along with germplasm exchange employing encapsulation [41, 67, 71, 73]. Substantial energies have been made for *in vitro* plantlet regeneration exploiting diverse strategies of micropropagation in giloe [8, 9, 19, 36] nevertheless to our knowledge till date no, protocol for synthetic seed production has been reported in giloe. Thus, in current study, an effort has been made to calculate the optimum quantity of encapsulation matrix and hardening solution for encapsulation of nodal segments and suitable concentrations and combinations of plant growth regulators to be supplied in culture medium for germination of encapsulated nodal segments.

2. MATERIALS AND METHODS

2.1 Explant Source

In vitro cultures of the *Tinospora cordifolia* were started from plant material grown at net house at Department of Plant Molecular Biology & Biotechnology, College of Agriculture, RVSKVV, Gwalior ($26^0$13'5.8332" N $78^0$10'58.1916" E) in cultivated soil beds.

2.2 Culture Media

MS [81] basal medium verbalized by Murashige & Skoog (1962) was used as basal medium in current study. In addition, MS basal macro and micro salts, vitamins and 7.5 gl^{-1}agar, cytokinin *viz*., BAP in varying concentrations (as sole) as well as in combination with an auxin *i.e*., NAA were supplied to MS media for culture establishment. Readymade basal medium and all other ingredients were procured from Hi-media Laboratories, Mumbai, India.

2.3 Preparation of Culture Media

All initial culture media were made employing readymade basal MS medium (HiMidia^{TM}) and amended with diverse types of plant growth regulators in different concentrations and combinations, 30.0 gl^{-1} sucrose and the final volume was kept to 1000 ml and pH was adjusted to 5.8±0.1 with 1N KOH solution. After adjusting the pH, agar @ 7.5 gl^{-1} was incorporated to the media as a semi-solidifying agent. Warm culture media, still in liquid state was dispensed into baby food bottles (50-60 ml / bottle) and culture tubes (15-20 ml / tube) followed by autoclaving at 121 $^{\circ}$C under 15 psi pressure for 25 minutes. Nevertheless, in case of pouring in petridishes, autoclaved warm culture media was bestowed into pre-sterilized 100x17 mm glass petridishes (25-30 ml/dish) under aseptic conditions of Laminar Flow Clean Air Cabinet.

2.4 Culture Establishment

Shoot tip explants collected from net house planted plants were washed thoroughly under running tap water chased by adding a few drops of detergent (Tween-20) pursued by surface sterilization with 0.1% (w/v) mercuric chloride for 5 min followed by rinsing 3 to 4 times with sterile double distilled water and inoculated on MS medium supplemented with plant growth regulators. Periodic subculturing at an interval of 28 days was carried out to uphold the proliferative shoot cultures. These cultures were further utilized for experimentation of development of artificial seeds.

2.5 Preparation of Gel Matrix

The encapsulation was endeavored by suspending the nodal segments on modified MS medium (devoid of calcium chloride) added with different concentrations (1, 2.0, 2.5 and 3% m/v) of sodium alginate and 0.5 M sucrose. This mixture was dispersed with a micropipette into different concentrations of calcium chloride solution (50, 75 and 100 mM) and left for 30 min for complex formation and encapsulation. The sodium alginate and calcium chloride solutions were prepared and sterilized by autoclaving at 121 °C and 1.05 kg/cm^2 pressure for 25 min.

2.6 Encapsulation of Nodal Explants

Nodal segments (0.3-0.5 cm) were excised from *in vitro* proliferated multiple shoots aseptically, dried employing sterile filter papers and encapsulated by mixing the freshly excised individual node micro cuttings in different concentrations of sodium alginate solution (1-3%w/v). The encapsulated explants were then thrown down in assorted concentrations of complexing solution *viz.,* calcium chloride in range of 50-100 mM using cut micropipette tips of 0.5 cm diameter. Each beaded drop comprising single nodes were left in calcium chloride solution for 30 min for polymerization. The calcium chloride solution was prudently decanted off and the encapsulated beads retrieved were washed 3-4 times with sterile double distilled water, blotted dry on sterilized filter paper and inoculated on MS nutrient medium amended with diverse concentrations of BA or BA in combinations with NAA to enable shoot organogenesis.

2.7 Culture Conditions

The cultures were sustained in culture tubes and kept in the culture room at a temperature regime of 25±2°C, 65±5% Relative Humidity and at a photoperiodic regime of 16/8 hr light/dark periods provided by cool white fluorescent tubes of intensity 1600-2000 lux. Regenerated multiple shoots having well developed roots were harvested from the culture tubes, washed under running tap water to eradicate sticking agar and transferred to plastic cups comprehending autoclaved sand. Primarily, the plastic cups were covered with thin polythene bags with minor holes to maintain humidity and incubated at 25±2°C. After two weeks, regenerants were transferred under net house conditions and maintained for further growth under 30±2°C and 65±5% RH for 15-30 days for adaptation. Acclimatized plants were then shifted to the field conditions.

2.8 Experimental Design and Data Analysis

Experiments were steered in CRD (completely randomized design) and the data were analysed as recommended by Snedecor and Cochran [82]. The significant difference between different treatments was experimented by Duncan's multiple range test (DMRT) at $p < 0.05$. The same letters in one treatment represent non-significant differences at $p < 0.05$.

3. RESULTS AND DISCUSSION

Synthetic seeds are encapsulated tissue culture resultant micro propagules that hold plant conversion ability and is used for conservation, germplasm exchange and dispersal. The employment of nodal segments as explant for synthetic seed production obliterates the chance of somaclonal variation [54]. Artificial seed production using nodal segments in *Tinospora cordifolia*, a plant of medicinal importance, fetching extinct due to high commercial exploitation is not yet documented.

3.1 Synthetic Seed Formation

The synthetic seeds are restricted by a defensive coating, which delivers essential protection during storage, handling and transportation. The coating could integrate plant nutrients or plant growth regulators that aid its conversion into plantlet [54]. In present investigation, nodal segments from the *in vitro* cultures were encapsulated employing sodium alginate (1, 2 2.5 and 3 5% m/v) in modified MS medium and calcium chloride (50, 75 and 100 200 mM). The beads were formed by exchange of ions, Na^+ ions of sodium alginate being exchanged with Ca^{++} ions forming calcium alginate. Encapsulation hardiness is determined by the optimal ion exchange of Na^+ and Ca^{++} ions, which may differ with propagules as well as with plant species [83, 84, 85].

Sodium alginate beads with encapsulated nodal segments speckled morphologically with respect to texture, shape and transparency with different concentrations of sodium alginate and calcium chloride (Table 1). Amongst the various concentrations of sodium alginate (1-3.0%) and calcium chloride (50-100 mM) applied in the research, a combination of 2.5% sodium alginate with 100 mM calcium chloride tracked by 2.5% sodium alginate in association with 75mM calcium chloride were demonstrated to be the best gel complexation medium and was suitable for the basis of safe quality beads which could be smoothly handled as well (Fig.1A). Alteration of encapsulated nodal segments into plantlets was effectively accomplished (Fig.1 B-C). Higher proportion of sodium alginate (3%) subdued the alteration of encapsulated due to toughness of beads while lower concentrations induced in the foundation of breakable encapsulated beads that were tough to grasp. The beads, which were formed commissioning 2.5% (w/v) sodium alginate solution was of uniform in size, clear, firm and round shaped and were showed perfect for further regeneration. Parallel results have also been reported by Sharma et al. [41] in *Bacopa monnieri,* by Castillo et al. [86] in *Carica papaya* and Prakash et al. [83] in *Plumbago rosea* whereas, successful germination in *Rhodiola kirilowii* at 4% and 5% of sodium alginate has also been possible [76].

3.2 Synthetic Seed Germination/ Regeneration

Higher germination/regeneration incidence has been recognized from synthetic seeds on MS medium amended with 2.0 mgl^{-1} BA in amalgamation with 0.2 mgl^{-1} NAA closely tracked by nutrient medium supplemented with 2.0 mgl^{-1} BA in association with 0.4 mgl^{-1} NAA and/or medium added with 1.0 mgl^{-1} BA in combination with 0.2 mgl^{-1} NAA. Therefore, it may be determined that for germination of synthetic seed culture medium amended with , cytokinins as alone supplemented in the MS medium (BA alone), did not bounce as much proliferation of shoots as with BA-NAA combination. This designates the synergistic effect of BA and NAA on shoot proliferation. Corresponding results have also been addressed by several other scientists in many crop plants [87-93, 8, 11, 12,13, 37, 83].

Variable percentage of germination shown after 21 days of inoculation (Table 2). In all cases synthetic seed germination occurred within 10-15 days of incubation primarily by bursting open sodium alginate matrix resulting in the formation of shootlets after 6-8 weeks of culture (Table 2). This was pursued by

the steady appearance of multiple shoots. These shoots were subsequently rooted (Fig. 1D) after transferring in to MS rooting medium supplemented with 1.0 mgl^{-1}IBA. Some of the artificial seeds failed to germinate and encapsulated nodes turned brown within the matrix. Similar reports have also been addressed by Sharma *et al*. [41].

Table 1. Effect of different concentrations of sodium alginate and calcium chloride on beads formation and germination from encapsulated explants

S. No.	$CaCl_2.H_2O$ concentration (m/M)	Sodium alginate concentration (%)	*Explants Responded (%)	Texture
1.	50	1	0.00^{g}	Failed to coat nodal explants
2.	75	1	4.80^{f}	Very fragile and soft to handle
3.	100	1	7.20^{e}	Fragile and soft
4.	50	2	8.66^{e}	Too soft and very fragile
5.	75	2	9.34^{e}	Soft and very fragile
6.	100	2	13.38^{d}	Poor bead formation
7.	50	2.5	24.28^{b}	Solid texture
8.	75	2.5	26.14^{b}	Uniform in size & solid
9.	100	2.5	34.58^{a}	Firm quality beads, clear, round and uniform in size
10.	50	3	14.28^{d}	Rigid & solid texture
11.	75	3	19.78^{c}	Solid
12.	100	3	17.27^{c}	Uniform in size, solid and short tail at the surface

a, b values within the column followed by different letters are significantly different, and the same letters are not different at a 5% probability level by Duncan's multiple range test.

Table 2. MS media supplemented with different concentrations and combinations of plant growth regulators for germination of synthetic seeds

S. No,	Culture media combination	Plant Growth regulators mgl^{-1}		Days taken to germinate	*Regeneration (%)	*Numbers of sheetlets
		BAP	NAA			
1.	MS	-	-	21 days	57.56^{h} ± 1.38	1.24^{i} ± 0.18
2.	MS0.5B	0.5	-	28 days	72.26^{f} ± 1.46	1.43^{h}± 0.32
3.	MSB	1.0	-	35 days	74.04^{ef} ± 1.56	1.63^{h}± 0.24
4.	MS1.5B	1.5	-	35 days	77.60^{d}± 1.58	1.93^{g} ± 0.34
5.	MS2B	2.0	-	40-45 days	82.63^{c}± 1.62	2.16^{g}± 0.40
6.	MS3B	3.0	-	45 days	80.32^{c}± 1.60	2.10^{g}± 0.38
7.	MSB0.2N	1.0	0.2	48-50 days	86.50^{b} ± 1.88	2.96^{cd}± 0.44
8.	MSB0.4N	1.0	0.4	48-50 days	88.50^{b}± 1.92	3.12^{c} ± 0.49
9.	MS2B0.2N	2.0	0.2	50 days	94.06^{a} ± 1.96	5.06^{a} ± 0.52
10.	MS2B0.4N	2.0	0.4	50 days	87.90^{b} ± 1.86	4.50^{b} ± 0.50
11.	MS3B0.2N	3.0	0.2	50 days	68.78^{g}± 1.44	2.32^{f}± 0.28
12.	MS3B0.4N	3.0	0.4	50 days	56.42^{h} ± 1.34	2.64^{e} ± 0.22

** Mean ± standard deviation. of three repeated experiments with ten replicates each. a, b Values within the column followed by different letters are significantly different, and the same letters are not different at a 5% probability level by Duncan's multiple range test.*

Higher germination proportion of synthetic seeds (deprived of storage) could be owing to the matrix, that not only enabled steady nutrient supply but also dwindling subtle tissue from any mechanical damage while handling and from desiccation. Plantlets regenerated from the encapsulated nodal segments were effectively transferred to pot (Fig.1E) and hardened off in polyhouse (Fig.1F) tracked by net house (Fig.1G) before transplanting in the field. The adapted plants showed normal growth and development.

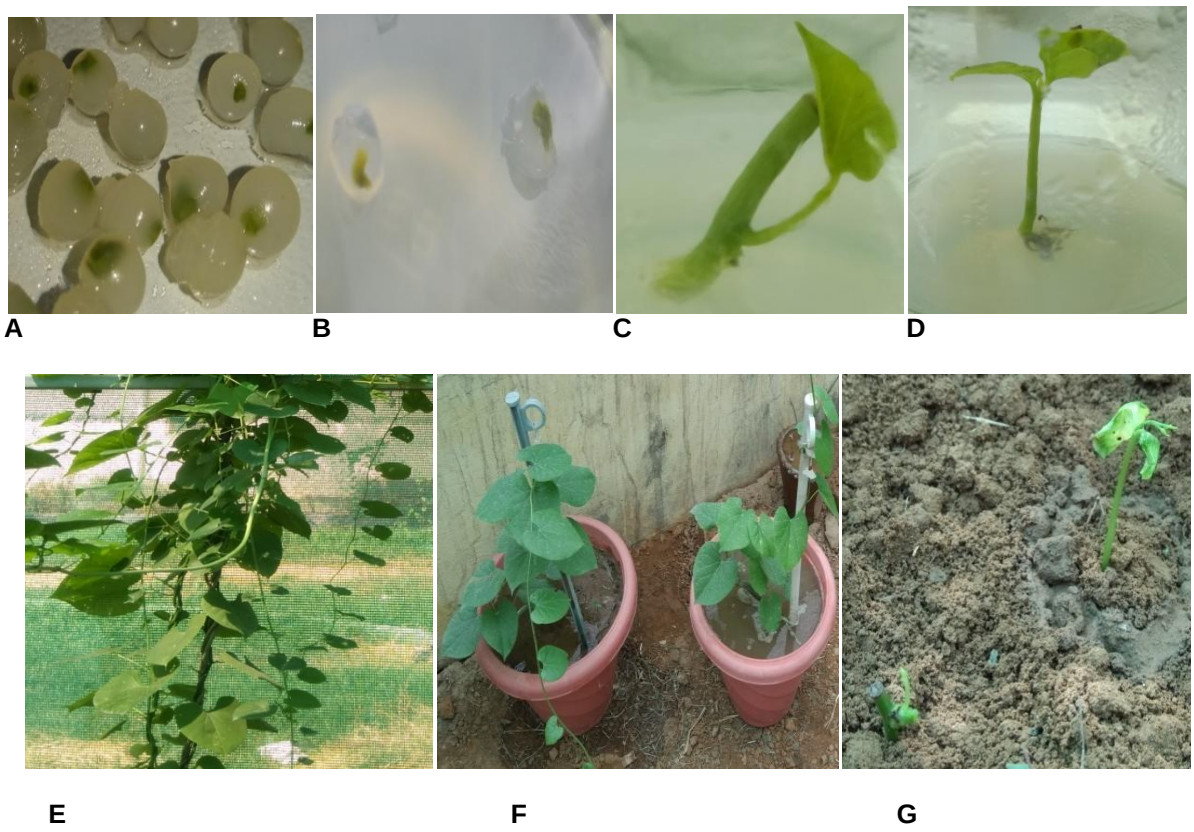


Fig. 1. Encapsulation of *in vitro* raised nodal segments of *Tinospora cordifolia* and germination A. Encapsulated nodal explants in 2.5% (w/v) sodium alginate solution; B-C. Emergence of shooting buds from encapsulated beads on MS2B.2N medium; D. *In vitro* rooting in MSIB medium; E. Acclimatize and hardening of plants in autoclaved sand; F. Plantlet in sterile soil after 4 weeks; and G. Plantlets transferred in net house conditions

The beads can potentially oblige as a reservoir of nutrients that may aid survival and speed up growth as earlier reported [94, 95, 41]. The alginate matrix containing nutrients reduced the viscosity and ability of the gel to form solid beads. Propagation through encapsulation of vegetative propagules has been reported in many medicinally significant plant species like *Bacopa monnieri* [41], *Withania somnifera* [66]. *Plumbago rosea* [83] and *Tylophora indica* [96].

4. CONCLUSIONS

This is first report of synthetic seed production in this imperative medicinal plant of high worth. In this investigation, synthetic seed production process in giloe (*Tinospora cordifolia*) has been standardized. The established method delivers good technique to regenerate plantlets of ‘elite’ clone and conservation for short-term storage in this significant medicinal plant in demand. Henceforth, this technique can be considered as an auspicious approach for the exchange and distribution of *giloe* between laboratories.

NOTE

The study highlights the efficacy of "Ayurveda" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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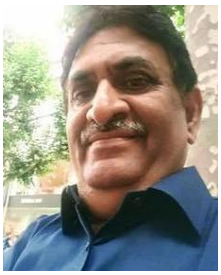
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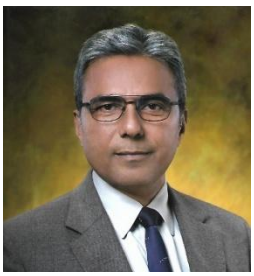
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Chapter 10
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Morpho-physiological Variations and Genetic Components Analysis in *Brassica juncea* (Linn.) Czern & Coss

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ABSTRACT

Study Objectives: Assessment of morpho-physiological genetic variation and analysis of genetic components may contribute a vital role in the preservation and enhancement of the embattled crop species.
Study Design: The current study consisted 196 Indian mustard genotypes together with checks. The genotypes were assessed on the basis of diverse morpho-physiological parameters. All the genotypes were grown in randomized block design with two replications.
Place and Duration of the Study: Field experiment was carried out in Rabi 2016-17 and 2017-18 at the experimental field of Department of Genetics & Plant Breeding, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, M.P., India.
Methodology: The investigation was steered to record different morphological and physiological parameters that play a crucial part in discriminating the embattled genotypes.
Results: Owing to current investigation, the highest genotypic and phenotypic coefficients of discrepancy were recognized for seed yield per plant trailed by harvest index and numbers of silique per plant. The highest heritability and genetic advance were found in seed yield per plot, followed by harvest index, biological yield per plot, days to 50% flowering, length of the main raceme, number of silique per plant, seed yield per plant, number of seeds per silique, 1000-seed weight, and number of silique per the main raceme.
Conclusions: Based on principal component analysis, 15 principal components were evinced while cluster analysis gave 16 clusters. The highest inter-cluster distance was demonstrated between cluster 9 and cluster 16 which advises that the hybridization programme seeing parents from these clusters is believed to be given a higher incidence of better-wanted combination(s) for development of advantageous genetic materials.

Keywords: Indian mustard; genetic variability; correlation; quantitative traits; principle component analysis.

1. INTRODUCTION

Brassica juncea (Linn.) is the furthermost important oilseed crop of India. It is being cultivated in several states along with Rajasthan, Haryana, Punjab, Madhya Pradesh, Uttar Pradesh, West Bengal, Gujarat, Bihar, Jharkhand, Assam, Jammu Kashmir, Uttaranchal, Chhattisgarh, Orissa and Maharashtra of India. Mustard is mostly a self- pollinating crop, nevertheless, about one third cross-pollination has also been noticed under normal field conditions [1]. Seeds of this important crop is used as source of oil, which is further employed in the devising of an array of industrial products including herbal drugs *etc* [2-6]. The above-mentioned characteristics and importance of this crop make it necessary to evaluate it for its quantitative and qualitative improvement [7,8].

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Crop improvement programme mostly depends upon a selection which is further decided by the convenience and incidence of genetic differences in the population of a demanding crop species [9,10]. This selection hangs on heritability, selection intensity, and the genetic advance of targeted characters of the crop [2]. Heritability determines the indentation of semblance between the phenotypic and genetic value. Genetic advance is the improvement in the mean of selection individual over the base population. These outcomes help the breeders to proceed for adaptation of appropriate breeding methods in the crop enhancement scheme. The diverse quantitative and qualitative parameters differ in their association with yield [7,3,4,11-14]. Utmost of the quantitative traits are controlled by multiple genes. So, there are probabilities of manifestation of an incessant variation in quantitative characters. These traits are governed by the environment. Correlation studies deliver a better understanding of the implication of diverse traits associated with yield. The traits which have enormously heritable and positively correlated with yield could be used in the indirect selection for yield. When the characters are related indirectly, path coefficient analysis proved helpful to pearl out direct and indirect reasons of connotation among the dissimilar variables. Consequently, the evidence of direct and indirect possessions of different traits on yield is momentous in selection. In view of the above realities, the present investigation was started by including some germplasm lines and varieties of Indian mustard for genetic components and Euclidean distance cluster analysis for yield and its accrediting traits along with determining the incidence of substantial changeability among diverse genotypes escorted by the analysis of diverse breeding parameters for yield and its attributing traits for further application in breeding schemes.

2. MATERIALS AND METHODS

The present study was undertaken on a total of 196 Indian mustard genotypes. Experimental materials were acquired from the Zonal Agricultural Research Station, Morena, RVSKVV, Gwalior M.P. (AICRP on Rapeseed and Mustard) and IARI, New Delhi. These were collected from different parts of India and the world. All the genotypes were grown in randomized block design with two replications in Rabi 2016-17 and 2017-18 at the experimental field of Department of Genetics & Plant Breeding, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, M.P., India. Each genotype was grown in a plot of one row of 2-meter length with an arrangement of 30 cm apart between rows and 15 cm plant to plant. The crop was captivated with protective irrigations and recommended packages of practices right through the growing season. Five randomly selected plants from each treatment were marked for captivating the observations for the parameters, *viz*., plant height (cm), numbers of primary and secondary branches per plant, days to 50 percent flowering, days to maturity, length of the main raceme (cm), numbers of silique per the main raceme, numbers of silique per plant, silique length (cm), numbers of seeds per silique, seed yield per plant (g) and per plot (g), 1000-seed weight (g), biological yield and harvest index (%) for analysis of mean performance.

Genotypic (GCV) and phenotypic coefficient of variation (PCV) was computed as per formula given by Burton [15]; heritability in the broad sense (h^2) as proposed by Burton and De [16] and genetic advance as per the method designated by Johnson et al. [17]. The correlation coefficients were premeditated to know the degree of association of a character with yield and also among the yield accrediting components. Genotypic and phenotypic correlations were calculated by way of the formula described by Weber and Moorthy [18] and Miller [19]. The portion of direct and indirect donations of different traits to the total correlation coefficients with yield was evaluated through path coefficient analysis as suggested by Wright [20,21] and suggested by Dewey and Lu [22]. Principal component analysis was carried out on PAST v3.14 software based on correlation matrix (normalized variance-covariance) as the variables are calm in different units; this recommends normalizing all variables using division by their standard deviations.

3. RESULTS AND DISCUSSION

Assessment of genetic diversity in a crop is a fundamental work since hybrids between lines of different bases generally; display better heterosis than those between closely associated parents. The preference of genetically divergent parents for hybridization is a principal jaw of any crop enhancement arrangement for getting anticipated segregates. Analysis of variance was found substantial for most of the traits that advised the occurrence of a considerable amount of variation in

explored materials for further enhancement of different traits (Table 1). Pooled variances for two years revealed that genotypes were enormously and significantly differing for all the inspected parameters. Plant height arrayed between 224 cm (PM-25) to 343.95 cm (MRNJ-127) with a mean worth of 296.90 cm. The number of primary branches per plant was lowest at 7.8 for genotype MRNJ-73 to13.0 for genotype Karishma, with an average number of 10.55. While maximum numbers of secondary branches (20.65) per plant were formed by genotype IDM-42 whereas minimum worth (11.45) was documented for genotype RB-50. Days to 50% flowering was evidenced to be lowest 53.70 for genotype MRNJ-102 while it was highest (105.53) for genotype IDM10, with a mean performance of 72.42 days. Genotype JD-6 required fewer days to mature (222.75), while, the maximum days to maturity was evinced for genotype MRNJ- 56 (295.7days). The length of the main raceme was ranged between 86.5cm to 185.4cm. Genotype MRNJ-138 had the highest length of the main raceme closely tracked by a group of three genotypes including MRNJ-90, MRNJ- 125 and IDM-12 with *at par* performance whereas the lowest value was exhibited by genotype MRNJ-138. The numbers of silique/main raceme were varied from 58.23 (MRNJ-134) to 125.03 (MRNJ-35), with an average of 89.98. Silique per plant ranged between 215.15 (MRNJ-44) to 586.8 (MRNJ-73), with a mean worth of 366.31. Length of silique was documented to be highest at 12.77 for genotype MRNJ-48 while it was lowest at 6.92 for genotype MRNJ-135. Maximum seeds per silique (40.07) were perceived for genotype MRNJ-15 while; the lowest worth (17.75) was recorded for genotype MRNJ-131. Seed yield per plant was ranged between 11.17g to 40.54g, with an average value of 21.95g. Genotype MRNJ-118 formed maximum seed per plant, nevertheless, the lowest value was documented for genotype MRNJ-11. The analogous conclusions were also evidenced by Shekhawat et al. [23], Akabari et al. [24], Salam et al. [25], Sikarwar et al. [26] Bafra et al. [2] and Dawar et al. [27]. Genotype L-6 exhibited the highest 1000-seed weight (16.14g) while; the lowest magnitude (7.05g) was documented for genotype MRNJ-6. Genotype MRNJ-60 had a maximum harvest Index (65.61%), while a minimum worth (16.77%) was evidenced for genotype MRNJ-26. According to the verdicts of various investigations, *Brassica* genotypes may own variation owing to their sowing time and genetic makeup. These issues may affect the plant height, the number of branches, silique, seed yield, seed weight and crude oil content as well. The explanations behind the modifications may be temperature, humidity and precipitation also [28-30]. The results of the current study that each parameter designated for the analysis has established its appropriateness to analyze genetic variability extant among Indian mustard genotypes.

Other trait the coefficient of variation was deliberated at both genotypic and phenotypic levels also (Table 2). The maximum genotypic coefficient of variation was recorded for seed yield per plant tracked by harvest index and numbers of silique per plant. Present results are in harmony with the outcomes of previous researchers including Akbar et al. [31] for high seed yield per plant and low for plant height; Dawar et al. [27] for higher seed yield per plant. The highest extent of the phenotypic coefficient of variation was documented for seed yield per plant followed by harvest index and number(s) of silique per plant. Plant height and days to maturity displayed very low PCV values. These results are in endorsement with the significances of Gangapur et al. [32] who specified low PCV for days to maturity and plant height. Tripathi et al. [33] conveyed maximum PCV for seed yield per plant (g), moderate worth for number(s) of primary branches, length of the main raceme, number(s) of siliqua per the main raceme, number(s) of seeds per siliqua, 1000-seed weight and low for days to maturity. Synerm et al. [34] also detected moderate PCV for the number(s) of seeds per siliqua and low for plant height and days to maturity. Moderate for days to 50% flowering and number(s) of primary branches by Amsalu et al. [35] while highest for seed yield per plant, moderate for length of main raceme and low for days to maturity by Singh et al. [36] also described.

Besides, heritability and genetic advance are obliging in selecting superior individuals. Higher heritability (Table 2) has been evinced for seed yield per plot, harvest index, biological yield per plot, days to 50% flowering, length of the main raceme, numbers of silique per plant, seed yield per plant, plant height, numbers of seeds per silique, 1000-seed weight and numbers of silique per the main raceme. Nevertheless, silique length, numbers of primary and secondary branches displayed low estimates of heritability. Earlier, Lodhi et al. [37] have attested moderate heritability for days to maturity; Akabari et al. [24] for number (s) of siliqua per plant and seed yield per plant; Salam et al. [25] for number(s) of seeds per siliqua and seed yield per plant; Sikarwar et al. [26] for 1000- seed weight; Dawar et al. [27] for number(s) of siliqua per plant and number (s) of seeds per siliqua and Singh et al. [36] for days to 50 % flowering and number(s) of silique per the main raceme.

Table 1. Mean performance for yield and its attributes

Parameters Genotypes	Plant height (cm)	Numbers of primary branches	Numbers of secondary branches	Days to 50% flowering	Days to maturity	Length of main raceme (cm)	Numbers of silique per main raceme	Numbers of silique per plant	Silique length (cm)	Seed yield per plant (g)	Seed yield per plot (g)	Seed yield per plot (g)	1000-seed weight (g)	Biological yield per plot (g)	Harvest index
MRNJ-1	37.5	135.5	134.7	5.9	8.7	65.9	47.2	224.6	5.8	16.5	4.1	1118.2	164.9	9.5	14.8
MRNJ-2	36.1	135.1	138.8	5.3	7.6	64.4	52.3	206.1	5.5	13.8	3.7	1215.5	235.4	15.6	19.4
MRNJ-3	39.0	137.6	145.6	5.6	7.6	64.9	54.9	236.2	4.9	12.8	4.1	1261.8	154.9	8.3	12.4
MRNJ-4	32.3	131.2	134.3	5.6	10.3	65.4	56.0	209.5	4.7	16.6	4.2	1036.2	196.4	12.7	19.0
MRNJ-5	40.5	138.9	137.6	5.9	8.6	71.2	49.5	224.3	5.5	11.5	4.1	1390.2	149.4	8.4	10.8
MRNJ-6	36.6	135.7	132.6	5.7	6.7	50.1	52.5	194.6	4.7	14.8	3.5	1101.1	191.3	7.6	17.2
MRNJ-7	34.9	133.8	138.6	5.1	7.7	72.2	53.0	202.3	4.7	10.5	4.5	1145.6	288.3	10.0	25.4
MRNJ-8	34.9	134.1	135.8	5.4	9.2	57.6	55.0	125.9	4.2	12.3	3.5	1317.5	158.1	7.6	12.1
MRNJ-9	33.1	132.9	138.9	5.6	8.6	61.1	55.3	164.2	4.9	10.5	4.1	1288.6	163.1	7.4	12.6
MRNJ-10	30.9	129.7	138.6	5.6	7.1	67.5	47.9	182.1	5.3	12.0	5.5	1090.1	174.3	8.6	16.0
MRNJ-11	29.4	130.9	141.8	5.6	7.7	64.3	44.2	153.6	4.6	10.4	4.6	1234.7	155.2	5.6	12.7
MRNJ-12	27.9	141.9	138.1	5.5	9.3	65.3	42.8	175.6	5.1	12.8	3.6	1187.1	180.8	10.2	15.3
MRNJ-13	38.3	140.8	142.4	5.5	6.9	69.0	43.7	205.6	4.2	15.7	5.5	1354.6	178.3	12.8	13.1
MRNJ-14	33.8	139.6	142.6	5.6	8.9	66.9	44.5	191.6	5.1	12.8	3.7	1286.6	147.6	7.7	11.4
MRNJ-15	30.3	140.0	136.4	5.3	6.1	64.8	48.0	163.0	5.0	20.0	4.4	1357.1	225.4	6.8	16.8
MRNJ-16	32.6	143.9	136.8	5.9	6.9	64.6	51.0	143.4	5.3	17.8	4.2	1298.6	295.8	8.8	22.8
MRNJ-17	30.8	139.6	141.1	5.9	7.9	52.2	51.9	206.7	5.1	12.1	3.8	1339.1	200.5	10.1	15.3
MRNJ-18	32.8	141.4	141.9	6.1	5.9	68.3	50.2	158.1	5.4	14.8	4.6	1102.7	198.1	6.9	18.1
MRNJ-19	30.5	137.4	139.1	5.6	7.5	66.1	48.8	203.0	4.8	12.6	4.2	1399.6	179.8	11.7	12.9
MRNJ-20	29.3	142.0	139.8	5.3	9.2	64.6	50.5	229.3	4.7	13.9	5.3	1301.7	150.1	11.7	11.4
MRNJ-21	30.6	138.6	140.1	5.5	6.8	66.1	44.0	161.7	5.4	15.2	3.5	1182.7	163.5	9.7	13.9
MRNJ-22	27.9	135.9	145.1	5.2	6.6	84.7	42.9	208.2	4.8	14.0	5.0	1410.6	179.3	12.2	12.6
MRNJ-23	30.1	136.8	147.9	5.8	7.1	76.2	38.7	220.1	5.4	12.5	4.2	1423.7	163.1	8.6	11.4
MRNJ-24	29.6	126.2	146.4	5.1	9.1	64.6	38.4	159.4	4.2	12.8	4.4	1423.1	153.4	9.8	10.8
MRNJ-25	28.1	121.0	148.4	5.3	9.4	63.1	41.7	211.1	4.8	14.1	4.3	1147.9	272.8	10.6	23.8
MRNJ-26	32.9	130.4	139.6	5.0	8.4	67.8	47.7	162.3	5.7	14.0	4.5	1819.6	153.5	8.4	8.4
MRNJ-27	32.1	129.3	146.2	5.3	6.5	64.1	56.5	205.0	4.7	15.0	4.8	1483.9	192.8	11.9	13.2
MRNJ-28	32.8	129.9	143.2	5.1	8.4	81.8	49.8	118.6	4.2	13.6	4.6	1269.6	198.3	6.9	15.8
MRNJ-29	32.1	130.5	145.6	6.0	8.9	64.6	50.4	217.5	4.7	14.6	5.0	1271.0	189.9	11.0	15.0
MRNJ-30	30.3	130.6	143.8	4.6	8.5	69.0	41.2	193.2	3.7	15.5	5.3	1316.9	178.5	8.1	13.7
MRNJ-31	34.1	127.4	146.1	5.9	8.5	66.2	45.7	213.1	4.7	11.8	5.8	1351.5	151.3	16.3	11.4
MRNJ-33	27.6	122.8	144.1	4.6	5.9	65.7	43.7	222.4	4.9	13.8	4.6	1104.9	155.4	10.7	14.1
MRNJ-34	35.1	132.6	147.4	5.6	8.3	65.1	47.0	167.1	5.4	16.8	3.5	1576.7	160.0	11.5	10.1
MRNJ-35	29.9	129.4	147.1	5.3	8.4	67.4	62.5	177.6	4.5	12.7	6.5	1267.1	151.5	9.1	12.1

Parameters Genotypes	Plant height (cm)	Numbers of primary branches	Numbers of secondary branches	Days to 50% flowering	Days to maturity	Length of main raceme (cm)	Numbers of silique per main raceme	Numbers of silique per plant	Silique length (cm)	Seed yield per Plant (g)	Seed yield per plot (g)	Seed yield per plot (g)	1000-seed weight (g)	Biological yield per plot (g)	Harvest index
MRNJ-36	33.6	130.0	147.6	5.3	7.7	83.7	53.5	229.9	5.2	13.0	7.3	1364.0	271.5	10.6	20.0
MRNJ-37	39.7	137.8	150.4	5.8	6.0	57.9	54.7	208.2	4.5	14.2	4.3	1331.1	146.3	20.2	11.0
MRNJ-38	39.9	136.9	149.2	5.3	7.0	82.8	59.3	226.3	4.1	11.7	4.5	1274.9	206.9	12.2	16.3
MRNJ-39	33.9	132.6	149.1	5.3	6.9	74.6	50.8	166.0	4.8	14.0	4.4	1144.5	155.2	10.2	13.7
MRNJ-40	38.1	135.1	150.1	5.6	6.5	73.4	53.8	203.8	5.9	12.1	5.4	1184.0	248.5	13.6	21.2
MRNJ-41	31.8	129.6	149.7	5.3	6.8	66.8	52.7	220.1	5.5	13.0	5.9	1283.1	142.4	13.1	11.0
MRNJ-42	34.8	132.8	147.8	5.0	7.2	72.1	50.0	198.7	5.4	10.0	4.3	1373.7	171.1	12.8	12.5
MRNJ-43	39.3	135.8	149.1	5.0	7.9	66.1	51.0	212.1	3.6	14.9	4.6	1235.7	150.3	11.3	12.1
MRNJ-44	45.1	141.8	149.4	5.3	7.8	65.1	46.7	107.6	5.1	13.2	4.4	1088.2	154.9	7.5	14.4
MRNJ-45	39.0	136.2	149.8	5.8	9.0	84.7	45.5	144.0	5.4	11.5	5.1	1456.6	149.6	9.2	10.3
MRNJ-46	38.6	135.6	149.7	4.3	6.7	68.6	54.8	121.7	4.6	11.3	4.3	1282.7	164.3	12.6	12.9
MRNJ-47	40.6	137.7	149.6	5.6	8.3	67.8	49.0	233.1	5.2	11.7	4.4	1304.6	179.7	7.6	13.9
MRNJ-48	38.6	135.3	150.2	5.5	7.4	65.6	48.2	208.1	6.4	13.3	4.9	1412.1	156.5	9.9	11.1
MRNJ-49	34.6	132.4	149.8	5.2	7.0	65.7	56.0	117.8	5.5	13.8	5.4	1044.2	181.5	15.2	17.4
MRNJ-50	33.1	131.8	149.8	5.3	7.3	72.6	52.1	137.1	5.4	13.5	4.5	1119.1	190.8	8.4	16.9
MRNJ-51	32.9	133.4	150.1	4.3	6.8	71.9	46.7	116.2	5.5	13.1	5.3	1318.1	153.4	8.2	11.9
MRNJ-52	32.6	131.1	156.1	4.9	7.1	60.0	47.7	131.7	4.6	15.6	4.2	1188.1	145.7	10.8	12.4
MRNJ-53	38.1	134.1	153.8	5.3	6.8	71.8	40.7	222.3	4.9	10.1	6.1	1150.7	210.1	15.3	18.4
MRNJ-54	43.6	139.4	150.8	4.9	7.5	62.6	45.3	133.1	4.1	11.4	5.5	1028.3	177.6	9.9	17.3
MRNJ-55	39.8	137.1	155.1	5.7	7.7	59.4	50.8	141.2	5.1	13.7	5.2	1325.6	155.5	7.3	11.9
MRNJ-56	50.6	147.9	163.9	5.6	7.5	56.4	56.2	118.0	3.9	10.5	5.4	1350.6	177.2	8.9	13.1
MRNJ-57	39.6	137.1	155.4	5.3	7.0	61.7	60.8	131.9	4.7	10.5	4.9	1418.6	180.0	8.2	12.8
MRNJ-58	37.3	134.3	154.3	5.4	6.5	62.6	51.9	206.9	5.5	12.6	5.2	1323.2	175.1	11.0	13.2
MRNJ-59	34.8	132.8	154.2	5.4	7.8	68.2	55.8	134.3	5.0	14.3	4.7	1143.7	147.9	8.9	13.3
MRNJ-60	40.1	137.3	156.5	5.4	8.8	66.0	53.3	124.1	4.2	12.0	4.4	1388.1	449.4	11.3	32.8
MRNJ-61	40.8	135.8	155.7	5.3	9.0	63.0	52.5	109.6	4.7	12.6	5.2	1326.1	170.1	10.7	12.3
MRNJ-62	30.5	130.9	166.5	5.0	8.3	74.8	55.1	128.0	5.5	13.9	5.8	1378.6	154.5	8.7	11.3
MRNJ-63	35.0	132.6	151.1	5.0	7.3	83.2	46.2	114.9	5.2	14.4	5.4	1538.7	150.7	8.3	9.6
MRNJ-64	35.3	131.9	152.6	5.1	7.2	66.7	44.8	186.8	6.2	13.6	6.4	1290.6	181.0	10.2	14.2
MRNJ-65	36.8	133.6	152.0	5.4	6.6	52.6	42.2	134.7	4.6	12.0	5.8	1296.1	155.6	7.7	12.1
MRNJ-66	34.3	132.1	148.1	4.9	6.5	59.8	45.5	246.8	5.6	13.8	6.0	1316.1	149.1	6.7	11.5
MRNJ-67	38.3	134.9	143.9	5.4	7.6	58.7	40.6	134.5	5.2	13.3	4.7	1382.7	197.4	11.7	14.3
MRNJ-68	50.1	144.3	145.4	5.0	7.0	69.0	38.6	109.3	5.4	13.0	5.5	1367.7	210.2	7.4	15.6
MRNJ-69	36.5	132.6	150.0	5.7	8.6	59.5	42.8	147.6	5.2	12.4	6.1	1609.6	171.9	7.3	10.8
MRNJ-70	33.3	131.6	142.2	5.5	8.3	59.2	46.7	182.4	4.6	12.8	4.6	1422.7	161.9	8.4	11.3
MRNJ-71	32.6	130.8	149.2	5.2	7.7	56.7	60.8	161.9	5.5	12.4	5.1	1360.7	159.6	10.9	11.8

Parameters Genotypes	Plant height (cm)	Numbers of primary branches	Numbers of secondary branches	Days to 50% flowering	Days to maturity	Length of main raceme (cm)	Numbers of silique per main raceme	Numbers of silique per plant	Silique length (cm)	Seed yield per plant (g)	Seed yield per plot (g)	Seed yield per plot (g)	1000-seed weight (g)	Biological yield per plot (g)	Harvest index
MRNJ-72	36.1	131.4	145.9	5.0	6.2	52.8	38.0	227.7	4.9	11.9	5.6	1146.6	170.4	12.3	15.2
MRNJ-73	40.4	136.3	147.4	3.9	8.8	54.6	42.6	293.4	3.7	13.9	5.8	1480.6	193.4	16.0	13.2
MRNJ-74	34.7	131.9	141.0	4.7	6.6	60.3	44.3	222.6	4.8	12.7	5.2	1541.0	183.3	9.9	12.0
MRNJ-75	34.6	130.8	147.6	5.4	7.5	73.0	48.4	169.6	5.9	13.5	5.6	1610.9	196.1	11.9	12.2
MRNJ-76	35.7	132.7	149.0	5.5	6.8	56.2	50.8	164.9	4.3	12.9	4.4	1101.9	185.5	14.8	17.4
MRNJ-77	50.9	144.7	147.2	5.3	6.2	57.8	55.0	135.6	4.3	12.9	5.7	1083.4	150.6	12.7	13.8
MRNJ-78	28.4	129.4	147.5	5.5	6.5	51.2	54.9	145.5	4.8	12.6	5.9	1254.7	150.0	9.8	12.0
MRNJ-79	36.6	138.1	145.7	5.6	8.0	54.6	58.0	187.3	4.5	12.9	6.6	1126.5	150.8	11.0	13.7
MRNJ-80	41.4	140.8	150.1	5.5	8.1	70.1	60.0	210.5	4.1	11.4	4.2	1315.2	187.9	13.2	14.4
MRNJ-81	37.3	139.1	148.6	5.3	7.2	62.8	55.7	187.6	4.8	12.6	5.9	1241.4	159.9	13.5	12.8
MRNJ-82	32.1	133.8	147.0	5.4	7.7	67.7	48.7	206.9	4.7	16.5	4.8	1462.5	179.5	10.6	12.3
MRNJ-83	37.8	139.1	147.6	5.3	6.5	54.2	49.1	146.4	4.1	13.5	5.9	1437.5	182.3	12.8	12.6
MRNJ-84	36.3	138.6	144.0	5.7	7.0	61.8	50.8	187.9	4.4	11.2	6.3	1489.5	183.9	13.2	12.5
MRNJ-85	40.3	142.1	139.8	5.3	7.7	55.3	50.0	228.6	4.3	12.9	5.4	1114.9	193.8	12.6	17.2
MRNJ-86	39.9	140.6	145.8	5.4	8.5	58.7	49.1	163.6	5.4	13.7	4.7	1201.5	164.6	14.0	13.7
MRNJ-87	35.0	136.4	142.8	5.5	7.0	69.2	57.7	179.9	4.7	11.6	5.2	1668.7	188.7	10.2	11.4
MRNJ-88	36.4	137.8	144.7	5.6	6.8	71.8	44.6	170.1	4.3	15.0	5.1	1206.9	176.5	7.6	14.8
MRNJ-89	33.1	134.7	143.7	5.1	6.1	63.2	52.7	143.3	4.2	13.2	6.5	1458.9	175.9	10.4	12.1
MRNJ-90	32.2	131.6	145.4	5.3	6.5	90.2	58.0	204.4	4.8	11.9	4.5	1135.0	151.0	8.1	13.4
MRNJ-91	34.3	136.9	144.6	6.0	8.1	58.1	46.2	131.6	4.1	12.3	5.0	1264.9	160.8	8.6	12.8
MRNJ-92	33.3	134.4	146.3	5.1	9.5	81.6	60.0	136.1	4.3	11.8	4.6	1062.5	145.6	15.4	13.8
MRNJ-93	34.6	136.3	146.0	5.8	7.6	71.8	47.7	129.4	4.3	12.7	5.4	1227.7	182.4	11.4	15.2
MRNJ-94	35.2	132.6	144.7	6.1	7.6	72.1	42.4	230.8	4.7	14.0	4.4	1308.9	149.4	7.5	11.4
MRNJ-95	34.3	130.5	143.4	4.8	7.8	52.2	39.3	137.7	4.0	15.3	5.3	1374.7	186.4	14.6	13.6
MRNJ-96	38.5	134.4	145.1	5.3	6.2	58.2	34.9	125.3	4.6	12.3	5.7	1216.6	190.5	7.2	15.8
MRNJ-97	36.0	133.8	144.1	5.3	7.1	60.9	42.3	122.1	5.4	12.7	4.2	1279.2	166.4	6.5	13.0
MRNJ-98	34.3	133.1	145.4	5.5	7.6	70.2	43.0	141.7	4.4	13.9	5.7	1109.9	158.3	8.7	14.1
MRNJ-99	37.0	133.2	147.3	5.0	6.8	82.8	39.3	144.4	5.3	17.4	4.2	1361.6	151.1	7.9	11.7
MRNJ-100	35.2	133.7	143.3	5.5	6.7	80.7	39.9	201.0	4.9	14.7	5.9	1434.4	169.4	11.6	12.0
MRNJ-101	34.6	131.1	144.8	5.1	6.7	70.2	39.2	112.3	4.1	15.5	6.1	1098.9	151.6	7.6	13.8
MRNJ-102	26.9	126.4	145.3	5.3	6.4	65.6	42.2	113.6	5.1	13.4	5.1	1190.8	134.7	11.2	11.5
MRNJ-103	39.5	137.4	143.7	4.6	8.5	67.7	33.5	218.9	5.5	12.6	4.3	1307.9	161.0	17.2	12.2
MRNJ-104	35.3	131.6	143.8	4.8	9.7	43.3	38.3	206.2	5.2	13.4	5.9	1185.1	152.6	9.1	13.0
MRNJ-105	34.3	130.6	144.9	5.4	9.5	65.6	36.7	234.9	5.2	16.4	5.1	1078.7	154.4	16.2	14.5
MRNJ-106	32.5	131.3	142.5	5.5	8.5	57.8	33.4	205.1	3.7	14.3	4.3	1293.0	145.1	13.7	11.1
MRNJ-107	37.3	134.3	145.6	5.5	8.0	53.2	40.5	212.0	4.9	12.5	4.2	1327.0	153.6	10.6	11.6

Parameters Genotypes	Plant height (cm)	Numbers of primary branches	Numbers of secondary branches	Days to 50% flowering	Days to maturity	Length of main raceme (cm)	Numbers of silique per main raceme	Numbers of silique per plant	Silique length (cm)	Seed yield per plant (g)	Seed yield per plot (g)	Seed yield per plot (g)	1000-seed weight (g)	Biological yield per plot (g)	Harvest index
MRNJ-108	35.5	133.8	145.9	5.1	7.3	64.4	40.9	174.6	3.7	12.4	3.7	1321.9	150.0	17.4	11.5
MRNJ-109	32.0	131.3	143.5	5.2	8.3	83.7	45.8	125.1	4.7	15.3	4.9	1144.0	190.0	7.7	16.5
MRNJ-110	32.3	130.7	148.2	5.5	6.3	72.3	37.2	213.1	4.4	10.3	6.2	1319.0	206.2	9.6	15.6
MRNJ-111	36.2	133.3	145.3	5.0	8.1	73.7	44.0	165.0	3.9	12.2	4.5	1425.7	158.8	13.4	11.2
MRNJ-112	40.6	136.6	146.1	5.6	7.1	74.7	34.7	183.6	4.2	14.5	5.2	1433.0	148.9	16.2	10.3
MRNJ-113	39.8	137.4	144.2	4.7	8.5	68.8	44.4	225.7	4.0	10.9	4.6	1322.7	191.5	15.1	14.6
MRNJ-114	37.7	134.3	145.3	5.3	7.2	68.2	37.8	141.8	5.6	16.1	4.9	1141.7	174.9	8.9	15.3
MRNJ-115	37.2	133.1	142.4	5.2	7.6	57.5	40.5	148.1	5.4	16.3	6.1	1311.5	164.5	12.5	12.4
MRNJ-116	40.4	138.1	152.6	5.2	8.0	53.9	41.7	178.1	4.5	11.3	4.4	1221.9	138.8	8.4	11.5
MRNJ-117	39.6	133.4	151.8	5.5	7.9	62.2	40.7	213.0	5.4	12.1	5.8	1467.1	169.9	11.3	11.9
MRNJ-118	35.4	132.9	148.9	4.7	8.0	57.7	43.0	218.6	5.5	12.3	4.5	1465.4	154.8	20.3	10.6
MRNJ-119	34.4	130.8	146.8	5.1	7.0	85.6	38.3	231.4	5.2	12.4	5.1	1313.1	147.3	16.8	11.3
MRNJ-120	40.4	136.6	149.6	4.8	6.8	69.3	33.0	157.3	5.1	13.1	5.4	1093.5	165.0	9.9	15.1
MRNJ-121	35.5	132.1	147.6	4.7	8.0	65.2	32.9	207.1	5.4	12.8	5.3	1226.4	173.5	17.4	14.3
MRNJ-122	41.3	139.2	147.3	4.8	7.6	59.1	33.3	135.3	4.5	13.4	5.3	1127.6	176.3	6.8	15.5
MRNJ-123	40.3	138.4	149.7	5.1	8.8	55.7	30.0	151.4	5.1	13.7	4.9	1053.0	193.2	11.1	18.4
MRNJ-124	40.7	137.1	147.3	4.7	7.4	65.2	35.9	127.6	4.8	11.9	5.0	1175.8	150.5	8.0	13.1
MRNJ-125	37.5	135.6	149.3	4.2	7.6	89.7	38.4	210.8	4.4	14.4	5.2	1089.4	143.0	12.1	13.2
MRNJ-126	36.4	134.4	165.3	5.9	8.2	58.8	39.3	149.7	4.8	16.0	5.5	1267.8	155.2	8.5	12.5
MRNJ-127	34.2	131.6	172.0	5.2	5.9	62.6	35.7	134.9	4.4	10.0	5.8	1304.9	163.3	6.0	12.4
MRNJ-128	31.5	130.2	167.9	5.1	8.0	62.6	37.4	148.1	4.8	13.6	5.1	1202.4	154.2	11.1	12.8
MRNJ-129	32.1	130.5	165.7	4.4	7.6	81.7	37.0	225.6	4.3	14.0	7.2	1099.4	149.5	10.4	13.6
MRNJ-130	38.0	133.4	170.5	5.5	8.6	63.4	36.7	224.3	4.2	11.7	4.9	1379.4	145.2	10.2	10.6
MRNJ-131	36.5	132.9	161.0	5.2	8.2	55.3	39.7	215.1	4.2	8.9	5.4	1034.0	143.7	11.2	13.7
MRNJ-132	35.6	133.6	150.1	4.7	6.8	63.3	38.2	128.1	4.4	12.5	4.5	1333.4	153.9	9.5	11.6
MRNJ-133	29.9	128.2	151.3	5.6	8.9	61.7	41.0	213.9	4.5	13.5	3.9	1372.4	181.0	18.6	13.1
MRNJ-134	36.6	134.4	151.9	5.1	8.8	53.6	37.7	213.5	4.5	15.6	4.6	1239.9	149.3	16.0	12.2
MRNJ-135	34.1	131.9	153.0	4.7	7.0	85.6	33.4	218.6	3.5	14.5	5.5	1105.4	144.1	12.4	13.2
MRNJ-136	37.9	133.6	149.2	5.2	8.2	75.2	40.2	205.6	3.6	11.7	4.8	1453.4	183.2	11.4	12.7
MRNJ-137	35.5	131.6	153.1	6.0	8.1	63.2	38.5	156.6	4.4	13.1	5.2	1213.4	144.9	9.6	12.1
MRNJ-138	38.5	135.6	145.6	5.4	6.6	92.7	39.5	185.3	3.7	10.6	6.9	1136.9	148.4	7.6	13.1
MRNJ-139	39.0	136.5	154.4	4.6	6.5	63.7	33.3	163.9	5.3	13.7	6.8	1237.2	158.9	7.5	12.8
MRNJ-140	38.5	135.9	153.4	5.7	7.0	72.7	39.4	215.2	3.5	12.5	4.6	1322.4	155.5	16.7	11.7
MRNJ-141	39.0	135.1	155.8	5.4	8.3	61.8	40.9	219.3	4.4	14.1	6.5	1037.4	188.9	15.8	18.3
MRNJ-142	37.0	131.9	151.5	5.4	9.2	65.5	38.9	226.6	5.7	15.4	4.7	1300.4	153.9	15.3	11.9
MRNJ-143	37.0	133.4	148.6	5.3	7.6	66.1	37.0	254.4	5.3	14.8	7.7	1067.4	219.7	16.2	18.6

Parameters Genotypes	Plant height (cm)	Numbers of primary branches	Numbers of secondary branches	Days to 50% flowering	Days to maturity	Length of main raceme (cm)	Numbers of silique per main raceme	Numbers of silique per plant	Silique length (cm)	Seed yield per Plant (g)	Seed yield per plot (g)	Seed yield per plot (g)	1000-seed weight (g)	Biological yield per plot (g)	Harvest index
MRNJ-144	36.9	133.1	149.2	5.1	8.8	65.1	34.5	205.2	4.2	13.3	5.8	1074.3	186.8	14.2	17.8
MRNJ-145	40.2	137.7	150.3	5.0	7.4	56.9	34.3	233.1	4.4	12.8	5.5	1423.5	153.3	11.5	10.8
IDM-2	40.1	136.6	150.3	5.0	8.4	60.2	38.4	161.9	5.4	14.0	6.8	961.8	154.9	13.7	16.0
IDM-8	40.6	135.9	152.8	5.4	6.5	57.9	36.3	195.6	5.3	13.3	5.3	1111.1	173.6	12.8	15.9
IDM-10	52.8	145.3	150.6	5.5	8.8	52.5	31.7	185.5	4.0	12.9	5.3	1048.5	150.6	6.4	14.6
IDM-11	50.5	142.1	149.8	5.2	8.3	54.7	40.7	172.7	4.4	13.3	7.3	1160.1	185.4	10.3	16.3
IDM-12	40.8	135.4	149.8	5.2	7.8	89.6	37.4	212.1	3.8	11.6	6.8	1016.5	157.4	12.2	15.5
IDM-15	52.3	142.8	152.0	4.9	9.1	54.5	33.5	167.0	5.5	12.4	7.4	1170.9	127.5	11.2	10.6
IDM-16	40.3	135.1	149.8	5.5	8.1	72.3	44.0	223.9	4.5	12.4	6.7	1193.0	158.4	11.2	13.4
IDM-25	36.3	131.8	148.0	5.9	9.6	62.2	32.5	227.4	4.6	12.6	5.3	1121.9	187.9	8.8	16.8
IDM-31	39.5	137.2	151.6	5.5	7.4	62.2	32.2	206.9	4.2	12.9	4.8	1029.3	155.5	11.2	15.2
IDM-41	31.1	135.4	155.9	4.8	8.9	54.5	36.5	230.1	4.2	15.1	4.8	980.4	195.9	8.6	20.3
IDM-42	33.0	132.3	150.8	5.0	10.3	54.2	34.8	227.4	5.4	13.0	4.3	1155.9	155.8	12.4	13.4
IDM-53	35.8	134.1	148.1	4.9	9.6	52.6	35.4	243.2	4.2	13.0	6.1	1296.0	152.7	10.8	11.7
IDM-58	35.6	135.3	150.2	4.7	6.9	72.1	34.0	202.6	5.4	14.8	6.2	1057.6	192.1	10.5	18.5
IDM-64	39.6	135.2	153.1	5.7	6.8	64.2	36.5	230.1	4.7	14.4	7.4	988.1	155.1	9.0	17.1
IDM-66	41.0	134.1	152.6	4.9	9.0	70.9	43.0	242.9	4.7	14.1	6.1	1195.9	155.3	10.4	13.0
IDM-67	37.5	136.0	153.8	5.2	8.1	61.7	34.5	124.1	4.9	15.6	5.7	1240.0	163.6	6.4	13.1
IDM-69	37.0	134.1	150.4	5.4	7.1	64.2	36.2	118.1	4.5	13.7	7.3	1426.9	155.5	7.7	10.9
Rohini	35.8	132.2	153.6	5.9	7.8	72.0	39.3	232.0	5.2	12.7	7.1	1135.9	177.7	14.0	15.8
Maya	36.0	132.5	155.1	5.5	9.3	69.1	41.6	234.1	4.9	14.1	5.2	1014.4	180.5	13.9	17.6
RVM-1	30.2	129.9	152.6	4.9	8.2	70.7	41.3	221.6	4.8	13.3	5.5	1192.9	140.5	10.8	11.9
RVM-2	39.5	135.6	154.9	4.9	7.9	75.6	39.2	230.4	5.2	13.6	7.4	1182.6	151.8	17.7	12.9
JM-1	34.5	131.6	153.0	5.4	7.6	79.6	41.6	209.9	5.4	12.6	5.9	1080.9	171.4	17.5	18.1
JM-2	36.7	133.7	156.4	5.3	8.1	65.1	36.8	213.6	4.0	11.8	5.7	1269.9	161.0	17.0	12.9
JM-3	40.6	133.2	158.8	5.1	8.4	80.0	44.3	227.1	4.7	14.4	4.9	1227.2	187.1	19.4	15.4
NRCDR-2	36.0	133.1	158.8	6.0	7.4	55.6	40.7	160.6	4.8	13.1	5.9	1193.5	183.2	12.1	17.8
GM-2	39.6	135.3	157.6	5.1	6.1	63.2	40.5	180.6	4.6	13.0	6.1	817.5	192.2	10.6	25.2
JD-6	29.1	111.4	153.8	5.2	7.7	64.7	43.2	233.3	5.4	12.2	7.1	804.0	166.5	14.8	22.7
CS-54	28.3	129.3	156.7	4.7	7.4	61.1	47.0	215.7	5.7	13.1	5.7	1385.0	181.2	10.9	13.1
PM-26	30.5	128.3	125.9	5.8	7.5	48.0	45.0	232.9	5.1	13.7	6.8	1401.9	140.4	10.5	10.1
PM-25	27.5	129.2	112.0	4.8	6.7	51.1	40.5	233.2	5.2	10.3	4.9	1108.1	157.4	11.0	14.6
DRMR-150-35	35.0	131.0	151.8	6.0	7.6	85.6	51.8	134.4	5.3	14.0	5.5	1431.2	164.2	8.6	11.5
DRMRIJ-31	34.0	138.0	151.9	5.5	7.5	67.7	46.6	144.6	4.5	14.3	5.7	1096.9	173.8	11.2	15.9
RB-50	43.3	142.0	153.0	4.8	5.7	74.6	48.2	124.6	6.0	15.5	5.4	1414.5	175.3	9.4	12.6
NRCHB-101	29.0	132.9	152.0	4.5	7.3	74.2	44.5	134.1	5.0	15.3	4.8	1169.1	166.3	10.0	14.4

Parameters Genotypes	Plant height (cm)	Numbers of primary branches	Numbers of secondary branches	Days to 50% flowering	Days to maturity	Length of main raceme (cm)	Numbers of silique per main raceme	Numbers of silique per plant	Silique length (cm)	Seed yield per plant (g)	Seed yield per plot (g)	Seed yield per plot (g)	1000-seed weight (g)	Biological yield per plot (g)	Harvest index
PM-28	27.0	130.5	158.7	5.2	6.5	68.6	46.2	155.4	5.1	14.0	6.4	1308.0	174.0	9.6	13.1
RH-74.9	29.0	139.8	152.6	4.8	6.9	77.9	49.8	158.5	5.5	13.3	6.4	1391.9	141.2	11.6	10.3
Kranti	33.8	133.0	145.5	4.3	7.0	61.6	50.6	182.8	5.5	12.7	6.6	1076.9	155.3	9.1	15.0
MC-25	47.6	139.6	155.2	4.8	7.6	67.8	52.9	244.6	5.0	15.1	5.6	1150.9	183.3	11.2	16.2
L-4	37.1	134.7	152.8	4.4	6.5	62.2	50.8	239.7	4.7	12.3	6.5	1006.0	165.0	13.4	16.5
L-6	40.8	134.8	155.4	4.7	7.5	63.1	48.2	175.5	5.6	12.5	8.1	1231.9	164.0	11.4	13.4
ISC-3	39.5	137.9	154.8	5.7	5.9	60.6	48.8	231.1	5.5	11.9	6.5	1323.9	164.7	12.0	12.4
ISC-12	39.7	136.4	150.7	5.3	6.8	64.3	49.3	217.8	4.7	12.3	6.0	1142.7	142.3	8.9	12.5
ISC-17	43.0	143.4	151.5	5.2	8.2	65.1	50.3	176.1	4.8	14.1	5.6	1332.4	153.5	8.0	11.4
ISC-18	34.0	139.4	151.0	5.4	6.8	55.4	41.2	223.2	5.5	13.4	7.6	957.4	145.8	9.3	15.6
ISC-20	35.7	135.3	151.8	5.2	7.8	53.6	47.9	137.3	4.9	12.8	6.2	965.5	179.3	11.0	18.5
ISC-23	36.0	141.1	156.0	6.0	6.2	55.8	50.8	128.1	5.0	13.2	5.8	842.8	147.7	7.9	17.5
PM-21	39.0	141.1	144.0	5.9	6.1	52.1	49.1	138.2	5.2	14.4	7.9	1143.5	150.2	8.1	13.5
PM-22	39.0	143.1	147.1	5.0	8.6	58.6	51.7	163.5	5.0	13.0	5.4	1048.8	181.3	10.3	17.5
PM-24	41.8	134.7	145.2	6.2	6.2	64.7	61.2	211.4	5.0	14.3	6.4	991.5	153.2	9.0	15.9
PM-29	39.6	142.6	136.8	6.2	6.2	55.3	57.6	228.0	6.0	11.3	6.7	1080.0	235.1	10.4	21.7
PM-30	35.3	133.2	152.6	6.4	8.3	64.3	50.3	137.6	5.7	13.8	7.9	1176.1	158.5	9.2	13.9
Karishma	38.5	134.4	152.0	6.5	8.3	83.7	55.4	206.5	5.8	12.1	6.4	1115.6	157.7	12.4	14.2
Mean	**36.1**	**134.66**	**148.45**	**5.28**	**7.61**	**65.66**	**45.04**	**183.16**	**4.83**	**13.7**	**5.35**	**1242.4**	**171.5**	**10.98**	**14.14**
CV	**0.017**	**0.007**	**0.009**	**0.04**	**0.05**	**0.027**	**0.040**	**0.036**	**0.056**	**0.04**	**0.062**	**0.041**	**0.021**	**0.053**	**0.054**
CD	**3.44**	**5.52**	**7.79**	**1.33**	**2.32**	**10.07**	**10.23**	**37.04**	**1.53**	**3.32**	**1.86**	**286.60**	**20.89**	**3.29**	**4.28**

Table 2. Pooled estimates of GCV, PCV, heritability and genetic advance for 15 quantitative characters over 2 years

S. No.	Characters	GCV (%)	PCV (%)	h^2	GA	GA (%)
1	Plant height (cm)	4.47	4.99	80.26	12.27	8.26
2	Numbers of primary branches	1.58	11.41	1.92	0.02	0.45
3	Numbers of secondary branches	3.59	17.49	4.21	0.11	1.52
4	Days to 50% flowering	12.72	13.12	94.05	9.20	25.42
5	Days to maturity	2.81	3.714	57.59	5.93	4.40
6	Length of main raceme (cm)	13.63	14.08	93.74	17.85	27.19
7	Numbers of silique per main raceme	14.36	18.22	62.08	10.49	23.31
8	Numbers of silique per plant	21.61	22.45	92.60	78.46	42.84
9	Silique length (cm)	7.32	15.32	22.81	0.34	7.20
10	Numbers of seeds per silique	10.89	12.89	71.38	2.51	18.96
11	Seed yield per plant (g)	26.72	28.03	90.93	5.76	52.50
12	Seed yield per plot (g)	18.95	19.04	99.07	66.68	38.87
13	1000- seed weight (g)	16.67	19.91	70.05	1.53	28.74
14	Biological yield per plot (g)	12.61	12.92	95.28	315.16	25.36
15	Harvest index (%)	22.52	22.84	97.20	6.47	45.75

The approximation of genetic advance (Table 2) was documented to be highest for seed yield per plant, harvest index, numbers of silique per plant, seed yield per plot, 1000-seed weight, length of the main raceme, days to 50% flowering, biological yield per plot and numbers of silique per the main raceme. It was low for plant height, silique length, days to maturity, the numbers of primary and secondary branches. Analogous declarations were also conveyed low GA for days to maturity by Bind et al. [38]; for days to maturity and plant height by Lodhi et al. [37] and for days to maturity by Akabari et al. [21]. Moderate were experimented for the number(s) of seeds per siliqua by Bibi et al. [39].

Correlation analysis spring a clear representation of traits association which is shared as a consequence of linkage, pleiotropy, physiological relationship in developmental path. The quantifiable dimension of a specific trait clarifies diverse variability characters. Days to 50% flowering exhibited a significant positive correlation with days to maturity (Table 3), plant height and 1000-seed weight. Parallel results were documented by Rathod et al. [40] for days to 50% flowering as they reported a significant positive correlation with days to maturity and plant height, days to maturity with numbers of primary branches, plant height with a number(s) of secondary branches. Amsalu et al. [35] documented significant positive correlation for days to 50 % flowering with days to maturity. Devi et al. [41] authenticated significant positive correlation for days to 50 % flowering with days to maturity and for plant height with number (s) of secondary branches while Raliya et al. [42] for days to 50% flowering with days to maturity and 1000-seed weight. The numbers of primary branches proved highly significantly positively correlated with numbers of silique per the main raceme, numbers of secondary branches, seed yield per plot, harvest index and silique length. The numbers of secondary branches had a significant positive correlation with numbers of silique per plant, seed yield per plant and numbers of seeds per silique. The numbers of silique per the main raceme exhibited a significant positive correlation with silique length. The numbers of silique per plant showed a significant positive correlation with seed yield per plant. The numbers of seeds per silique showed positive and negative correlations. However, all were found non-significant.

Furthermore, days to 50% flowering had a significant positive correlation with days to maturity and plant height (Table 4). Plant height, had a significant positive correlation with 1000-seed weight, while, significant negative correlation was recognized with numbers of silique per the main raceme. The numbers (s) of primary and secondary branches had a significant positive correlation with the numbers of silique per the main raceme. Number (s) of silique per main raceme had significant and positive correlation with seed yield per plot (0.167), biological yield per plot and silique length. The number(s) of silique per plant displayed a significant positive correlation with seed yield per plant and silique length had a significant positive correlation with the numbers of seeds per silique. Seed yield per plot confirmed significant positive correlation with harvest index. Current outcomes are in promise with the verdicts of Doddabhimappa et al. [43] where they evidenced a significant positive correlation between plant height with 1000-seed weight. For days to 50% flowering a significant positive correlation with days to maturity and for plant height, a significant positive correlation with 1000-seed

weight was conveyed by Gupta et al. [44]. While, Kumar et al. [45] reported a significant negative correlation for days to 50% flowering with a length of the main raceme, number(s) of seeds per siliqua and 1000-seed weight and for days to maturity with a length of the main raceme, number(s) of siliqua per plant and seed yield per plant (g) whereas in the investigation of Raliya et al. [42] number(s) of siliqua per plant showed a significant positive correlation with seed yield per plant (g).

Among other analyzed parameters, genotypic path coefficient analysis revealed an extreme positive direct effect of numbers of silique per plant on seed yield per plant tracked by harvest index, plant height, days to 50% flowering, number(s) of seeds per silique, biological yield per plot and number(s) of silique per main raceme (Table 5). Days to maturity showed maximum positive indirect effect via the number(s) of secondary branches followed by days to 50% flowering. Numbers of primary branches exhibited a positive indirect effect on seed yield per plant through 1000 -seed weight, harvest index, length of the main raceme and numbers of silique per the main raceme. Number (s) of secondary branches demonstrated maximum positive indirect effect via 1000-seed weight, while negative indirect effect via the number(s) of primary branches. Number(s) of silique per the main raceme showed a positive indirect effect through the number(s) of secondary branches, whereas negative indirect effect through the number(s) of primary branches. Number(s) of silique per plant exhibited a positive indirect effect via the number (s) of primary branches. Silique length showed a positive indirect effect through the number (s) of secondary branches, number(s) of seeds per silique, days to maturity, harvest index, number(s) of silique per the main raceme, length of the main raceme and biological yield per plot. Similar findings were also documented for the numbers of siliqua per plant and per the main raceme and the numbers of seeds per siliqua as they exerted a positive direct effect on seed yield per plant. Afrin et al. [46] reported that plant height and the numbers of siliqua per plant had a positive direct effect on seed yield per plant. Yadav et al. [47] found that seed yield per plant had a positive direct effect on the numbers of siliqua per plant. Dawar et al. [27] reported a positive direct effect on the numbers of siliqua per plant, plant height, numbers of seeds per siliqua on seed yield per plant and a negative direct effect on siliqua length and Gupta et al. [44] documented that harvest index, numbers of seeds per siliqua, plant height had a positive direct effect on seed yield per plant and length of the main raceme and 1000-seed weight had a negative direct effect with seed yield per plant.

Phenotypic path coefficient examination (Table 6) interpreted that the highest positive direct effect of seed yield per plant was positively and directly depended up on number(s) of silique per plant, days to 50% flowering, harvest index, plant height, biological yield per plot, number(s) of secondary branches, length of main raceme, number(s) of silique per main raceme and number(s) of seeds per silique. Similarly, Yadav et al. [47] reported that numbers of siliqua per plant directly affected seed yield per plant. In a different investigation, Dawar et al. [27] demonstrated that seed yield per plant was positively and directly affected by numbers of siliqua per plant, plant height and numbers of seeds per siliqua. Days to maturity showed maximum positive indirect effect via days to 50% flowering, harvest index, 1000-seed weight, number(s) of silique per main raceme, silique length and number(s) of seeds per silique. Numbers of secondary branches exhibited maximum positive indirect effect through numbers of silique per plant, 1000-seed weight, numbers of primary branches, biological yield per plot and numbers of seeds per silique. Number(s) of silique per plant exhibited maximum positive indirect effect through days to maturity, number(s) of secondary branches, harvest index, number(s) of primary branches, length of main raceme, seed yield per plot and silique length. In the same way, Kardam et al. [48] reported that numbers of siliqua per plant, numbers of seeds per siliqua and numbers of siliqua per main raceme had positive direct effect on seed yield per plant. Gupta et al. [44] documented that seed yield per plant had positively and directly affected by harvest index, numbers of secondary branches per plant, and numbers of seeds per siliqua.

Principal components of the data over pooled years (Table 7, Fig. 1) were analyzed and found that the first principle component contributed 13.55% variation for plant height, numbers of primary branches, days to 50% flowering, days to maturity, numbers of silique per the main raceme, silique length, 1000-seed weight, seed yield per plot and harvest index. The second principle component contributed a total of 12.56% having plant height, numbers of secondary branches, days to 50% flowering, days to maturity, numbers of silique per plant, 100-seed weight and seed yield per plant. The principal component three accounted for 12.19 % of divergence and among those values

recorded for numbers of secondary branches, length of the main raceme, numbers of silique per plant, numbers of seeds per silique, seed yield per plant and per plot and harvest index. The total contribution of the 4th component was 9.48 % while the component 5th explained 7.98 % of the total variation. The six[th] component estimated 7.71% variance and the 7th principal component accounted for 6.77 % of diversity. The eigh[th] component possessed 6.07 % variance by plant height, number(s) of primary and secondary branches, length of main raceme while the 9th principal component accounted for 5.67 % of divergence. The total contribution of the 10th principal component was 4.65 %, created by plant height, number(s) of primary branches, number (s) of silique per plant and principal component 11th showed 4.59% variance. The 12th component contributed 3.76 %, 13th principal component 3.03%, 14th contributed1.82 % and 15th component showed 0.11 % variance.

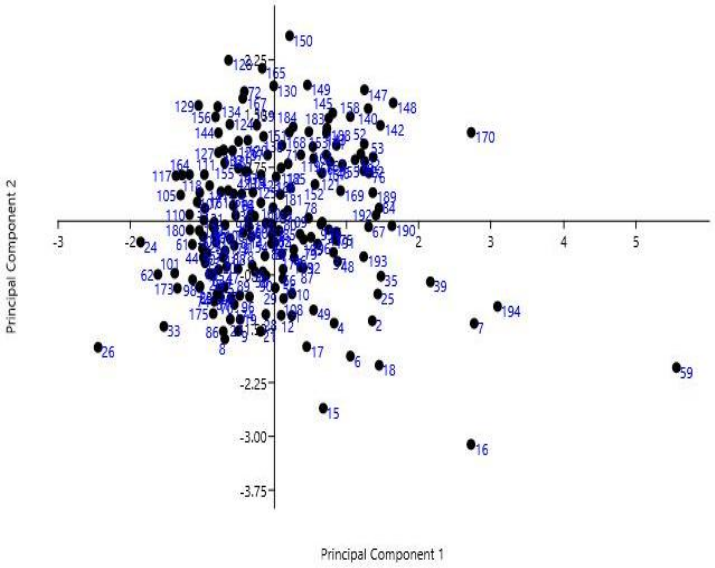


Fig. 1. Scatter plot for the genetic relationship among 196 Indian mustard as revealed by first second principal components

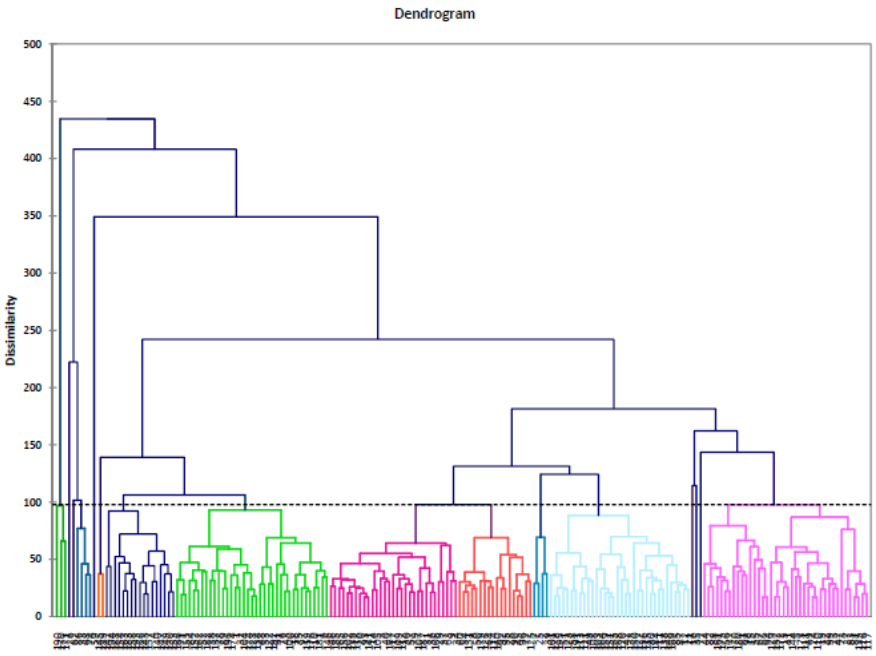


Fig. 2. Dendogram of pooled Morpho-physiological traits using XLSTAT software

Table 3. Genotypic correlation coefficient for 15 quantitative traits over pooled years

Parameters	Days to 50% flowering	Days to maturity	Plant height (cm)	Numbers of primary branches	Numbers of secondary branches	Length of main raceme cm)	Numbersof siliqueper mainraceme	Numbers of silique per plant	Silique length (cm)	Numbersof seeds per silique	1000-seed weight(g)	Biological yield per plot (g)	Seed yield per plot(g)	Seed yield per plant (g)	Harvest index (%)
Days to 50% flowering	1														
Days to maturity	0.750**	1													
Plant height (cm)	0.236**	-0.033NS	1												
Numbers of primary branches	-0.074NS	1.084**	-0.623**	1											
Numbers of secondary branches	0.013NS	-0.554**	0.113*	0.979**	1										
Length of main raceme (cm)	-0.114*	-0.152**	0.114*	-0.311**	-0.278**	1									
Numbers of silique per main raceme	-0.097NS	0.190**	-0.207**	1.249**	-0.959**	0.075NS	1								
Numbers of silique per plant	-0.050NS	-0.172**	-0.099NS	-0.067NS	0.522**	0.019NS	-0.175**	1							
Silique length (cm)	-0.186**	-0.151**	-0.154**	0.315**	-0.813**	-0.029NS	0.253**	-0.047NS	1						
Numbers of seeds per silique	-0.162**	-0.018NS	-0.132**	-0.179**	0.138**	0.044NS	-0.141**	-0.102*	0.217**	1					
1000- seed weight (g)	0.210**	-0.009NS	0.346**	-0.478**	-0.843**	-0.019NS	-0.203**	0.094NS	0.189**	-0.082NS	1				
Biological yield per plot(g)	-0.111*	-0.024NS	-0.104*	-0.052NS	0.007NS	0.085NS	0.135**	-0.085NS	0.055NS	-0.014NS	-0.279**	1			
Seed yield per plot (g)	0.000NS	0.085NS	-0.109*	0.446**	-0.011NS	0.034NS	0.201**	-0.004NS	0.041NS	0.025NS	-0.118*	0.038NS	1		
Seed yield per plant(g)	0.027NS	-0.161**	0.094NS	-0.686**	0.497**	0.048NS	-0.106*	0.436**	-0.152**	-0.031NS	-0.001NS	-0.028NS	0.038NS	1	
Harvest index(%)	0.049NS	0.030NS	-0.020NS	0.404**	-0.047NS	-0.029NS	0.078NS	0.057NS	0.056NS	0.030NS	0.083NS	-0.562**	0.787**	0.057NS	1

Table 4. Phenotypic correlation coefficient for 15 quantitative traits over pooled years

Parameters	Days to 50% flowering	Days to maturity	Plant height (cm)	Numbers of primary branches	Numbers of secondary branches	Length of main raceme (cm)	Numbers of silique per main raceme	Numbers of silique per plant	Silique length (cm)	Numbers of seeds per silique	1000-seed weight (g)	Biological yield per plot (g)	Seed yield per plot (g)	Seed yield per plant (g)	Harvest index (%)
Days to 50% flowering															
Days to maturity	0.551**														
Plant height (cm)	0.217**	-0.027^{NS}													
Numbers of primary branches	0.006^{NS}	0.032^{NS}	-0.080^{NS}												
Numbers of secondary branches	0.016^{NS}	-0.032^{NS}	-0.018^{NS}	-0.012^{NS}											
Length of main raceme (cm)	-0.130**	-0.131**	0.090^{NS}	-0.040^{NS}	-0.067^{NS}										
Numbers of silique per main raceme	-0.070^{NS}	0.073^{NS}	-0.132**	0.188**	-0.036^{NS}	0.056^{NS}									
Numbers of silique per plant	-0.044^{NS}	-0.143**	-0.090^{NS}	-0.019^{NS}	0.136**	0.015^{NS}	-0.115*								
Silique length (cm)	-0.068^{NS}	-0.014^{NS}	-0.039^{NS}	0.087^{NS}	-0.043^{NS}	-0.004^{NS}	0.105*	-0.002^{NS}							
Numbers of seeds per silique	-0.125*	0.005^{NS}	-0.075^{NS}	0.001^{NS}	0.011^{NS}	0.030^{NS}	-0.074^{NS}	-0.055^{NS}	0.105*						
1000- seed weight (g)	0.176**	-0.029^{NS}	0.254**	-0.000^{NS}	-0.117*	-0.013^{NS}	-0.079^{NS}	0.093^{NS}	0.078^{NS}	-0.070^{NS}					
Biological yield per plot(g)	-0.105*	-0.024^{NS}	-0.094^{NS}	0.020^{NS}	0.012^{NS}	0.082^{NS}	0.130*	-0.077^{NS}	0.036^{NS}	-0.019^{NS}	-0.211**				
Seed yield per plot (g)	-0.000^{NS}	0.064^{NS}	-0.098^{NS}	0.055^{NS}	0.015^{NS}	0.032^{NS}	0.167**	-0.004^{NS}	0.015^{NS}	0.024^{NS}	-0.097^{NS}	0.035^{NS}			
Seed yield per plant (g)	0.029^{NS}	-0.128*	0.066^{NS}	-0.088^{NS}	0.105*	0.049^{NS}	-0.064^{NS}	0.413**	-0.047^{NS}	-0.036^{NS}	0.016^{NS}	-0.020^{NS}	0.036^{NS}		
Harvest index(%)	0.047^{NS}	0.028^{NS}	-0.016^{NS}	0.047^{NS}	-0.012^{NS}	-0.030^{NS}	0.058^{NS}	0.053^{NS}	0.002^{NS}	0.027^{NS}	0.063^{NS}	-0.568**	0.775**	0.049^{NS}	

Table 5. Genotypic path-coefficient direct and indirect effects of various quantitative characters on seed yield per plant over pooled years

Parameters	Days to 50% flowering	Days to maturity	Plant height (cm)	Numbers of primary branches	Numbers of secondary branches	Length of main raceme (cm)	Numbers of silique per main raceme	Numbers of silique per plant	Silique length (cm)	Numbers of seeds per silique	1000-seed weight (g)	Biological yield per plot (g)	Seed yield per plot (g)	Harvest index	Genotypic correlation with seed yield per plant (g)
Days to 50% flowering	0.172	-0.098	0.049	0.025	-0.006	0.028	-0.004	-0.036	0.049	-0.018	-0.142	-0.006	0.000	0.013	0.027^{NS}
Days to maturity	0.129	-0.130	-0.007	-0.371	0.251	0.037	0.009	-0.124	0.040	-0.002	0.006	-0.001	-0.006	0.008	-0.161**
Plant height (cm)	0.041	0.004	0.207	0.213	-0.051	-0.028	-0.010	-0.071	0.041	-0.014	-0.233	-0.006	0.007	-0.005	0.094^{NS}
Numbers of primary branches	-0.013	-0.141	-0.129	-0.342	-0.443	0.076	0.058	-0.048	-0.083	-0.020	0.322	-0.003	-0.029	0.109	-0.686**
Numbers of secondary branches	0.002	0.072	0.023	-0.335	-0.452	0.068	-0.044	0.375	0.216	0.015	0.568	0.0004	0.001	-0.013	0.497**
Length of main raceme (cm)	-0.020	0.020	0.024	0.106	0.126	-0.245	0.003	0.014	0.008	0.005	0.013	0.005	-0.002	-0.008	0.048^{NS}
Numbers of silique per main raceme	-0.017	-0.025	-0.043	-0.427	0.434	-0.018	0.046	-0.126	-0.067	-0.016	0.137	0.007	-0.013	0.021	-0.106*
Numbers of silique per plant	-0.009	0.022	-0.020	0.023	-0.236	-0.005	-0.008	0.719	0.012	-0.011	-0.063	-0.005	0.0003	0.015	0.436**
Silique length (cm)	-0.0320	0.020	-0.0319	-0.108	0.368	0.007	0.012	-0.034	-0.265	0.024	-0.127	0.003	-0.003	0.015	-0.152**
Numbers of seeds per silique	-0.028	0.002	-0.027	0.061	-0.062	-0.011	-0.007	-0.073	-0.058	0.110	0.056	-0.001	-0.002	0.008	-0.031^{NS}
1000- seed weight (g)	0.036	0.001	0.072	0.163	0.381	0.005	-0.009	0.068	-0.050	-0.009	-0.674	-0.015	0.008	0.023	-0.001^{NS}
Biological yield per plot (g)	-0.019	0.003	-0.022	0.018	-0.003	-0.021	0.006	-0.061	-0.015	-0.002	0.188	0.053	-0.002	-0.152	-0.028^{NS}
Seed yield per plot (g)	0.000	-0.011	-0.023	-0.153	0.005	-0.008	0.009	-0.003	-0.011	0.003	0.080	0.002	-0.065	0.213	0.038^{NS}
Harvest index (%)	0.008	-0.004	-0.004	-0.138	0.021	0.007	0.004	0.041	-0.015	0.003	-0.056	-0.030	-0.051	0.270	0.057^{NS}

Residual effect = 0.599

Table 6. Phenotypic path-coefficient direct and indirect effects of various characters on seed yield per plant over pooled years

Parameters	Days to 50% flowering	Days to maturity	Plant height (cm)	Numbers of primary branches	Numbers of secondary branches	Length of main raceme (cm)	Numbers of silique per main raceme	Numbers of silique per plant	Silique length (cm)	Numbers of seeds per silique	1000-seed weight (g)	Biological yield per plot (g)	Seed yield per plot (g)	Harvest index	Phenotypic correlation with seed yield per plant (g)
Days to 50% flowering	0.1124	-0.0687	0.0190	-0.0005	0.0006	-0.0038	-0.0009	-0.0179	0.0020	-0.0008	-0.0093	-0.0070	0.00001	0.0041	0.029^{NS}
Days to maturity	0.0620	-0.1247	-0.0024	-0.0023	-0.0013	-0.0039	0.0010	-0.0582	0.0004	0.00003	0.0015	-0.0016	-0.0014	0.0025	-0.128*
Plant height (cm)	0.0244	0.0034	0.0875	0.0058	-0.0007	0.0027	-0.0018	-0.0367	0.0011	-0.0005	-0.0134	-0.0063	0.0021	-0.0015	0.066^{NS}
Numbers of primary branches	0.0007	-0.0040	-0.0070	-0.0723	-0.0005	-0.0012	0.0025	-0.0076	-0.0025	0.00001	0.00001	0.0013	-0.0012	0.0042	-0.088^{NS}
Numbers of secondary branches	0.0018	0.0040	-0.0016	0.0009	0.0404	-0.0020	-0.0005	0.0555	0.0012	0.0001	0.0062	0.0008	-0.0003	-0.0011	0.105*
Length of main raceme (cm)	-0.0147	0.0163	0.0079	0.0029	-0.0027	0.0294	0.0008	0.0062	0.0001	0.0002	0.0007	0.0055	-0.0007	-0.0026	0.049^{NS}

Parameters	Days to 50% flowering	Days to maturity	Plant height (cm)	Numbers of primary branches	Numbers of secondary branches	Length of main raceme (cm)	Numbers of silique per main raceme	Numbers of silique per plant	Silique length (cm)	Numbers of seeds per silique	1000-seed weight (g)	Biological yield per plot (g)	Seed yield per plot (g)	Harvest index	Phenotypic correlation with seed yield per plant (g)
Numbers of silique per main raceme	-0.0078	-0.0091	-0.0116	-0.0136	-0.0015	0.0016	0.0135	-0.0468	-0.0030	-0.0005	0.0041	0.0087	-0.0036	0.0052	-0.064^{NS}
Numbers of silique per plant	-0.0049	0.0178	-0.0079	0.0013	0.0055	0.0004	-0.0016	0.4076	0.0001	-0.0003	-0.0049	-0.0052	0.0001	0.0047	0.413^{**}
Silique length (cm)	-0.0077	0.0017	-0.0035	-0.0063	-0.0017	-0.0001	0.0014	-0.0007	-0.0288	0.0007	-0.0041	0.0024	-0.0003	0.0002	-0.047^{NS}
Numbers of seeds per silique	-0.0141	-0.0006	-0.0066	-0.0001	0.0004	0.0009	-0.0010	-0.0223	-0.0030	0.0061	0.0037	-0.0013	-0.0005	0.0024	-0.036^{NS}
1000- seed weight (g)	0.0198	0.0036	0.0223	0.0000	-0.0047	-0.0004	-0.0011	0.0379	-0.0022	-0.0004	-0.0527	-0.0141	0.0021	0.0056	0.016^{NS}
Biological yield per plot (g)	-0.0118	0.0029	-0.0082	-0.0014	0.0005	0.0024	0.0018	-0.0314	-0.0010	-0.0001	0.0111	0.0668	-0.0008	-0.0504	-0.020^{NS}
Seed yield per plot (g)	-0.00004	-0.0080	-0.0086	-0.0040	0.0006	0.0010	0.0023	-0.0016	-0.0004	0.0002	0.0051	0.0024	-0.0218	0.0688	0.036^{NS}
HarvestI ndex (%)	0.0052	-0.0035	-0.0014	-0.0034	-0.0005	-0.0009	0.0008	0.0216	-0.0001	0.0002	-0.0033	-0.0379	-0.0169	0.0888	0.049^{NS}

Residual = 0.79

Table 7. Principal components for morpho-physiological traits over pooled years

Parameters	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15
Plant height (cm)	0.024	0.406	-0.118	-0.239	0.153	-0.278	-0.014	0.487	0.274	0.051	0.317	-0.169	0.454	0.114	-0.003
Numbers of primary branches	0.139	-0.279	-0.108	0.084	0.153	0.402	-0.207	0.551	-0.315	0.405	0.030	-0.273	-0.118	-0.026	-0.012
Numbers of secondary branches	-0.067	0.095	0.218	0.464	-0.234	0.014	-0.092	0.565	0.208	-0.303	-0.194	0.369	-0.147	0.107	0.017
Days to 50% flowering	0.271	0.318	-0.432	0.270	0.080	-0.001	0.245	0.039	0.008	-0.068	-0.107	-0.017	-0.088	-0.688	-0.016
Days to maturity	0.275	0.023	-0.502	0.308	-0.009	0.025	0.299	-0.087	-0.159	-0.077	-0.060	-0.005	0.097	0.658	0.045
Length of main raceme (cm)	-0.099	-0.046	0.131	-0.236	0.356	-0.445	0.298	0.257	-0.482	-0.197	-0.383	-0.014	-0.131	0.018	0.010
Numbers of silique per main raceme	0.113	-0.427	-0.115	-0.007	0.438	0.071	-0.054	0.029	-0.035	-0.354	0.432	0.484	0.173	-0.117	0.001
Numbers of silique per plant	-0.017	0.231	0.398	0.230	0.256	0.380	0.149	-0.120	-0.179	0.113	-0.244	0.102	0.610	-0.040	-0.003
Silique length (cm)	0.014	-0.202	-0.001	-0.337	0.032	0.470	0.397	0.127	0.423	-0.376	-0.216	-0.287	-0.018	0.017	-0.014
Numbers of seeds per silique	-0.030	-0.137	0.095	-0.129	-0.547	0.015	0.588	0.170	-0.235	0.204	0.319	0.228	0.123	-0.123	-0.0002
Seed yield per plant (g)	-0.016	0.265	0.364	0.256	0.318	0.083	0.311	-0.061	0.010	-0.025	0.507	-0.212	-0.454	0.119	-0.003
Seed yield per plot (g)	0.491	-0.258	0.232	0.089	0.092	-0.277	0.128	-0.014	0.312	0.287	-0.131	0.042	0.005	0.027	-0.574
1000-seed weight (g)	0.132	0.371	-0.088	-0.436	0.158	0.266	0.013	0.018	0.066	0.297	-0.122	0.568	-0.316	0.148	0.00020
Biological yield per plot (g)	-0.385	-0.268	-0.128	0.211	0.262	-0.164	0.255	0.007	0.393	0.448	-0.121	0.101	-0.007	-0.023	0.422
Harvest index	0.629	-0.050	0.283	-0.080	-0.074	-0.107	-0.058	-0.012	0.035	-0.020	-0.025	-0.049	0.006	-0.025	0.700

Table 8. Grouping of 196 Indian mustard genotypes in various clusters

Clusternumber	Numbersof genotypes	Name of genotypes
1	37	MRNJ-1, MRNJ-6, MRNJ-10, MRNJ-18, MRNJ-33, MRNJ-39, MRNJ-44, MRNJ-49, MRNJ-53, MRNJ-54, MRNJ-72, MRNJ-76, MRNJ-77, MRNJ-79, MRNJ-85, MRNJ-90, MRNJ-92, MRNJ-98, MRNJ-101, MRNJ-105, MRNJ-120, MRNJ-123, MRNJ-125, MRNJ-129, MRNJ-135, MRNJ-138, IDM-8, IDM-25, IDM-42, Rohini, DRMRIJ-31, Kranti, PM-25, MC-25, ISC-12, PM-22 and Karishma
2	4	MRNJ-2, MRNJ-7, MRNJ-25 and MRNJ-40
3	31	MRNJ-3, MRNJ-9, MRNJ-14, MRNJ-17, MRNJ-20, MRNJ-29, MRNJ-31, MRNJ-30, MRNJ-35, MRNJ-37, MRNJ-38, MRNJ-41, MRNJ-47, MRNJ-58, MRNJ-64, MRNJ-66, MRNJ-80, MRNJ-94, MRNJ-103, MRNJ-106, MRNJ-107, MRNJ-108, MRNJ-110, MRNJ-113, MRNJ-119, MRNJ-140, MRNJ-142, IDM-53, JM-2, ISC-3 and ISC-17
4	17	MRNJ-4, MRNJ-131, MRNJ-143, MRNJ-141, IDM-12, MRNJ-144, IDM-10, IDM-31, IDM-41, IDM-58, IDM-64, Maya, JM-1, L-4, ISC-18, PM-24 and PM-29
5	40	MRNJ-5, MRNJ-13, MRNJ-15, MRNJ-19, MRNJ-22, MRNJ-23, MRNJ-24, MRNJ-27, MRNJ-42, MRNJ-48, MRNJ-45, MRNJ-56, MRNJ-57, MRNJ-62, MRNJ-67, MRNJ-68, MRNJ-70, MRNJ-71, MRNJ-74, MRNJ-82, MRNJ-83, MRNJ-84, MRNJ-89, MRNJ-95, MRNJ-99, MRNJ-100, MRNJ-111, MRNJ-112, MRNJ-117, MRNJ-118, MRNJ-130, MRNJ-133, MRNJ-136, MRNJ-145, IDM-69, CS-54, PM-26, DRMR-150-35, RB-50 and RH-74.9
6	18	MRNJ-8, MRNJ-28, MRNJ-46, MRNJ-51, MRNJ-55, MRNJ-61, MRNJ-65, MRNJ-78, MRNJ-91, MRNJ-93, MRNJ-96, MRNJ-97, MRNJ-115, MRNJ-126, MRNJ-127, MRNJ-132, IDM-67 and PM-28
7	34	MRNJ-11, MRNJ-12, MRNJ-21, MRNJ-43, MRNJ-50, MRNJ-52, MRNJ-59, MRNJ-81, MRNJ-86, MRNJ-88, MRNJ-102, MRNJ-104, MRNJ-109, MRNJ-114, MRNJ-116, MRNJ-121, MRNJ-122, MRNJ-124, MRNJ-128, MRNJ-134, MRNJ-137, MRNJ-139, IDM-11, IDM-15, IDM-16, IDM-66, RVM-1, RVM-2, JM-3, NRCDR-2, NRCHB-101, L-6, PM-21 and PM-30
8	1	MRNJ-16
9	1	MRNJ-26
10	4	MRNJ-34, MRNJ-69, MRNJ-75 and MRNJ-87
11	1	MRNJ-36
12	1	MRNJ-60
13	1	MRNJ-63
14	1	MRNJ-73
15	2	IDM-2 and ISC-20
16	3	GM-2, JD-6 and ISC-23

Table 9. Mean performance of individual clusters for 15 quantitative characters

Parameters Class	Plant height (cm)	Numbers of primary branches	Numbers of secondary branches	Days to 50% flowering	Days to maturity	Length of main raceme (cm)	Numbers of silique per main raceme	Numbers of silique per plant	Silique length (cm)	Seed yield per plant (g)	Seed yield per plot (g)	Seed yield per plot (g)	1000-seed weight (g)	Biological yield per plot (g)	Harvest index
1	146.79	5.23	7.45	36.68	134.85	67.22	45.16	187.20	4.79	13.20	10.97	166.60	5.39	1104.68	15.22
2	143.97	5.33	7.79	34.29	131.25	68.26	50.18	205.82	5.23	12.62	12.44	261.24	4.48	1173.21	22.45
3	147.46	5.37	7.81	35.79	134.69	65.63	46.29	212.42	4.74	12.79	11.98	164.54	5.00	1305.83	12.64
4	150.23	5.35	7.78	37.88	135.57	64.39	41.80	218.64	4.73	13.37	11.84	176.08	6.07	1029.39	17.30
5	147.90	5.29	7.41	35.70	134.89	66.97	45.65	178.41	4.86	13.35	11.01	170.10	5.18	1414.94	12.10
6	150.61	5.25	7.38	35.35	133.85	62.63	45.12	131.21	4.73	13.08	8.71	165.54	5.22	1285.78	12.94
7	150.03	5.20	7.82	36.71	135.02	64.43	42.96	169.87	4.91	13.65	10.69	160.64	5.53	1190.69	13.67
8	136.80	5.85	6.93	32.56	143.88	64.55	51.01	143.38	5.30	17.80	8.84	295.81	4.19	1298.58	22.79
9	139.55	5.00	8.35	32.85	130.43	67.78	47.74	162.25	5.65	13.96	8.42	153.48	4.54	1819.55	8.39
10	146.94	5.53	7.84	35.32	133.09	66.68	48.96	166.04	5.28	13.58	10.21	179.17	5.11	1616.44	11.11
11	147.63	5.30	7.73	33.60	129.95	83.65	53.51	229.93	5.20	12.95	10.62	271.53	7.29	1364.03	20.01
12	156.48	5.43	8.80	40.06	137.30	65.95	53.28	124.10	4.25	11.97	11.34	449.38	4.42	1388.13	32.81
13	151.10	5.00	7.30	35.02	132.55	83.15	46.24	114.90	5.17	14.40	8.32	150.65	5.44	1538.65	9.63
14	147.43	3.90	8.75	40.37	136.25	54.60	42.55	293.40	3.67	13.88	15.96	193.35	5.83	1480.63	13.17
15	151.04	5.08	8.08	37.92	135.96	56.89	43.13	149.59	5.14	13.41	12.36	167.07	6.52	963.64	17.27
16	155.78	5.45	6.67	34.87	129.24	61.20	44.86	180.65	4.99	12.80	11.07	168.77	6.35	821.42	21.83

The multivariate analysis based on Euclidean distance cluster analysis (Fig. 2) was used for divergence analysis. Based on these values, 16 clusters were formed (Table 8). Outcomes of cluster examination exposed that the cluster 5 was the major which contain 40 genotypes tracked by cluster - 1 (37 genotypes), cluster-7 (34 genotypes), cluster-3 (31 genotypes), cluster-6 (18 genotypes), cluster-4 (17 genotypes), cluster-2 and cluster-10 (each having 4 genotypes), cluster-16 (3 genotypes), cluster- 15 (2 genotypes) and cluster 8, 9, 11, 12, 13 and 14 (one genotype each). The supreme inter-cluster distance was evidenced between cluster-9 and cluster-16 (998.7) followed by cluster-9 and cluster-15 (856.4), cluster-10 and cluster-16 (795.4), cluster-4 and cluster-9 (792.7), cluster-13 and cluster-16 (720.9), cluster-1 and cluster-9 (715.5), cluster-14 and cluster-16 (669.5), cluster-2 and cluster-9 (656.9), cluster-10 and cluster-15 (653.3) and cluster-12 and cluster-16 (635.1). The minimum inter-cluster distance (82.4) was documented between clusters-1 and 4. However, the intra-cluster distance was noted as zero.

Additionally, the cluster means from various parameters (Table 9) revealed that cluster-8 had the highest mean value for days to maturity (143.88), number(s) of primary branches (5.85) and number(s) of seeds per silique (17.80). Correspondingly; genotypes encompassed in cluster-9 chronicled the highest mean value for silique length (5.65) and biological yield per plot (1819.55). The cluster-11 exposed maximum values for the length of the main raceme (83.65), silique per the main raceme (53.51) and 1000-seed weight (7.29). The cluster-12 had high mean worth for plant height (156.48), number(s) of secondary branches (8.80), seed yield per plot (449.38) and harvest index (32.81) while, the cluster-14 displayed the highest mean worth for days to 50 % flowering (40.37), silique per plant (293.40) and seed yield per plant (15.96). The preference of genetically differed parents for hybridization schemes is an authoritative feature of any crop enhancement program to realize desirable sergeants [49-54]. Meanwhile the credentials of morphological, biochemical or molecular data-based variation are imperative for effective assessment and application of genotypes in a breeding scheme, screening is the only valid way to advance new genotypes for cultivation [49,55-80]. It is established that the mustard genotypes used in this investigation have high genetic variability which makes them potential genotypes to foster new cultivars through future mustard breeding. This is the first description on such a big set of Indian mustard genotypes.

4. CONCLUSION

The deduction of the current investigation may, owing to findings of genetic components, correlation and path analysis associated to diverse morpho-physiological parameters, it is encouraged that the genotypes with having higher worth with wanted connotation may be employed to advance the seed yield per plant (g) of Indian mustard genotype (s) by means of hybridization and selection. From the investigation of cluster analysis for morpho-physiological parameters various parents fit in to cluster 9 *viz*., MRNJ-26 and cluster 16 *i.e*., GM-2, JD-6 and ISC-23 may be employed in the mustard breeding scheme for receiving a higher benefit.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 1
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Biotechnological Interventions to Combat against Charcoal Rot and *Rhizoctonia* Root Rot Diseases of Soybean [*Glycine max* (L.) Merrill]

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ABSTRACT

Soybean is a prime legume in a world's oilseed cultivation set-up. Yield of soybean hampered by several biotic and abiotic stresses including drought. Among different biotic stresses, Charcoal rot and *Rhizoctonia* root rot are the leading diseases for a huge damage in soybean production and still there is no prominent work has been done effectually to address these problems. Biotic stresses can be controlled by using an integrated management approach either by cultural practices including crop rotation, tillage, irrigation, or chemical control like seed treatments could be employed to diminish damage instigated by fungal pathogen in soybeans. The current study was conducted with the objective to characterize soybean genotypes for yield and its accrediting characters along with validation of gene-based SSR molecular markers against charcoal rot and *Rhizoctonia* root rots diseases. On the basis of different traits, genotypes *viz.,* JS335, JS20-69, JS97-52, KDS980 and KDS992 were found to be the most divergent and promising genotypes and may be employed as parents in future hybridization programme to breed tolerance/ resistance against *Rhizoctonia* root rot and charcoal rot by means of conventional and/or molecular breeding approaches.

Keywords: Molecular breeding; hybridization; fungal diseases; oil seed; SSR markers.

1. INTRODUCTION

Soybean [*Glycine max* (L.) Merr] (2n=40) is a commercially imperative dicot legume in a world's oilseed cultivation scenario, having a prominent position in terms of high productivity, profitability and maintaining soil fertility too [1-5]. On account of its miscellaneous usages and limitless benefits, it is rightly called as "golden bean", "miracle bean" or "wonder crop" of the 20th century [4]. Soybean contributes to our country's economy and foreign earnings by donating 42 percent and 25 percent of national oilseeds and edible oil production, respectively. [6-11]. It encompasses essential amino acids predominantly glycine, tryptophan and lysine, analogous to cow’s milk and animal proteins. After the United States, Brazil, Argentina, and China, India ranked fourth in terms of worldwide soybean producing area (11 million ha) and fifth in terms of production (11 million metric tonnes). Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, and Andhra Pradesh are India's top soybean-producing states, with 96 percent of production subsidized in decreasing order of production.

Yield of soybean hampered by several biotic [12-16] and abiotic stresses including drought [17-21]. Comprehensive gamut biotic stress which decreases both yield and seed quality is Charcoal rot instigated by the soil borne polyphagous fungus *Macrophomina phaseolina* (Tassi) Goid. This

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was first detected in the United States in 1949 [22]. Contagion arises in all plant parts of prone soybean genotypes of this disease. Infection primarily flinches in the roots which ultimately spread to the entire plant. The yield loss can go up to 80% in severe cases [23]. The second most destructive biotic stress is *Rhizoctonia* root and hypocotyl rot, caused by *Rhizoctonia solani*. Losses up to 35% is reported due to *Rhizoctonia* root rot disease in epidemic circumstances. Still there is no prominent work has been carried out to effectively encounters these problems. As such more concentrated efforts are required to direct towards to work upon this aspect employing diverse tactics. Biotic stresses can be controlled by using an integrated management approach either by cultural practices including crop rotation, tillage, irrigation, or chemical control like seed treatments could be employed to diminish damage instigated by fungal pathogen in soybeans; however, none of these of practices has been executed for controlling the disease [24-25] effectively. So, development of resistant/tolerant varieties may be an effective means of disease controlling on the basis of eco-friendly, cost benefit and easily available and use by farmer in production purpose. Still, we are lacking of resistant/tolerant varieties, however moderately resistant cultivars are currently commercially available [25].

Screening is first step for selection of desired parents to make crosses and develop resistant variety with genetic background of popular variety. For development of resistant variety either conventional or marker assisted selection approaches [26-40] can be applied but conventional method needs more time and highly affected by environment, generation after generation we have to fix the characters for a particular genotype and environment interaction as result the whole process needed an efficient labor with more screening cycles. Meanwhile marker assisted selection is an indirect selection process where a trait of interest is selected based on marker (morphological, biochemical or DNA/RNA variation) linked to a trait of interest may be attempted. In soybean, an array of molecular markers has been employed by various scientist [41-48] throughout the world to facilitate marker assisted selection to develop resistant /tolerant varieties against different biotic and abiotic stresses. Considering these facts in mind, the present investigation was carried out with the objective to identify soybean genotypes resistant against charcoal rot and *Rhizoctonia* root rots diseases by help of gene-based SSR molecular markers.

1.1 Study Objectives

The present investigation was carried out with the aim to identify soybean genotypes resistant/tolerant against charcoal rot and *Rhizoctonia* root rots diseases by employing gene-based SSR molecular markers.

2. MATERIALS AND METHODS

2.1 Plant Material

A set of 53 soybean genotypes (Table 1) were chosen for screening against charcoal rot and *Rhizoctonia* root rot diseases. These genotypes were acquired from Jawaharlal Nehru Agricultural University, Jabalpur and Rajmata Vijayaraje Scindia Agricultural University, Gwalior. The genotypes having conflicting responses *viz.,* susceptible, tolerant and resistant reaction towards charcoal rot and *Rhizoctonia* root rot diseases. The field experiments were steered at Research Farm, Department of Plant Molecular Biology & Biotechnology and molecular work at Plant Molecular Biology Laboratory, Department of Plant Molecular Biology & Biotechnology, College of Agriculture, RVSKVV, Gwalior M.P., India.

2.2 Morpho-physiological Studies

Among morpho-physiological parameters plant height (cm), number(s) of primary branches per plant and leaf area (cm^2 / plant) with the help of Automatic Leaf Area Meter were measured. Among post-harvest parameters the number(s) of pods per plant, number(s) of seed per pod, yield per plant (g), biological yield/plant (g) and harvest index (%) were computed. Harvest index was worked out by the formula given by Donald and Hamblin [49].

2.3 Molecular Characterization

Genomic DNA isolation from young leaves was carried out by modified CTAB methods as suggested by Murray and Thompson [50] with minor modification as proposed by Tiwari *et al.* [51]. Extracted DNA was purified and quantified with the help of Nanodrop spectrophotometer. Quantified DNA samples were diluted up to the concentration of 25ng/ µl. Highly polymorphic gene-linked SSR molecular markers four for *Macrophomina phaseolina* (Table 2) and five for *Rhizoctonia solani* (Table 2) were employed to screen across 53 soybean genotypes. The primers were synthesized by Integrated DNA Technologies. Polymerase chain reaction was performed in 10µl reaction mixture comprising of 10X dream *Taq* buffer (1µl), 5U/µl *Taq* DNA polymerase, 15µl dNTP (1mM), 6.75µl NFW, 0.5 µl of forward and reverse primers each (10 pM) and 50 ng/µl of genomic DNA in a Thermocycler (BIO-RAD). Following parameters were followed to amplify the templates: initial denaturation at 94°C for 1 min followed by 35 cycles of denaturation at 94°C for 40 sec, annealing 52-55°C for 90 sec, extension at 72°C for 60 sec. The final extension was performed at 72°C for 7 min.

2.4 Data Analysis

The genetic profile of 53 soybean genotypes was scored on the basis of difference in allele size using SSR markers. The major allele frequency, polymorphism information content (PIC) and genetic distance-based clustering was accomplished with Unweighted Pair Group Method for Arithmetic average (UPGMA) tree using Power Marker v3.25 software [52] and the dendrogram was constructed using MEGA 4.0 software [53]. SSR data was again imperiled to cluster analysis tracked by bootstrap analysis with 1000 permutations for all the genotypes using Mega 4.0 software. The population structure for 53 soybean genotypes comprising between genotype and susceptible and resistance one was inferred using Structure 2.3.4 software [54]. The structure harvests were envisaged employing Structure Harvester from which Evanno plots were constructed [55]. Duncan's Multiple Range Test (DMRT) (P=0.05) was used to assess differences among clusters for significance by using SPSS ver. 19.0 software.

3. RESULTS AND DISCUSSION

The presence of significant variability and its mode of inheritance for high grain yielding and its attributing traits may be helpful to achieve desired goal in crop improvement programme. Furthermore, this study also aimed to screen tolerant/resistant genotypes of soybean against *Rhizoctonia* root rot *and* charcoal rot for future improvements of crop.

3.1 Morpho-physiological Characterization

Plant height fluctuated between 43.47cm (NRC-2) to 111.01cm (NRC- 76) with an average of 69.90 cm. Genotypes NRC-76 and RVS-76 were proved significantly taller over other genotypes. Days to flower initiation were ranged between 35.50 days (JS20-34) to 45.50 days (AMS-MS-58) with a mean value of 38.21 days. Genotypes JS93-05 tracked by MACSNRC-1575, JS20-94, NRC-76 and SP37 documented significantly earlier for flower initiation. Days to 50% flowering was varied between 43.5 days (JS 20-98) to 54.0 days (AMS-MS-58) with a mean worth of 46.95 days. Genotype RVS 2007-6 intimately trailed by genotypes SL-1123, JS20-94, NRC-76, NRC-131, SP37, NRCSL-1, KDS 992 and NRC -125 significantly started early 50% flowering. Days to maturity was documented in range of 65.5 days (SL-1068) to 92.5 days (JS-93-05) with a mean value of 76.07 days. Genotypes JS95-60, NRC-131 and NRC-125 performed significantly early in terms of maturity. Number(s) of primary branches per plant was varied between 1. 55 (MACSNRC-1575) to 8.0 (NRC-76) with a mean worth of 5.22. Genotypes NRC-76, NRC-131 and AMSMBC-18 produced significantly maximum numbers of primary branches. Leaf area was covered in range of 12.85 cm^2 (PS-1092) to 78.03 cm^2 (NRC-131) with a mean value of 35.93 cm^2.Genotype AMS-100-39 closely tracked by genotypes MACSNRC-1575 and NRC-131 were occupied significantly more leaf area. Chlorophyll content was varied between 2.2350 mg/ml.

(NRC-76) to 14.4 mg/ml (MACS-725) with a mean worth of 7.38 mg/ml. Genotypes RVS 2001-4 intimately followed by genotypes RVS-14 and MACS725 showed significantly higher chlorophyll a content. While, Chlorophyll b content was ranged between 1.03 mg/ml (VLS-94) to 25.25 mg/ml (JS-20-94) with a mean value 10.39 mg/ml. Genotypes JS20-69 and RVS-14 synthesized significantly higher magnitude of chlorophyll b. Numbers of seed per pod was varied in range of 1.65 (MAS-MS-58) to 3.8 (JS-95-60). Genotypes JS 93-05 and JS 95-60 showed significantly higher numbers of seed per pod. Whereas, numbers of pods per plant was varied between 6.20 (NRC-2) to 45.23 (JS-335) with a mean worth of 23.56. Genotypes JS20-29, AMS-MS-58 and JS335 formed significantly higher numbers of pod per plant. Yield per plant was ranged from 14.51g (PS-1613) to 106.05g (JS20-29) with an average of 56.47g. Genotypes KDS980, JS335, JS97-52 and JS 20-29 gave significantly higher yield. The value of biological yield per plant was ranged between 68.0g (JS-20-71) to 210.0 g (JS-20-29) with a mean value of 115.82g. Genotypes KDS980, JS335 and JS20-29 produced significantly higher biological yield. The average value of harvest index was documented as 48.31 with an extent of dispersion from 21.11 (SP-37) to 64.82(JS 97- 52). Genotypes RSC-10-71 and JS 97-52 exhibited significantly higher harvest index.

3.2 Molecular Characterization

Biological slants for the control of soil-borne diseases are not very efficacious owing to diverse factors, for instance variability in performance and poor efficacy under optimal conditions for disease development, stemming from the complex and dynamic host plant × pathogen × biocontrol agent × environment interactions [56]. Due to non-availability of root rot resistant cultivars has made the use of chemicals an inescapable necessity. As such, genetic improvement by breeding for resistance is the only defensible option. Genetic resistance is the most effective, cost-efficient and environment friendly method for disease control [57]. Plant breeders have mostly relied on germplasm resources and crop wild relatives for useful genetic variants to create novel gene combinations in crop improvement programmes [58].

Recent breeding tactics such as marker-assisted selection (MAS) are more efficient and accurate for targeted trait improvement. In the current learning a set of five gene-linked SSR molecular markers were employed for validation of resistant gene linked with *Rhizoctonia* root rot across fifty-three soybean genotypes. Among five SSR primers, marker Satt693 was found to be monomorphic and remaining four was polymorphic. Parallel results have also been reported by Zhao et al. [59] with soybean genotypes where three SSR markers, *viz*., Satt281, Satt177 and Satt245 were found to be linked with *Rhizoctonia* root rot resistant gene in segregating population. The primer which exhibited highest gene diversity and PIC values was Satt232 while the lowest gene diversity and PIC values were documented for the primer Satt245. Gene diversity varied in range of 0.69 (Satt281) to 0.49 (Satt245). The major allele frequency varied between 0.3 (Satt232) to 0.5 (Satt245) with a mean value of 0.4646 (Table 5). Zhao *et al*. [59] stated that marker-assisted selection coupled with phenotypic selection in later generations, facilitate to the development of soybean cultivars resistant to *Rhizoctonia* root and hypocotyl rot.

The soybean genotype displaying the genetic relationships are showed by SSR based UPGMA tree. Total 53 genotypes were grouped into 5 clusters (Table 6; Fig. 2). Cluster 1 is grouped with two resistance genotypes *i.e*., JS- 20 69 and NRC-2 with other 9 genotypes and cluster 2 having genotype JS93-05 which is known as resistant genotype including 2 moderately susceptible genotypes JS335 and JS-20-29 tracked by 6 other genotypes. Cluster 3 contains genotypes JS20-34 and JS20-71 that are putative resistance along with a susceptible genotype *namely* NRSCL-1 including other 11 genotypes. Whereas cluster 4 formed groups with moderately susceptible genotypes *viz*., JS20-98, JS, 97-52 and NRC-86, tolerant genotype PS1092 and resistive genotypes *I e.,* JS 95-60, JS20-116 and JS20-94 with 8 other genotypes and cluster 5 having resistant genotypes *i.e*., KDS-992, PS-1613and EC-457286 a susceptible genotype *namely*: RSC-1071. Susceptible parents contributing resistance alleles to fungal pathogen as well so all susceptible genotype(s) under grouped of resistance genotype(s) may will have the resistance source of gene. This has already been testified by Babu et al. [60], Tomar et al. [61], Mengistu et al. [62], Silva et al. [63], Quazi et al. [64] and Upadhyay et al. [15]. The reports emphasized that genomic

regions harboring resistance to charcoal rot in soybean and it may facilitate breeding and molecular engineering progress to combat charcoal rot disease in the future. Grouping of resistant genotypes along with sensitive genotypes is occurring because of a smaller number of primers used in the investigation. Other reason behind this could be, with time of evolution gene fragment has lost its effect of resistance and although at genomic level, genotype is resistant but disease symptoms appear under field conditions. There is a pressing requirement of more number(s) of primers to be employed for accomplishment more appropriate and consistent outcome to screen genotypes against disease.

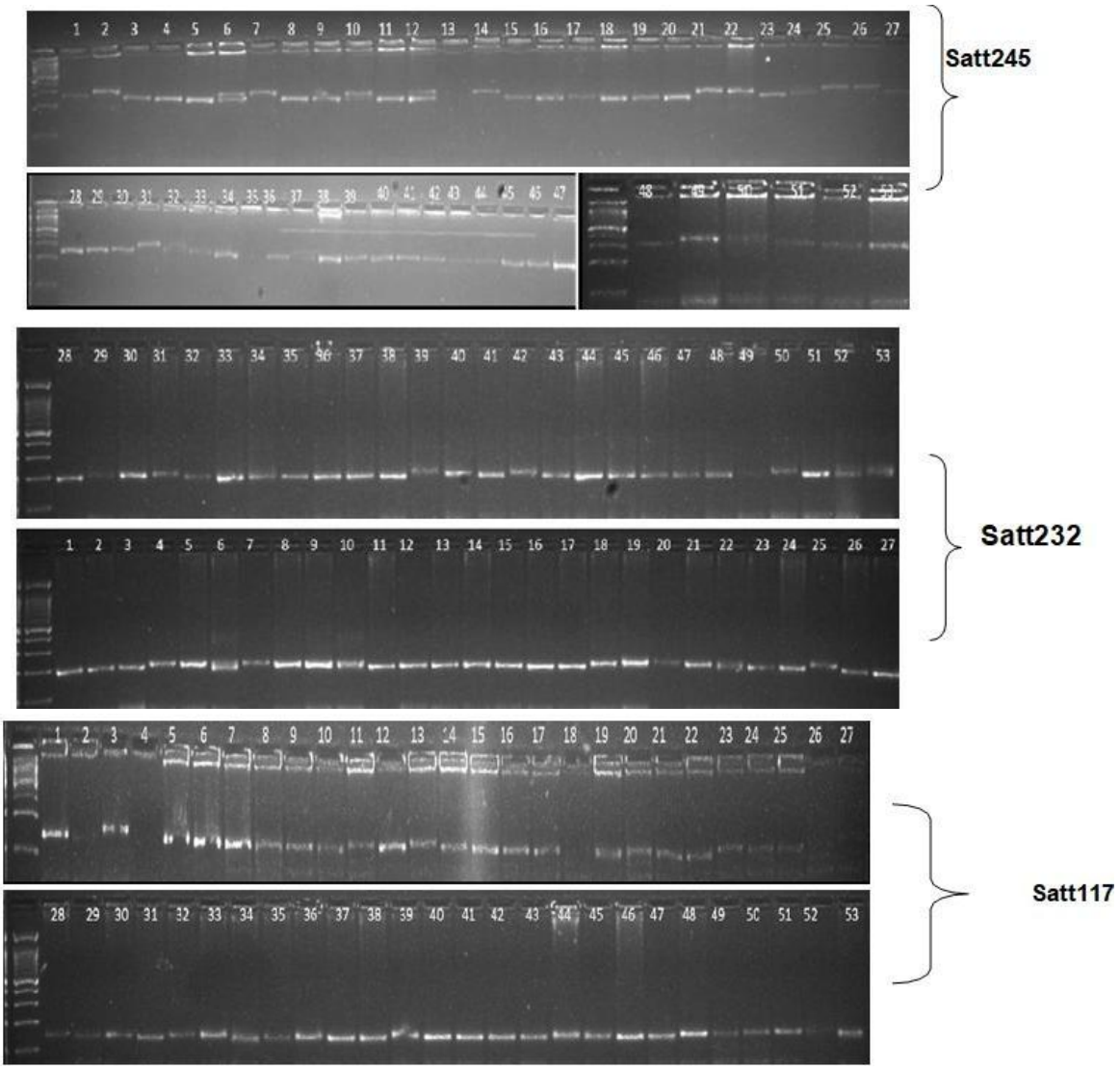


Fig. 1. Gel image of soybean germplasm representing polymorphic SSR Primer

Note: 1. JS 20-29, 2. JS 20-69, 3. JS 335,4. JS 20-98,5. JS 20-94,6. JS 93-05,7. JS 20-116 8. JS 95-60,9. JS 97-52, 10.JS20-84,11. JS 20-34,12. JS 20-71,13. RVS 2007-6,14. RVS 2011-35,15. RVS 2001-4,16. RVS-14, 17.RVS-24, 18.RVS-18, 19.NRC-76, 20.NRC-86, 21.NRC-130, 22. NRC-131, 23. NRC-147, 24. AMSMBC - 18, 25. AMS-100-39, 26. MACS–1520,27. MACSNRC-1575, 28. RSC-10-52, 29.SL-1123, 30.SL-1068, 31. AGS111,32.EC457286,33. MACS725, 34. SP37, 35.NRC-125, 36. NRC-132, 37. NRC-134, 38. NRC SL-1, 39.PS1092,40.PS1613, 41. AMS2014-1, 42. KDS992**,** 43.VLS-94, 44.SKF-SPS -11,45. RVS76, 46. NRC127, 47. KDS980, 48.G-29, 49.RSC-10-70, 50.RSC-10-71, 51.NRC-2,52. MAUCS-1520 and 53.AMS-MS-58

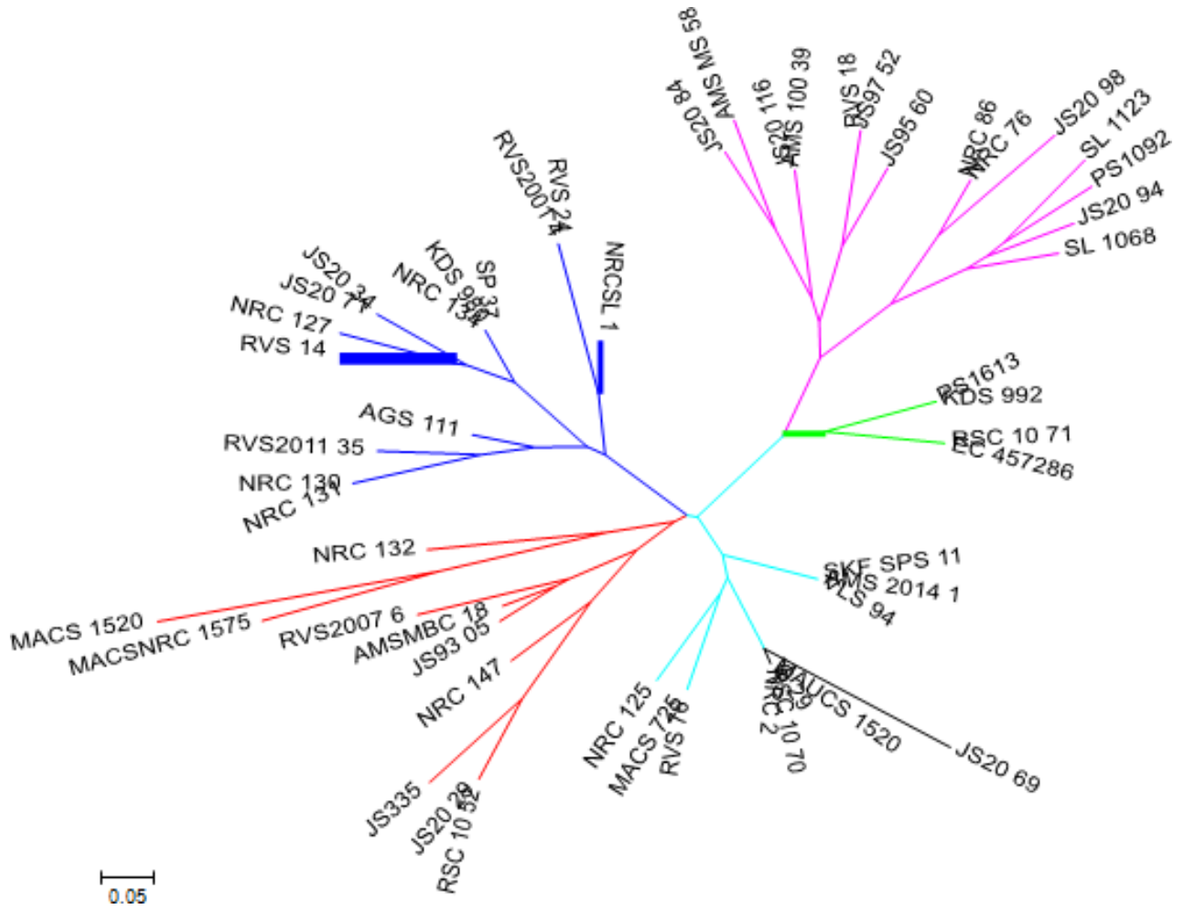


Fig. 2. UPGMA tree (rotational form) based on similarity index of 4 SSR markers for 53 soybean genotypes using banding pattern analysis

Table 1. List of soybean genotypes with their parentage

S. No.	Genotypes	Source/Pedigree	S. No.	Genotypes	Source/Pedigree
1.	JS 20-29	JS 97-52 x JS 95-56	28.	RSC-10-52	NRC 37X JS335
2.	JS 20-69	JS 97-52 x SL 710	29.	SL -1123	Selection from AGS751
3.	JS 335	JS 78-77 x JS 71-05	30.	SL-1068	SL755XSL525
4.	JS 20-98	JS 97-52x JS SL710	31.	AGS 111	Germplasm accession
5.	JS 20-94	JS 97-52 x JS 20-02	32.	EC457286	Germplasm accession
6.	JS 93-05	Selection from PS 73-22	33.	MACS725	JS93-05X MAUS71
7.	JS 20-116	JS 97-52 x JSM 120 A	34.	SP 37	Not known selection
8.	JS 95-60	Selection from PS 73-22	35.	NRC -125	EC54688xps1044
9.	JS 97-52	PK 327 x L 129	36.	NRC-132	JS97-52X PI086023
10.	JS 20-84	JS 98-63 x PK 768	37.	NRC-134	NRC7XAGS191
11.	JS 20-34	JS 98-63 x PK 768	38.	NRC SL-1	JS335XSL525
12.	JS 20-71	JS 97-52 x JS 90-5-12-1	39.	PS 1092	PS1042 x MACS 450
13.	RVS 2007-6	JS 20-10 x MAUS162	40.	PS 1613	PS1225XPS1042
14.	RVS 2011-35	JS 335 X PK 1042	41.	AMS 2014-1	AMS99-33XH6P5
15.	RVS 2001-4	JS 93-01x EC 390981	42.	KDS 992	JS93-05XEC241780
16.	RVS -14	JS 93-05x EC 390981	43.	VLS -94	VL Soya59X VS2005-1
17.	RVS -24	J.P 120 x JS 335	44.	SKF-SPS -11	Not known selection
18.	RVS -18	JSM110XJSM66	45.	RVS 76	MAUS-162XJSM-66
19.	NRC- 76	NRC-37XL-27	46.	NRC127	JS97-52XPI542044
20.	NRC -86	RKS15XEC481309	47.	KDS980	JS93-05XAMS1
21.	NRC- 130	EC390977XEC538828	48.	G-29	Germplasm
22.	NRC -131	EC390977XEC538828	49.	RSC-10-70	JS335X Bragg
23.	NRC -147	Germplasm accessions C210	50.	RSC-10-71	Bragg XJS335
24.	AMSMBC -18	Mutant of Bragg	51.	NRC-2	Induced mutant of Bragg
25.	AMS-100-39	Mutant of JS93-05	52.	MACS-15-20	NRC37XMohetta
26.	MACS – 1520	EC241780XMACS330	53.	MACS-58	JS2 x Improve pelican
27.	MACSNRC-1575	PI542044XJS9305			

Table 2. Molecular markers used for screening against *Rhizoctonia* root rot and Charcoal rot

Primer	Forward 5'-3'	Reverse 5'-3'
Satt281	AAG CTC CAC ATG CAG TTC AAA AC	TGC ATG GCA CGA GAA AGA AGT A
Satt177	CGT TTC ATT CCC ATG CCA ATA	CCC GCA TCT TTT TCA ACC AC
Satt245	AAC GGG AGT AGG ACA TTT TAT T	GCG CCT CCT GAA TTT CAA AGA ATG AAG A
Sat_232	GCG CGT CCT TTA TTT AAT TTA ATA TGA A	GCG TGG CTT TGC TAA TAA TGA ATG AT
Sct_028	TCGCCGGTACAAAAG	CGAATGAACAAACA
Satt512	AACGTCTTCAAGTCAAGTGCCTACA	GCCCACATAGTTTTCATTTTTCTCCA
S60211-TB	GAAGATCCTAACACGATGGCCG	TTCGTTGTTTCCTTCATTGCCG
Sat_117	TTTGGCAGTTTCTTGTAG	GCTGGATCGCAGTTA
S63880-CB	AGTCCTCCTCGCCAACAACAAC	TTCATTTCATTTCCAAGCGGT

Table 3. Analysis of variance for yield traits in genotypes of soybean

Source of variation	D.f	Plant height	Days to flower initiation	Days to 50% flowering	Days to maturity	Numbers of primary branches /plant	Leaf Area (cm^2)	Chlorophyll a (mg/ml)	Chlorophyll b (mg/ml)	Numbers of seeds / pod	Numbers of pods / plant	Grain yield / plant (g)	Biological yield / plant (g)	Harvest index
Replications	1	20.65	0.01	42.35	9.66	13.62	25.44	100.51	46.21	0.38	35.59	40.33	70.95	161.11
Genotypes	52	459.08**	30.59**	26.12**	110.47**	4.60**	468.72**	16.73**	106.98**	0.33**	224.09**	1118.48**	3691.20**	86.79**
Error	52	16.38	1.07	2.54	2.76	1.02	2.81	1.51	1.93	0.04	3.87	3.40	4.42	13.71

*Note: * and ** significance at 1% and 5% probability level*

Table 4. Mean observations on Grains yields and its components of different Genotype in soybean

S. N	Genotype	Plant height (cm)	Days to flower initiation	Days to 50% flowering	Days to maturity	No. of primary branches /plant	Leaf Area (cm^2)	Chloro-phyll a (mg/ml)	Chloro-phyll b (mg/ml)	No. of seeds / pod	No. of pods / plant	Grain yield/ plant (g)	Bio. yield/ plant (g)	Harves tindex
1	JS 20-29	71.5	38.5	48.5	87.0	4.0	15.2	8.0	17.5	2.5	42.5	106.1	210.7	50.4
2	JS 20-69	8s4.8	35.5	43.0	85.0	5.0	33.8	7.7	21.8	2.8	31.7	89.7	173.8	50.7
3	JS 335	76.1	38.5	48.0	89.5	6.2	13.4	5.6	17.5	1.8	45.2	94.2	187.9	50.1
4	JS 20-98	70.1	35.0	43.5	94.0	5.6	32.9	8.3	21.4	2.7	16.8	44.7	88.0	50.9
5	JS 20-94	83.3	36.5	47.0	86.5	5.1	36.8	7.7	25.3	2.5	19.4	48.5	94.9	51.2
6	JS 93-05	71.8	36.0	46.0	92.5	3.8	36.2	10.7	2.9	3.6	19.4	69.7	137.7	50.6
7	JS 20-116	86.0	34.5	44.5	76.5	4.0	35.0	8.1	14.0	2.8	32.5	90.3	180.0	50.2
8	JS 95-60	55.7	35.0	44.0	69.0	2.2	31.0	4.1	7.0	3.8	12.4	47.0	94.1	49.9
9	JS 97-52	73.2	35.5	44.5	81.0	6.6	20.7	10.4	16.8	2.7	35.4	95.5	155.6	64.8
10	JS 20-84	43.2	33.5	44.5	66.5	2.4	21.8	4.6	6.9	2.5	29.0	71.3	141.1	50.5
11	JS 20-34	46.4	34.5	44.5	66.5	2.9	20.6	9.5	16.4	2.7	16.6	44.9	87.1	51.5
12	JS 20-71	60.2	33.5	40.5	68.5	5.3	20.3	11.4	19.4	2.5	7.6	18.9	38.7	48.9
13	RVS 2007-6	89.3	35.5	46.5	67.0	2.9	22.0	11.1	14.5	2.4	20.7	49.3	97.8	50.4
14	RVS 2011-35	83.7	34.5	42.0	72.0	5.3	35.0	7.1	13.7	2.6	26.2	68.2	135.6	50.3
15	RVS 2001-4	73.5	33.5	44.5	81.5	6.3	34.1	12.7	21.6	2.3	21.6	49.7	97.3	51.1
16	RVS -14	78.7	34.5	44.0	70.5	4.3	53.4	13.7	23.0	2.3	35.8	82.1	163.5	50.2
17	RVS -24	86.3	35.0	44.5	81.0	4.5	41.2	11.3	12.7	2.7	24.9	67.1	132.5	50.6
18	RVS -18	78.6	34.5	44.5	85.0	2.0	44.2	6.5	20.2	2.7	22.5	60.8	120.9	50.3
19	NRC- 76	111.0	36.5	47.0	71.5	6.0	52.3	2.2	15.4	2.6	7.9	20.3	42.3	48.0
20	NRC -86	66.3	34.5	45.5	76.0	6.4	51.3	3.6	9.4	2.8	20.4	57.2	113.5	50.4
21	NRC- 130	67.2	33.5	41.0	67.0	2.7	30.8	5.5	16.4	2.4	26.6	63.9	126.0	50.7
22	NRC -131	80.8	34.5	47.0	69.0	6.1	78.0	10.3	7.3	2.2	32.5	71.0	140.5	50.5
23	NRC -147	84.0	35.5	43.5	70.5	5.4	44.4	4.6	12.8	2.1	25.3	53.1	104.6	50.8
24	AMSMBC -18	83.2	34.5	41.5	89.0	6.1	52.8	11.2	7.1	2.0	32.4	64.0	126.7	50.5
25	AMS-100-39	75.8	35.5	43.5	87.0	5.0	73.3	2.5	10.6	2.2	39.2	86.2	171.3	50.3
26	MACS – 1520	76.0	38.5	49.0	87.5	7.8	62.5	9.6	4.3	2.6	32.5	84.6	166.7	50.7
27	MACSNRC-1575	74.0	36.0	46.0	86.0	1.6	75.5	6.1	9.5	2.5	31.0	77.2	152.5	50.6
28	RSC-10-52	82.7	39.5	50.0	74.0	6.7	30.4	4.4	9.4	2.0	30.9	61.1	121.0	50.5
29	SL -1123	66.3	39.0	46.5	68.0	5.1	48.3	5.4	21.2	2.8	12.4	34.7	96.2	36.1

30	SL-1068	56.9	35.5	44.0	65.5	6.9	56.2	6.5	21.5	2.0	22.3	44.5	110.3	40.3
31	AGS 111	42.8	39.0	48.0	71.0	3.9	27.9	9.3	5.9	2.3	23.9	54.7	107.5	51.0
S.N	**Genotype**	**Plant height (cm)**	**Days to flower initiation**	**Days to 50% flowering**	**Days to maturity**	**No. of primary branches /plant**	**Leaf Area (cm^2)**	**Chloro-phyll a (mg/ml)**	**Chloro-phyll b (mg/ml)**	**No. of seeds / pod**	**No. of pods / plant**	**Grain yield/ plant (g)**	**Bio. yield/ plant (g)**	**Harvestindex**
32	EC457286	57.5	39.5	48.5	71.0	5.3	26.4	7.4	8.0	1.9	23.4	44.5	90.8	49.0
33	MACS725	79.2	35.0	42.5	74.5	3.2	40.9	14.4	11.4	2.8	12.5	34.9	87.4	39.9
34	SP 37	73.7	37.5	47.0	82.0	6.3	38.4	6.6	6.8	2.8	6.8	19.0	89.9	21.1
35	NRC -125	66.7	41.0	47.5	69.5	5.9	30.7	9.7	8.8	2.7	15.8	42.6	104.4	40.8
36	NRC-132	68.0	44.0	50.0	74.0	6.5	14.0	10.6	2.4	2.0	21.3	42.1	104.1	40.5
37	NRC-134	53.7	42.0	49.0	75.0	5.5	16.1	3.7	14.5	2.7	6.9	18.6	42.3	43.8
38	NRC SL-1	49.8	38.5	47.0	70.0	6.3	31.1	9.8	19.4	2.5	20.2	50.3	99.2	50.7
39	PS 1092	42.2	40.0	49.0	72.5	4.1	12.9	5.8	3.7	2.4	17.4	41.7	82.1	50.9
40	PS 1613	69.9	33.5	42.5	70.5	4.6	29.5	8.8	2.1	1.7	8.5	14.5	46.0	31.5
41	AMS 2014-1	57.3	43.0	46.0	75.0	5.9	53.8	6.1	2.6	1.9	33.2	63.0	123.4	51.0
42	KDS 992	53.0	43.5	47.0	77.0	6.3	43.2	6.6	3.3	2.4	14.2	33.7	66.8	50.6
43	VLS -94	53.8	44.5	53.0	70.5	5.1	26.7	6.5	1.0	2.6	20.8	54.0	105.9	51.0
44	SKF-SPS -11	56.6	45.5	54.0	70.5	5.6	27.5	5.8	1.5	2.6	23.3	60.2	118.4	50.9
45	RVS 76	109.3	43.5	53.5	73.5	4.6	29.3	5.5	2.8	2.1	10.2	21.3	54.0	39.4
46	NRC127	81.8	43.0	49.5	77.0	5.4	39.6	6.8	1.1	2.4	30.0	72.1	142.2	50.7
47	KDS980	79.5	43.5	54.0	80.0	5.5	36.2	3.5	1.3	2.5	37.7	94.1	187.0	50.3
48	G-29	71.5	42.0	53.0	74.0	2.2	30.9	5.5	3.1	2.7	16.2	43.7	85.4	51.3
49	RSC-10-70	59.9	44.0	52.5	72.5	5.2	35.7	5.5	1.1	2.7	32.0	84.0	166.2	50.6
50	RSC-10-71	62.1	43.0	50.0	76.0	4.0	23.0	5.5	1.8	3.2	7.4	23.3	44.2	53.2
51	NRC-2	43.5	43.0	51.5	77.0	4.0	23.3	2.5	4.8	2.6	6.2	15.8	51.1	31.0
52	MACS-1520	63.4	44.5	50.5	73.0	5.5	39.2	8.3	2.9	2.5	34.0	84.8	167.6	50.6
53	AMS-MS-58	73.3	45.5	54.0	75.5	5.0	24.3	6.3	3.3	1.7	40.7	66.3	131.2	50.5
Mean		69.9	38.2	47.0	76.1	5.2	35.9	7.4	10.4	2.5	23.6	56.5	115.8	48.3
SE(m)		2.9	0.7	1.1	1.2	0.7	1.2	0.9	1.0	0.1	1.4	1.3	1.5	2.6
CD5%		8.1	2.1	3.2	3.3	2.0	3.4	2.5	2.8	0.4	0.9	3.7	4.2	7.4
CV%		5.8	2.7	3.4	2.2	19.3	5.0	16.6	13.4	7.6	8.4	3.3	1.8	7.7

Table 5. Summary of polymorphic SSR markers representing PIC value and other analysis data

Marker	Major Allele Frequency	Allele No	Gene Diversity	PIC
satt281	0.3962	4	0.6921	0.6359
satt177	0.4340	4	0.6401	0.5671
satt245	0.6226	3	0.4956	0.4047
satt232	0.4057	4	0.6769	0.6127
Mean	0.4646	3.75	0.6262	0.5551

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Table 6. Genotype in different clusters based on UPGMA tree

Cluster	Number of genotypes	Genotypes
1	11	JS 20-69, MAUCS-1520, RSC-10-17, VLS-94, , G-29, NRC-2, RVS-16, NRC-125, MACS-725, SKF SPS-11 and AMS-2014-1
2	10	RSC-10-52, JS-20-29, JS335, NRC-147, JS-93-05, AMAMBC-18, RVS-2007-6, MACSNRC-1575, MACS-1520 and NRC-132
3	14	NRC-131, NRC-130, RVS-2011-35, AGS-111, RVS-14, NRC-127, JS-20-71, JS-20-34, NRC-134, KDS-980, SP-37, RVS-2001-4, RVS-24 and NRCSL-1
4	14	JS-20-84, AMS MS-58, JS-20-116, AMS100-39, RVS-18, JS-97-52, JS-95-60, NRC-86, NRC-76, JS-20-98, SL-1123, PS-1092, JS-20-98, SL-1123, PS-1092, JS-20-94 and SL-1068
5	4	KDS 992, PS 1613, RSC-10-71 and EC457286,

Another most abolishing fungal disease of soybean is charcoal rot. Its management approaches in soybean include cultural methods, seed-applied fungicide and biological control, but these have not been effectively or widely adopted and have provided limited control [65]. In such circumstances, genetic resistance may be the most practicable and supportable method to accomplish charcoal rot [24]. Complete resistance to *M. phaseolina* is not reported in any plant species, but identification of partial resistance has been documented in soybean, including moderately resistant cultivars, for example DT97-4290, used as a disease check standard [66]. However, investigations into commercially available germplasm lines and their general response to the fungus have not been extensively evidenced. In current study, a set of five gene-linked SSR molecular markers against charcoal rot *viz*., Sct_028, Satt512, S60211-TB, Sat_177 and S63880-CB linked with charcoal rot were tested across 53 soybean genotypes and only one primer *i.e.,* S60211 showed polymorphism among them.

4. CONCLUSION

The highest percent contribution towards genetic divergence was recorded by biological yield per plant. On the basis of divergent traits genotype *viz.,* JS335, JS 20-69, JS97-52, KDS980 and KDS 992 were found to be most divergent and promising genotypes and they may be employed as parents in future hybridization programme. Genotype(s) that gained resistance /tolerance against *Rhizoctonia* root rot and charcoal rot may be used further in molecular breeding programmes to develop resistant/tolerant cultivars.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Analysis of Genetic Differences in fatty Acids and Oil Contents among Brassica *juncea* (Linn.) Czern & Coss Genotypes

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ABSTRACT

Study Objectives: The experimentation was steered to assess erraticism among 188 Indian mustard genotypes owing to assorted biochemical parameters *viz*., palmitic, oleic, linoleic, linolenic and erucic acids accompanied by oil composition.
Location of Study: Experiment was carried out at Department of Genetics and Plant Breeding, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, India
Results: Analysis of variance designated the presence of considerable extent of variation among premeditated Indian mustard genotypes which advocate better promises for their enhancement. Genotypic and phenotypic coefficient of variation was investigated to be higher for oleic acid pursued by erucic and palmitic acids. Erucic, oleic, palmitic, linoleic and linolenic acids had determined heritability and genetic advance. Significant negative correlation of erucic acid was recognized with palmitic, oleic, linoleic and linolenic acids. Genotypic and phenotypic path coefficient analysis had the higher positive direct effect of palmitic acid on erucic acid, while highest negative direct effect on erucic acid was demonstrated by linoleic, oleic, linolenic acids and oil content. Genetic deviation employing Euclidean distance cluster congregated the genotypes into eighteen diverse groups. Amongst entirely considered biochemical strictures, erucic acid was investigated to be low in 9, modest in 57 and higher in 122 genotypes. In group investigation of qualitative parameters, extreme inter cluster distance was documented between cluster 18 (Karishma) and cluster 12 (Maya). Consequently, these genotypes may be employed as parentages in Indian mustard breeding arrangement for enhancement of various qualitative parameters.

Keywords: Indian mustard; biochemical parameters; correlation coefficient; path coefficient; quantitative traits; principle component analysis.

1. INTRODUCTION

Brassica genus is uninterruptedly acquiring industrial standing owing to the incidence of oil annoying species for instance *Brassica juncea*, *B. carinata*, *B. rapa* and *B. napus* in it [1]. Explicit nutritional standards are elementary standards to choose the oil for comestible and industrial purposes. Among all the mustard species *B. juncea* has acquired higher adaptableness as oilseed crop in India, China and Pakistan attributable to higher oil contents up to 44% [2-4]. In addition to oil content diverse biochemical traits for instance essential and non-essential fatty acids are also well-thought-out to warrant industrial along with nutritional reputation of mustard oil [5-7]. Among different nutritive strictures of edible oil fatty acids similar to oleic, linolenic, erucic, palmitic and linoleic acids is very significant. Indian mustard (*B. juncea*) genotypes have been testified with higher portions of erucic

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acid and glucosinolates in the oil part [4]. Low erucic acid comprising mustard genotypes are favorite due to cardiac glitches with high erucic acid [5-7]. Industrial qualities of oils are also imperative for their application in the production of detergents, cosmetics, lubricants, hydraulic oils or bio-diesel and other non-edible items [8,7,3]. Therefore, it is important to advance superior mustard cultivars with essential assets for human ingesting along with industrial applications [9].

Shelf- life of oil and food sweltering abilities are usually dependent on higher mono and low poly-unsaturated besides very low levels of saturated fatty acid [10,3]. To accomplish the supposed ideas, the researchers elaborated in mustard crop enhancement prerequisite to recognize various genotypes with each other in view of embattled apprehensions [11-16] with enough heritability and selection standards. To comprehend the level of association between two parameters commonly correlation analysis steered. Many investigations have been led on correlation analysis for quantitative traits in crops together with Canola [17] along with in *B. campestris, B. napus, B. carinata* and *B. juncea* [18-20]. In the parallel way, path analysis has also been applied widely by investigators for the enhancement of crops [21]. For the present study, genotypic and phenotypic correlations were assessed to engender significant and convenient evidence for the imminent development scheme of *Brassica*. Accordingly, this examination was commenced to screen the Indian mustard germplasm lines in view of diverse biochemical traits that further may be added in *Brassica* improvement scheme to breed optimal array of fatty acid encompassing cultivars.

2. MATERIALS AND METHODS

A total of 188 Indian mustard genotypes obtained from the Zonal Agricultural Research Station, Morena, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya (RVSKVV), Gwalior, India (AICRP on Rapeseed and Mustard) and Indian Agricultural Research Institute, New Delhi were employed in current study (Table1). All chosen genotypes were grown in randomized block design with two replications during *Rabi* 2016-17 at the experimental field, Department of Genetics & Plant Breeding, College of Agriculture, RVSKVV, Gwalior. Each genotype was grown in a plot of single row of 2-meter length with an arrangement of 30 cm apart between rows and 15 cm plant to plant. The crop was given protective irrigations and suggested package of practices to grow vigorous crop throughout the growing period. Seeds were analyzed for the diverse biochemical parameters, *i.e*., palmitic, oleic, linoleic, linolenic and erucic acids together with oil percentage for analysis of mean performance, genotypic (GCV) and phenotypic coefficient of variation (PCV) as per formula prearranged by Burton [22]; heritability in broad sense (h^2) as proposed by Burton and De [23] and genetic advance as per method designated by Johnson et al*.* [24]. Fatty acid analysis was conducted at Quality Lab, Division of Genetics, Indian Agricultural Research Institute, New Delhi and the oil extraction at Department of Soil Science and Agricultural Chemistry, College of Agriculture, RVSKVV, Gwalior, India by employing Soxhlet method. The profiling of fatty acid was steered by employing Gas-Liquid Chromatography (GLC). Fatty acid methyl esters (FAME) of oil sample from each genotype were extracted. Gas chromatography (Perkin Elmer Claurus 500) fitted with megabore column (30 meter long and 0.53mmicro) fitted with OV-101, equipped with a Flame Ionization Detector (FID) was used for FAME analysis. The surroundings sustained were Column temperature: 150^0C-270^0C, Injector temperature: 250^0C and Detector temperature: 250^0C. Ultrapure nitrogen gas was experienced as transporter. GLC was automated for the temperature at the rate of 10^0C per minute increase and finally it was upholded at 270^0C. In gas chromatographs, area under each peak is calculated automatically, it can be computed by measuring the peak height and width at half height (Triangulation method). After calculating total peak area for each sample, percent area under each peak was calculated that would spring percentage of matching fatty acid.

Genotypic and phenotypic correlations were computed by using the formula given by Weber and Moorthy [25] and Miller [26]. Principal component analysis was performed on PAST v3.14 software depending upon correlation matrix (normalized variance-covariance) since the variables are premeditated in different units; this recommends standardizing all variables by means of division by their standard deviations.

3. RESULTS AND DISCUSSION

Disparities in genetic components aid breeders to edge an efficacious hybridization programme with embattled traits owing to their use in the estimation of inherent distance among or between the genotypes [27-33]. These analyses also support in the selection of various auspicious parents to pledge crossing programme [34]. In current study, variance analysis designated subsistence of substantial sum of variability amongst diverse genotypes for all the selected biochemical traits *viz*., palmitic, oleic, linoleic, linolenic and erucic acids in conjunction with oil content.

3.1 Mean Performance of Selected Parameters

In the current investigation, six biochemical parameters were analyzed of 188 Indian mustard genotypes. The percentage of different fatty acids in the oil from Indian mustard seeds is depends upon different hereditary features of cultivars chosen for the study [35]. Palmitic acid arrayed from 3.94 to13.27%, with an average worth of 6.82% (Table1). Comparable ranges for palmitic acid have also been testified formerly by various investigators [36-43]. The genotypes of Indian mustard with high palmitic acid are considered better for industrial purposes for the manufacture of soap and other similar substances owing to skin-care and moisturizing belongings of palmitic acid [44]. Nevertheless, the part of palmitic acid has been conveyed in several basic usual occupations at cellular as well as tissue levels [45]. In the current investigation, genotype IDM-67 had maximum palmitic acid content (13.27%) followed by ISC-12 (12.17%) and PM28 (12.05%) so, these mustard genotypes may have potential for industrial applications. While, minimum (3.94%) palmitic acid was documented in genotype MRNJ-21.

Table 1. Values of different biochemical parameters in 188 Indian mustard genotypes

Genotypes	Source	Palmitic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Erucic acid (%)	Oil %
MRNJ1	ZARS, Morena	7.4	13.8	25.3	11.6	36.0	31.5
MRNJ2	ZARS, Morena	6.1	17.3	22.3	10.7	35.2	33.5
MRNJ3	ZARS, Morena	7.1	18.4	25.0	11.7	30.1	34.5
MRNJ4	ZARS, Morena	7.2	19.7	24.7	10.8	29.0	33.5
MRNJ5	ZARS, Morena	7.6	19.6	23.0	10.7	29.2	37.0
MRNJ6	ZARS, Morena	5.8	17.8	16.4	8.9	40.0	36.5
MRNJ7	ZARS, Morena	7.8	14.3	26.0	11.7	33.9	36.5
MRNJ8	ZARS, Morena	8.6	21.5	29.0	11.6	21.3	34.5
MRNJ9	ZARS, Morena	7.4	22.3	26.2	11.0	25.2	34.5
MRNJ10	ZARS, Morena	4.9	15.0	20.8	10.7	37.3	35.0
MRNJ11	ZARS, Morena	4.8	13.9	20.2	10.3	37.6	32.5
MRNJ12	ZARS, Morena	6.1	6.5	23.0	14.3	35.3	32.5
MRNJ13	ZARS, Morena	4.7	18.4	20.5	11.3	33.7	33.0
MRNJ14	ZARS, Morena	4.6	14.7	19.7	12.6	37.1	34.5
MRNJ15	ZARS, Morena	5.8	14.2	19.7	12.0	38.5	33.5
MRNJ16	ZARS, Morena	6.6	8.3	22.4	14.0	34.0	34.0
MRNJ17	ZARS, Morena	5.6	15.8	21.8	11.0	34.0	36.0
MRNJ18	ZARS, Morena	4.2	15.3	17.0	10.3	40.1	36.0
MRNJ19	ZARS, Morena	5.8	10.3	20.3	10.0	39.3	34.0
MRNJ20	ZARS, Morena	5.0	16.1	19.1	10.3	36.2	33.0
MRNJ21	ZARS, Morena	3.9	15.5	19.8	10.4	35.8	34.5
MRNJ-22	ZARS, Morena	4.0	14.6	18.3	9.3	37.9	31.5
MRNJ-23	ZARS, Morena	4.9	8.6	21.1	11.8	40.4	30.5
MRNJ-24	ZARS, Morena	4.4	15.8	18.4	11.8	37.3	32.5
MRNJ-25	ZARS, Morena	5.3	26.8	25.1	11.4	21.5	34.5
MRNJ-26	ZARS, Morena	4.8	16.2	20.3	10.4	35.4	35.0
MRNJ-27	ZARS, Morena	4.7	18.3	23.1	11.4	29.7	37.0
MRNJ-28	ZARS, Morena	4.2	19.1	21.2	10.3	29.9	34.5
MRNJ-29	ZARS, Morena	4.3	20.5	20.4	11.1	32.7	32.5
MRNJ-30	ZARS, Morena	4.2	32.2	23.1	12.0	16.1	33.5

Genotypes	Source	Palmitic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Erucic acid (%)	Oil %
MRNJ-33	ZARS, Morena	4.8	16.3	19.9	10.4	36.2	37.0
MRNJ-34	ZARS, Morena	6.5	14.4	24.9	11.2	30.4	35.5
MRNJ-35	ZARS, Morena	5.5	19.8	21.1	10.2	34.8	36.5
MRNJ-36	ZARS, Morena	5.4	17.5	19.4	9.2	37.4	37.5
MRNJ-37	ZARS, Morena	5.5	19.2	20.2	10.0	32.7	37.5
MRNJ-38	ZARS, Morena	7.4	10.3	23.3	9.9	36.8	34.5
MRNJ-39	ZARS, Morena	6.0	14.7	24.0	9.9	33.5	36.5
MRNJ-40	ZARS, Morena	5.7	19.3	21.1	10.6	31.3	38.5
MRNJ-41	ZARS, Morena	7.0	10.5	24.0	9.2	37.2	34.5
MRNJ-42	ZARS, Morena	6.8	17.5	22.0	8.3	35.1	35.5
MRNJ-43	ZARS, Morena	6.3	19.9	22.6	10.0	30.9	36.0
MRNJ-44	ZARS, Morena	6.1	17.7	20.9	9.8	35.1	37.5
MRNJ-45	ZARS, Morena	7.4	18.4	28.6	10.1	25.9	38.5
MRNJ-46	ZARS, Morena	5.7	18.1	22.8	10.8	30.3	38.5
MRNJ-47	ZARS, Morena	5.7	16.4	21.6	9.4	35.8	36.5
MRNJ-48	ZARS, Morena	5.7	17.4	21.1	10.8	33.8	35.0
MRNJ-49	ZARS, Morena	7.4	17.7	25.6	9.8	30.1	34.0
MRNJ-50	ZARS, Morena	6.2	22.1	23.8	10.0	25.5	35.0
MRNJ-51	ZARS, Morena	5.3	14.7	19.9	8.9	39.6	36.0
MRNJ-52	ZARS, Morena	5.7	15.4	20.4	10.7	35.3	35.5
MRNJ-53	ZARS, Morena	6.2	10.2	21.0	10.1	37.3	34.0
MRNJ-54	ZARS, Morena	6.1	8.8	19.7	9.0	42.4	33.0
MRNJ-55	ZARS, Morena	6.3	15.7	21.9	10.1	33.4	33.5
MRNJ-56	ZARS, Morena	6.8	10.7	20.0	9.2	38.4	35.5
MRNJ-57	ZARS, Morena	6.7	12.5	19.1	9.5	38.0	37.0
MRNJ-58	ZARS, Morena	7.6	16.4	24.2	11.7	29.6	35.5
MRNJ-59	ZARS, Morena	7.5	10.5	27.3	11.8	31.1	32.0
MRNJ-60	ZARS, Morena	5.7	14.3	21.2	11.6	33.6	31.5
MRNJ-61	ZARS, Morena	5.2	12.8	21.9	11.2	35.9	32.0
MRNJ-62	ZARS, Morena	6.2	15.2	24.9	13.0	28.5	35.0
MRNJ-63	ZARS, Morena	5.7	10.5	24.0	14.1	33.7	34.5
MRNJ-64	ZARS, Morena	4.4	14.4	19.3	12.7	36.1	34.0
MRNJ-65	ZARS, Morena	8.1	18.3	27.1	13.2	24.0	34.5
MRNJ-66	ZARS, Morena	6.1	10.6	23.7	13.2	34.1	36.0
MRNJ-67	ZARS, Morena	6.4	10.0	24.9	12.4	32.2	32.5
MRNJ-68	ZARS, Morena	6.1	16.0	23.4	12.1	29.5	30.5
MRNJ-69	ZARS, Morena	5.3	15.8	20.2	10.3	34.2	32.5
MRNJ-70	ZARS, Morena	6.4	8.7	21.2	12.5	37.1	34.5
MRNJ-71	ZARS, Morena	6.8	16.3	23.0	12.3	31.1	34.5
MRNJ-72	ZARS, Morena	5.4	16.7	18.8	9.8	39.1	30.5
MRNJ-73	ZARS, Morena	7.2	18.8	22.9	12.2	29.2	32.5
MRNJ-74	ZARS, Morena	6.3	16.3	21.6	10.4	32.3	32.5
MRNJ-75	ZARS, Morena	4.8	15.3	19.4	11.0	35.1	30.5
MRNJ-76	ZARS, Morena	5.7	16.3	22.4	11.3	31.0	31.5
MRNJ-77	ZARS, Morena	5.6	14.5	20.3	13.0	33.3	33.5
MRNJ-78	ZARS, Morena	4.6	15.3	19.9	11.9	36.3	32.5
MRNJ-79	ZARS, Morena	5.5	15.7	18.7	10.9	38.2	32.5
MRNJ-80	ZARS, Morena	6.3	18.8	22.9	11.0	30.3	33.5
MRNJ-81	ZARS, Morena	7.2	22.5	22.8	10.1	26.5	35.5
MRNJ-82	ZARS, Morena	6.2	17.2	23.9	12.9	28.7	37.5
MRNJ-83	ZARS, Morena	5.4	14.1	18.8	10.6	38.3	35.5
MRNJ-84	ZARS, Morena	6.1	15.2	21.8	11.0	35.4	33.5
MRNJ-85	ZARS, Morena	5.8	16.0	23.6	10.9	32.3	34.5
MRNJ-86	ZARS, Morena	6.7	16.8	22.3	9.8	31.9	31.5
MRNJ-87	ZARS, Morena	7.4	16.2	24.9	10.3	30.9	31.5
MRNJ-88	ZARS, Morena	7.1	17.8	23.6	10.9	30.9	32.5
MRNJ-89	ZARS, Morena	7.4	17.6	26.0	11.8	26.3	33.0
MRNJ-90	ZARS, Morena	8.4	10.9	29.7	13.0	27.1	34.0
MRNJ-91	ZARS, Morena	5.8	20.2	22.0	9.6	29.4	33.5
MRNJ-92	ZARS, Morena	6.7	22.5	24.9	11.9	23.8	31.0
MRNJ-93	ZARS, Morena	7.5	19.0	23.9	11.0	27.1	31.5

Genotypes	Source	Palmitic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Erucic acid (%)	Oil %
MRNJ-94	ZARS, Morena	8.2	13.1	25.5	10.9	31.3	33.0
MRNJ-95	ZARS, Morena	8.3	13.1	24.1	11.1	31.3	31.0
MRNJ-96	ZARS, Morena	7.5	17.4	23.7	10.0	31.1	33.5
MRNJ-97	ZARS, Morena	8.5	12.7	27.7	12.0	29.1	35.0
MRNJ-98	ZARS, Morena	8.8	9.6	27.8	11.3	31.2	20.5
MRNJ-99	ZARS, Morena	6.4	16.3	22.1	11.1	32.8	36.0
MRNJ-100	ZARS, Morena	6.0	14.5	21.6	10.0	37.0	36.0
MRNJ-101	ZARS, Morena	5.3	16.0	20.7	10.2	35.3	33.5
MRNJ-102	ZARS, Morena	6.5	17.8	22.6	10.0	33.0	34.5
MRNJ-103	ZARS, Morena	7.4	18.8	25.9	9.9	28.0	35.0
MRNJ-104	ZARS, Morena	8.1	19.8	26.7	10.0	28.1	33.5
MRNJ-105	ZARS, Morena	8.2	18.9	27.6	10.8	26.3	34.5
MRNJ-106	ZARS, Morena	5.5	18.1	22.0	10.0	33.0	33.5
MRNJ-107	ZARS, Morena	7.0	19.5	27.3	13.3	23.6	32.5
MRNJ-108	ZARS, Morena	7.2	10.8	28.1	14.3	27.2	33.5
MRNJ-109	ZARS, Morena	5.8	18.5	23.5	12.2	28.3	33.5
MRNJ-110	ZARS, Morena	5.4	16.4	20.8	11.6	34.4	35.5
MRNJ-111	ZARS, Morena	7.6	22.0	26.2	10.8	23.9	35.5
MRNJ-113	ZARS, Morena	7.5	21.1	26.9	12.3	23.4	37.5
MRNJ-114	ZARS, Morena	8.3	19.7	28.0	10.8	24.9	38.5
MRNJ-115	ZARS, Morena	7.4	16.1	24.2	11.8	32.8	37.0
MRNJ-116	ZARS, Morena	7.1	18.9	24.2	11.8	27.8	36.5
MRNJ-117	ZARS, Morena	7.3	18.4	25.2	13.7	27.2	35.5
MRNJ-118	ZARS, Morena	6.5	17.1	23.0	11.3	32.8	35.5
MRNJ-119	ZARS, Morena	7.4	18.7	25.0	13.2	25.2	31.5
MRNJ-120	ZARS, Morena	8.8	11.9	29.4	13.2	26.9	32.5
MRNJ-121	ZARS, Morena	7.4	19.1	25.0	10.4	29.1	33.5
MRNJ-122	ZARS, Morena	10.3	11.7	32.6	12.3	27.1	33.5
MRNJ-123	ZARS, Morena	8.1	13.7	25.1	10.0	34.0	33.5
MRNJ-124	ZARS, Morena	7.8	9.7	29.3	10.8	30.7	32.5
MRNJ-125	ZARS, Morena	5.0	9.9	20.0	10.0	41.7	32.5
MRNJ-126	ZARS, Morena	9.7	14.4	32.8	10.2	20.7	33.5
MRNJ-127	ZARS, Morena	5.4	17.1	22.2	12.4	29.8	33.5
MRNJ-128	ZARS, Morena	7.4	10.7	28.0	14.3	26.6	34.5
MRNJ-129	ZARS, Morena	8.3	8.9	27.3	14.0	29.7	35.5
MRNJ-130	ZARS, Morena	7.1	19.6	21.1	12.7	32.1	36.5
MRNJ-131	ZARS, Morena	8.1	22.3	23.7	11.2	27.0	37.5
MRNJ-135	ZARS, Morena	9.7	12.0	28.0	13.4	24.9	32.5
MRNJ-136	ZARS, Morena	9.4	11.8	27.6	12.6	30.7	33.5
MRNJ-137	ZARS, Morena	8.3	20.8	24.0	13.2	26.6	33.0
MRNJ-138	ZARS, Morena	6.4	17.8	23.7	12.3	29.7	35.5
MRNJ-139	ZARS, Morena	8.3	11.0	25.0	12.0	33.9	34.5
MRNJ-140	ZARS, Morena	8.9	12.5	28.6	11.0	28.9	35.0
MRNJ-142	ZARS, Morena	9.4	11.5	28.2	12.3	30.6	36.5
MRNJ-143	ZARS, Morena	7.4	16.8	23.0	13.7	29.4	37.5
MRNJ-144	ZARS, Morena	7.2	17.0	22.9	12.8	30.3	35.5
MRNJ-145	ZARS, Morena	6.1	11.8	18.9	10.0	25.9	36.5
IDM-2	ZARS, Morena	7.3	14.7	25.9	13.9	30.2	35.5
IDM-8	ZARS, Morena	8.3	15.0	26.8	10.6	30.0	33.0
IDM-10	ZARS, Morena	8.9	13.7	24.9	9.0	37.2	34.5
IDM-11	ZARS, Morena	8.6	13.7	25.9	9.6	34.7	33.5
IDM-12	ZARS, Morena	9.0	13.1	28.7	11.0	32.8	36.5
IDM-15	ZARS, Morena	7.6	14.6	27.1	13.9	30.4	35.5
IDM-16	ZARS, Morena	7.2	15.2	23.9	11.8	36.2	34.5
IDM-25	ZARS, Morena	8.2	11.6	22.6	10.7	42.6	32.5
IDM-31	ZARS, Morena	8.5	14.0	27.7	10.1	34.7	35.5
IDM-41	ZARS, Morena	4.6	16.9	19.9	10.9	43.8	36.5
IDM-42	ZARS, Morena	7.7	17.4	28.4	11.0	25.6	33.0
IDM-53	ZARS, Morena	8.1	11.2	27.0	12.1	35.0	34.5
IDM-58	ZARS, Morena	7.2	20.0	22.0	10.9	33.8	31.5
IDM-64	ZARS, Morena	8.5	11.0	26.0	12.2	33.8	33.5

Genotypes	Source	Palmitic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Erucic acid (%)	Oil %
IDM-66	ZARS, Morena	9.2	9.9	26.5	9.4	34.7	35.5
IDM-67	ZARS, Morena	13.3	11.0	35.6	11.2	22.2	34.5
IDM-69	ZARS, Morena	7.6	12.0	25.1	12.9	30.2	32.5
NRCDR-2	DRMR, Bharatpur	7.4	10.8	26.2	13.0	36.7	40.5
NRCHB-101	DRMR, Bharatpur	8.1	9.7	27.3	14.0	36.2	38.5
DRMRIJ-31	DRMR, Bharatpur	7.1	10.9	23.6	12.9	39.9	37.5
DRMR-150-35	DRMR, Bharatpur	6.2	13.7	24.0	12.0	35.0	35.5
RVM-1	RVSKVV, Gwalior	7.1	6.1	23.8	11.2	41.7	35.5
RVM-2	RVSKVV, Gwalior	4.8	8.3	18.6	11.3	45.1	37.5
JM-1	RVSKVV, Gwalior	5.3	12.3	18.9	10.4	46.2	40.5
JM-3	RVSKVV, Gwalior	7.5	14.5	19.7	11.2	41.1	38.5
Rohini	CSAUAT, Kanpur	4.0	16.0	16.8	9.7	37.8	40.5
Maya	CSAUAT, Kanpur	4.5	6.1	15.0	10.1	51.4	38.5
GM-2	SDAU,Banaskantha	5.5	12.0	20.6	12.9	41.0	39.5
L-4	Canada	10.7	12.5	31.2	14.0	24.2	33.0
L-6	Canada	6.1	11.1	22.7	11.6	36.9	33.5
CS-54	CSSRI, Karnal	5.9	12.9	21.7	12.1	41.4	40.5
RB-50	CCS, Hisar	7.4	11.3	25.6	13.0	35.8	40.5
RH-74.9	CCS, Hisar	5.1	14.1	23.7	10.0	42.3	39.5
MC-25	Rasi seed company	7.3	13.4	24.3	11.7	29.9	29.5
ISC-3	Rasi seed company	9.2	13.4	29.0	11.0	30.2	33.5
ISC-12	Rasi seed company	12.2	14.8	37.3	10.4	19.6	31.5
ISC-17	Rasi seed company	11.0	15.1	31.7	11.3	24.8	33.5
ISC-18	Rasi seed company	7.4	37.1	32.8	11.4	27.0	33.5
ISC-20	Rasi seed company	7.9	13.3	25.2	9.9	32.7	33.5
ISC-23	Rasi seed company	8.7	16.0	24.8	9.0	34.0	35.5
JD-6	IARI, New Delhi	5.6	9.8	20.3	11.2	31.7	39.5
PM-25	IARI, New Delhi	8.6	8.8	28.2	15.3	1.0	38.5
PM-26	IARI, New Delhi	6.3	10.1	25.2	9.8	1.6	38.5
PM-28	IARI, New Delhi	12.1	9.2	28.8	15.3	1.1	40.5
PM-21	IARI, New Delhi	10.0	12.1	30.8	13.1	1.1	35.5
PM-22	IARI, New Delhi	6.9	11.8	36.2	11.1	1.1	35.5
PM-24	IARI, New Delhi	6.9	22.8	31.0	14.0	1.2	34.5
PM-29	IARI, New Delhi	6.2	21.6	32.6	9.6	0.9	36.5
PM-30	IARI, New Delhi	5.5	25.6	26.4	13.3	1.1	36.5
Karishma	IARI, New Delhi	7.4	23.2	35.9	16.8	1.2	37.5
Mean		6.82	15.36	24.01	11.34	30.99	34.68
Sem		0.163	0.214	0.410	0.270	0.299	0.535
CV		0.471	0.619	1.186	0.780	0.863	0.0081
$CD_{0.05}$		4.792	2.790	3.421	4.761	1.931	1.528

The oleic acid content was differed in range of 6.06 to 37.1% with a mean value of 15.36%. Comparable findings concerning range of oleic acid have been reported previous [46,38,40,41,42] and [46]. In respect of genotypic difference, genotype ISC-18 formed maximum oleic acid (37.1%) tracked by genotypes MRNJ-30 (32.21%), MRNJ-25 (26.82%) and PM30 (25.6%) whereas, minimum was recorded in genotype RVM-1 (6.06%). Higher concentrations of oleic acid are elegant nutritionally authoritative for human diet since it advances the strength of High-density lipoproteins and declines low density lipoproteins in human body. Moreover, raised oleic acid constituent in seed oil delivers thermo-stability and makes it suitable for food preparation [47]. Oleic acid not only playing a noteworthy part in increasing the ability of cooking oil but also better seed oil for industrial purposes [48].

Linoleic and linolenic acids are omega-6 and omega-3 fatty acid congruently. Both of these are well-thought-out as poly-unsaturated fatty acids. These are principally significant fatty acids as human body cannot produce them and they must be taken from food ingredients. Linoleic acid fluctuated between 14.9 to 37.33%, with an average worth of 24.01%. Among all investigated genotypes, genotype ISC-12 displayed the highest linoleic acid percent (37.33%) lagged by genotype PM22 (36.2%) while, the lowest magnitude was displayed by genotype Maya (14.97%). Reports are

available on the consequence of the ingesting of high linoleic acid eatable oil on blood cholesterol and anticipation from atherosclerosis [49]. Dupont et al. [50] described the part of linoleic acid on maintenance of the decency of the skin, cell membranes and the immune system. Reports are also available on damaging properties of ingesting of high linoleic acid on human health due to its role as an enhancer of inflammation. Data are also stayed on their role in cancer growth [51,52]. Nevertheless, more reliable researches are needed to approve the conceivable role of linoleic acid as a causative agent of cancer. The exclusion of *trans* fatty acids from several food substances has obliquely generated in the enhancement of oil seed cultivars [53]. Linolenic acid arrayed between 8.29 to 16.75% with a mean worth of 11.34%. Likewise, Lavkopr et al. [54] found 3.3 - 13.1% linolenic acid in *Brassica* cultivars during their investigation. Joughi et al. [41] and Shyam and Tripathi [43] have also been reported almost parallel range of linolenic acid among various *Brassica* genotypes. In our investigation, maximum linolenic acid was recorded in genotype LES-39 (16.75%) tracked by genotypes PM28 (15.3%) and PM-25 (15.25%) whereas, the lowest worth (8.29%) was demonstrated by genotype MRNJ-42. Low linolenic acid comprehending canola cultivars have been found superior in terms of storage and frying constancy. Linolenic acid is known as a meaningfully imperative fatty acid but its incidence may be a motive of off flavor and rancidity of the oil [40]. Linoleic and linolenic acid usually play central role in the amalgamation of membrane lipids. They are also the precursor of prostaglandins, thromboxanes and leukotrienes which are important signaling molecules. The bigger numbers of double bonds speed up the oxidation progression and oils rich in linoleic and linolenic acid decline quickly upon connection to air at raised temperatures, with a reduction of quality for human feeding [55].

Erucic acid belongs to monosaturated fatty acid which is a restrictive factor of mustard seed oil for human along with animal attributable to their indigestibility in both. Sometimes erucic acid also works as a toxic material [56]. In the current investigation, erucic acid content speckled between 0.92 to 51.44% with an average worth of 30.99%. Among investigated Indian mustard genotypes, the highest level of erucic acid was recognized in genotype Maya (51.44%) convinced by genotypes JM-1 (46.24%), RVM-2 (45.06%) and IDM-41 (43.75%) whereas, PM29 had minimum erucic acid value. Alike to current study, Kumar et al. [57] reported the range of percentage of erucic acid between 0.5-57.3 in the genotypes of *B. juncea*. Nevertheless, range of erucic acid contents was found to be high (35.7-51.4) in Indian mustard varieties [58]. Mustard varieties with optimum level of erucic acid could be established with the help of breeding methods. Some of the mustard cultivars with optimal level of erucic acid are under cultivation in several nations [59]. Mustard oil rich in erucic acid is considered offensive for feeding in human diet owing to its anti-nutritional possessions. It has been also conveyed as a causative agent of lipidosis in children. However, mustard oil rich in erucic acid are favored to produce numerous commercial foodstuffs for instance wonderful eminence lubricants, emulsifiers, plastics, textile softeners, coatings, biodiesel and surfactants [60]. Energies are also in advancement for the expansion of low erucic acid (less than 2%) encompassing *B. juncea* cultivars with the aid of breeding tactics to take the Indian mustard cultivars in the group of international quality norms. For the submission of breeding method, the evidence on number of genes and their inheritance that switch level of erucic acid in mustard is pre-requisite. Formerly, Saini et al. [61] confirmed the role of two genes controlling erucic acid level in *B. juncea*. In our investigation, oil of nine genotypes *viz*., PM-25 (1.00), PM-26 (1.55), PM-28 (1.14), PM-21 (1.05), PM-22 (1.13), PM-24 (1.18), PM-29 (0.93), PM-30 (1.08) and Karishma (1.24) had less than 2 per cent erucic acid proportion. While, fifty-seven genotypes displayed moderate erucic acid content having 2 to 30 %. However, 122 genotypes had high erucic acid content greater than 30%. Prior, Saini et al. [61] and Rai et al. [44] also reported diversity in erucic acid content among Indian mustard genotypes.

Oil percentage may be given weightage to *Brassica* seeds only when if they have greater nutritional qualities like better blend of saturated and unsaturated fatty acids. In our research, oil percentage ranged between 20.5 to 40.5% with a mean worth of 34.68%. In a analogous investigation conducted by Tahira et al. [4], range of oil contents was investigated between 22.02 to 41.69% in *B. juncea* genotypes of Pakistan. Parallel range (27.9 to 41.72%) of oil contents in *B. juncea* have been evidenced by Mandal et al. [62]. In the current study, genotypes NRCDR-2, JM-1, Rohini, CS-54, RB-50 and PM28 exhibited the highest oil percentages followed by genotypes GM-2, RH74.9 and JD6 (39.5) while MRNJ98 (20.5%) exhibited minimum oil percentage. The differences in oil content may be due to the variations in genetic constituents of genotypes along with environmental influences. The verdicts of the current research highlight prodigious potentials for the selection of investigated

genotypes as parents in future breeding scheme owing to higher variability in oil contents [63]. Nevertheless, in a previous report low variability for oil content was also reported among Indian mustard genotypes [58].

3.2 Variation in Genetic Parameters

Before starting a hybridization programme, a plant breeder desires to choose a sketch of work and for this it is essential to judge genetic parameters of target crop with diverse statistical characteristics like genetic advance, coefficient of variation and heritability. In the present research, several of the embattled parameters had high proportion of genetic variation with progressive standards of variation of genotypic coefficient. Oleic acid had highest GCV and PCV (Table 2) tracked by erucic and palmitic acids. Whereas, linoleic and linolenic acids had moderate GCV and PCV values while, oil percentage displayed very low worth for both. Previous researches [20,38] carried out on mustard genotypes displayed comparable inclinations of GCV and PCV values. The broad sense heritability estimations for erucic acid (99.51) had owned higher heritability followed by oleic (99.05), palmitic (95.95), linoleic (95.63) and linolenic acids (86.94). Analogous statements have also been documented former by Khan et al. [46] as they documented maximum heritability for oleic and erucic acid whereas moderate for oil percentage. The ratio of genotypic to phenotypic variance is termed the heritability and if its expression is in the form of genetic advance, it is considered more beneficial for the investigations. In our research, genetic advance was found to be higher for oleic acid (58.28) trailed by erucic acid (56.71) nevertheless, it was evidenced to be low for oil percentage (8.77). In few other investigations, relative consequences of higher genetic advance were evidenced for oleic and linolenic acids [58, 38] while, Ali et al. [20] evidenced low genetic advance for oil percentage.

Among all targeted biochemical traits, palmitic acid exhibited significant positive correlation (Table 3) with linoleic and linolenic acid whereas, significant negative correlation with erucic and oleic acid after evaluation of genotypic and phenotypic correlation coefficients. In a preceding study, Tahira et al. [4] demonstrated comparable correlation of palmitic acid with linolenic acid. However, in opposite to our results, they documented positive relation of palmitic acid with oleic acid whereas they evidenced that palmitic acid had significant negative correlation with erucic acid which is parallel to our findings. The similar correlation between palmitic acid and erucic acid was documented by Chauhan et al. [58] also. Furthermore, oleic acid exhibited significant negative correlation with linolenic acid and erucic acid. The analogous correlation for oleic acid content with linolenic and erucic acid contents was evidenced by Kumar et al [57] but, they documented negative correlation of oleic acid content with linoleic acid as well. Our verdicts designate that linoleic and linolenic acids both had significant negative correlations with erucic acid whereas linoleic acid examined significant positive correlation with linolenic acid.

Table 2. Genetic parameters of variations for biochemical parameters

Parameters	Mean	Range		PCV (%)	GCV (%)	h^2	GA	GA (%)
		Min.	Max.					
Palmitic acid (%)	6.82	3.94	13.27	23.74	23.25	95.95	3.20	46.92
Oleic acid (%)	15.37	6.07	37.10	28.56	28.43	99.05	8.96	58.28
Linoleic acid (%)	24.01	14.97	37.34	16.36	16.00	95.63	7.74	32.24
Linolenic acid (%)	11.35	8.29	16.75	13.18	12.29	86.94	2.68	23.60
Erucic acid (%)	30.99	0.93	51.44	27.66	27.60	99.51	17.58	56.71
Oil (%)	34.76	29.50	41.00	8.32	5.95	51.18	3.04	8.77

During present investigation, erucic acid content was measured as dependent variable whereas path coefficient analysis was performed. The analysis (Table 4) designated that palmitic acid had maximum positive direct effect on erucic acid, while highest negative direct effect on erucic acid was imposed by linoeic acid followed by oleic and linolenic acids along with oil percentage. The traits having significant correlation counts with erucic acid were only considered for the analysis of the indirect effect. Palmitic acid exhibited maximum positive indirect effect *via* oleic acid and oil percentage, while negative indirect effect *via* linoeic and linolenic acid. Oleic acid presented maximum positive indirect effect *via* linolenic acid and oil percentage whereas negative indirect effect by means of palmitic and linoeic acid. Linoeic acid displayed maximum positive indirect effect through

palmitic acid and oil percentage whereas, negative indirect effect through oleic and linolenic acid. In previous research carried out by Tahira et al. [4] on mustard genotypes, genotypic path coefficient analysis exposed positive direct effect of palmitic acid and negative direct effect of oil percentage, oleic and linoleic acid on erucic acid. In the comparable means, during phenotypic path coefficient analysis, negative direct effect of oleic and linoleic acid on erucic acid was evidenced [58]. In the principal component examination, the first principal component had donated 41.19 % of the total for the biochemical traits with linoleic, palmitic, linolenic and oleic acids. Second principal component assessed 20.38 % disparities and third component described for 17.13 % dissimilarities. The entire involvement of fourth principal components was 13.41 %, counting palmitic, oleic and linoleic acids with oil percentage and erucic acid (Table 5). Fifth principal component donated a total of 5.57 % variation and sixth component elucidated 2.29 % of the total difference.

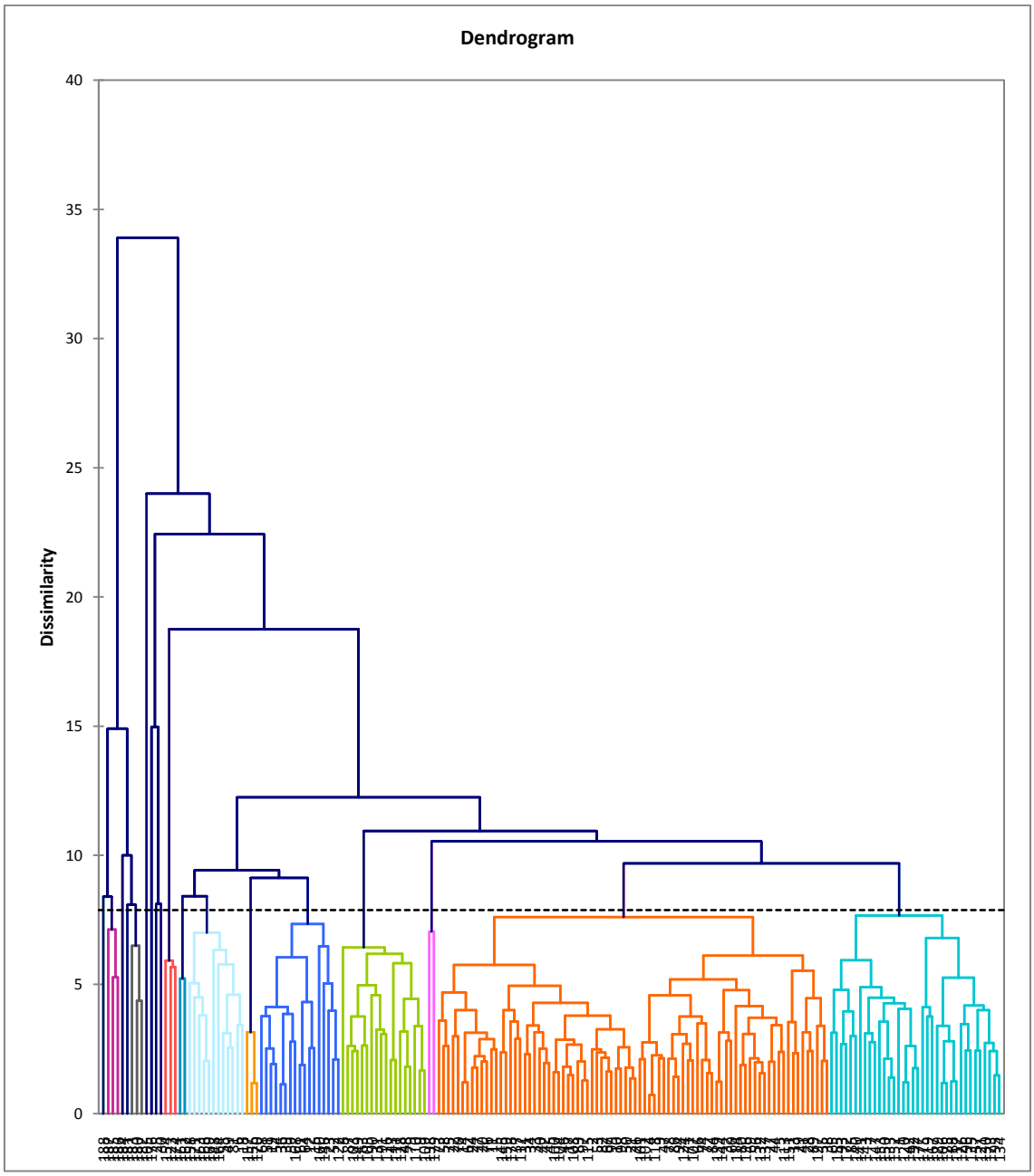


Fig. 1. Dendrogram of 188 Indian mustard genotypes based on biochemical traits using XLSTAT software

Table 3. Genotypic and phenotypic correlation coefficient for biochemical parameters

Parameter	Palmitic acid (%)		Oleic acid (%)		Linoeic acid (%)		Linolenic acid (%)		Erucic acid (%)		Oil (%)	
	GCC	PCC	GCC	PCC	GCC	PCC	GCC	PCC	GCC	PCC	GCC	PCC
Palmitic acid (%)	1	1										
Oleic acid (%)	-0.173**	-0.170**	1	1								
Linoeic acid (%)	0.798**	0.768**	0.053	0.05	1	1						
Linolenic acid (%)	0.248**	0.224**	-0.117*	-0.110*	0.381**	0.354**	1	1				
Erucic acid (%)	-0.402**	-0.394**	-0.325**	-0.322**	-0.685**	-0.669**	-0.390**	-0.365**	1	1		
Oil (%)	-0.094	-0.063	-0.011	-0.008	-0.106*	-0.064	0.073	0.03	-0.037	-0.027	1	1

Table 4. Genotypic path-coefficient direct and indirect effects of various biochemical parameters on erucic acid

Parameters	Palmitic acid (%)	Oleic acid (%)	Linoeic acid (%)	Linolenic acid (%)	Oil (%)	Correlation
Genotypic path-coefficient						
Palmitic acid (%)	0.213	0.046	-0.628	-0.042	0.009	-0.402**
Oleic acid (%)	-0.037	-0.267	-0.041	0.020	0.001	-0.325**
Linoeic acid (%)	0.170	-0.014	-0.787	-0.064	0.010	-0.685**
Linolenic acid (%)	0.053	0.031	-0.300	-0.168	-0.007	-0.390**
Oil (%)	-0.020	0.003	0.084	-0.012	-0.092	-0.037
Phenotypic path-coefficient						
Palmitic acid (%)	0.124	0.049	-0.530	-0.040	0.004	-0.394**
Oleic acid (%)	-0.021	-0.286	-0.035	0.020	0.0005	-0.322**
Linoeic acid (%)	0.095	-0.014	-0.691	-0.063	0.004	-0.669**
Linolenic acid (%)	0.028	0.031	-0.244	-0.178	-0.002	-0.365**
Oil (%)	-0.008	0.002	0.044	-0.005	-0.061	-0.027

Table 5. Principal components for biochemical parameters

Traits	PC1	PC2	P 3	PC4	PC5	PC6
Palmitic acid	0.506	0.275	-0.217	0.405	0.401	-0.545
Oleic acid	0.047	-0.857	0.104	0.035	0.499	-0.037
Linoeic acid	0.595	0.012	-0.111	0.196	0.031	0.771
Linolenic acid	0.356	0.202	0.256	-0.816	0.314	-0.046
Oil percentage	-0.006	0.170	0.915	0.355	0.080	0.040
Erucic acid	-0.511	0.346	-0.166	0.060	0.696	0.322

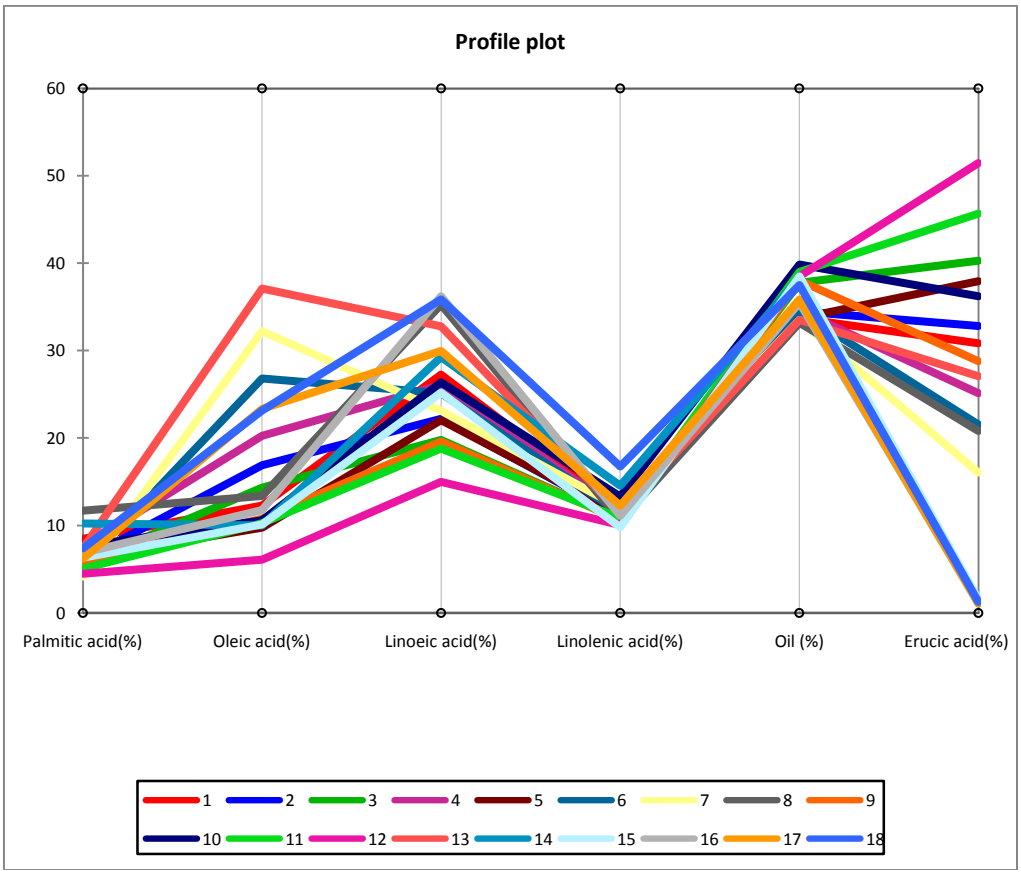


Fig. 2. Profile plot of 188 Indian mustard genotypes based on different biochemical traits

Table 6. Grouping of 188 Indian mustard genotypes in various clusters

Cluster number	No. of genotypes	Name of genotypes
1	36	MRNJ1, MRNJ7, MRNJ-59, MRNJ-67, MRNJ-87, MRNJ-90, MRNJ-94, MRNJ-95, MRNJ-97, MRNJ-98, MRNJ-108, MRNJ-120, MRNJ-122, MRNJ-123, MRNJ-124, MRNJ-128, MRNJ-129, MRNJ-135, MRNJ-136, MRNJ-139, MRNJ-140, MRNJ-142, IDM-8, IDM-10, IDM-11, IDM-12, IDM-31, IDM-53, IDM-64, IDM-66, IDM-69, L-4, MC-25, ISC-3, ISC-17 and ISC-20
2	82	MRNJ2, MRNJ3, MRNJ4, MRNJ5, MRNJ10, MRNJ11, MRNJ13, MRNJ14, MRNJ15, MRNJ17, MRNJ20, MRNJ21, MRNJ-22, MRNJ-24, MRNJ-26, MRNJ-27, MRNJ-28, MRNJ-29, MRNJ-33, MRNJ-34, MRNJ-35, MRNJ-36, MRNJ-37, MRNJ-39, MRNJ-40, MRNJ-42, MRNJ-43, MRNJ-44, MRNJ-46, MRNJ-47, MRNJ-48, MRNJ-49, MRNJ-52, MRNJ-55, MRNJ-58, MRNJ-60, MRNJ-62, MRNJ-64, MRNJ-68, MRNJ-69, MRNJ-71, MRNJ-72, MRNJ-73, MRNJ-74, MRNJ-75, MRNJ-76, MRNJ-77, MRNJ-78, MRNJ-79, MRNJ-80, MRNJ-82, MRNJ-84, MRNJ-85, MRNJ-86, MRNJ-88, MRNJ-91, MRNJ-96, MRNJ-99, MRNJ-100, MRNJ-101, MRNJ-102, MRNJ-103, MRNJ-104, MRNJ-106, MRNJ-109, MRNJ-110, MRNJ-115, MRNJ-116, MRNJ-117, MRNJ-118, MRNJ-121, MRNJ-127, MRNJ-130, MRNJ-138, MRNJ-143, MRNJ-144, IDM-2, IDM-15, IDM-16, IDM-58, DRMR-150-35 and ISC-23
3	12	MRNJ6, MRNJ18, MRNJ-51, MRNJ-57, MRNJ-83, IDM-41, DRMRIJ-31, JM-3, Rohini, GM-2, CS-54 and RH-749
4	18	MRNJ8, MRNJ9, MRNJ-45, MRNJ-50, MRNJ-65, MRNJ-81, MRNJ-89, MRNJ-92, MRNJ-93, MRNJ-105, MRNJ-107, MRNJ-111, MRNJ-113, MRNJ-114, MRNJ-119, MRNJ-131, MRNJ-137 and IDM-42
5	17	MRNJ12, MRNJ16, MRNJ19, MRNJ-23, MRNJ-38, MRNJ-41, MRNJ-53, MRNJ-54, MRNJ-56, MRNJ-61, MRNJ-63, MRNJ-66, MRNJ-70, MRNJ-125, IDM-25, RVM-1 and L-6
6	1	MRNJ-25
7	1	MRNJ-30
8	3	MRNJ-126, IDM-67 and ISC-12
9	2	MRNJ-145 and JD-6
10	3	NRCDR-2, NRCHB-101 and RB-50
11	2	RVM-2 and JM-1
12	1	Maya
13	1	ISC-18
14	3	PM-25, PM-28 and PM-21
15	1	PM-26
16	1	PM-22
17	3	PM-24, PM-29 and PM-30
18	1	Karishma

Cluster analysis gathered the investigated 188 Indian mustard genotypes into 18 diverse bunches (Table 6, Fig. 1). Cluster 2 had highest genotypes (82), followed by cluster 1 (36), cluster 4 (18), cluster 5 (17), cluster 3 (12), cluster 8, 10, 14 and 17 (3 genotypes each), cluster 9 and 11 (2 genotypes each) and cluster 6, 7, 12, 13, 15, 16 and 18 (1 genotype each). Just, few investigators evidenced parallel clustering of mustard genotypes [13-15] and other crop species depending up on morphological [64], biochemical [65-71] and molecular markers-based investigations [72-88]. The highest cluster mean value (11.70) for palmitic acid was evidenced in cluster 8 (Table 7) and lowest in cluster 7 (4.16). For oleic acid, cluster 13 had highest value (37.10), while it was lowest in cluster 12 (6.08). The highest and lowest worth for linoleic acid were evidenced in cluster 16 (36.20) and cluster 12 (14.97) correspondingly, whilst the highest and lowest cluster means were recognized for linolenic acid in cluster18 (16.75) and cluster 15 (9.80) correspondingly. Profile of biochemical parameters and scatter plot of grouping of genotypes have been presented (Fig. 2 and Fig. 3). Cluster 10 exhibited highest cluster mean (39.83) for oil percentage and lowest value (33.17) oil percentage was evidenced in the cluster 8. For erucic acid, highest value (51.44) was in cluster 12 and lowest in cluster 17 (1.06). Inter cluster distance (Table 8) is imperative to choose the genotypes as parents for upcoming breeding scheme. During present investigation, the highest distance (57.46) was evidenced between cluster 18 and cluster 12 tracked between cluster 17 and cluster 12 (55.45), cluster 16 and cluster 12 (55.04), cluster 14 and cluster 12 (53.02), cluster 15 and cluster 12 (51.12),

cluster 18 and cluster 11 (49.72), cluster 16 and cluster 11 (47.99), cluster 17 and cluster 11 (47.93), cluster 14 and cluster 11 (46.25) and cluster 12 and cluster 7 (45.06). However, the lowest value (6.95) of inter cluster distance was confirmed between clusters 3 and 11.

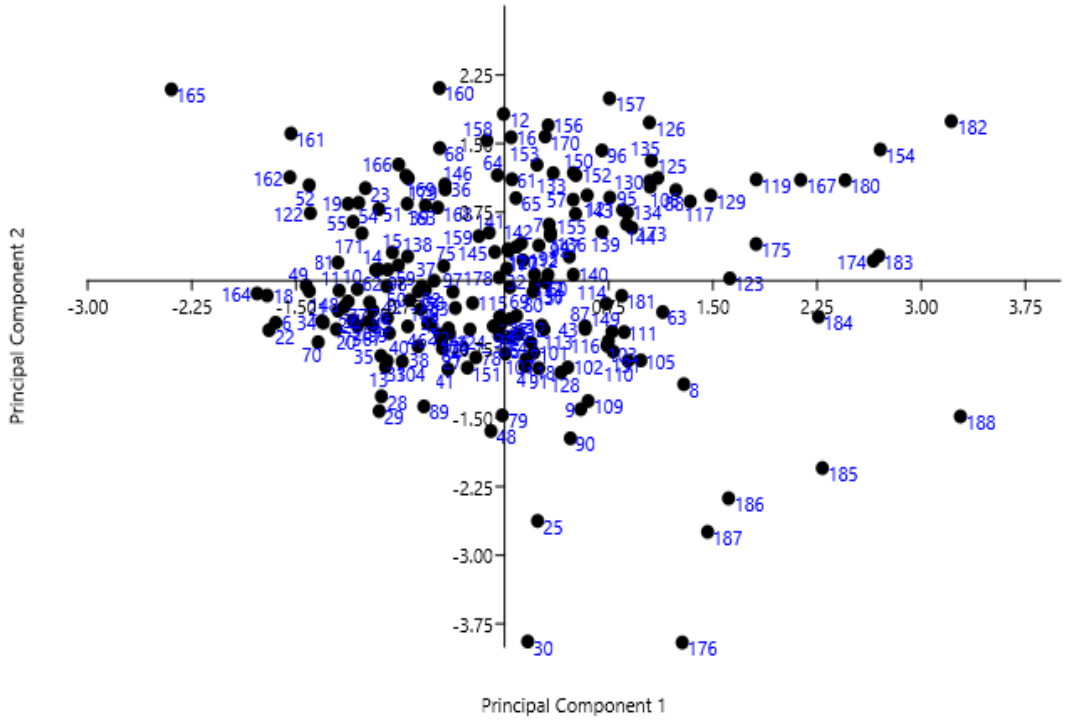


Fig. 3. Scatter plot of the genetic relationship among 188 *Brassica juncea L.* genotypes as revealed by first and second principal components

Table 7. Mean performance of individual clusters for 6 biochemical parameters

Class	Palmitic acid (%)	Oleic acid (%)	Linoeic acid (%)	Linolenic acid (%)	Oil (%)	Erucic acid (%)
1	8.46	12.26	27.23	11.70	33.69	30.84
2	6.06	16.89	22.20	11.07	34.49	32.81
3	5.59	14.32	19.77	10.66	37.75	40.27
4	7.59	20.25	26.08	11.52	34.56	25.10
5	6.26	9.74	22.04	11.29	33.71	37.92
6	5.26	26.82	25.13	11.39	34.50	21.53
7	4.16	32.22	23.11	12.03	33.50	16.05
8	11.70	13.39	35.25	10.58	33.17	20.82
9	5.86	10.78	19.59	10.60	38.00	28.78
10	7.61	10.57	26.37	13.31	39.83	36.23
11	5.05	10.27	18.79	10.86	39.00	45.65
12	4.49	6.08	14.97	10.06	38.50	51.44
13	7.37	37.10	32.79	11.45	33.50	27.05
14	10.21	10.02	29.27	14.55	38.33	1.06
15	6.30	10.10	25.20	9.80	38.50	1.55
16	6.85	11.75	36.20	11.10	35.50	1.13
17	6.20	23.32	29.95	12.28	35.83	1.06
18	7.35	23.15	35.85	16.75	37.50	1.24

Table 8. Inter and intra-cluster distance of 188 genotypes

Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0.00	7.58	13.21	9.97	9.41	17.72	25.55	13.34	9.56	8.58	18.31	25.58	25.76	30.47	29.97	31.14	32.04	33.32
2		0.00	8.90	9.39	8.84	15.33	22.84	18.98	8.54	10.25	15.55	23.15	23.59	33.90	32.40	35.04	33.35	35.57
3			0.00	17.88	6.99	23.39	30.66	26.02	12.03	9.45	6.95	14.76	29.75	41.02	39.34	42.60	41.58	43.59
4				0.00	17.13	7.90	15.72	13.01	12.69	15.76	24.53	32.33	18.28	26.89	26.05	27.40	24.63	26.64
5					0.00	23.91	31.46	22.60	10.48	8.11	10.02	16.55	31.37	38.19	36.85	39.52	40.14	41.97
6						0.00	8.11	18.09	18.81	22.79	30.27	38.03	14.15	27.71	26.43	27.74	21.40	24.13
7							0.00	24.16	25.67	30.66	37.53	45.06	15.79	28.67	27.17	28.72	18.99	22.64
8								0.00	19.31	19.82	31.24	38.51	25.03	21.96	23.27	20.51	23.61	23.56
9									0.00	10.74	16.95	23.65	29.89	29.95	27.83	32.38	32.26	34.88
10										0.00	12.64	20.09	29.55	35.44	34.93	36.80	37.83	38.60
11											0.00	8.17	36.03	46.25	44.60	47.99	47.93	49.72
12												0.00	43.71	53.02	51.12	55.04	55.45	57.46
13													0.00	38.24	38.28	36.47	29.68	30.24
14														0.00	7.40	9.07	14.31	15.15
15															0.00	11.61	14.52	18.28
16																0.00	13.22	12.89
17																	0.00	7.68
18																		0.00

4. CONCLUSIONS

In supposition, the Indian mustard genotypes disclosed higher genetic erraticism for the levels of investigated biochemical traits. In forthcoming days, if our target is to tailor Indian mustard genotypes with superior oil quality then we should focus to increase the oleic acid to more than 50 percent, linoleic and linolenic acid below 40% and 14% correspondingly. Cluster analysis delivers the information about inter cluster distance and maximum inter cluster distance was evidenced between cluster 18 (Karishma) and cluster 12 (Maya). Subsequently, these genotypes can be considered as parents in mustard breeding. Owing to pattern attained for genetic variability among the investigated genotypes for diverse investigated parameters, it is determined that low erucic acid encompassing genotypes may be established through either direct selection or by hybridization method with low erucic acid possessing genotypes as parent. The incidence of variability among a huge set of Indian mustard genotypes demonstrating great options of the enhancement aiming nutritional along with industrial significance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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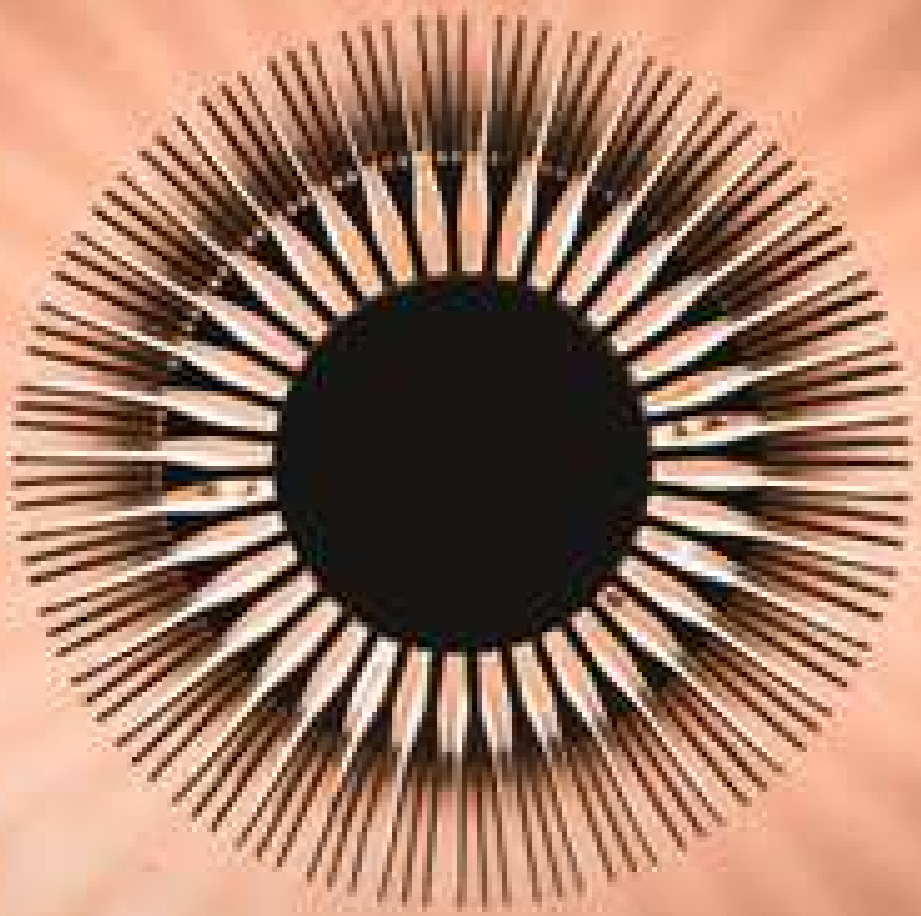
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Characterization of Soybean Genotypes on the Basis of Yield Attributing Traits and SSR Molecular Markers

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ABSTRACT

Soybean is well-thought-out to be a main crop as an important foundation of nutrients to humans and animals. The current investigation has been executed to recognize different soybean genotypes on account of diverse morpho-physiological traits and SSR molecular markers. Data for different morpho-physiological traits were documented from experiment conducted under field conditions in RBD design whereas molecular work was conducted in laboratory with 32 microsatellite markers to analyze the existence of possible diversity among different soybean genotypes. Morpho-physiological investigation evidenced the incidence of substantial magnitude of variability. Phylogenetic tree based on morpho-physiological traits grouped the genotypes into major and minor cluster. Major cluster had fifty genotypes while minor cluster had only three genotypes. Among polymorphic 32 microsatellite markers, the highest genetic diversity (0.66) was documented for the marker Satt520 whilst lowest (0.037) for the marker Satt557 with an average of 0.35. The highest PIC value also was 0.59 prearranged by same marker *viz*., Satt520 and lowest 0.036 by marker Satt557. An average major allele frequency was 0.69 while, an average PIC value was 0.32. Microsatellite markers-based data also congregated the genotypes into one major and one minor cluster. Molecular analysis based on microsatellite markers confirms the presence of genetic variability among genotypes under the investigation. Data obtained from the present research may contribute towards improvement of soybean genotypes to advance high yielding varieties by considering assorted genotypes with good agronomical traits in breeding scheme.

Keywords: Breeding; genetic diversity microsatellites; sustainable agriculture; soybean; variability.

1. STUDY OBJECTIVES

The present study was conducted to characterize different soybean genotypes on the basis of different morpho-physiological traits and SSR molecular markers.

2. INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is among one of the major crops, disbursed as human foods as well as animal feed [1-6]. It is also an ironic foundation of indispensable amino acids in addition to oil. The role of soybean and its components as therapeutic agents, antioxidants, isoflavones *etc*. has been recognized. Multipurpose nature of soybean makes it valuable in the field of industrial formulations, agriculture sector and pharmaceuticals [7-13]. Due to its high protein content, its flour is commonly

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employed to complement the nutritional worth of cereal flours lacking perilous amino acids like sorghum and maize [14-19]. This designates significant upsurge in demands of soybean in the future owing to rapid growth in the population of India along with the world. This demand can be contented with high yielding varieties and this can be achieved through hybridization of genetically dissimilar parental genotypes.

A random mating population base with extensive genetic diversity is required to breed new varieties. Soybean has narrow genetic base due to its self-pollinating nature [20]. Plant breeders have found exertion in creation of genetic variation through hybridization procedure in soybean owing to small size and fragile nature of flowers. This makes the emasculation process of flower very tough [21]. The level of genetic variability present between and among developed elite genetic resources and commercialized soybean varieties has not been done properly so far. As a result, determining genetic diversity among released varieties and elite genotypes is critical for guaranteeing proficient selection and proposal of diverse lines as parents for expansion, commercialization and future breeding drives to get better soybean yield, quality along with pest and disease resistance [22-29].

Knowledge of the genetic diversity among and between existing soybean genotypes can help breeders to comprehend the structure of germplasm lines and prediction of best parental combinations [27]. This estimate may be proved appropriate to accomplish the goal of high yield along with augmentation of genetic variability of breeding stock for the selection in future. The developed or released variety should also be genetically diverse from the existing varieties of the same crop. Assessment of genetic variability also aids the breeders in crop variety protection [28].

The employment of morphological and agronomic parameters, isozymes, pedigree data and molecular markers have all been done to judge genetic diversity among different soybean accessions [1,12, 25-28]. Moreover, the application of pedigree information is constrained by uncertain or mislaid data along with prospect of data entry errors. Because the usage of isozymes confines the amount of data accessible, DNA markers could be employed in genetic diversity investigations for more accurate information [29].

Morpho-physiological features have been used to analyze variability and relatedness between and among crop varieties and genetic stocks. Nevertheless, molecular markers have been testified as reliable tools in an array of crop species including soybean [30-46]. Numerous molecular markers have been employed to illustrate soybean genotype(s) as they are usually free from environmental conditions and bargain a new dimension, exactness and brilliance in confession of germplasm line(s). An array of molecular markers *viz.*, Random Amplified Polymorphic DNA, Inter Simple Sequence Repeats, Amplified Fragment Length Polymorphism and Simple Sequence Repeat have been engaging to study genetic diversity in soybean [47] [21-28]. Among all the revealed markers, SSRs have been widely employed in plants [27, 36-46] owing to their higher level of polymorphisms, higher polymorphic information content (PIC), co-dominant inheritance and distribution in the entire genome [1, 32-33]. Use of morphological characteristics and molecular markers in the detection of inherited variability is essential for the managing crop genetic stocks [39-40]. The current investigation was accomplished to screen soybean genotypes on the basis of yield attributing traits and microsatellite molecular markers.

3. MATERIALS AND METHODS

The current study was confined 53 *Glycine max* (L.) Merrill genotypes (Table 1). The seeds were acquired from College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur and Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, India. The field experiment was conducted at the experimental field, Department of Plant Molecular Biology & Biotechnology and the laboratory work at Plant Molecular Biology Laboratory, Department of Plant Molecular Biology & Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, India during Kharif 2018-19. Crop was sown on dated 27th July 2018. The experiment was designated in Randomized Block Design in three rows and two replications and row to row distance was kept 30 cm. Fertilizer was applied in the ratio of 20 N: 60 P_2O_5: 20 K_2O:20S kgha^{-1}.

Table 1. List of soybean genotypes with their parentage

S No	Genotypes	Source/Pedigree	S No	Genotypes	Source/Pedigree
1.	JS 20-29	JS 97-52 x JS 95-56	28	RSC-10-52	NRC37 x JS335
2.	JS 20-69	JS 97-52 x SL 710	29	SL -1123	Selection from AGS751
3.	JS 335	JS 78-77 x JS 71-05	30	SL-1068	SL755 x SL525
4.	JS 20-98	JS 97-52 x JS SL710	31	AGS 111	Germplasm accession
5.	JS 20-94	JS 97-52 x JS 20-02	32	EC457286	Germplasm accession
6.	JS 93-05	Selection from PS 73-22	33	MACS725	JS93-05 x MAUS71
7.	JS 20-116	JS 97-52 x JSM 120 A	34	SP 37	Not known selection
8.	JS 95-60	Selection from PS 73-22	35	NRC -125	EC54688 x ps1044
9.	JS 97-52	PK 327 x L 129	36	NRC-132	JS97-52 x PI086023
10.	JS 20-84	JS 98-63 x PK 768	37	NRC-134	NRC7 x AGS191
11.	JS 20-34	JS 98-63 x PK 768	38	NRC SL-1	JS335 x SL525
12.	JS 20-71	JS 97-52 x JS 90-5-12-1	39	PS 1092	PS1042 x MACS 450
13.	RVS 2007-6	JS 20-10 x MAUS162	40	PS 1613	PS1225 x PS1042
14.	RVS 2011-35	JS 335 x PK 1042	41	AMS 2014-1	AMS99-33 x H6P5
15.	RVS 2001-4	JS 93-01 x EC 390981	42	KDS 992	JS93-05 x EC241780
16.	RVS -14	JS 93-05 x EC 390981	43	VLS -94	VL Soya59 x VS2005-1
17.	RVS -24	J P 120 x JS 335	44	SKF-SPS -11	Not known selection
18.	RVS -18	JSM110 x JSM66	45	RVS 76	MAUS-162 x JSM-66
19.	NRC- 76	NRC-37 x L-27	46	NRC127	JS97-52 x PI542044
20.	NRC -86	RKS15 x EC481309	47	KDS980	JS93-05 x AMS1
21.	NRC- 130	EC390977 x EC538828	48	G-29	Germplasm
22.	NRC -131	EC390977 x EC538828	49	RSC-10-70	JS335 x Bragg
23.	NRC -147	Germplasm accessions C210	50	RSC-10-71	Bragg x JS335
24.	AMSMBC -18	Mutant of Bragg	51	NRC-2	Induced mutant of Bragg
25.	AMS-100-39	Mutant of JS93-05	52	MACS-15-20	NRC37 x Mohetta
26.	MACS – 1520	EC241780 x MACS330	53	MACS-58	JS2 x Improve pelican
27.	MACSNRC-1575	PI542044 x JS9305			

3.1 Morpho-physiological Parameters

Leaf area of three leaves of each experimental plant was measured by Automatic Leaf Area Meter in cm^2 at a 30 days interval from 30 DAS to 60 DAS.The mean numbers of five randomly selected plants were recognized for numbers of primary brances per plant. Number(s) of days required for beginning of flowering was considered from the date of sowing for days to flowering. Numbers of days needed for 50 per cent of the plants to flower taken for 50 % flowering observation. Total number of seeds of 5 uprooted plants from each plot was documented and then the mean number of seeds per plant was calculated for numbers of seeds per plant. Total weight of plants with pods of 5 uprooted plants from each plot were recorded and then the average weight per plot was computed for weight of plant with pod. The pods harvested from five observations threshed discretely and grain yield was documented and the mean was worked out. Hundred-seed weight (SW) recorded based on randomly chosen five plants of each plot by counting weight and averaging of 100 seeds. The weight of all harvested plant parts of 5 observational plants was recorded before threshing including the dry weight of leaves, stems and pods. Then the average biomass per plant was computed. The data were analysed as suggested by Snedecor and Cochran [48].

3.2 SSR Molecular Marker Analysis

Forty-eight simple sequence repeats primer pairs, distributed diagonally the amalgamated linkage map of soybean [49] were acquired from Imperial Life Sciences Pvt. Ltd, Gurgaon, Haryana, India. Genomic DNA was extracted from young leaves of 10 plants from each genotype perusing the Cetyl Trimethyl Ammonium Bromide (CTAB) method as suggested by Saghai-Maroof et al. [50] with slight amendments as advocated by Tiwari et al. [51]. DNA was quantified by inspecting the concentration of ethidium bromide-stained DNA bands on 0.8 % agarose gels. Final concentration was adjusted to 50 ng μl^{-1} for later usages in PCR analysis. DNA was amplified by PCR in a total volume of 10 µl comprising 25 ng template DNA, 1×buffer (75 mM Tris.HCl (pH 9.0), 50 mM Kull, 20 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 200 µM of each dNTP, 5 pmol each of forward and reverse SSR primers and 1-unit *Taq* polymerase (Fermentas). PCR reactions were accomplished in a Bio-Rad Thermocycler. Cycling parameters were initial denaturation step at 94 °C for 5 min, pursued by 94°C, 30 s, 52–58 °C, 30 s and 72 °C, 30 s. This cycle was repeated 35 times, tracked by 5 min final extension at 72 °C. The amplified artifacts were separated on 3.5 % agarose gels and perceived by ethidium bromide staining. Allele sizes were estimated in comparison with 100 bp DNA ladder procured from Fermentas.

3.4 Data Scoring

The PCR products produced by SSR were examined by scoring qualitatively for presence or absence of bands. A genetic similarity between the genotypes was quantified by the similarity coefficient. In instance of SSRs, Polymorphism Information Content (PIC) was computed perusing the equation: $PIC_j = 1 - \sum_{i=1}^{n} P_i^2$ Where, i = the i^{th} allele of the j^{th} marker, n = the number of alleles at the j^{th} marker and p = allele frequency.

4. RESULTS AND DISCUSSION

4.1 Morpho-physiological Characterization

The analysis of variance given in Table 2 magnificently designated presence of considerable extent of variations among 53 soybean genotypes for different yield accrediting traits. Maximum leaf area in cm sq/plant was documented for genotype: AMS-100-39 (75.18304cm sq/plant) intimately tracked by genotype MACSNRC-1575 (73.65035cm sq/plant) whilst the lowest area was covered by genotype PS1092 (11.02047 cm sq/plant) closely pursued by a group of three statically *at par* genotypes, *viz.,* NRC-132 (12.09476 cmsq/plant), SL-1123 (13.31485 cmsq/plant) and JS335 (14.65448 cm sq/plant). Number (s) of primary branches per plant ranged between 2.3-6.1 with maximum numbers in genotype RSC-10-52 (6.1) familiarly followed by genotype SL-1068 (6.0), whereas the lowest numbers of primary branches (2.3) were evidenced for the genotypes *viz*., RVS 2011-35, JS 93-05, JS 20-34 and RVS2007-6.

Table 2. Mean performance of different morpho-physiological traits of soybean genotypes

S No	Parameter Genotypes	Leaf area (cm sq/plant)	Numbers of primary branches per plant	Days to initial flowering	Days to 50% flowering	Days to pod formation	Numbers of seeds per pod	Weight of plant with pod (g)	Grain yield per plant (g)	100- seed weight (g)	Biological Yield
1.	JS 20-29	15.16	3.3	38.5	48.5	49.2	2.5	25.05	16.07	10.30	24.70
2.	JS 20-69	33.58	3.9	35.5	43.0	49.0	2.8	14.06	15.16	9.19	17.87
3.	JS 335	14.65	4.7	38.5	48.0	45.4	1.8	28.20	14.54	10.10	30.64
4.	JS 20-98	31.89	5.7	35.0	43.5	47.1	2.7	41.80	12.34	9.79	32.21
5.	JS 20-94	37.04	37	36.5	47.0	50.6	2.5	26.04	16.17	11.23	30.82
6.	JS 93-05	39.14	2.7	36.0	46.0	36.5	3.6	24.42	16.54	10.88	27.43
7.	JS 20-116	32.91	4.3	34.5	44.5	50.4	2.8	25.56	11.14	9.77	26.50
8.	JS 95-60	30.91	3.3	35.0	44.0	37.4	3.8	12.60	10.57	10.07	15.35
9.	JS 97-52	22.03	5.2	35.5	44.5	50.5	2.7	19.52	17.57	10.10	21.57
10.	JS 20-84	22.86	2.5	33.5	44.5	42.5	2.5	18.12	10.24	9.48	18.70
11.	JS 20-34	19.70	2.7	34.5	44.5	41.1	2.7	21.34	10.35	9.58	21.29
12.	JS 20-71	20.38	4.7	33.5	40.5	44.6	2.5	18.06	10.80	9.47	18.62
13.	RVS 2007-6	20.18	2.7	35.5	46.5	47.6	2.4	33.60	11.74	9.32	31.32
14.	RVS 2011-35	33.41	2.3	34.5	42.0	45.7	2.6	31.30	10.29	9.80	27.75
15.	RVS 2001-4	33.22	5.5	33.5	44.5	45.1	2.3	14.30	14.54	10.10	16.72
16.	RVS -14	54.71	3.7	34.5	44.0	44.7	2.3	13.96	12.59	9.12	18.00
17.	RVS -24	39.76	3.3	35.0	44.5	48.2	2.7	17.84	10.95	9.36	19.55
18.	RVS -18	44.36	2.8	34.5	44.5	47.1	2.7	54.12	13.13	9.12	22.96
19.	NRC- 76	48.06	3.9	36.5	47.0	49.9	2.6	22.00	13.03	9.05	21.61
20.	NRC -86	52.13	5.2	34.5	45.5	49.1	2.8	43.84	12.38	9.38	23.32
21.	NRC- 130	29.04	3.3	33.5	41.0	41.9	2.4	26.40	21.67	15.70	27.90
22.	NRC -131	76.25	2 8	34.5	47.0	45.8	2.2	12.24	11.23	9.85	15.33
23.	NRC -147	43.91	4.9	35.5	43.5	48.3	2.1	95.44	14.93	10.37	28.57
24.	AMSMBC -18	50.54	4.2	34.5	41.5	50.4	2.0	17.80	7.52	6.60	19.70
25.	AMS-100-39	75.18	4.5	35.5	43.5	50.5	2.2	19.00	11.57	9.18	37.10
26.	MACS – 1520	62.38	5.1	38.5	49.0	49.7	2.6	53.60	10.61	8.42	49.00
27.	MACSNRC-1575	73.65	3.4	36.0	46.0	43.6	2.5	35.00	14.74	13.65	35.30
28.	RSC-10-52	29.30	6.1	39.5	50.0	42.5	2.0	33.20	16.15	9.79	32.15
29.	SL -1123	13.31	5.1	39.0	46.5	50.6	2.8	64.60	14.25	13.09	63.63
30.	SL-1068	53.67	6.0	35.5	44.0	50.4	2.0	58.20	12.10	11.91	61.40
31.	AGS 111	25.17	3.7	39.0	48.0	50.0	2.1	26.36	10.95	10.43	32.11
32.	EC457286	26.85	5.5	39.5	48.5	49.6	1.2	43.20	9.65	8.80	44.38
33.	MACS725	42.05	3.8	35.0	42.5	51.7	1.6	41.80	11.37	10.83	46.60
34.	SP 37	39.57	5.1	37.5	47.0	49.8	1.7	61.20	11.60	10.00	67.70

S No	Parameter Genotypes	Leaf area (cm sq/plant)	Numbers of primary branches per plant	Days to initial flowering	Days to 50% flowering	Days to pod formation	Numbers of seeds per pod	Weight of plant with pod (g)	Grain yield per plant (g)	100- seed weight (g)	Biological Yield
35.	NRC -125	29.69	5.4	41.0	47.5	50.4	1.7	75.40	18.48	11.20	62.53
36.	NRC-132	12.09	5.3	44.0	50.0	51.0	1.3	63.20	10.48	6.85	62.80
37.	NRC-134	14.20	5.3	42.0	49.0	48.8	1.5	51.80	14.91	9.56	33.74
38.	NRC SL-1	27.48	3.9	38.5	47.0	52.5	1.5	49.96	12.61	10.25	39.82
39.	PS 1092	11.02	3.0	40.0	49.0	47.3	1.5	27.80	14.00	11.11	26.72
40.	PS 1613	28.32	3.9	33.5	42.5	49.1	0.96	30.60	12.20	10.70	32.01
41.	AMS 2014-1	62.22	5.6	43.0	46.0	52.4	1.2	30.20	13.25	10.06	29.20
42.	KDS 992	39.96	5.4	43.5	47.0	51.5	1.6	44.40	15.48	11.47	34.42
43.	VLS -94	24.59	4.5	44.5	53.0	53.9	1.7	12.20	9.85	8.68	14.30
44.	SKF-SPS -11	28.68	4.8	45.5	54.0	51.5	1.7	41.66	11.74	10.30	27.54
45.	RVS 76	28.13	4.6	43.5	53.5	51.1	1.4	47.16	14.89	10.13	34.90
46.	NRC127	38.30	4.8	43.0	49.5	50.1	1.5	30.48	10.91	8.66	27.79
47.	KDS980	45.01	5.0	43.5	54.0	55.1	1.5	70.90	13.32	9.25	69.61
48.	G-29	29.98	3.1	42.0	53.0	48.8	1.7	12.56	13.57	11.90	16.60
49.	RSC-10-70	33.71	5.8	44.0	52.5	44.9	2.5	25.05	11.70	10.15	22.38
50.	RSC-10-71	24.57	4.2	43.0	50.0	42.5	2.8	14.06	9.89	8.88	16.85
51.	NRC-2	22.94	4.4	43.0	51.5	44.3	2.5	28.20	10.33	9.31	34.36
52.	MACS-15-20	37.15	5.2	44.5	50.5	42.0	2.5	41.80	11.61	10.18	38.12
53.	MACS-58	22.52	4.6	45.5	54.0	44.2	1.7	26.04	12.23	9.71	35.67
Range		11.02-76.25	2.3-6.1	33.5-45.5	40.5-54.0	36.5-55.1	0.96-3.8	12.20-95.44	7.52-21.67	6.60-15.70	14.30-69.61
SE (m)		0.691	0.623	0.730	1.127	1.114	0.452	0.175	0.740	0.379	2.747
$CD_{0.05}$		1.964	1.769	2 074	3.201	3.164	1.284	0.498	2.052	1.078	7.801

It is evidenced that the productivity of soybean is mainly dependent on flowering and maturity periods. Days to flowering play an authoritative role in the productivity of crops and were found to be positively correlated with seed yield [52-55]. The longer days to 50% flowering trait was found to be allied with the higher economic productivity in several investigations. Kachare et al. [1] demarcated flowering comportment as the time and position of first flower and measured it to be an vital trait of soybean cultivars. Days to initial flowering differed significantly in range of 33.5-45.5 days with maximum in genotype MACS-58 (45.5 days) and SKF-SPS-11(45.5 days) decisively pursued by genotypes VLS-94 (44.5 days) and MACS-15-20 (44.5 days), however the minimum days (33.5) to initiate flowering was taken by genotypes JS20-84, JS20-71, RVS 2001-4, NRC-130 and PS1613. Days to 50% flowering also varied suggestively in range of 40.5-54.0 days. Highest 54 days was taken by genotypes KDS980, MACS-58 and SKF-SPS-11, however the minimum days (40.5) affianced by genotype JS20-71 precisely chased by genotypes NRC-130 (41 days). It was also evidenced that around 70% of the genotypes display flower initiation within 30-40 days and were considered as early flowering genotypes.

Pod initiation is an imperative phenological stage sensitive to environmental conditions and regulates the crop yield [56-57]. In the current research, range of days taken to pod formation differed between 36.5-55.1 days with highest by genotype KDS980 (55.1 days) lagged by genotypes VLS -94 (53.9 days), NRC SL-1 (52.5 days) and AMS 2014-1 (52.4 days), whereas the minimum days to pod formation was engaged by genotype JS 93-05 (35 days) familiarly tracked by genotype JS 95-60 (37 days). Earlier variation in numbers of days to pod initiation among genotypes were also documented by Jadhav [58], Kachare [19], Karam et al*.* [59], Meena [55] and Wei et al. [60].

Significant variations were experimented for number(s) of pods per plant and number(s) of seeds per pod among the 53 genotypes. Numbers of seeds per pod differed significantly in an array of 0.96-3.8. Maximum numbers of seeds per pod was formed by genotype JS 95-60 (3.8) persuaded by JS 93-05 (3.6). whilst, the minimum numbers of seeds per pod was formed by genotype PS1613 (0.96) meticulously tracked by genotypes EC457286 (1.2), AMS 2014-1(1.2), NRC-132 (1.3) and RVS 76 (1.4). In preceding investigations, difference in numbers of seeds per pod among different genotypes were also evidenced by Kachare [19], Meena [55], Feroud et al. [61], Jain et al. [62], Onemi [63], Oya et al*.* [64] and Salimi et al. [65].

Weight of plant with pod speckled significantly in range of 12.20 g to 95.44g. Maximum weight of plant with pod was recognized for the genotype NRC-147 (95.44 g) pursued by NRC-125 (75.40 g) and KDS980 (70.90 g). However, the minimum weight of plant with pod was recorded in genotype NRC-131 (12.24 g) straggled by two genotypes *namely*: G-29 (12.56 g) and RSC-10-71 (14.06 g).

Grain yield per plant ranged from 7.52-21.67g. Maximum with genotype NRC-130 (21.67 g) perused by JS97-52 (17.57 g), whreas the minimum grain yield per plant was noted with genotype AMSMBC-18 (7.52 g) dawdled by two genotypes *viz.,* VLS-94 (9.85 g) and EC457286 (9.65 g). Difference in grain yield also reported by Kachare [19] and Meena [55] in earlier investigations.

Hundred-seed weight is the eventually decider yield component linked with higher yield, therefore, occupies crucial position in the selection criteria for improving yield potential of soybean. In the present investigation, 100- seed weight was varied between 6.60-15.70 g. Maximum 100-seed weight was recognized with genotype NRC-130 (15.70 g) pursued by genotypes MACS-1575 (13.65 g), SL-1123 (13.09 g), SL-1068 (11.91 g), G-29 (11.90 g), KDS 992 (11.47 g), JS 20-94 (11.23 g), NRC -125 (11.20 g) and PS 1092 (11.11 g), whereas the minimum was documented in genotype AMSMBC-18 (6.60 g) lagged by genotypes NRC-132 (6.85 g), MACS- 1520 (8.42 g), NRC-127 (8.66 g), VLS -94 (8.68 g), EC457286 (8.80 g) and RSC-10-71 (8.88 g). Seed index is an imperative yield component directly associated to seed yield in soybean. In previous studies, differences in 100-seed weight among different soybean genotypes also reported by Kachare [19], Meena [55], Oya et al. [64], Manavalan et al. [66], Angra et al. [67], Demirtas et al*.* [68], Maleki et al. [69] and Khan et al. [70].

Biological yield is a protuberant yield component which determines the fortune of seed yield in soybean. Kunert et al. [71] and Ghanbari et al. [72] specified that biological yield is highly correlated with seed yield in soybean. Significant variations were also documented for biological yield among the

genotypes. Biological yield varied between 14.30 and 69.61, maximum with genotype KDS980 (69.61) intimately followed by genotypes SP37 (67.70), SL-1123 (63.63), NRC-132(62.80), NRC-125 (62.53) and SL-1068 (61.40), whereas the minimum biological yield was documented with genotype VLS -94 (14.30) pursued by genotypes NRC -131 (15.53), JS 95-60 (15.35), G-29 (16.60), RSC-10-71 (16.85) and JS 20-69(17.87).

4.2 Hierarchical Cluster Analysis

Interpreting to the hierarchical cluster analysis and the traits values (Table 2), the active expression profile was depicted and is presented in Fig. 1. Multivariate analysis constructed on diversity was accomplished *via* the UPGMA. A dendrogram of 53 genotypes of soybean acquired with simple flexible linkage was constructed appointing different morpho-physiological traits of soybean genotypes. Agglomerative genotype hierarchical clustering assemblage the genotypes into two major cluster MC-1 and MC-2. Later MC-1 was segmented into two sub clusters in that NRC-147 presented as a distinct group (Fig. 1). Afterward, in MC-2 cluster, NRC-86, NRC-131 and AMS-100-39 genotypes are presented as an out group. This outline of clustering hereafter, show blended propensity of diverse morpho-physiological parameters of genotypes.

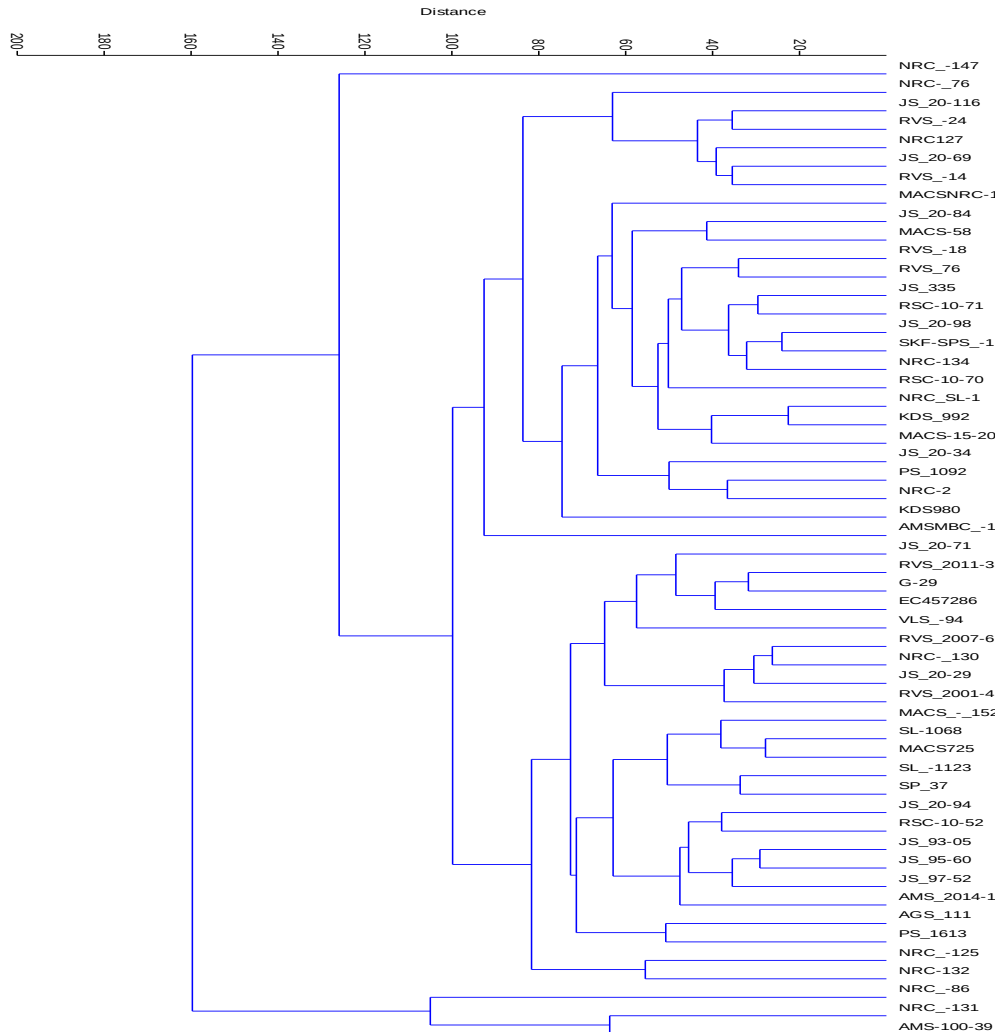


Fig. 1. Dendrogram based on different morpho-physiological traits showing relationship among 53 soybean genotypes

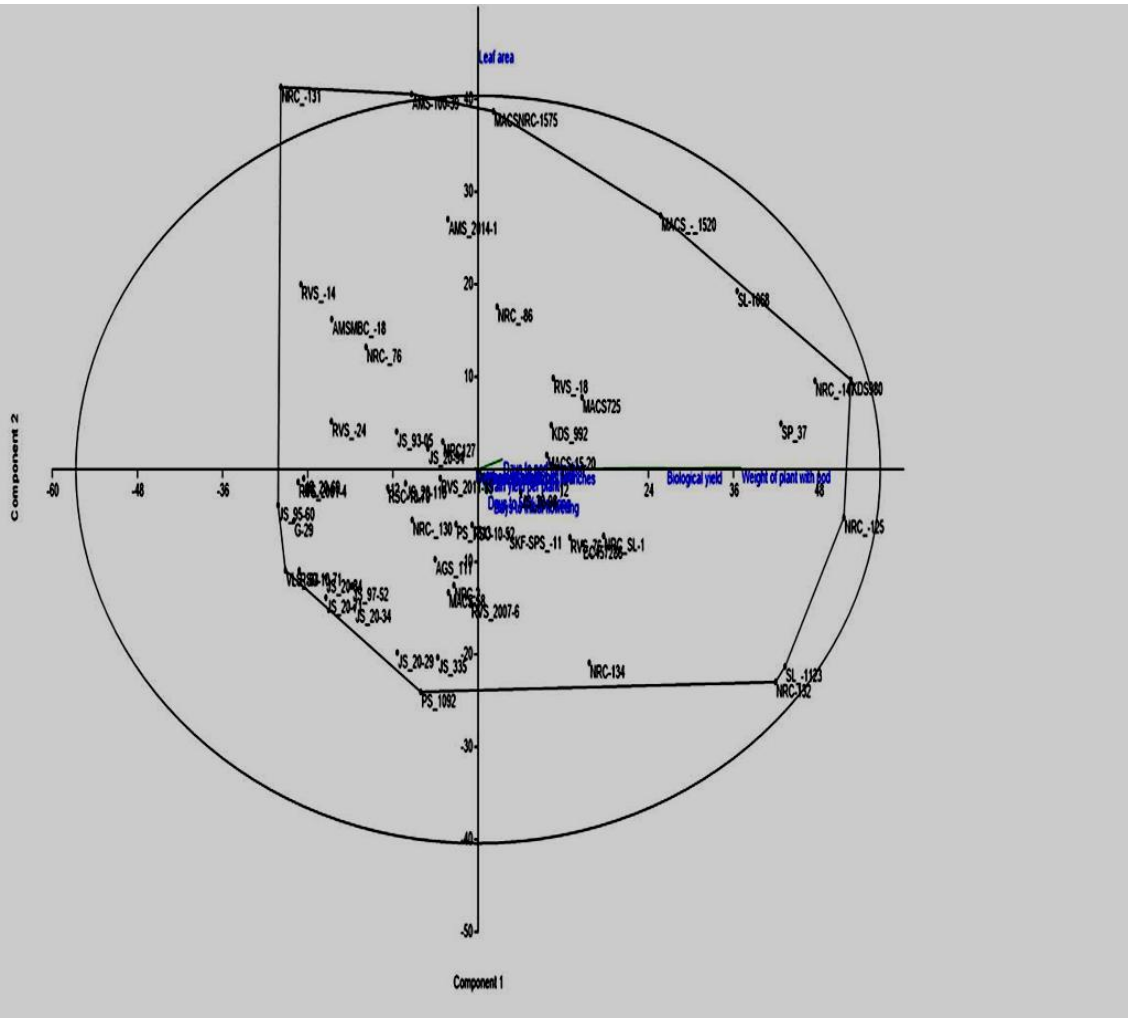


Fig. 2. Principal component analysis based on different morpho-physiological traits showing relationship among soybean genotypes

4.3 Principal Component Analysis

Principal component analysis (PCA) was executed by considering morpho-physiological variables concomitantly. The plan of variations confirmed by the PCA designated by correlation coefficients administered for pair-wise connotation of the parameters. The PCA correlation exemplified that which genotype controlled higher and lower content occupying unique position in the direction of the graph (Fig. 2). Among ten variables, leaf area content has the highest variability (57.95 %).

4.4 SSR Markers-based Characterization

Diversity assessment of any plant species is an exact initiator for improvement of the crop since it bids base line data to right selection of parental genotypes for a varietal improvement programme [27, 28, 31]. Hybridization between genotypes belonging to the parallel cluster will not prime to anticipated segregates as all the genotypes with genetic similitude set into same cluster, while genotypes with genetic distance departed into clusters representing better diversity between the clusters [73] [22]. Molecular markers-based profiling has been the idyllic preference of hybridization as it verified its consistency and validity without influence of environmental conditions on it [1,12,22,27]. Molecular markers have also been employed for finding of specific trait linked markers [1,12,21]. These

investigations are beneficial in the cataloguing of crop populations into assorted grouping which may be obliging in expansion of gene pool.

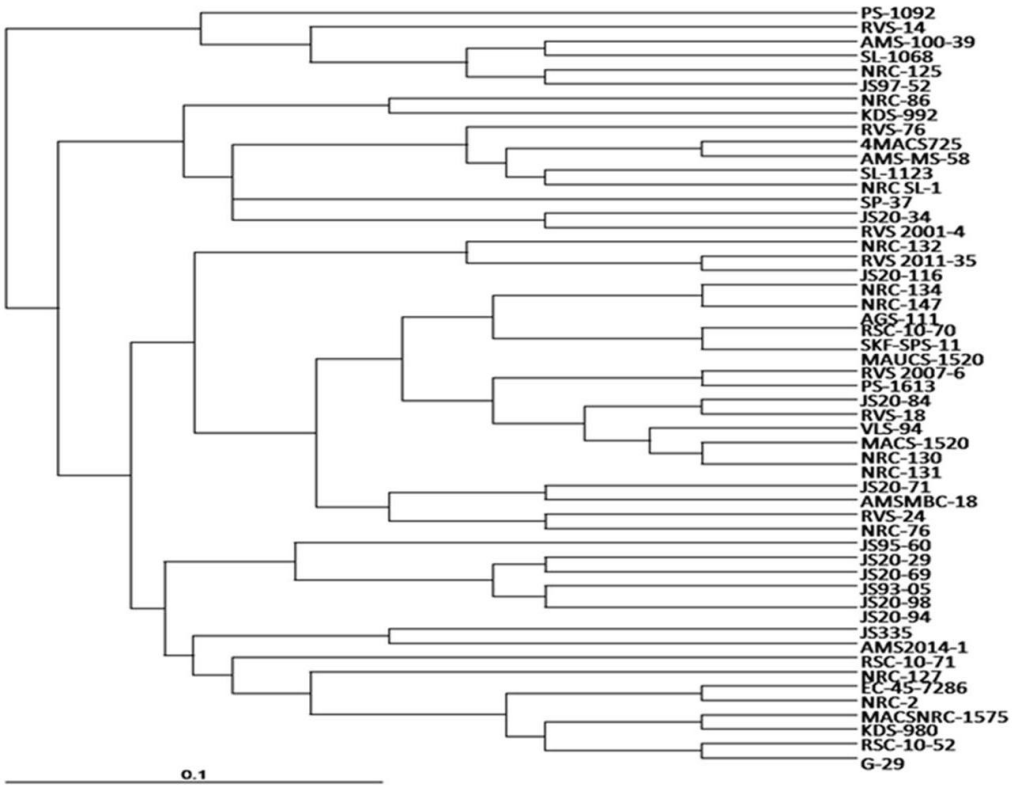


Fig. 3. Dendrogram representing SSR based relationship among 53 genotypes of soybean

A total of forty-eight SSR markers were endeavored to amplify 53 soybean genotypes designated under the present investigation in preliminary experiment. Primarily only twenty SSR markers amplified the template DNA across all 53 genotypes. Nevertheless, rest markers were exasperated two more times with diverse annealing temperatures and lastly thirty-two SSR markers were effectively amplified across all soybean genotypes. These all thirty-two SSR markers (66.67%) were found to be polymorphic. Whilst, in earlier investigation, Bisen et al. [27] documented less than 50% (23 out of 50 SSR markers) amplification and polymorphism proficiency of SSR markers in soybean. This may be because trial of only one annealing temperature during PCR course.

The total numbers of alleles amplified with all SSR markers were 76 and mean polymorphic alleles were 2.38. Out of thirty-two microsatellites, two markers *viz*., Satt119 and Satt154 amplified maximum four alleles and ten markers were found to be able to amplify only three alleles each. Remaining markers were found to be able to amplify only two alleles. The highest major allele frequency (0.98) was documented for the marker Satt557 chased by 0.95 in Satt518 whilst the lowest (0.36) for the marker Satt520. An average major allele frequency was documented 0.69. The highest genetic diversity (0.66) was confirmed by marker Satt520 while the lowest (0.04) was in Satt557. An average genetic diversity was evidenced 0.35. Among all thirty-two SSR molecular markers the highest PIC value was 0.67 demonstrated by marker Satt119 and the lowest 0.04 by marker Satt557 with a mean worth of 0.32 (Table 3). Similar to the current results, the polymorphism of SSR loci professed in this investigation tie with the former data of Bisen et al. [27] and PIC values were in promise with earlier verdict of Sahu et al. [22]. Valliyodan et al. [74] also evidenced an average PIC value of 0.36 with SSR markers in soybean. According to various other investigators, PIC values were varied between 0.199 to 0.87 [27]. Higher value of PIC designates the incidence of several alleles in every locus, and is also imperative in the detection of molecular markers-based analysis of variation [21].

Table 3. Details of SSR markers used for diversity analysis among soybean genotypes

Marker	Forward 5'-3'	Reverse 5'-3'	AN	MAF	GD	PIC
Sat_044	AAAAAATATTTATAGGTTACATGTG	TTACCACTAAGAATTAGGTCTAA	2	0.66	0.45	0.35
Sat_171	GCGCTCCTCTTTTTTTCACTTTC	GCGCGTGGGATTTTGGTATTTTT	2	0.57	0.49	0.37
Sat_205	GCGCCTTTTCGTCTGTTCTGTTC	GCGAGCTTTTAAAAATTTAGAAATCAAT	2	0.83	0.28	0.24
Sat_375	GCGTGTTAATGATTGCATAAGGTTCG	GCGTGTCAAAAGAAACTCAATAAAGAAAAAT	2	0.87	0.23	0.20
Satt174	TTTCATTTCTTTGCCTTCT	TTCGTAGTCCGTCTTTCAT	2	0.89	0.21	0.18
Satt226	GCGAAACAACTCACTTAAGCAATACAT	GCGTCCTCCTACCTTTCTTATC	3	0.58	0.51	0.39
Satt244	GCGCCCCATATGTTTAAATTATATGGAG	GCGATGGGGATATTTTCTTTATTATCAG	2	0.60	0.48	0.36
Satt500	GCGAACGACCATGATAATCACA	GCGCTCATTTGAAAGCATTGTTATA	3	0.75	0.38	0.32
Satt520	GCGGTGTGCAAGAGTGACA	GCGCATTTGGACTTTCTA	3	0.36	0.66	0.59
Satt540	CTGGCGAATCAAGCTTTGTAAC	CCGTGATTGCGAAGAGGATATT	2	0.68	0.44	0.34
Satt551	GAATATCACGCGAGAATTTTAC	TATATGCGAACCCTCTTACAAT	2	0.77	0.35	0.29
Satt557	GCGGGATCCACCATGTAATATGTG	GCGCACTAACCCTTTATTGAA	2	0.98	0.04	0.04
Satt119	TGTGCCAGTGTTGATAGTTA	CTGATCCCCAATAAATCTG	4	0.78	0.56	0.67
Satt245	AACGGGAGTAGGACATTTTATT	GCGCCTCCTGAATTTCAAAGAATGAAGA	2	0.58	0.33	0.43
Satt281	AAGCTCCACATGCAGTTCAAAAC	TGCATGGCACGAGAAAGAAGTA	3	0.83	0.54	0.29
Sat_076	GCGTAATTAACACCAATATATGACATG	GCGGGGTTAAAAATTCAAAATGT	3	0.67	0.45	0.44
Satt510	GCGAGTTTCGCCGTTACCACCTCAGCTT	CCCTCTTATTTCACCCTAAGACCTACAA	2	0.59	0.32	0.22
Satt114	GGGTTATCCTCCCCAATA	ATATGGGATGATAAGGTGAAA	3	0.69	0.29	0.32
Satt275	GCGGGATAATTGGTTTTACGAAAATGC	GCGCCTAATCACCTAAAAAAACGTTTA	2	0.85	0.21	0.23
Satt280	GCGGAATCTGCTTATTCATTGTGTG	GCGCCATGCTGTAACACGTCAAT	2	0.77	0.19	0.25
Satt292	CGGAATTAGAACTCCAGTAAAGA	GCGAGGCCAACATTGAAAAGT	2	0.93	0.33	0.19
Satt130	TAAACGAAATTTAGTTTTAAGACT	TGAATGGCTAAAAACGTGATT	3	0.53	0.43	0.37
Satt042	GACTTAATTGCTTGCTATGA	GTGGTGCACACTCACTT	2	0.68	0.33	0.30
Satt442	CCTGGACTTGTTTGCTCATCAA	GCGGTTCAAGGCTTCAAGTAGTCAC	2	0.49	0.19	0.28
Satt154	AGATACTAACAAGAGGCATAAAACT	AAAGAAACGGAACTAATACTACATT	4	0.88	0.58	0.66
Satt518	GCGCATATCAAATTGCATATAAAAATACG	GCGGGAATATAAAATAAAAATGCTCACTT	2	0.95	0.27	0.26
Satt418	GCGAAAGCACATATGGGTTTGAAT	GCGAGGGCATATATATGATGAGGTA	3	0.82	0.35	0.31
Satt235	GCGGGCTTTGCCAAGAAGTTT	GCGGTGAGGCTGGCTATAAG	3	0.65	0.31	0.29
Satt236	GCGTGCTTCAAACCAACAAACAACTTA	GCGGTTTGCAGTACGTACCTAAAATAGA	3	0.55	0.34	0.38
Satt184	GCGCTATGTAGATTATCCAAATTACGC	GCCACTTACTGTTACTCAT	2	0.64	0.44	0.29
Satt686	ACGGAAAATAAATGAAACTAAGA	GCGCTATCAGATAGAGAAGCAGAAGAAT	2	0.67	0.32	0.31
Total			76	22.09	11.3	10.16
Average			2.38	0.69	0.35	0.32

AN-Allele numbers, MAF-Major allele frequency, GD-Genetic diversity, PIC-Polymorphism information content

Due to high level of reproducibility and co-dominant inheritance, SSR markers have been experienced for distinguishing genotypes and inspecting genetic relationships among 53 soybean genotypes. Microsatellites have been used for genetic diversity analysis among soybean genotypes by several researcher groups [22,27,73]. The current investigation with 53 genotypes together with a variety of imperative cultivars from India is the important research so far, to depict the variation at molecular level. Thirty-two SSR markers used in this investigation offered valuable suggestion about genetic diversity present in soybean genotypes as they were linked with genotypes. For remarkable genetic diversity analysis, allele frequency, genetic diversity and polymorphism information content for each SSR locus were calculated. The PIC values were usually good for all the SSR loci experienced with a mean worth of 0.32. Two SSR loci discovered PIC values higher than 0.6 and, Satt154 and Satt119 molecular markers were distinguished because of their relatively higher polymorphism (four alleles). The average numbers of alleles per locus in our analysis was lesser than the past research carried out by Kaewwongwal et al. [75] where it was 9.05. While, Bisen et al. [27] reported an average of 1.97 alleles per locus across 38 Indian soybean genotypes. This higher rate of SSR polymorphism may be attributed to the designated set of SSR markers which were earlier experienced for polymorphism among a set of genotypes. However, the lower allele number and PIC values intitles low allelic diversity in current set of soybean accessions. The SSR allelic diversity famed among soybean genotypes in this investigation was low as compared to earlier study [76].

The UPGMA cluster analysis was accomplished commissioning SSR data. The clustering was done based on genetic similarity between and among investigated soybean genotypes. Primarily 53 soybean cultivars were alienated into two clusters one minor and one major (Fig. 3). Minor cluster confined six genotypes, *namely.,* PS-1092, RVS-14, AMS-100-39, SL-1068, NRC-125 and JS97-52. Among these six genotypes, genotypes RVS-14 and AMS-100-39 have similar parental source. Genotype PS-1092 exhibited lower resemblance with remaining of the five genotypes and assembled distantly. This may be owing to its geographical origin and parentage also. The major cluster contained 47 genotypes and further splinted into two groups one minor and one major. Minor group had ten genotypes, *i.e*., NRC-86, KDS-992, PS-1613, MACS725, AMS-MS-58, SL-1123, NRC-SL-1, SP-37, JS20-34 and RVS2001-4. The major group contained 37 genotypes and it was later strapped into two sub groups. Major sub group contained 21 genotypes including NRC-132, RVS2011-35, JS20-116, NRC-134, NRC-147, AGS-111, RSC-10-70, SKF-SPS-11, MACS-1520, RVS2007-6, PS-1613, JS20-84, RVS-18, VLS-94, NRC130, NRC-131, JS20-71, AMSMBC-18, RVS-24 and NRC-76. Among these genotypes, NRC130 and NRC131 demonstrated higher similarity with each other and grouped meticulously. This may be due to the similarity in their parentage (EC390977 x EC538828). However minor group had 16 genotypes *viz*., JS95-60, JS20-29, JS20-69, JS93-05, JS20-98, JS20-94, JS335, AMS2014-1, RSC-10-71, NRC-127, EC-45-72-86, NRC-2, MACSNRC-1575, KDS980, RSC-10-52 and G-29. This clustering designates the assemblage of seven soybean varieties *i.e.,* JS95-60, JS20-29, JS20-69, JS93-05, JS20-98, JS20-94, JS335 developed in the same centre. Among these seven varieties four *viz*., JS20-29, JS20-69, JS20-98 and JS20-94 share one of the common parents *namely* JS97-52. Comparable clustering was evidenced in earlier investigations conducted on microsatellite-based diversity analysis among Indian soybean genotypes [77] [1,12,22, 27]. The clustering of large numeral of soybean germplasm lines in a single cluster designates that soybean germplasm accumulation is having high genetic association among genotypes.

In current study, the genotypes grouped in different clusters having large genetic distance could be employed as parental entrants for cross breeding to yield progeny with additional value or heterotic effect from each parent. In contrast, the genotypes grouped in the same cluster confirmed their close genetic relation and should not be used as parents in cross-breeding to prevent the incidence of inbreeding depression. Inbreeding depression, the reverse of heterosis, is the decreased progeny vigour owing to the increased homozygosity level as a result of crosses between two individuals with close genetic relationship [78]. Inbreeding depression in plants may cause the plants to be stressed, which is recognized by the decrease of plant height, less vigour, sensitive to pest and disease attack, decrease of fruit number and increased fruit abortion and the presence of several unwanted characters owing to the combination of recessive alleles [79]. Overall, the results of this investigation recommended the prospect to select parental lines for heterotic crosses in breeding programme employing microsatellites.

When we likened morpho-physiological and microsatellite markers-based clustering pattern of the soybean genotypes under the current investigation, no close association was evidenced. The minor harmony between morpho-physiological and microsatellite dendrogram may be because of limited expression of morpho-physiological characters in the field conditions. Limited association between both of the data may also be owing to substantial significances of environment on these characters and use of less numbers of microsatellite markers. Markers are not enough to epitomize whole genome of soybean so, more numbers of markers with widespread coverage may be advantageous in vibrant understanding of connotation between morpho-physiological and molecular data.

5. CONCLUSIONS

Genetic diversity analysis based on yield attributing traits and SSR markers authorizes the existence of variations among soybean genotypes under the investigation. Genetic diversity identified among soybean genotype collection in present study dictates the stipulation of enlargement genetic diversity by acquainting more exotic germplasm lines along with engagement of wild relatives. The ideal method for breeding is genetic diversity analysis based on molecular markers, owing to its legitimacy and dependability. The assorted genotypes identified in current study may contribute as basis of new alleles in soybean breeding programme.

Data Availability Statement: The data presented in this study is available within this chapter.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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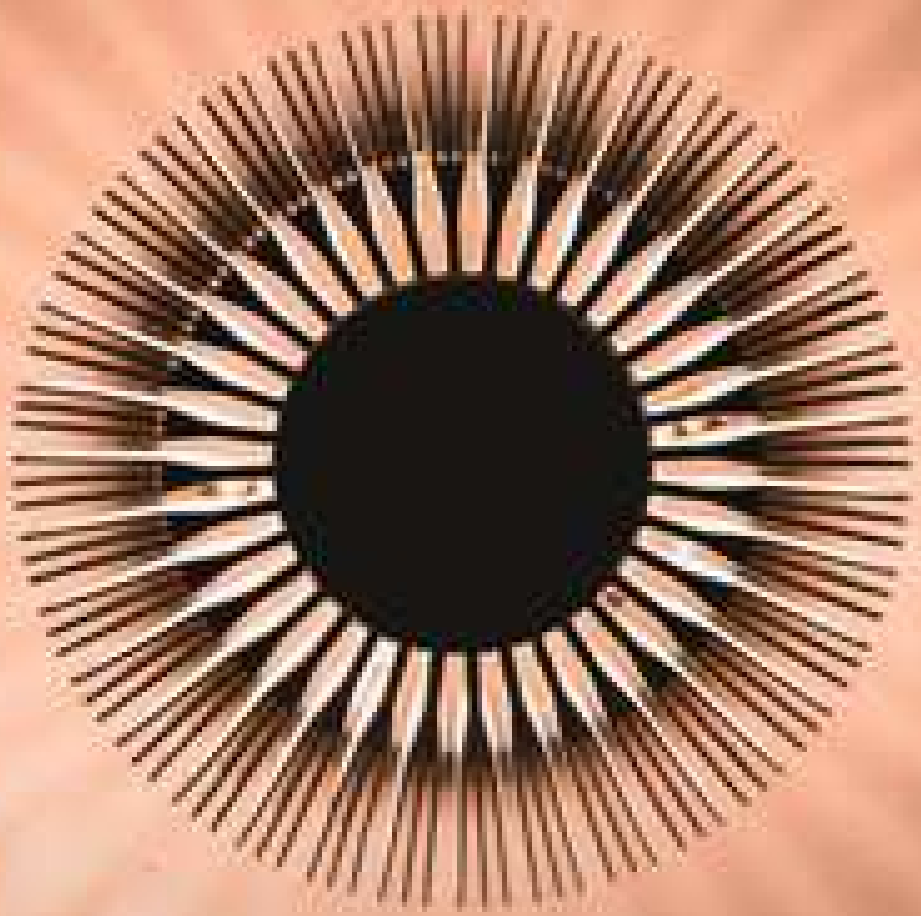
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Chapter 4
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Screening of Soybean Genotypes against Drought on the Basis of Gene-Linked Microsatellite Markers

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ABSTRACT

Soybean is elegant to be a key crop attributable to its significant contribution as vegetable oil and protein in human diet. Nevertheless, inopportunely, its production has been exaggeratedly declined due to the ordinariness of drought related stress. In present investigation, total 12 SSR molecular markers were employed for screening of 53 soybean genotypes to determine the effectiveness of existing markers in genetic diversity analysis as well as their validation on the basis of their connotation with drought tolerant gene. Among applied drought tolerance gene-linked SSR molecular markers, the highest genetic diversity (0.6629) was documented with marker Satt520 while lowest (0.0370) was for the marker Satt557 with an average of 0.3746. The highest PIC value was 0.5887 prearranged by similar markers *viz*., Satt520 and lowest 0.0363 by Satt557 with the mean worth of 0.3063. Dendrogram constructed owing to banding profile of used markers was able to victimize some putative drought tolerant genotypes *i.e*., JS97-52, JS95-60 from rest of the genotypes. The findings of the current investigation may contribute towards improvement of soybean to bread drought tolerant varieties in future.

Keywords: Climate change; molecular diversity; drought; microsatellites; sustainable agriculture; water stress.

1. STUDY OBJECTIVES

The current chapter was intended to determine the efficacy of existing SSR molecular markers in genetic diversity analysis in addition to their validation based on their implication with drought tolerant gene in soybean genotypes.

2. INTRODUCTION

Soybean is the imperative crops owing to its application as a spring of vegetable oil along with proteins throughout the world [1-9]. Production and productivity of soybean has been declined due to different biotic [10-12] and abiotic [13-18] stresses. Among different abiotic factors, drought is prime stress which envisaged to be increased in future [19] as well. It is a serious issue because of its role in reduction of production of important crops including soybean. Accessibility of passable water provisions in growth along with development of plants. But, alteration in weather is a leading reason of drought conditions in many portions of the world. Drought stress may mainly harm to the susceptible crop varieties. So, it is desirable to recognize drought tolerant varieties among the available varietal resources or advance a new variety with tolerant mechanism against drought to develop drought tolerant varieties.

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Identification or selection of a drought tolerant genotype is plausible through morpho-physiological parameters [20-21,18,14] under field environments, biochemical traits [22,15-17] and biotechnological tools [23-28,13] with variable degree of accomplishment. Nevertheless, abundant problems may distress the recital of a genotype/variety throughout field trials and may mislead the precise identification. Owing to these limitations an array of molecular markers has been applied to be acquainted with drought tolerant genotype/variety as they are frequently free from ecological influences. Numerous studies have been conducted to categorize genotypes/varieties of crop plants including soybean have been employing different classes of dominant as well as co-dominant molecular markers *viz.*, Random Amplified Polymorphic DNA, Inter Simple Sequence Repeats, Amplified Fragment Length Polymorphism and Simple Sequence Repeat to investigate genetic diversity in soybean [29-36,10-11,26-28]. Among all the cited markers, SSRs have been extensively used in different crop plants for study of diversity and identification of linked gene against different biotic and abiotic stresses because of their higher level of polymorphisms, higher polymorphic information content (PIC), co-dominant inheritance and dispersal in the whole genome [37-49]. The present investigation was accomplished to screen putative drought tolerant soybean genotypes based on gene -linked SSR molecular markers.

3. MATERIALS AND METHODS

The present investigation was conducted with 53 *Glycine max* (L.) Merrill genotypes (Table 1) with diverse reactions to drought *viz*: susceptible and tolerant as investigated during previous studies [13-18], [25]. The seeds were acquired from College of Agriculture, JNKVV, Jabalpur, RAK, College, Sehore and Zonal Agricultural Research Station, Morena, RVSKVV, Gwalior, Madhya Pradesh, India. The laboratory work was carried out at Plant Molecular Biology Laboratory, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, India. Leaf samples of each of the genotype collected after 20 days after sowing for genomic DNA extraction.

3.1 SSR Molecular Marker Analysis

Genomic DNA from young leaves was extracted employing CTAB method [50] with minor modifications as suggested by Tiwari et al. [51]. Extracted DNA was evaluated qualitatively and quantitatively by employing Nano spectrophotometer. Quantified DNA samples were diluted up to 15ng/ µl for further analysis. Primarily, a total of 20 SSR molecular markers (Table 2) were selected on the basis of published literature for screening of drought tolerant and susceptible genotypes and procured from Imperial Life Sciences Pvt. Ltd, Gurgaon, Haryana, India. Diluted DNA was amplified by PCR in a total volume of 10 µl encompassing 25 ng template DNA, 1×buffer (75 mM Tris.HCl; pH 9.0), 50 mM KCl, 20 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 200 µM of each dNTP, 5 pmol SSR primers and 1-unit *Taq* DNA polymerase (Fermentas). PCR reactions were performed in a Bio-Rad thermocycler. Cycling parameters were initial denaturation step at 94°C for 5 min, tracked by 94°C for 30 s, 52–58°C for 30 s and 72 °C for 30 s. This cycle was repeated 35 times, trailed by 5 min final extension at 72°C. The amplified artifacts were separated on 3.5% agarose gels and detected by ethidium bromide staining. Allele sizes were estimated in comparison with 100 bp DNA ladder procured from Fermentas.

3.2 Data Analysis

The PCR harvests engendered by SSR were examined by scoring qualitatively for presence or absence of bands. A genetic similarity between the genotypes was computed by the similarity coefficient. In illustration of SSRs, Polymorphism Information Content (PIC) was computed deliberating to Anderson et al. [52] perusing the equation: $PIC_j = 1 - \sum_{i=1}^{n} P_i^2$ Where, i = the i^{th} allele of the j^{th} marker, n = the number of alleles at the j^{th} marker and p = allele frequency.

4. RESULTS AND DISCUSSION

Drought stress distresses plant growth and development at each phase of life [53]. Molecular characterization and discrimination of drought tolerant and susceptible genotypes/varieties of soybean

are imperative for further development of tolerant varieties with higher yield potential. Discrimination owing to molecular data authorizes the actual diversity and genetic distance present among/ between genotypes. Earlier, various studies have been conducted to screen soybean genotypes for specific traits with the use of molecular markers as seed related traits [33], YMV [10], charcoal rot and *Rhizoctonia* root rot [11], drought [25-26] and in other crops like pearl millet [20-21].

Table 1. List of soybean genotypes with their parentage

S. No.	Genotypes	Source/Pedigree	S. No.	Genotypes	Source/Pedigree
1.	JS 20-29	JS 97-52 x JS 95-56	28.	RSC-10-52	NRC 37X JS335
2.	JS 20-69	JS 97-52 x SL 710	29.	SL -1123	Selection from AGS751
3.	JS 335	JS 78-77 x JS 71-05	30.	SL-1068	SL755XSL525
4.	JS 20-98	JS 97-52x JS SL710	31.	AGS 111	Germplasm accession
5.	JS 20-94	JS 97-52 x JS 20-02	32.	EC457286	Germplasm accession
6.	JS 93-05	Selection from PS 73-22	33.	MACS725	JS93-05X MAUS71
7.	JS 20-116	JS 97-52 x JSM 120 A	34.	SP 37	Not known selection
8.	JS 95-60	Selection from PS 73-22	35.	NRC -125	EC54688xps1044
9.	JS 97-52	PK 327 x L 129	36.	NRC-132	JS97-52X PI086023
10.	JS 20-84	JS 98-63 x PK 768	37.	NRC-134	NRC7XAGS191
11.	JS 20-34	JS 98-63 x PK 768	38.	NRC SL-1	JS335XSL525
12.	JS 20-71	JS 97-52 x JS 90-5-12-1	39.	PS 1092	PS1042 x MACS 450
13.	RVS 2007-6	JS 20-10 x MAUS162	40.	PS 1613	PS1225XPS1042
14.	RVS 2011-35	JS 335 X PK 1042	41.	AMS 2014-1	AMS99-33XH6P5
15.	RVS 2001-4	JS 93-01x EC 390981	42.	KDS 992	JS93-05XEC241780
16.	RVS -14	JS 93-05x EC 390981	43.	VLS -94	VL Soya59X VS2005-1
17.	RVS -24	J.P 120 x JS 335	44.	SKF-SPS -11	Not known selection
18.	RVS -18	JSM110XJSM66	45.	RVS 76	MAUS-162XJSM-66
19.	NRC- 76	NRC-37XL-27	46.	NRC127	JS97-52XPI542044
20.	NRC -86	RKS15XEC481309	47.	KDS980	JS93-05XAMS1
21.	NRC- 130	EC390977xEC538828	48.	G-29	Germplasm
22.	NRC -131	EC390977xEC538828	49.	RSC-10-70	JS335X Bragg
23.	NRC -147	Germplasm accessions C210	50.	RSC-10-71	Bragg XJS335
24.	AMSMBC -18	Mutant of Bragg	51.	NRC-2	Induced mutant of Bragg
25.	AMS-100-39	Mutant of JS93-05	52.	MACS-15-20	NRC37XMohetta
26.	MACS – 1520	EC241780XMACS330	53.	MACS-58	JS2 x Improve pelican
27.	MACSNRC-1575	PI542044XJS9305			

A total of twenty drought tolerance linked SSR molecular markers were endeavored to amplify 53 soybean genotypes primarily (Table 2) but out of them only twelve SSR markers (Table 3) were efficaciously amplified across all the genotypes. All twelve SSR markers were found to be polymorphic. Similar to this, Bisen et al. [34] also documented less than 50% (23 out of 50 SSR markers) amplification and polymorphism efficiency of SSR markers in Indian soybean. The mean polymorphic alleles were 2.25. Out of twelve, three SSR markers *viz*., Satt226, Satt500 and Satt520 amplified maximum three alleles each and the rest of the markers were found to be able to amplify only two alleles each. The highest major allele frequency (0.9811) was evidenced for the Satt557 marker tracked by 0.8868 for the marker Satt174 whilst the lowest (0.3585) for the marker Satt520. The average major allele frequency was 0.7123. The highest genetic diversity (0.6629) was demonstrated by marker Satt520 whereas the lowest (0.0370) was by marker Satt557. The average genetic diversity was 0.3746. Among all twelve SSR molecular markers the highest PIC value was 0.5887 given by Satt520 (Fig.1) and the lowest 0.0363 by Satt557 with an average PIC value of 0.3063. Similar to the present finding, the polymorphism of SSR loci apparent in this study matched with the earlier data of Bisen et al*.* [34] and PIC values were in agreement with previous result of Sahu et al. [31]. Hipparagi et al. [54] reported an average PIC value of 0.36 for the SSR markers in soybean. According to various other researchers, PIC values were ranged between 0.199 to 0.87 [55]

[32]. Higher value of PIC indicates the presence of various alleles in every locus, and is also important in the identification of molecular markers-based analysis of variability [56,34].

Due to high level of reproducibility and co-dominant inheritance, SSR molecular markers have been experienced for distinguishing genotypes and investigating genetic relationships among 53 soybean genotypes. Microsatellites have been employed for genetic diversity analysis among soybean genotypes by various research groups [57,58,31]. The present investigation conducted with 53 genotypes including a variety of imperative cultivars from India is the important study so far, to characterize the variation at molecular level. The twelve SSR markers employed in this research obtainable valuable evidence about genetic diversity present in soybean genotypes as they were linked with genotypes. For impressive genetic diversity analysis, number of alleles, polymorphic alleles, polymorphism percentage and effective number of alleles, allele frequency, genetic diversity and polymorphism information content for each SSR locus were calculated. The PIC values were generally good for all the SSR loci tested with an average of 0.266. One SSR loci revealed PIC values higher than 0.5 and, Satt510 was notable owing to its relatively higher polymorphism (four alleles). The average numbers of alleles per locus in our analysis was lesser than the past experiment conducted by Kaewwongwal et al. [59] where it was 9.05. However, Bisen et al. [34] detected an average of 1.97 alleles per locus across 38 soybean genotypes. This high rate of SSR polymorphism may be attributed to the selected set of SSR markers which were formerly tested for polymorphism among a set of genotypes. However, the lower allele number and PIC values designates low allelic diversity in present set of soybean accessions. The SSR allelic diversity distinguished among soybean genotypes in this experimentation was low comparison to earlier investigation [60].

The UPGMA cluster analysis was accomplished employing SSR data. The clustering was done owing to genetic similarity present between and among studied soybean genotypes. Initially 53 soybean cultivars were divided into two clusters one minor and one major (Fig. 2). Minor cluster delimited six genotypes, *namely*: JS97-52, JS95-60, JS93-05, RVS-14, MACS-58 and NRC-2. Among these six genotypes, genotype JS97-52 was alone and rest of the five genotypes displayed similarity with each other. The clustering of immense numeral of soybean germplasm lines in a single cluster entitles that soybean germplasm lines grouping is having high genetic affiliation among genotypes. Among all 53 genotypes, genotype JS97-52 formed a separate sub cluster and in an earlier investigation it has been testified as drought tolerant genotype during field experiment as well as gene expression analysis [18,25]. In his experiment, genotype JS95-60 was also found as drought tolerant variety. Correspondingly in current investigation, genotype JS95-60 has shown comparable banding pattern as in genotype RVS-14 with markers Satt174 and Sat_205. These markers have been reported drought tolerant gene linked markers in soybean by researchers in their investigations [61,62]. The similar banding pattern designates drought tolerant nature of genotype RVS-14.

Table 2. List of SSR markers used for screening of soybean genotypes

S.No.	Primers	Forward 5'-3'	Reverse 3'-5'	References
1	Satt383	CGATCTAACACGC ATATTTCCTCTG	CTTCCCTAATATTGGCA ACCTCTATG	[63]
2	Satt557	GCGGGATCCACCA TGTAATATGTG	GCGCACTAACCCTTTAT TGAA	[53]
3	Satt577	CAAGCTTAAGTCT TGGTCTTCTCT	GGCCTGACCCAAAACTA AGGGAAGTG	[64]
4	Sat_171	GCGCTCCTCTTTT TTTCACTTTC	GCGCGTGGGATTTTGG TATTTTT	[61]
5	Satt321	CACCGTCGTAAAA ACTGTGTCGT	GCGTGTCAAAGAGTTTT AGACATC	[61]
6	Satt244	GCGCCCCATATGT TTAAATTATATGGAG	GCGATGGGGATATTTTC TTTATTATCAG	[61]
7	Satt393	CAAGCCCATAAAC GAAATAA	GCTCGGCTTGGCTTGTT TACTA	[63]
8	Satt520	GCGGTGTGCAAGA GTGACA	GCGCATTTGGACTTTCT A	[61]

S.No.	Primers	Forward 5'-3'	Reverse 3'-5'	References
9	Satt540	CTGGCGAATCAAG CTTTGTAAC	CCGTGATTGCGAAGAG GATATT	[61]
10	Satt547	GCGCTATCCGATC CATATGTG	TGATTTCGCTAGGTAAA ATCA	[61]
11	Satt551	GAATATCACGCGA GAATTTTAC	TATATGCGAACCCTCTTAC AAT	[61]
12	Satt286	GCGGCGTTAATTT ATGCCGGAAA	GCGTTTGGTCTAGAATA GTTCTCA	[61]
13	Sat_312	GCGCCTCCCATTA CTTCGGATTAGTTA	GCGAACGCAACAAATAA TCAAACATC	[65]
14	Sat_044	AAAAAATATTTATA GGTTACATGTG	TTACCACTAAGAATTAG GTCTAA	[65]
15	Satt226	GCGAAACAACTCA CTTAAGCAATACAT	GCGTCCTCCTACCTTTC TTATC	[61]
16	Sat_375	GCGTGTTAATGAT TGCATAAGGTTCG	GCGTGTCAAAAGAAACT CAATAAAGAAAAAT	[61]
17	Satt174	TTTCATTTCTTTGC CTTCT	TTCGTAGTCCGTCTTTCAT	[61]
18	Sat_205	GCGCCTTTTCGTC TGTTCTGTTC	GCGAGCTTTTAAAAATT TAGAAATCAAT	[62]
19	Satt489	GCGTGTGCTTGCT TCTCTTAGACTGACT	GCGTACTACTTACCCTG TTTGTCTAAAA	[62]
20	Satt500	GCGAACGACCATG ATAATCACA	GCGCTCATTTGAAAGCA TTGTTATA	[66]

Table 3. Different parameters analyzed with drought linked SSR markers in soybean

Marker	Major allele frequency	Allele No.	Gene diversity	PIC value
Sat_044	0.6604	2	0.4486	0.3480
Sat_171	0.5660	2	0.4913	0.3706
Sat_205	0.8302	2	0.2820	0.2422
Sat_375	0.8679	2	0.2293	0.2030
Satt174	0.8868	2	0.2008	0.1806
Satt226	0.5849	3	0.5005	0.3928
Satt244	0.6038	2	0.4785	0.3640
Satt500	0.7547	3	0.3788	0.3199
Satt520	0.3585	3	0.6629	0.5887
Satt540	0.6792	2	0.4357	0.3408
Satt551	0.7736	2	0.3503	0.2889
Satt557	0.9811	2	0.0370	0.0363
Mean	**0.7123**	**2.25**	**0.3746**	**0.3063**

Major cluster confined forty-seven genotypes and this cluster was further alienated into two sub clusters, one major and one minor. Minor group had ten genotypes including MACS-15-20, RSC10-70, SKF-SPS-11, RVS-76, KDS980, KDS992, RSC-10-71, JS335, RVS2011-35 and RVS2007-6. Among these ten genotypes, genotypes KDS992 and KDS980 shared common parent *i.e.*, JS93-05. The major sub cluster contained 37 genotypes and it was later splinted into two sub groups. Major sub group contained 21 genotypes *viz.,* MACSNRC-1575, NRC-147, AGS111, AMS100-39, MACS1520, JS20-94, NRC86, EC457286, NRC125, PS-1613, VLS94, SL-1068, RSC10-52, NRC130, G-29, JS20-34, JS20-84, MACS575, NRC SL-1, PS-1092 and NRC127 while minor cluster had genotypes *viz.*, SP37, SL-1123, NRC76, AMSMBC-18, NRC131, NRC134, RVS18, RVS24, AMS2014-1, RVS2001-4, JS20-98, JS20-69, JS20-71, JS20-116, NRC-132 and JS20-29. Similar clustering was found in preceding investigations carried out on microsatellite-based diversity analysis among Indian soybean genotypes [30,31].

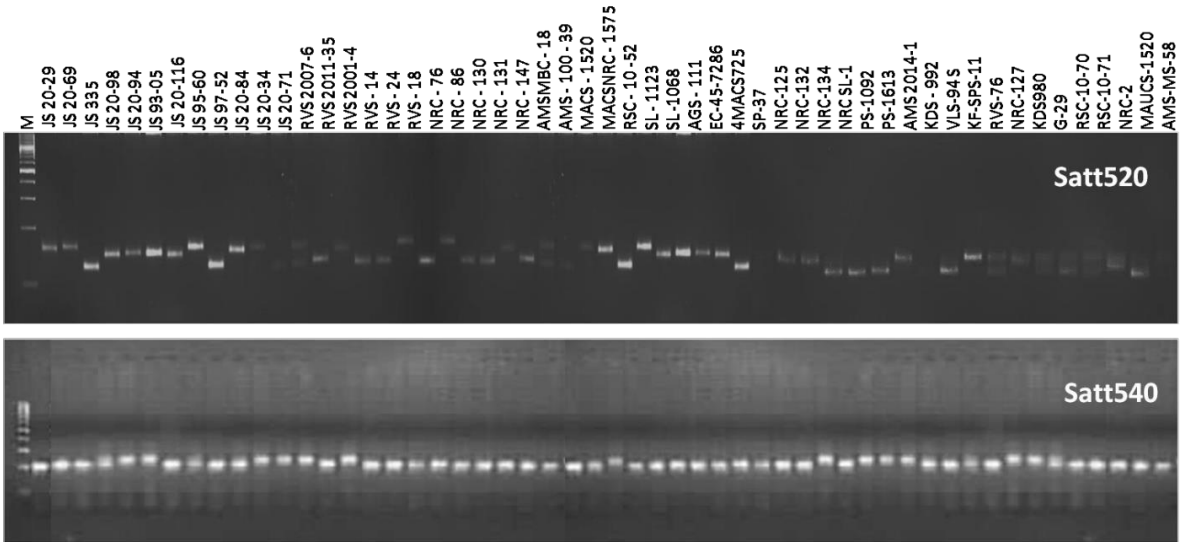


Fig. 1. Electrophoretic banding pattern of template DNA samples of soybean genotypes amplified with SSR markers

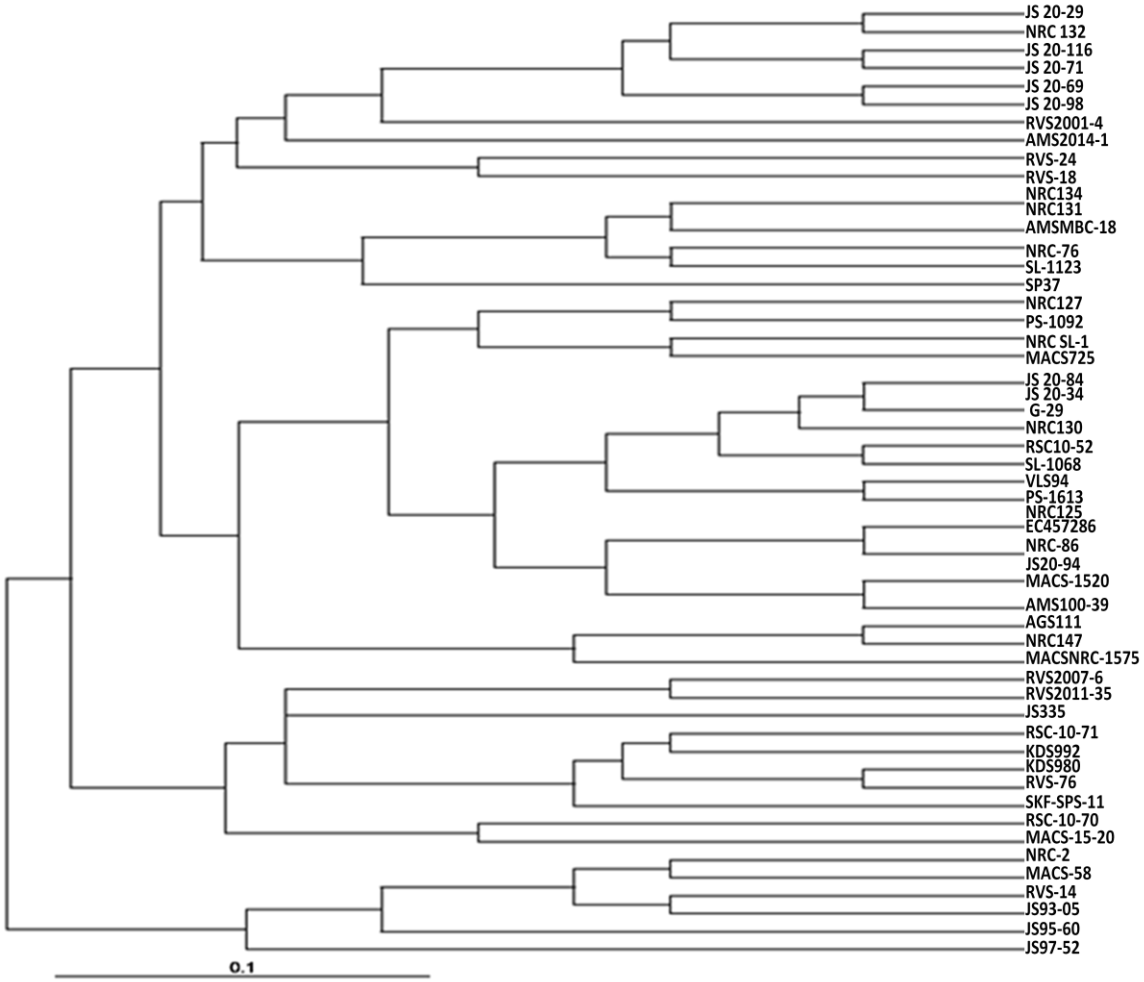


Fig. 2. Dendrogram representing SSR markers-based relationship among genotypes of soybean

5. CONCLUSION

The clusters formed in the current investigation based on SSR molecular markers data were able to distinguish few droughts tolerant genotypes from rest of the susceptible genotypes. The grouping of the genotypes also designates clustering of most of the genotypes conferring to their centers of development. Some of the genotypes showing higher similarity were also developed with the use of common parents during hybridization programme. These results confirm the efficiency of SSR molecular markers to discriminate the genotypes according to their genetic makeup as well as targeted characters.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Identification of Low and High Erucic Acid Containing Genotype(S) in Indian Mustard Employing Molecular Markers

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ABSTRACT

In traditional *Brassica* oilseeds, the incidence of erucic acid is well-thought-out as anti-nutritional issue for human ingesting as these reasons toxic possessions on the heart at higher enough dosages. As such there is a pressing necessity to restrain the erucic acid content and develop varieties having low erucic acid through conventional and/or molecular breeding approaches. Genetic enhancement of crops can be augmented when there is a broad genetic variation and information on these genetic possessions is available. The current investigation was carried out with 48 *Brassica* genotype (s) with the intention to recognize genotype (s) with low and high erucic acid content on the basis of molecular markers. For the investigation, a total of 50 SSR molecular markers were chosen for the amplification of genomic DNA. Out of these, only 23 SSR molecular markers were found to be polymorphic. A total of 109 alleles were recognized with an average of 4.47 alleles per locus for polymorphic SSR markers. Genetic diversity ranged between 0.55 for marker Na10-D07 to 0.77 for marker BRMS-098 with a mean worth of 0.68. Polymorphism information content (PIC) value of the markers varied from 0.51 for SSR Na10-D07 to 0.73 for the primer BRMS-098 with an average value of 0.62. The dendrogram was constructed and major three clusters were formed. Most of the genotypes were clustered conferring to the sites they established. Genotypes evidenced with low erucic acid content displayed higher similarity and grouped together. Mustard genotypes recognized with higher genetic variability with convenient traits may be employed for crop improvement programmes in future.

Keywords: Indian mustard; fatty acid; oil quality; erucic acid, SSR markers.

1. STUDY OBJECTIVES

The present study was conducted with 48 *Brassica* genotype (s) with the aim to distinguish genotype(s) with low and high erucic acid content based on employment of molecular markers.

2. INTRODUCTION

India is the world's third-largest producer of mustard seeds. Indian mustard [*Brassica juncea* (Linn.) Czern & Coss] is an ordinary amphidiploid (2n=36) that is the outcome of an interspecific cross between *Brassica campestris* (2n=20) and *Brassica nigra* (2n=16) followed by natural chromosomal doubling [1]. In India, it is mostly grown for its oil seeds. India, as one of the world's largest oilseed producers, contributes around 7% of the worldwide total. Oilseed harvests are also important in the Indian economy. Foremost rapeseed mustard producing countries of the world are Canada, China, France, Germany, Poland, UK, India, Australia, Russia and Ukraine [2-3]. In 2016-17, the total area, production, and productivity of rapeseed mustard cultivation in India were 6.65 million hectares, 7.10

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million tonnes, and 1069 $kgha^{-1}$, respectively. It was sown in 0.723 million hectares in M.P. and produced 0.607 million tonnes with 840 kgh^{-1} productivities in 2016-2017 [2-3]. In traditional *Brassica* oilseeds, the occurrence of erucic acid is considered as anti-nutritional factor for human consumption that causes toxic effects on the heart at higher enough doses [4-7,3]. As such there is need to minimize the erucic acid content and have low erucic acid genotypes through breeding and biotechnological tools. Genetic enhancement of crops can be augmented when there is a broad genetic variation and information on these genetic possessions is available [8]. Research on *Brassica* germplasm lines could enhance the edible oil production and nutritional benefits of these crops. The collection of these genetic resources and the assessment of genetic diversity within and between these resources should be given priority for varietal improvement [3-4,8].

Molecular markers are beneficial contrivances for sleuthing dissimilarity within different cultivars [9]. There are several advantages of the DNA based diversity analysis techniques including highly reproducible result, can be done at any stage of growth, only a small amount of plant tissue is required, trait for insect and disease resistance including qualitative traits can be designated as testified in many crop species by several scientists all over the world including Indian mustard [10-32]. DNA based diversity analysis authorize the consistent chasing of beneficial trait during varietal selection. DNA based diversity analysis is the only practical or available technique for ensuring the presence of multiple beneficial genes in a single variety. Owing to above facts in mind, the current investigation was commenced with the intend to detect *Brassica* genotype(s) for low and high erucic acid content by employing molecular markers.

2. MATERIALS AND METHODS

2.1 Experimental Material

Experimental material comprises of forty-eight Indian mustard genotypes collected from the Zonal Agricultural Research Station, Morena, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior M.P., India (AICRP on Rapeseed and Mustard) and IARI, New Delhi, India. These were assembled from diverse portions of India and abroad (Table 1). These genotypes were chosen depend on their high and low erucic acid containing aptitudes from 196 genotypes based on biochemical analysis work carried out by Shyam and Tripathi [2].

2.2 Molecular Analysis

For isolation of genomic DNA, fresh leaf tissue was acquired from 3-4-week-old plants cultivated in the field. DNA was isolated using modified CTAB method of Murray and Thompson [33] with slight modifications as suggested by Tiwari et al. [34]. Highly polymorphic random SSR (Table 2), gene based SSR (Table 2) and gene-based CAPS molecular markers (Table 2) were employed in present investigation. The primers were synthesized by Integrated DNA Technologies. Polymerase chain reaction was performed in 10µl reaction mixture comprising of 10X dream *Taq* buffer (1µl), 5U/µl *Taq* DNA polymerase, 15µl dNTP (1mM), 6.75µl NFW, 0.5 µl of forward and reverse primers each (10 pM) and 50 ng/µl of genomic DNA in a Thermocycler (BIO-RAD). Following parameters were tailed to amplify the templates: Initial denaturation at 94°C for 1 min tracked by 35 cycles of denaturation at 94°C for 40 sec (denaturation), annealing at 52-55°C for 90 sec, extension at 72°C for 60 sec. The final extension was performed at 72°C for 7 min.

2.3 Data Analysis

Amplicons were separated on 3% agarose gel through electrophoresis and later gels were exposed to visualization with UV image analyzer and their images were considered for further scoring of band. The major allele frequency, polymorphism information content (PIC) and genetic distance-based clustering was accomplished with Unweighted Pair Group Method for Arithmetic average (UPGMA) tree using Power Marker v3.25 software [35] and the dendrogram was constructed using MEGA 4.0 software [36]. SSR data was again imperiled to cluster analysis tracked by bootstrap analysis with 1000 permutations for all the genotypes using Mega 4.0 software. The population structure for 48 genotypes was inferred using Structure 2.3.4 software [37]. The structure harvests were envisaged

employing Structure Harvester from which Evanno plots were constructed [38]. Duncan's Multiple Range Test (DMRT) (P=0.05) was employed to evaluate differences among clusters for significance by using SPSS ver. 19.0 software.

Table1. List of mustard genotypes along with source used in present experimentation

S. No.	Genotypes	Germplasm/Varieties	Origins/Sources	Country of origin
1	MRNJ-1	Germplasm	ZARS, Morena (M.P.)	India
2	MRNJ-2	Germplasm	ZARS, Morena (M.P.)	India
3	MRNJ-3	Germplasm	ZARS, Morena (M.P.)	India
4	MRNJ-4	Germplasm	ZARS, Morena (M.P.)	India
5	MRNJ-5	Germplasm	ZARS, Morena (M.P.)	India
6	MRNJ-6	Germplasm	ZARS, Morena (M.P.)	India
7	MRNJ-7	Germplasm	ZARS, Morena (M.P.)	India
8	MRNJ-8	Germplasm	ZARS, Morena (M.P.)	India
9	MRNJ-9	Germplasm	ZARS, Morena (M.P.)	India
10	MRNJ-10	Germplasm	ZARS, Morena (M.P.)	India
11	MRNJ-11	Germplasm	ZARS, Morena (M.P.)	India
12	MRNJ-12	Germplasm	ZARS, Morena (M.P.)	India
13	MRNJ-13	Germplasm	ZARS, Morena (M.P.)	India
14	MRNJ-14	Germplasm	ZARS, Morena (M.P.)	India
15	MRNJ-15	Germplasm	ZARS, Morena (M.P.)	India
16	MRNJ-16	Germplasm	ZARS, Morena (M.P.)	India
17	MRNJ-17	Germplasm	ZARS, Morena (M.P.)	India
18	MRNJ-18	Germplasm	ZARS, Morena (M.P.)	India
19	MRNJ-19	Germplasm	ZARS, Morena (M.P.)	India
20	MRNJ-20	Germplasm	ZARS, Morena (M.P.)	India
21	MRNJ-21	Germplasm	ZARS, Morena (M.P.)	India
22	MRNJ-22	Germplasm	ZARS, Morena (M.P.)	India
23	MRNJ-23	Germplasm	ZARS, Morena (M.P.)	India
24	MRNJ-24	Germplasm	ZARS, Morena (M.P.)	India
25	MRNJ-25	Germplasm	ZARS, Morena (M.P.)	India
26	ISC-3	RIL	Rasi seed company, Gururam (Haryana)	India
27	JM-2	Variety	RVSKVV, Gwalior (M.P.)	India
28	RVM-1	Variety	RVSKVV, Gwalior (M.P.)	India
29	RVM-2	Variety	RVSKVV, Gwalior (M.P.)	India
30	Rohini	Variety	CSAUAT, Kanpur (U.P.)	India
31	Maya	Variety	CSAUAT, Kanpur (U.P.)	India
32	NRCDR-2	Variety	DRMR, Bharatpur (Rajasthan)	India
33	DRMRIJ-31	Variety	DRMR, Bharatpur (Rajasthan)	India
34	DRMR-150-35	Variety	DRMR, Bharatpur (Rajasthan)	India
35	GM-2	Variety	SDAU, Banaskantha (Gujarat)	India
36	CS-54	Variety	CSSRI, Karnal (Haryana)	India
37	RB-50	Variety	CCSHAU, Hisar (Haryana)	India
38	RH749	Variety	CCSHAU, Hisar (Haryana)	India
39	JD-6	Variety	IARI, New Delhi	India
40	L-4	Germplasm	Canada	Canada
41	L-6	Germplasm	Canada	Canada
42	PM-25	Variety	IARI, New Delhi	India
43	PM-21	Variety	IARI, New Delhi	India
44	PM-22	Variety	IARI, New Delhi	India
45	PM-24	Variety	IARI, New Delhi	India
46	PM-29	Variety	IARI, New Delhi	India
47	PM-30	Variety	IARI, New Delhi	India
48	LES-39	Variety	IARI, New Delhi	India

Table 2. List of SSR and CAPS markers with their sequences used in the study

S. No.	Marker name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Random	*SSR markers*		
1	BRMS-093	TCCAAGTAGACCGAATCAAGAGAGT	ATAAATCGAACCTGAAACCATGTCT
2	BRMS- 098	TGCTTGAGACGCTGCCACTTTGTTC	CATTCCTCCCCACCACCTTCACATC
3	BRMS- 240	CAAGAGTATTTGTGTGGGTTGACTC	AAATAACGAACGGAGAGAGAGAGAG
4	BRMS- 324	AACTTAACCGAAACCGAGSTAGGTG	AATCTCGAAATTCATCGACTTCCTC
5	CB -10065	CGGCAATAATGGACCACT	CGGCTTTCACGCAGACTTCG
6	SORF -73	CGTGGGCCAAGCTTAGATTA	CGTTCAAGAAGACACAGATCAAA
7	SR- 7223	AGGACCCGACTTTCCTTGTT	ACCAAACTCGGCGTACAAAT
8	SR- 9222	CACCGAACAAAACTGAGGGT	CGTTTCACTGCGTTCTACCA
9	SR 94102	ATCCCCAAACTACCCTCACC	AGGATGAGCAAAGGAAAGCA
10	SR-9447	AAATTCGAAAATGCAAACGG	CCAATCTTGGAACAATAGAAGATG
11	OI 10-CO 5	GGCTACAAAATGTTTGATAAGCTCT	ACCTGAAAGAGAGGCTACACAT
12	SSR Na10-B08	AGAGAAAAACACTTCCCGCC	GTGAGCTTTGCGAAACACG
13	SSR Na10-B10	GTCGGGTTTGAGTGAGTTGG	CATCGCAGATCCTTCTCTCC
14	SSR Na10-B11	TTTAACAACAACCGTCACGC	CTCCTCCTCCATCAATCTGC
15	SSR Na10-C01	TTTTGTCCCACTGGGTTTTC	GGAAACTAGGGTTTTCCCTTC
16	SSR Na10-C03	TTGGGTGTCTTTGTTACCCC	ACCGAGAAGACTGATACGGG
17	SSR Na10-C06	TGGATGAAAGCATCAACGAG	ATCAATCAACACAAGCTGCG
18	SSR Na10-C08	GTTTGGTTCAGAGGCAGAGG	CTATCGCTGCAGAAGAAGGG
19	SSR Na10-D03	ATGATTTGCCTTGAAATGCC	GATGAAACAATAACCTGAGACACAC
20	SSR Na10-D07	CTACTTTGATGGACACTTGCC	TCTGAAGTTGATTAGTCGGTCC
21	SSR Na10-D08	TCCATTCATTAAAATCGGCG	TTCTGATCCCTTTCTCTCCC
22	SSR Na10-D09	AAGAACGTCAAGATCCTCTGC	ACCACCACGGTAGTAGAGCG
23	SSR Na10-D11	GAGACATAGATGAGTGAATCTGGC	CATTAGTTGTGGACGGTCGG
Gene specific SSR markers			
24	FAE1	ATGACGTCCGTTAACGTA	AAGACTTGTCGTCAGCTCCA
25	Sal-SRK-I	GATTATCTCGTGTCTGAATG	GGTAATGTCGAATCTCTCCT
CAPS markers			
26	CAPS1265	ACGTTAGGTCCGTTGATTCTTC	GGGTATCTGTCGATGCAATGT
27	CAPS237	TAACCATCGCTCCACTCTTTG	TCAAGAAGTCAAGCCACGAC

3. RESULTS AND DISCUSSION

Molecular breeding is one of the noteworthy and fast approaches for crop improvement in mustard [39-40]. High quality DNA was employed for amplification of highly polymorphic SSR across 48 genotypes of Indian mustard. A total of 50 SSR markers set were amplified with DNA samples of two Indian mustard genotypes in preliminary experiment. Out of 50 SSR markers, a total of twenty-seven were found monomorphic and 23 were polymorphic. So, these 23 polymorphic markers were amplified across 48 Indian mustard genotypes.

The demonstrative gel exemplified amplified monomorphic and polymorphic banding patterns of selected primers. A total 109 alleles were amplified across 23 markers among all the 48 genotypes (Table 3). Diversity analysis of Indian mustard genotypes was done with 23 highly polymorphic SSR markers. A total of 109 alleles were recognized with an average of 4.47 alleles per locus for polymorphic SSR markers. Genetic diversity varied from 0.55 for SSR marker Na10-D07 to 0.77 for marker BRMS-098 with a mean value of 0.68. Polymorphism information content (PIC) value of the markers ranged between 0.51 for SSR marker Na10-D07 to 0.73 for primer BRMS-098 with a mean value of 0.62. The results were similar with the results of Yadav et al. [41], studied genetic diversity among 30 Indian mustard genotypes using 20 ISSR primers showing 156 amplified bands with an average of 7.8 bands per primer out of which 115 were p o l y m o r p h i c. Size of amplified fragments ranged from 100 bp to 1500 bp and number ranged between 4 to 14. The primer BRMS-098 showed highest gene diversity (0.77) and PIC value (0.73) which is representing 77% polymorphism probability of the marker. The lowest gene diversity and PIC value (0.51) was documented for the primer Na10-D07, indicating 51% polymorphism. All 23 primers representing PIC value more than 50%, so these primers can further be used for diversity analysis. Similarly, Fayyaz et al. [42] also employed 90 genotypes for molecular characterization employing 24 microsatellite markers, but only 12 SSR primer combinations generated a total of 33 alleles, of that 32 were polymorphic, whereas only one had monomorphic locus. The average number(s) of polymorphic alleles per locus was 2.66. The PIC values arrayed from 0.395 for primer Ra2-E03 to 0.726 for primer BRMS-019 with an average genetic diversity of 0.584 per locus. Recently, Habib et al. [43] presented diversity among 20 *Brassica juncea* genotypes using 15 SSR primer pairs. A total of 79 polymorphic alleles with an average of 5.26 alleles per locus were generated. The polymorphism information content (PIC) values varied between 0.41 to 0.94 reflecting the presence of high allelic variation. The magnitude of similarity coefficient was found to be the maximum amongst pair-wise combinations of genotypes.

The dendrogram was arranged based on scoring done for the entire polymorphic SSR markers. Major three clusters were formed *i.e*., C-I, C-II and C-III, in which cluster C-I consisted of 4 genotypes (Fig.1; Table 4), while Cluster C-II had 9 genotypes. Among these nine genotypes three *namely*: JM-2, RVM-1 and RVM-2 have been developed at Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh. Cluster C-III contained 35 genotypes including MRNJ-24, MRNJ-23, MRNJ-22, L-6, L-4, MRNJ- 14, MRNJ-11, MRNJ-15, MRNJ-19, MRNJ-18, MRNJ-21, MRNJ-20, MRNJ-17, MRNJ-16, MRNJ-13, MRNJ-12, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-10, MRNJ-5, MRNJ-4, MRNJ-3, MRNJ-2, MRNJ-1 and MRNJ-6 germplasm lines representing same place of their origin (Zonal Agriculture Research Station, Morena, Madhya Pradesh, India) except L-4 and L-6. Further genotypes PM-25, PM-22, PM-21, PM-29, PM-24, LES-39, PM-30 are notified varieties and congregated together with higher similarity index. These all varieties have been developed at Indian Agricultural Research Institute, New Delhi having low erucic acid content. These all varieties are favored for timely sown under rainfed conditions. In these varieties, Pusabold, Pusa Agrani, Pusa Barani, Pusa Jagannath is involved as one of the parents. Likewise, in a previous study conducted by Vinu et al. [44] with 44 mustard genotypes, four clusters were designed. Among them first cluster encompassed eight varieties developed at IARI, New Delhi. In six of these varieties, except two early maturing varieties *i.e*., Pusa Agrani and Pusa Tarak, Varuna is involved as one of the parents directly or through the ancestry. The result of the present examination displays the usefulness of SSR markers in identification of the close pedigree association in the studied genotypes. Previously, a similar finding concerning efficiency of SSR markers in determination of genetic variability have also been stated by Plieske and Struss [45]. Related types of investigations on application of SSR markers have also been accomplished in *B. napus* by Hopkins et al. [46]. In the current investigation, good quality

polymorphic markers were found that can be utilized for the identification of genotypes with useful traits for future breeding tenacities. With the help of these markers, genes introgression can be done to desirable genetic backgrounds for the improvement of Indian mustard. The population structure of the 48 Indian mustard genotypes was assessed employing STRUCTURE v2.3.3 software based on SSR molecular markers. The optimum K value was determined by using Structure Harvester, where the highest peak was observed at delta K = 3. The number of sub populations (K) was recognized based on maximum likelihood and delta K (dK) values, with into three groups (Fig. 2 and Fig. 3) using a membership probability threshold of 0.80. First population embodied by green colour contained 10 genotypes. Second population (red) signified 8 genotypes and third (blue) 11 genotypes. Admixture genome was represented by subpopulation of 5 genotypes green and red, 5 other genotypes represented green and blue, 3 genotypes signified all three population *i.e*., green, red and blue and 4 genotypes red and blue subpopulation. Molecular valuation of crop genetic variability using SSR molecular markers is important owing to their co-dominance nature and competence to divulge a high number of alleles per locus. It is enormously valuable for modern-day plant breeding [47]. The recognized assorted genotypes can be exploited for future breeding schemes in mustard.

Table 3. List of SSR and CAPS markers with their major allele frequency, allele number, gene diversity, heterozygosity and PIC values

Markers	Major Allele Frequency	Allele No.	Gene Diversity	Heterozygosity	PIC value
BRMS-093	0.48	6.00	0.71	0.00	0.68
BRMS- 098	0.33	6.00	0.77	0.00	0.73
BRMS- 240	0.33	4.00	0.74	0.00	0.69
BRMS- 324	0.56	4.00	0.60	0.00	0.55
CB -10065	0.46	5.00	0.69	0.00	0.65
SORF -73	0.42	5.00	0.69	0.00	0.63
SR- 7223	0.44	3.00	0.62	0.00	0.54
SR- 9222	0.52	4.00	0.63	0.00	0.57
SR 94102	0.38	5.00	0.74	0.00	0.70
SR-9447	0.52	6.00	0.64	0.00	0.59
OI 10-CO 5	0.44	4.00	0.62	0.00	0.55
SSR Na10-B08	0.31	6.00	0.75	0.77	0.71
SSR Na10-B10	0.42	4.00	0.71	0.83	0.66
SSR Na10-B11	0.35	5.00	0.73	0.00	0.68
SSR Na10-C01	0.54	4.00	0.60	0.00	0.53
SSR Na10-C03	0.44	5.00	0.65	0.00	0.58
SSR Na10-C06	0.38	6.00	0.75	0.00	0.71
SSR Na10-C08	0.40	4.00	0.69	0.00	0.63
SSR Na10-D03	0.48	4.00	0.63	0.00	0.56
SSR Na10-D07	0.65	5.00	0.55	0.00	0.51
SSR Na10-D08	0.44	5.00	0.68	0.00	0.62
SSR Na10-D09	0.38	4.00	0.68	0.00	0.62
SSR Na10-D11	0.51	5.00	0.65	0.44	0.60
Mean	0.44	4.74	0.68	0.09	0.62
Gene specific SSR markers					
FAE1	0.37	5.00	0.70	0.00	0.64
Sal-SRK-I	0.43	5.00	0.71	0.00	0.66
Mean	0.40	5.00	0.70	0.00	0.65
CAPS markers					
CAPS237	0.50	5.00	0.66	0.00	0.61
CAPS1265	0.31	4.00	0.74	0.00	0.69
Mean	**0.41**	**4.50**	**0.70**	**0.00**	**0.65**

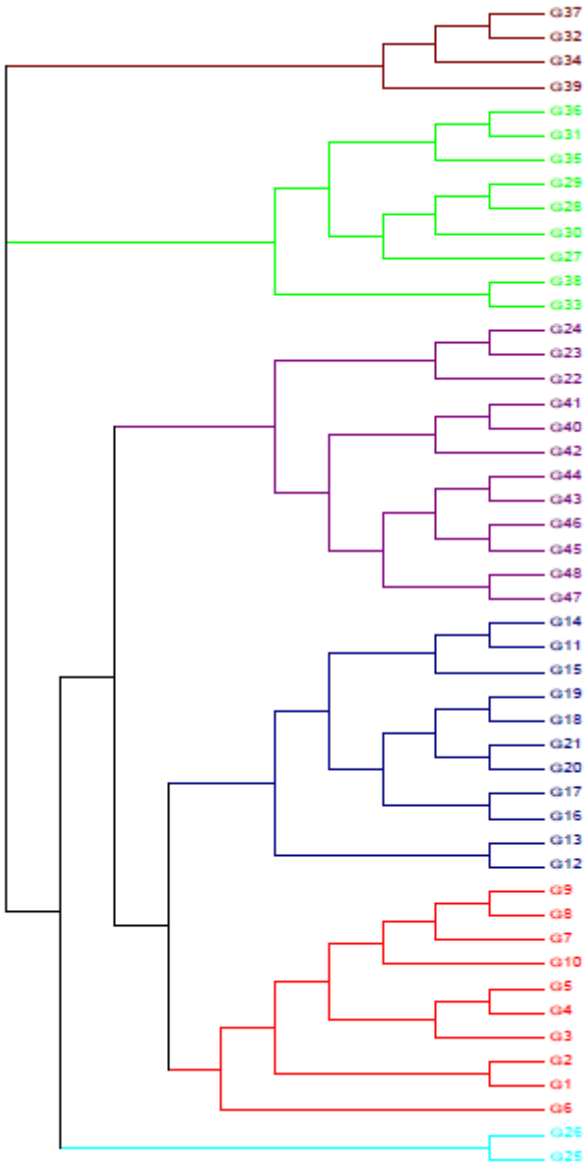


Fig. 1. Dendrogram of highly polymorphic SSR markers using power marker v3.25 software

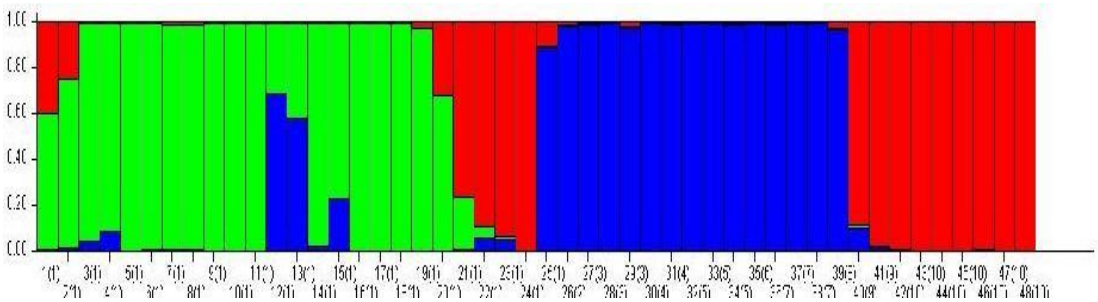


Fig. 2. Model based population structure plot with K = 3, using 28 primers

Table 4. Cluster groups of Indian mustard genotypes and their characteristics using highly polymorphic SSR markers

Major clusters	Sub clusters	No. of genotypes	Name of genotypes	Remark
C-I,		4	RB-50, NRCDR-2, DRMR150-35 and JD6	Suitable for rainfed and salinity condition (RB50), Suitable for rainfed & Irrigated conditions, high oil content, high temperature tolerant and tolerant to salinity (NRCDR-2), Suitable for early sown and rainfed condition, early maturity, tolerant to powdery mildew & *A. blight* (DRMR150-35), Drought (rainfed), suitable for early (September) and late (November) sowing under irrigated situations (JD6)
C-II	C-II (a)	7	CS-54, Maya, GM-2, RVM-2, RVM-1, Rohini and JM-2	Salinity tolerant (CS54), Suitable for irrigated condition and white rust resistance (Maya), Nontraditional areas (GM2), Early maturity and high oil content (RVM1), Suitable for rainfed and late sown conditions (RVM2), High oil content, drought tolerant and suitable for irrigated Condition (Rohini), Suitable for rainfed condition and white rust resistance (JM2)
	C-II (b)	2	RH749 and DRMRIJ-31	Suitable for irrigated condition (RH749), Suitable for timely sown and irrigated condition, bold seeded, high oil content and high yielding variety (DRMRIJ-31)
C-III	C-III (a)	33	MRNJ-24, MRNJ-23, MRNJ-22, L-6, L-4, PM-25, PM-22, PM-21, PM-29, PM-24, LES-39, PM-30, MRNJ-14, MRNJ-11, MRNJ-15, MRNJ-19, MRNJ-18, MRNJ-21, MRNJ-20, MRNJ-17, MRNJ-16, MRNJ-13, MRNJ-12, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-10, MRNJ-5, MRNJ-4, MRNJ-3, MRNJ-2, MRNJ-1 and MRNJ-6	Low erucic acid, suitable for timely sown irrigated conditions and very wider adaptability (PM21), Low erucic acid, suitable for timely sown and irrigated conditions (PM22), Low erucic acid, suitable for timely sown and irrigated conditions (PM24), High temperature tolerant, suitable for irrigated conditions and early sown (PM25), Low erucic acid, timely sown and irrigated conditions (PM29), Low erucic acid, timely sown and irrigated conditions (PM30) Low erucic acid, timely sown, irrigated condition and tolerance to white rust (LES39)
	C-III (b)	2	MRNJ-26 and MRNJ-25	

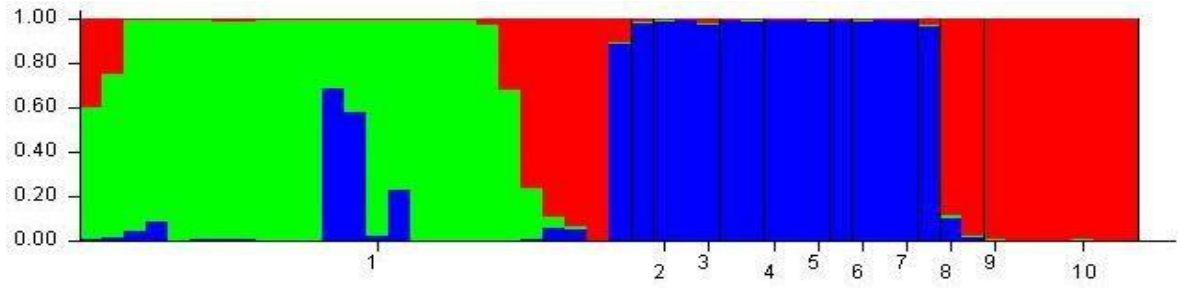


Fig. 3. Single line analysis based on population structure plot with K=3, using 28 primers

3.1 Screening for Erucic Acid Content in Indian Mustard Genotypes Using Gene Based SSR Markers

Erucic acid is one of the auspicious traits having versatile use and there is numerous information where molecular markers have been employed for screening and characterization [48-49]. Gene based SSR markers have been used for erucic acid and glucosinolate screening [50,49,9]. In the current investigation, two gene based SSR markers for erucic acid were employed to screen mustard germplasm lines. Maximum major allele frequency was 0.43 for marker Sal-SRK-I and minimum 0.37 for marker FAE1 with an average of 0.40. A total of 10 alleles were identified with an average of 5 alleles per locus for polymorphic SSR markers. Allele number of both markers were 5.00 and also mean was 5.00. Highest gene diversity was documented 0.71 for marker Sal-SRK-I and minimum 0.70 for marker FAE1 with an average value of 0.70. Maximum polymorphic information content was 0.66 for marker Sal-SRK-I and minimum 0.64 for marker FAE1 with a mean worth of 0.65. The primer which showed highest gene diversity (0.71) and PIC value (0.66) was observed in Sal-SRK-I, which is representing 71% polymorphism probability of the marker. The lowest gene diversity and PIC value (0.64) was documented for the primer FAE1, indicating 64% polymorphic nature of the marker. Both primers representing PIC value more than 50%, so these primers can further be used. The genetic relationships among Indian mustard genotypes were characterized. Major three clusters were formed *i.e*., C-I, C- II and C-III. Cluster I make a group of 4 diverse genotypes *namely*: CS-54, GM-2, DRMR-150-35 and DRMRIJ-31. Cluster II was also having 4 genotypes including MRNJ-6, MRNJ-5, RH-74.9 and RB-50. Cluster III had 40 genotypes (Fig. 4, Table 5).

In the present experimentation, gene specific markers were not able to differentiate all mustard genotypes according to their erucic acid content. Contrary, Sarikamis et al. [51] have got successful amplifications with genomic DNA from kale genotypes with the SSR marker $O_{l12}FO_2$. It was determined that $O_{l12}FO_2$ was polymorphic among kale genotypes. It was successful amplification of markers within different vegetable *Brassica*s. Further, Chandra et al. [52] estimated 37 diverse Indian mustard genotypes using 10 A and C genome specific SSR markers for the development of molecular profile. Singh et al. [53] used 453 SSRs on mustard genotypes to identify white rust resistance genotypes. The cluster analysis gave three major groups where white rust resistant genotypes were grouped in cluster 1, low quality genotypes in cluster 2 while the recipients were grouped in the cluster 3 indicating that grouping of genotypes based on SSRs corresponded. Similarly, Bharti et al. [49] used four SSR markers to characterize mustard genotypes for erucic acid and glucosinolate contents. All the genotypes were clustered in three groups. Cluster I had seven genotypes trailed by cluster II five and cluster III two genotypes. Recently, Kapadia et al. [41] screened molecular markers for powdery mildew resistance in Indian mustard. The molecular analysis for powdery mildew resistance in *Brassica spp.* was carried out with four females, three males, F_{1S} and F_{2S} generation. The SSR markers *viz,* OI10-B12 and OI10-C01 clearly distinguished between susceptible and resistant bulks of interspecific cross GM-3 x Pusa Swarnim. Monika et al. [54] screened out 200 SSR markers for polymorphism in two parental *Brassica juncea* genotypes *i.e*., RB 50, drought tolerant and Kranti, drought susceptible. Markers were able to differentiate drought tolerant and susceptible genotypes with low similarity coefficient. Bhatia and Alok [55] Gupta et al. [56]) and Yan et al. [57] documented that erucic acid trait is governed by two independent genes FAE1.1 and FAE1.2 in allotetraploids *B. napus and B. juncea*. Several earlier studies including Pandey et al. [58], Singh et al. [59], Cao et al. [60] and Lühs et al. [61] have also reported the presence of continuous variation in erucic acid content

in segregating populations of *B. juncea* and *B. napus*, respectively. Related to our study, Saini et al. [46] also documented that two SNPs in *FAE1.1* at position 591 and 1265 and one in *FAE1.2* at 237 were found polymorphic among low and high erucic acid containing genotypes.

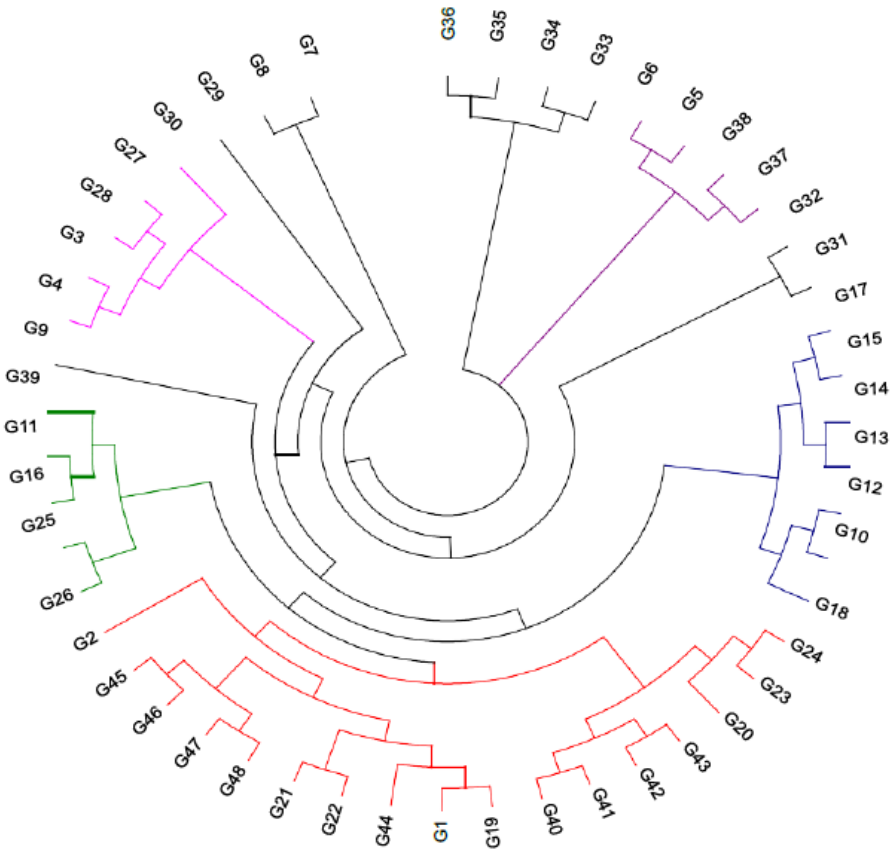


Fig. 4. Phylogenetic tree analysis of gene based SSR marker data analysis using power marker v3.25 software

3.2 Gene Based CAPS Markers for Screening of Erucic Acid Contents of Indian Mustard

Two gene-based CAPS markers for erucic acid were employed to screen mustard germplasm lines. Maximum major allele frequency was found 0.50 for CAPS237 and minimum 0.31 for CAPS1265 with an average of 0.41. A total of 9 alleles were recognized with an average of 4.5 alleles per locus for polymorphic SSR markers. Maximum gene diversity was consisted 0.74 for marker CAPS1265 and minimum was 0.66 for marker CAPS237 with an average of 0.70. Highest polymorphic information content was found 0.69 for marker CAPS1265 and lowest 0.61 for marker CAPS237 with a mean worth of 0.65. The primer which showed highest gene diversity (0.74) and PIC value (0.69) was observed in CAPS1265, which is representing 74% polymorphism probability of the marker. The lowest gene diversity and PIC value (0.61) was observed for the primer CAPS237, indicating 61% polymorphic nature of the marker.

Based on dendrogram, genetic relationships among Indian mustard genotypes were documented. Major three clusters *viz.,* C-I, C-II and C-III were formed. Cluster I had 2 genotypes. Cluster II confined 42 genotypes. While C-III consisted of 4 genotypes (Fig. 5, Table 6).

Table 5. Tabular representation of major and sub clusters formed based on gene based SSR markers

Major clusters	Sub clusters	No. of germplasm lines and varieties	Name of germplasm lines and varieties	Remark
C-I,		4	CS-54, GM-2, DRMR-150-35 and DRMRIJ-31	Salinity tolerant (CS54), Nontraditional areas (GM2), Suitable for early sown and rainfed condition, early maturity, tolerant to powdery mildew &*A. blight (*DRMR-150-35), Suitable for timely sown and irrigated condition, bold seeded, high oil content and high yielding variety (DRMRIJ-31)
C-II		4	MRNJ-6, MRNJ-5, RH-749 and RB-50	Suitable for irrigated condition (RH749), Suitable for rainfed and salinity condition (RB50)
C-III	C-III (a)	38	NRCDR-2, Maya, MRNJ-17, MRNJ-15, MRNJ-14, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-18, MRNJ-24, MRNJ-23, MRNJ-20, PM-21, PM-25, L-6, L-4, MRNJ-19, MRNJ-1, PM-22, MRNJ-22, MRNJ-21, LES-39, PM-30, PM-29, PM-24 MRNJ-2, ISC-3, MRNJ-25, MRNJ-16, MRNJ-11, JD-6, MRNJ-9, MRNJ-4, MRNJ-3, RVM-1, JM-2, Rohini and RVM-2	Suitable for rainfed & Irrigated conditions, high oil content, high temperature tolerant and tolerant to salinity (NRCDR-2), Suitable for irrigated condition and white rust resistance (Maya), Low erucic acid, suitable for timely sown irrigated conditions and very wider adaptability (PM21), Low erucic acid, suitable for timely sown and irrigated conditions (PM22), Low erucic acid, suitable for timely sown and irrigated conditions (PM24), High temperature tolerant, suitable for irrigated conditions and early sown (PM25), Low erucic acid, timely sown and irrigated conditions (PM29), Low erucic acid, timely sown and irrigated conditions (PM30) Low erucic acid, timely sown, irrigated condition and tolerance to white rust (LES39), Drought (rainfed) Suitable for early (September) and late (November) sowing under irrigated situations (JD6), Suitable for rainfed condition and white rust resistance (JM2), High oil content, drought tolerant and suitable for irrigated Condition (Rohini), Early maturity and high oil content (RVM1), Suitable for rainfed and late sown conditions (RVM2)
	C-III (b)	2	MRNJ-8 and MRNJ-7	

Table 6. Tabular representation of major and sub clusters formed based on CAPS markers

Major clusters	Sub clusters	No. of germplasm and varieties	Name of germplasm and varieties	Remarks
C-I,		2	JD6 and RB50	Drought (rainfed) Suitable for early (September) and late (November) sowing under irrigated situations (JD6), Suitable for rainfed and salinity condition (RB50)
C-II	C-II (a)	41	RH749, Maya, MRNJ-17, MRNJ-16, MRNJ-15, MRNJ-14, PM25, PM29, MRNJ-4, MRNJ-3, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-5, PM24, PM22, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-11, MRNJ-6, JM-2, ISC-3, L6, GM-2, DRMR150-35, DRMRIJ-31, LES-39, PM30, MRNJ- 2, MRNJ-1, MRNJ-22, MRNJ-25, MRNJ-20, MRNJ-18, MRNJ-19, PM-21, MRNJ-23, MRNJ-21, MRNJ-24 and RVM-1	Suitable for irrigated condition (RH749), Suitable for irrigated condition and white rust resistance (Maya), Low erucic acid, suitable for timely sown irrigated conditions and very wider adaptability (PM21), Low erucic acid, suitable for timely sown and irrigated conditions (PM22), Low erucic acid, suitable for timely sown and irrigated conditions (PM24), High temperature tolerant, suitable for irrigated conditions and early sown (PM25), Low erucic acid, timely sown and irrigated conditions(PM29), Low erucic acid, timely sown and irrigated conditions (PM30) Low erucic acid, timely sown, irrigated condition and tolerance to white rust (LES39), Suitable for rainfed condition and white rust resistance (JM2), Nontraditional areas (GM2), Suitable for early sown and rainfed condition, early maturity, tolerant to powdery mildew & *A. blight* (DRMR150-35), Suitable for timely sown and irrigated condition, bold seeded, high oil content and high yielding variety (DRMRIJ-31), Early maturity and high oil content (RVM1)
	C-II (b)	1	L-4	
C-III		4	RVM-2, Rohini, NRCDR-2 and CS- 54	Suitable for rainfed and late sown conditions (RVM2) High oil content, drought tolerant and suitable for irrigated condition (Rohini), Suitable for rainfed & Irrigated conditions, high oil content, high temperature tolerant and tolerant to salinity (NRCDR-2), Salinity tolerant (CS54)

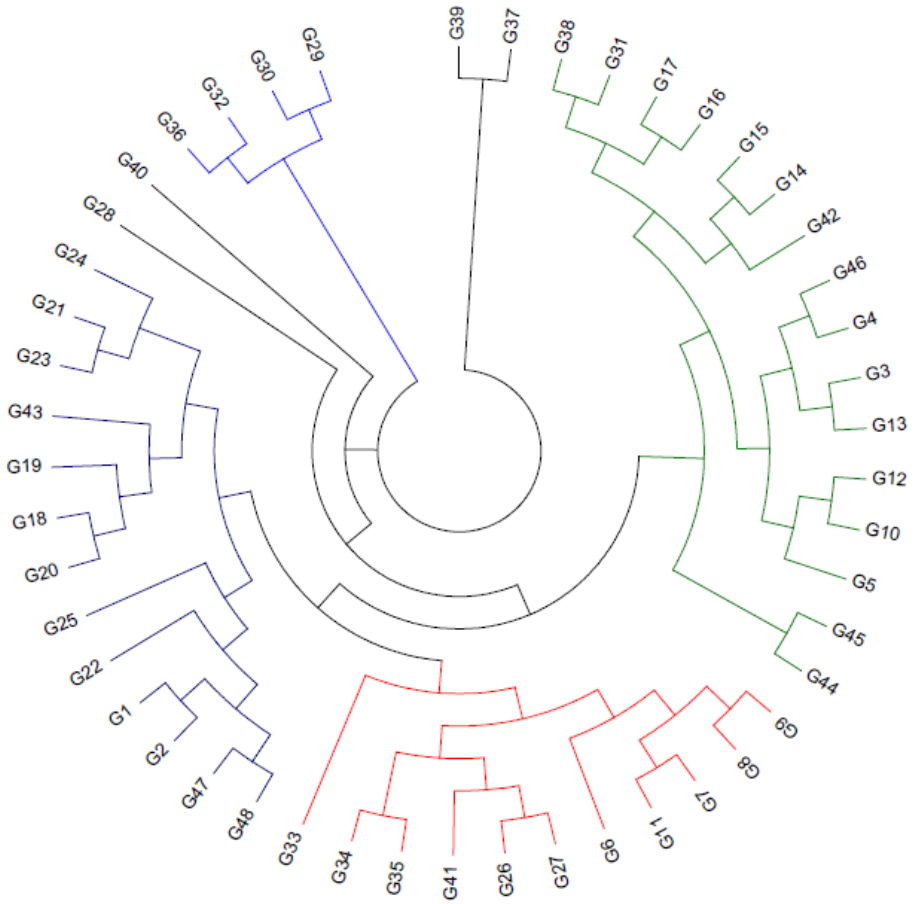


Fig. 5. Dendrogram of gene-based CAPS marker data analysis using power marker v3.25 software

The most interesting group was formed in cluster II (a), including 41 indigenous and exotic genotypes including RH749, Maya, MRNJ-17, MRNJ-16, MRNJ-15, MRNJ-14, PM25, PM29, MRNJ-4, MRNJ-3, MRNJ-13, MRNJ-12, MRNJ-10, MRNJ-5, PM24, PM22, MRNJ-9, MRNJ-8, MRNJ-7, MRNJ-11, MRNJ-6, JM-2, ISC-3, L6, GM-2, DRMR150-35, DRMRIJ-31, LES-39, PM30, MRNJ-2, MRNJ-1, MRNJ-22, MRNJ-25, MRNJ-20, MRNJ-18, MRNJ-19, PM-21, MRNJ-23, MRNJ-21, MRNJ-24 and RVM-1. RH749, Maya, PM25, PM29, PM24, PM22, JM-2, GM-2, DRMR150-35, DRMRIJ-31, LES-39, PM30 and VM-1 that are released varieties suitable for irrigated and rainfed condition, white rust resistance, high temperature tolerant, suitable for early sown, low erucic acid, timely sown, non-traditional areas, bold seeded, high oil content and high yielding, having early maturity and tolerant to powdery mildew and *A. blight,* so that these germplasm lines falling along with these varieties may be contained these characteristics features as well.

4. CONCLUSION

On the basis of the results of the present study, it can be concluded that SSR markers are the better tools to discriminate *B. juncea* genotypes based genetic variability present at molecular level. Information obtained on genetic variation based on microsatellite markers can be used to select diverse genotypes for future breeding purposes to minimize erucic acid content in Indian mustard.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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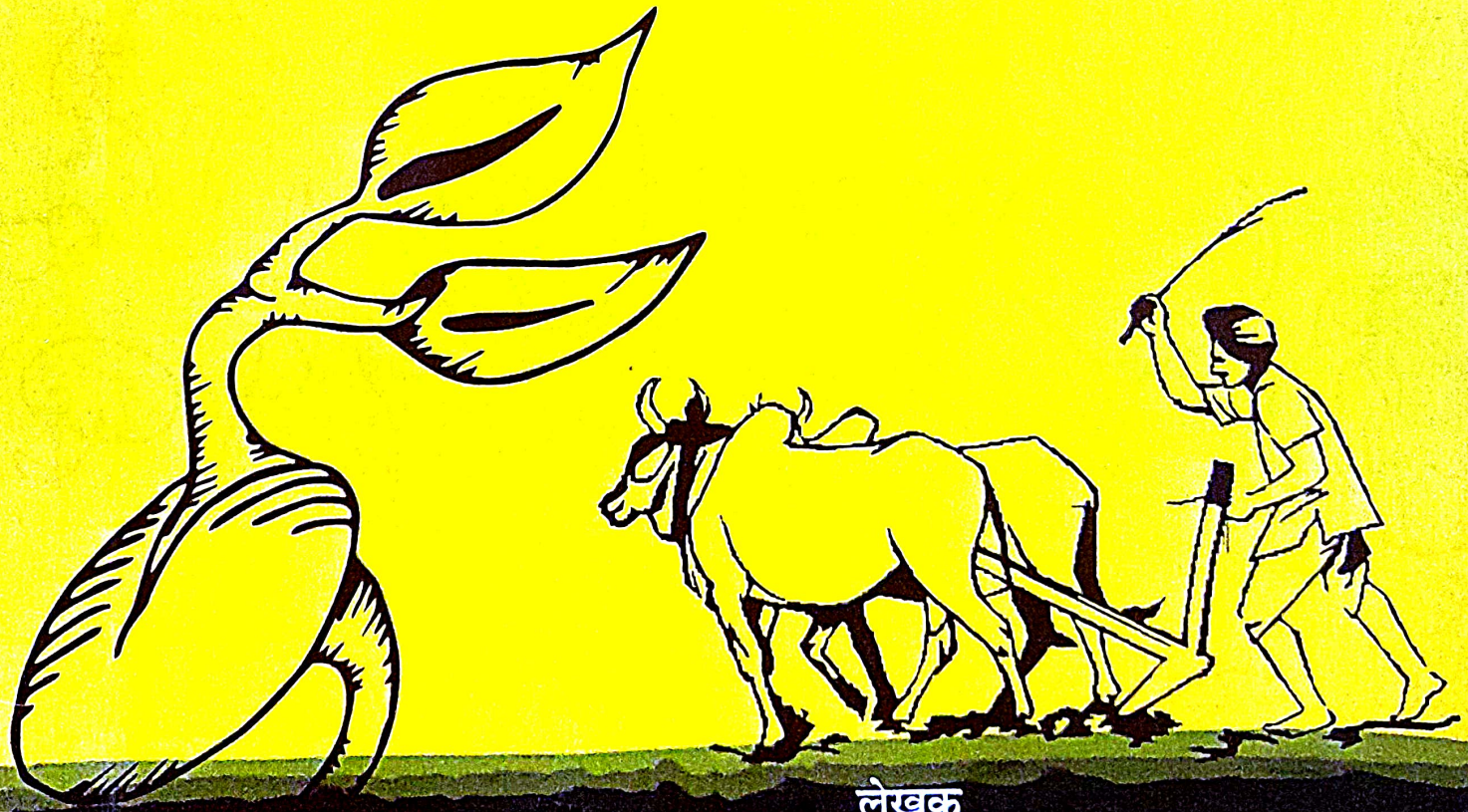


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डॉ. प्रवीण कुमार सिंह गुर्जर

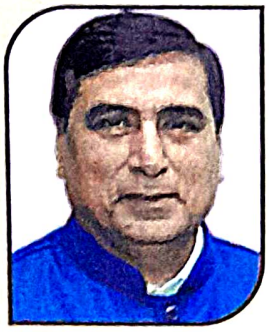


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डॉ. राजेश लेखी



(विभागाध्यक्ष)

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FUNDAMENTAL OF AGRICULTURAL EXTENSION EDUCATION

A PRACTICAL MANUAL

Dr. Rohan Sharma
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Department of Agricultural Extension
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RAJMATA VIJAYARAJE SCINDIA KRISHI VISHWA VIDYALAYA, GWALIOR - 474 002 (M.P.)

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Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (M.P.)

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HORTICULTURE

Basic Principles and Recent Approaches



Pranava Pandey
A.K. Pandey
Sanjeev Kumar



HORTICULTURE
Basic Principles and Recent Approaches

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Post Harvest Management of Horticultural Crops

Shailendra K Dwivedi, A. K. Pandey
Ankit Pandey and Vivek Tiwari

Fruits and vegetables account for nearly 90% of total horticulture production in the country. India is the second largest producer of fruits and vegetables in the world and the leader in several horticultural crops namely Mango, Banana, Papaya, Cashewnut, Arecanut, Potato and Okra. However, the nature of horticultural crops being such that it's not easy to make assessment of their production. These crops, especially vegetables are grown in small plots, fields or in the backyard of the houses; do not have single harvesting in most of the cases which makes their assessment difficult. Many horticultural crops have multiple pickings in a single season. Similarly many fruit trees are scattered, which do not count for assessment.

India has witnessed increase in horticulture production over the last few years. Significant progress has been made in area expansion resulting in higher production. Over the last decade, the area under horticulture grew by 2.6% per annum and annual production increased by 4.8%. During 2017-18, the production of horticulture crops was 311.71 Million Tonnes from an area of 25.43 Million Hectares. The production of vegetables has increased from 101.2 Million Tonnes to 184.40 Million Tonnes since 2004-05 to 2017-18 and production of fruits has increased from 50.9 Million Tonnes to 97.35 Million Tonnes since 2004-05 to 2017-18.

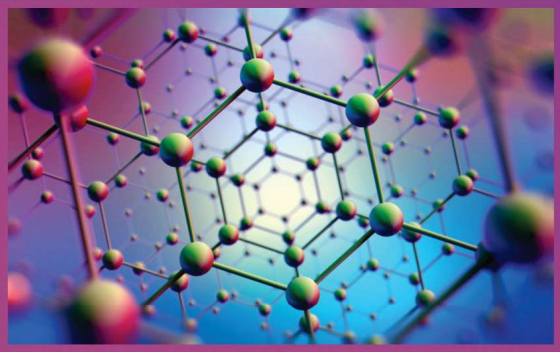
Fruits and vegetables provide health benefits and are important for the prevention of illnesses. Fruits and vegetables contain a variety of nutrients including vitamins, minerals and antioxidants. Eating the recommended amount of fruits and vegetables each day can reduce the risk of chronic diseases. The healthiest choices are fresh fruits or frozen without added sweeteners. Fruits are naturally

Innovations in Agricultural & Biological Engineering

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Volume 3: Trends, Nanomaterials, and Food Delivery



Editors

Megh R. Goyal | Santosh K. Mishra | Satish Kumar

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NANOTECHNOLOGY HORIZONS IN FOOD PROCESS ENGINEERING

Volume 3
Trends, Nanomaterials, and Food Delivery

Edited by
Megh R. Goyal, PhD, PE
Santosh K. Mishra, PhD
Satish Kumar, PhD

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CHAPTER 4

POSTHARVEST PHYSIOLOGY, VALUE CHAIN ADVANCEMENT, AND NANOTECHNOLOGY IN FRESH-CUT FRUITS AND VEGETABLES

NEERU DUBEY, SRISHTI CHITRANSHI, SHAILENDRA K. DWIVEDI, and ANSHU SHARMA

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ABSTRACT

In this fast-paced world, fresh-cut produce occupies high importance as both the partners are working, and preparing daily fresh vegetables is a task, which has given rise to usage of fresh-cut produce and its industry. Maintaining the quality of the fresh produce is a challenge as normal physiological and biochemical processes occur continuously and is enhanced by the wounding procedure. Wounding increases respiration rate, ethylene production in most of the produce, enhances cell wall deterioration while it is also dependent on harvest maturity and sanitizing treatment. Nanotechnology, with its advanced tools finds vast application in fresh produce shelf-life and value chain management in terms of application of nanoparticles for diseases control, packaging, controlling of growth of microorganisms, and development of nanosensors for continuous monitoring of the quality and spoilage.

4.1 INTRODUCTION

Fresh-cut produce is also referred to as 'pre-prepared,' 'lightly processed,' 'fresh-processed,' and 'partially processed.' The most common types of

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Nanotechnology Horizons in Food Process Engineering

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Although nanotechnology has revolutionized fields such as medicine, genetics, biology, bioengineering, mechanics, and chemistry, its increasing application in the food industry is relatively recent in comparison. Nanotechnology in the food industry is now being explored for creating new flavors, extending food shelf life, and improving food protection and nutritional value, as well as for intelligent nutrient delivery systems, "smart" foods, contaminant detection nanodevices and nanosensors, advanced food processing, antimicrobial chemicals, encapsulation, and green nanomaterials.

This new three-volume set addresses a multitude of topical issues and new developments in the field. Volume 1 focuses food preservation, food packaging, and sustainable agriculture, while Volume 2 looks at nanotechnology in food process engineering, applications of biomaterials in food products, and the use of modern nanotechnology for human health. The third volume explores the newest trends in nanotechnology for food applications for improving food delivery systems.

Together, these three volumes provide a comprehensive and in-depth look at the emerging status of nanotechnology in the food processing industry, explaining the benefits and drawbacks of various methodologies that will aid in the improvement and development of food product sourcing and food hygiene monitoring methods.

ABOUT THE EDITORS

Megh R. Goyal, PhD, PE, is a Retired Professor in Agricultural and Biomedical Engineering from the General Engineering Department in the College of Engineering at the University of Puerto Rico–Mayaguez Campus. He was proclaimed as the "Father of Irrigation Engineering in Puerto Rico for the twentieth century" by the ASABE, Puerto Rico Section, for his pioneering work on micro irrigation, evapotranspiration, agroclimatology, and soil and water engineering. A prolific author and editor, he has written more than 200 journal articles and several textbooks and has edited over 100 books.

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We are pleased to present this book entitled “Recent Advances in Agricultural Science and Technology for Sustainable India”. Ratnesh Kumar Rao, Secretary, Mahima Research Foundation and Social Welfare are not new to Agriculture students. With his vast experience in Academic activities, he has dealt this complex subject and edited, with practical approach and simple language, to meet the requirement of the students and teachers of Agriculture. The large gap between potential and current crop yields makes increased food production attainable. India's low agricultural productivity has many causes, including scarce and scant knowledge of improved practices, low use of improved seed, low fertilizer use, inadequate irrigation, conflict, absence of strong institutions, ineffective policies, lack of incentives and prevalence of diseases. Climate change could substantially reduce yields from rainfed agriculture in some countries. With scarcity of land, water, energy, and other natural resources, meeting the demands for food and fiber will require increases in productivity. Though this book is mainly deals with the agriculture research and education, it will also be very handy for those who desire to start Agricultural Research in Science and Technology. We are sure this will be accepted very much by the students, teachers, scientists and Stakeholders of Agriculture all over the India. We solicit your encouragement in this endeavour.

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Part I

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Recent Advances in Agricultural Science & Technology for Sustainable India

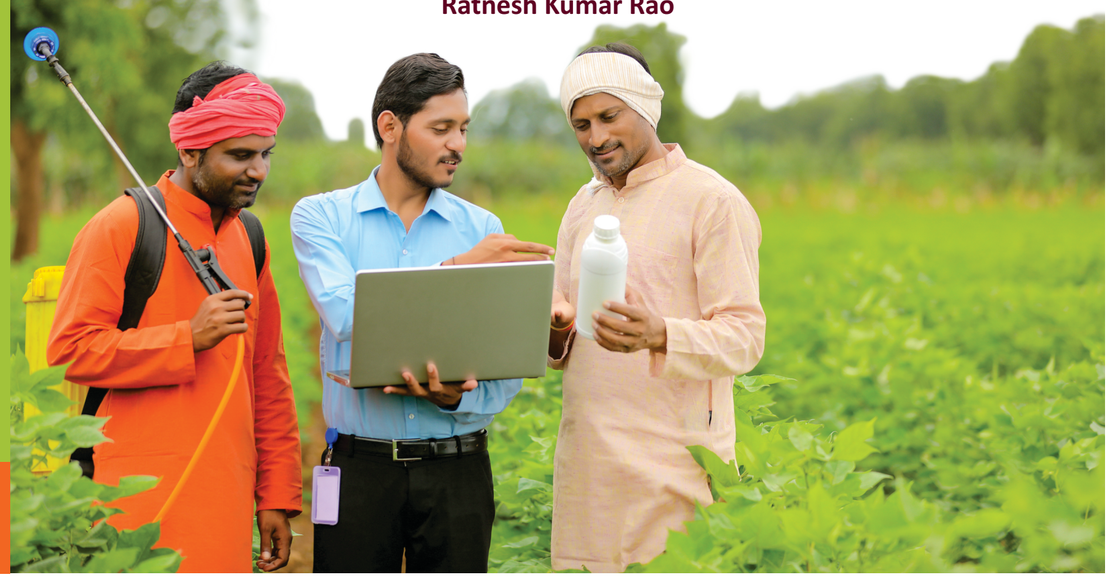
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CLIMATE CHANGE: PERSPECTIVES AND IMPACT ON INDIAN AGRICULTURE

Ekta Joshi[1], Priyadarshni A. Khambalkar[1], Akhilesh Singh[1], Pragati Agarwal[1], Neelam Singh[1], Shashi S. Yadav[1], D.S. Sasode[1], Popiha Bordoloi[2] and S. S. Bhadauria[1]

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The Intergovernmental Panel on Climate Change (IPCC) reported that humankind is causing global warming through the emission of greenhouse gases (GHGs), particularly carbon dioxide (CO_2) and methane (CH_4) (Stocker *et al.,* 2013). The GHGs play a vital role on the climate system by absorbing the long wave infrared radiation and causes warming the earth's atmosphere. Thus, radiation emitted from the surface through the atmospheric window is higher compared to radiation from the stratosphere (Schmithusen *et al*., 2015). CO_2 levels have been consistently increasing since preindustrial time and its daily mean value reached 400 ppm in May 2013 at the reference site of Mauna Loa, Hawaii (Monastersky, 2013). Most regions in the world are warming because of increasing concentrations of atmospheric CO_2 (Sid Perkins, 2015). This increase is caused by human activities, which is subsequently contributing to increase the earth's surface temperature (Huang *et al.,* 2015). Arid and semi-arid areas comprise about 30% of the Earth's land surface. Climate change and climate variability will likely have a significant impact on these regions. The variability of environmental factors may result in significant effects on regional climate and global climate (Wang *et al.,* 2010), especially the radiative forcing, via the biogeochemical pathways affecting the terrestrial carbon cycle. Global climate change has serious impacts on humans and ecosystems.

This climate change altered the atmospheric concentration which leads to warming of the global temperature by 0.7° C till the recent past. It has been estimated that the Climate Change will lead to increase in temperature between 1.8 and 4° C in the years to come. This increasing temperature has caused global warming and created consequent environmental changes (Singh *et al*., 2011) which lead to decreased precipitation and increased temperature. This climate change will have a significant impact in developing countries like India because of their low adaptation capacity. The agriculture sector is primarily vulnerable and may create risk to the rural population (IPCC, 2007). As it has been estimated by the World Bank that, if climate change continues unhindered, then average temperatures in India is likely to reach as high as 29.1° C by the end of the century (up from 25.1 °C currently). Increase in temperature of the earth may lead to decrease in the length of growing season and yield of most crops (Saseendran *et al.,* 2008; Lal *et al.*, 2020; Sharma *et al.*, 2020; Sagar *et al.*, 2019; Parth *et al.*, 2019; Sagar *et al.*, 2019; Rao, 2019; Sharma and Rao, 2019; Dharbale *et al.*, 2019; Nyath *et al.*, 2018; Singh *et al.*, 2018). The anticipated negative impact of global warming on the climate of India is large; influence livestock population, agriculture and related allied sectors and ecosystem. A wide range of studies have substantiated that agroforestry systems have unique opportunity to augment the carbon stocks in the terrestrial biosphere (IPCC, 2000; Albrecht and Kandji, 2003). It has been estimated that 63 million ha of land areas are suitable for agroforestry and making this system a quantitatively important carbon stock (Kandji *et al.,* 2012).

This article reviews the understanding of consequence of climate change on Indian agriculture, various environmental parameters, livestock's population and health parameter etc. to highlight some important aspects in particular. As the agriculture and animal production system which is vulnerable to climate change is itself a large contributor to potential global warming through emission of CO_2, methane and nitrous oxide. The paper also presents a comprehensive review of the effect of climate change and GHG emissions in Indian scenario.

Is Climate Change Really Happening?: What are the indicators? Climate Change is the long-term average of the weather in a given place. While the weather can change in minutes or hours, a change in climate is something that develops over longer periods of decades to centuries. Climate is defined not only by average temperature and precipitation but also by the type, frequency, duration, and intensity of weather events such as heat waves, cold spells, storms, floods, and droughts (Source: https://www.epa.gov/climate-indicators). Global Climate Change indicators The Earth's climate is



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We are pleased to present this book entitled “Recent Advances in Agricultural Science and Technology for Sustainable India”. Ratnesh Kumar Rao, Secretary, Mahima Research Foundation and Social Welfare are not new to Agriculture students. With his vast experience in Academic activities, he has dealt this complex subject and edited, with practical approach and simple language, to meet the requirement of the students and teachers of Agriculture. The large gap between potential and current crop yields makes increased food production attainable. India's low agricultural productivity has many causes, including scarce and scant knowledge of improved practices, low use of improved seed, low fertilizer use, inadequate irrigation, conflict, absence of strong institutions, ineffective policies, lack of incentives and prevalence of diseases. Climate change could substantially reduce yields from rainfed agriculture in some countries. With scarcity of land, water, energy, and other natural resources, meeting the demands for food and fiber will require increases in productivity. Though this book is mainly deals with the agriculture research and education, it will also be very handy for those who desire to start Agricultural Research in Science and Technology. We are sure this will be accepted very much by the students, teachers, scientists and Stakeholders of Agriculture all over the India. We solicit your encouragement in this endeavour.

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Part I

Recent Advances in Agricultural Science & Technology for Sustainable India



Recent Advances in Agricultural Science & Technology for Sustainable India

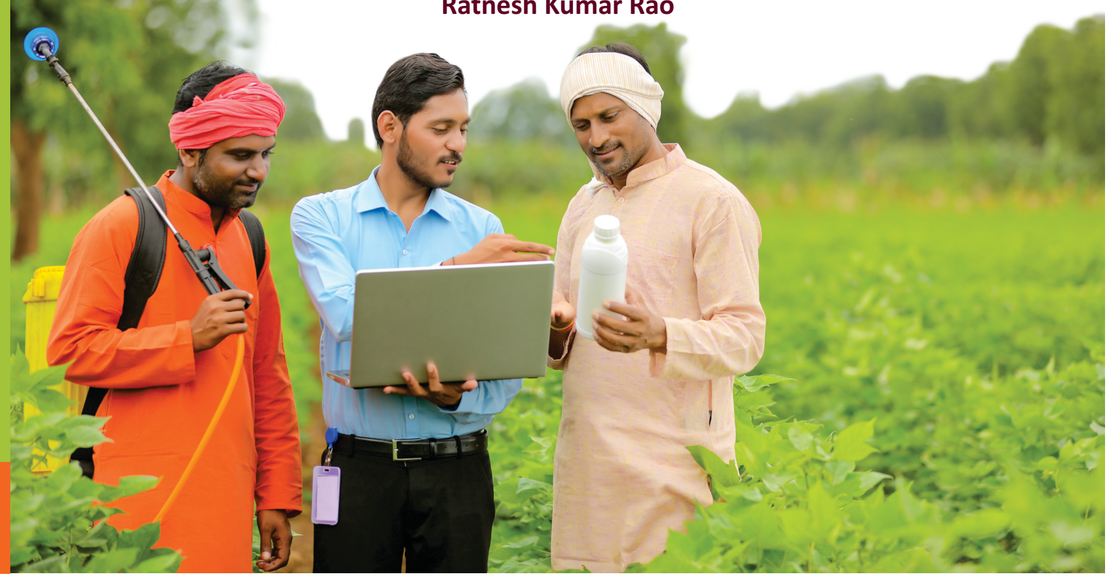
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AQUAPONICS: AN INNOVATIVE SUSTAINABLE FOOD PRODUCTION FARMING SYSTEM

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By 2050, the world population is estimated to increase to 9 billion. The expansive numbers of people are expecting to rely on agricultural sector including farming, fisheries, woodcrafts, and livestock. Natural calamities and crisis affect millions of people who depend on the primary sector. Indian farmers are exposed to many challenges resulting from low agricultural growth, sustainability concerns, and land degradation, as a large area of farmland has become infertile due to the overuse of fertilizers and pesticides. Conventional farming methods because of large usage of fertilizers for growing crops degraded the quality of the soil and local water sources. It is high time to overcome these challenges through innovative farming methods. For reducing poverty and attaining food security, expansion of agriculture sector is the most efficacious means. The technological and scientific advancement in the field of agriculture has opened a new era for the design and development of modern devices for plant health monitoring.

In order to meet human demand for aquatic products, total global aquatic production increased by 27.5% in 2010–2018, during which aquaculture and capture output increased by 29.8% and 10.4%, respectively (FAO, 2018). Aquaponics farming is a solution to overcome some of these challenges to an extent if the farmers are able to maintain the system with proper care and technical support. Aquaponics is an integrated system combination of aquaculture together with growing vegetables and crops without the use of fertilizers. Water circulates between the components of the system (fish tank, biofilter, hydroponic system), during which the feces of the fish become fertilizers for the plants (Nichols and Savidov, 2012).

Aquaponic systems have the potential to avoid some of the major resource inefficiencies present in conventional agriculture; however, achieving the resource efficiency as mentioned above, has been largely unquantified in scientific publications. Based on several past reviews, successful aquaponic operations must consider the impacts of system design (Palm *et al*., 2018), system water pH control (Tyson *et al*., 2011), aeration and filtration technologies (Danaher *et al*., 2013), acceptable nutrient ranges (Delaide *et al*., 2016), pairing of plant and fish species, microbial populations, nitrogen levels, quantity and type of feed (Endut *et al*., 2010), pest management and effective marketing. These factors are the main concern of those managing the system.

Thus aquaponics has emerged as a low-risk, high-profit farming method that requires only little maintenance and expenditure once it's set up and it also happens to be purely organic, chemical free, sustainable, no tilling the soil, no compost, no manure spreading, no tractor and no breaking back and sore joints, no cracked skin or needless exhaustion, no working to the bone for no reward, no trouble feeding the family. This is a globally accepted technology and adapted to a greater extent. Aquaponics has already made waves in the USA as well as other parts of the developed world.

Definition and Concept of Aquaponics: The term "aquaponics" is derived from the "aqua" in aquaculture and "ponics" in hydroponics (Marklin *et al*., 2013). Aquaponics is defined as the combination of hydroponics and recirculating aquaculture system which also plays a major role in minimizing the negative environmental impacts resulted from intensive fish farming and crop production. While aquaculture constitutes the breeding, rearing, and harvesting of fishes and other aquatic organisms in a controlled environment, hydroponics is a method of growing plants without soil by using mineral nutrient solutions in a water solvent. Aquaponics is an integrated sustainable method of farming by bringing aquaculture and hydroponics together in a single re-circulating system(Fig. 1).This process is the same closed-loop symbiotic combination as occurrs in nature, such as in a river or lake basin, where plants and fish live together in which fish wasteserves as nutrients for the plants, which in turn clean the water for the fish. In this type of integration, nutrients excreted by the fish through microbial activity (Zou *et al*., 2016) and the nitrifying bacteria in the biofilter

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Chapter 8
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Role of Biochemical and Antioxidant Enzymes Activities in Drought Tolerance in Soybean: A Recent Study

N. Mishra[1], M. K. Tripathi[1*], N. Tripathi[2], S. Tiwari[1], N. Gupta[1], A. Sharma[1] and M. K. Shrivastav[3]

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ABSTRACT

Soybean genotypes were divided into two groups based on biochemical profiling, anti-oxidant enzyme activities and protein profiling. Drought tolerance features were found in three genotypes *viz*., JS97-52, RVS-14, and JS95-60, based on different biochemical and antioxidant enzyme activities analysis among 53 diverse genotypes. The results obtained could aid in the improvement of soybean genotypes through the production of drought-tolerant genotypes using both conventional and molecular breeding approaches. These findings also provided a foundation for additional research employing advanced biotechnological technologies to investigate the drought tolerance mechanism in soybean crops.

Keywords: Antioxidant enzymes; Biochemical parameters; Drought; Protein profiling; Sustainable agriculture

1. INTRODUCTION

Changes in the environment are to blame for the emergence of new issues. Climate change is one of them, and it has a significant impact on agriculture. In most parts of the world, there is a high need for water for crop irrigation in order to meet grain production targets. More than 40% of people from 54 nations will face a major problem in the coming days as a result of the drought [1]. A crop species or genotype that is tolerant of low rainfall intensity, unpredictable distribution, and high temperatures would be critical for ensuring a long-term food supply for the world's ever-growing population. It is believed that by carefully crafting biotechnological ways to build a cultivar for such a suboptimal environment, food security in inhospitable regions will be maintained.

Soybean is also acknowledged as a ‘miracle crop’ due to over 40% protein and 20% oil [2-8]. It needs an adequate water supply for the duration of its growth and development course to accomplish better yields [9]. The plants of soybean have been found to be affected by drought at every stage of life [10-15]. Significant reductions in the levels of chlorophyll a, b, and total chlorophyll have been observed due to drought in soybean crop [16]. Plants develop various mechanisms to fight different stresses [17] and these mechanisms may be due to alteration in biochemical pathways. Numerous biochemical parameters have been exploited to recognize tolerant genotype (s) to drought [18-20]. Knowledge of the biochemical mechanisms for drought tolerance in vegetable-type soybeans is very limited. Plants have evolved a number of biochemical and physiological mechanisms to improve their tolerance to abiotic stress. These may include the employment of various antioxidative mechanisms that are enzymatic or non-enzymatic in nature [21-24].

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To distinguish the desired genotype(s), various biochemical parameters that are being utilized for selection are proline, membrane stability, total sugar, MDA, protein, antioxidant activities (catalase, glutathione reductase and peroxidase) etc. Earlier, it is reported that the antioxidant enzymes play major role in control, and removal of reactive oxygen species (ROS). The increased activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) have been observed in crops as well as soybean under water stress [11,12,18]. Enhancement of MDA content has also been recorded under water deficit in crops [18,19].

Identification of drought tolerant genotypes due to biochemical alterations may provide a basis for the development of new plant varieties using conventional [25] as well as molecular breeding approaches to fight water stress [26-29]. The current investigation was executed to monitor drought tolerant soybean genotypes based on manifestation of different biochemical parameters, antioxidant enzymatic activities and protein profiling.

2. MATERIALS AND METHODS

The present investigation was consisted of 53 soybean genotypes (Table 1) with diverse reactions to drought *viz*., susceptible and tolerant. The seeds were obtained from College of Agriculture, JNKVV, Jabalpur, RAK, College, Sehore and Zonal Agricultural Research Station, Morena, Madhya Pradesh, India.

The field experiment was conducted at the experimental field and the laboratory work at Biochemical Analysis Laboratory, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayraje Scindia Agricultural University, Gwalior, Madhaya Pradesh, India during *Kharif* 2018-19. The investigational region covered was fairly identical in term of topography and fertility. Gwalior has subtropical, semi-arid climate and chilly winters with random showers. The average rainfall was about 312.0 mm in July, 190.6 mm in August, 166.4 mm in September and 0.0 mm in the month of October respectively. Crop was shown on July 2019. Between 60^{th} to 70^{th} days of crop growth season neither rain has been received nor has irrigation given manually. Leaves were collected after 70 days of sowing from five random selected plants of each line for the analysis of diverse biochemical parameters. Completely Randomized Design (CRD) was adopted and the data were analyzed as per method suggested by Snedecor and Cochran [30].

2.1 Biochemical Analysis

Photosynthetic pigments (chlorophyll a, b and total) were quantified using UV-VIS spectrophotometer at 470, 645 and 663 nm absorbance and calculated according to Arnon's equation [31]. Proline content in leaves was determined as suggested by Bates et al. [32]. Estimation of sugar content (mgg^{-1} fresh weight) was estimated as methods adopted by Kachare [11] in soybean. Malondialdehyde test (Lipid peroxidation assay, nmol g^{-1}FW) estimation was exercised with the help of method developed by Stewart and Bewley [33]. Membrane stability index (MSI) was worked out as formulae suggested by Razzaq et al. [34]. Total seed protein content was estimated by the process of Bradford [35].

2.2 Determination of Antioxidant Enzyme Activity (Catalase, Glutathione Reductase and Peroxidase)

Sample preparation was done according to the method adopted by Kachare [11]. Ascorbate peroxidase (APX) activity (EC 1.11.1.11) was computed as per method of Nakano and Asada [36]. Catalase (CAT) activity (EC 1.11.1.6) was analyzed by the UV process as proposed by Aebi [37]. Glutathione reductase (GR) activity (EC 1.6.4.2) was estimated by the technique as explained by Smith et al. [38]. Guaiacol peroxidase (PDX) activity (EC 1.11.1.7) was computed by estimating the oxidation of guaiacol as protocol described by Rao et al. [39].

Table 1. List of soybean genotypes with their parentage

S. No.	Genotypes	Source/Pedigree	S. No.	Genotypes	Source/Pedigree
1.	JS 20-29	JS 97-52 x JS 95-56	28.	RSC-10-52	NRC 37X JS335
2.	JS 20-69	JS 97-52 x SL 710	29.	SL -1123	Selection from AGS751
3.	JS 335	JS 78-77 x JS 71-05	30.	SL-1068	SL755XSL525
4.	JS 20-98	JS 97-52x JS SL710	31.	AGS 111	Germplasm accession
5.	JS 20-94	JS 97-52 x JS 20-02	32.	EC457286	Germplasm accession
6.	JS 93-05	Selection from PS 73-22	33.	MACS725	JS93-05X MAUS71
7.	JS 20-116	JS 97-52 x JSM 120 A	34.	SP 37	Not known selection
8.	JS 95-60	Selection from PS 73-22	35.	NRC -125	EC54688xps1044
9.	JS 97-52	PK 327 x L 129	36.	NRC-132	JS97-52X PI086023
10.	JS 20-84	JS 98-63 x PK 768	37.	NRC-134	NRC7XAGS191
11.	JS 20-34	JS 98-63 x PK 768	38.	NRC SL-1	JS335XSL525
12.	JS 20-71	JS 97-52 x JS 90-5-12-1	39.	PS 1092	PS1042 x MACS 450
13.	RVS 2007-6	JS 20-10 x MAUS162	40.	PS 1613	PS1225XPS1042
14.	RVS 2011-35	JS 335 X PK 1042	41.	AMS 2014-1	AMS99-33XH6P5
15.	RVS 2001-4	JS 93-01x EC 390981	42.	KDS 992	JS93-05XEC241780
16.	RVS -14	JS 93-05x EC 390981	43.	VLS -94	VL Soya59X VS2005-1
17.	RVS -24	J.P 120 x JS 335	44.	SKF-SPS -11	Not known selection
18.	RVS -18	JSM110XJSM66	45.	RVS 76	MAUS-162XJSM-66
19.	NRC- 76	NRC-37XL-27	46.	NRC127	JS97-52XPI542044
20.	NRC -86	RKS15XEC481309	47.	KDS980	JS93-05XAMS1
21.	NRC- 130	EC390977XEC538828	48.	G-29	Germplasm
22.	NRC -131	EC390977XEC538828	49.	RSC-10-70	JS335X Bragg
23.	NRC -147	Germplasm accessions C210	50.	RSC-10-71	Bragg XJS335
24.	AMSMBC -18	Mutant of Bragg	51.	NRC-2	Induced mutant of Bragg
25.	AMS-100-39	Mutant of JS93-05	52.	MACS-15-20	NRC37XMohetta
26.	MACS – 1520	EC241780XMACS330	53.	MACS-58	JS2 x Improve pelican
27.	MACSNRC-1575	PI542044XJS9305			

2.3 Protein's Characterization

The extracted protein was used to analyze through SDS-PAGE method according to the steps suggested by Laemmli [40].

3. RESULTS

3.1 Biochemical Parameters

Variations among 53 soybean genotypes for studied biochemical traits with analysis of variance are shown in Table 2 which indicated that the alteration in their response against drought stress. Total Chlorophyll content in $mgml^{-1}$varied considerably in ranged of 38.13-59.87 mgml-1, with maximum in genotype JS 97-52 (59.87 $mgml^{-1}$) chased by genotypes: RVS-14 (56.29 mgml-1), JS20-94 (55.88 $mgml^{-1}$) and NRC-76 (55.07 $mgml^{-1}$). Whereas the minimum was documented in genotype JS20-84 (38.13 mgml-1) pursued by genotypes RVS2011-35 (40.01 $mgml^{-1}$), NRC-130 (41.21 $mgml^{-1}$) and MACS-1520 (41.58 mgml-1). Proline content varied significantly in range of 66.25-111.40 μgg^{-1} with highest in genotype JS 97-52 (111.40 μgg^{-1}), tracked by genotypes RVS-14 (103.45 μgg^{-1}), JS 95-60 (93.95 μgg^{-1}) and RVS 2001-4 (91.54 μgg^{-1}). However, the least showed in genotype JS335 (66.25 μgg^{-1}) followed by genotypes: JS 20-29 (67.90 μgg^{-1}), NRC-76 (68.05 μgg^{-1}), NRC-134 (68.15 μgg^{-1}) and SL-1123 (68.30 μgg^{-1}). Total Sugar in mgg^{-1}varied significantly between 2.35-5.45 mgg^{-1} with utmost in genotype JS97-52 (5.45 mgg^{-1}) which is pursued by genotypes RVS-14 (5.15 mgg^{-1}) and JS95-60 (5.15 mgg^{-1}) while lowest in genotype KDS992 (2.35 mgg^{-1}), tracked by genotype RSC-10-71 (2.45mgg^{-1}). MDA content in nmole gm^{-1}differed significantly in range of 42.65 to 60.80 with greatest in genotype JS 97-52 (60.80 nmole gm^{-1}) tracked by JS95-60 (57.22 nmole $gm^{-1)}$ and least amount (42.65 n mole gm^{-1}) in genotype RVS2007-6. Membrane stability is one of the major components in tolerance under stress. Highest membrane stability (MS) was texted in genotype JS97-52 (64.50 %) intimately trailed by genotypes RVS-14 (63.00%) and JS95-60 (61.00%), while minimum (29%) in genotype RSC-10-70. Protein synthesized in immature seeds varied significantly among 53 soybean genotypes in ranged of 34.4%-39.3% with upmost in genotype JS20-71 (39.30%) closely pursued by genotypes: AMS-100-39 (39.10%) and MACS725 (38.6%%). Whilst the lowest amount was documented in genotype JS95-60 (34.4%) tracked by genotypes JS 20-69 (34.5%) and SP 37 (34.8%).

3.2 Biochemical Parameters Based Hierarchical Cluster Analysis

The hierarchical cluster analysis and the content values are presented in Table 2, and the dynamic expression profile was determined and is shown in Fig. 1. Multivariate analysis based on diversity was performed using the UPGMA. The mean value of biochemical parameters of different genotypes falling in each cluster was presented in the generated dendrogram for distinguished into two major clusters *i.e.,* I and II. Cluster I divided into subclusters. These clusters further subdivided into minor clusters. Cluster I consisted genotypes RVS-2001-4 and KDS-980 as an out group and cluster II also consisted two genotypes *viz*, JS-97-52 and RVS-14 as an outgroup. The biochemical parameters illustrated similar pattern in both of these two genotypes, and they were clustered together.

3.3 Principal Component Analysis (PCA) of Biochemical Parameters

Principal component analysis (PCA) was done by considering biochemical variables simultaneously. The pattern of variations illustrated by the PCA described by correlation coefficients determined for pair-wise association of the traits. Genotypes JS-97-52 and RVS-14 situated at the unique position of the plot. The PCA correlation depicted those genotypes possessed higher and lower magnitudes of biochemical parameters occupying unique position towards the graph (Fig. 2).

3.4 Activities of Antioxidant Enzymes

The analysis of variance (Table 2 and Fig. 3) clearly indicated presence of ample variations among 53 soybean genotypes for all antioxidant enzymes activities. APX was found to be maximum in genotype

JS97-52 (2.21-unit mg^{-1} protein min-1) whereas minimum (0.23-unit mg^{-1} protein min^{-1}) was documented in genotype JS20-94. Catalase activity was found to be highest (0.97-unit mg^{-1} protein min^{-1}) in genotype JS 97-52 while lowest (0.30-unit mg^{-1} protein min^{-1}) was observed in genotype NRC-132. Highest Gluthione reductase (GR) activity was texted in genotype JS 97-52 (0.87-unit mg-1proteinmin^{-1}) narrowly pursued by genotypes: JS 95-60 (0.80-unit mg^{-1}protein min^{-1}), however, least in RVS-18 (0.22-unit mg^{-1}protein min^{-1}). Guaiacol peroxidase (PDX) ranged between 0.24-1.91-unit mg^{-1} protein min^{-1} with greatest (1.91-unit mg^{-1} protein min^{-1}) in genotype JS 97-52 and lowest (0.24-unit mg^{-1} protein min-1) in genotype JS20-98. Further, elevated PDX activity was examined in genotypes *i.e.,* JS 97-52, RVS 2001-4, NRC-76 and RVS-14.

3.5 Antioxidant Activities Based Cluster Analysis

In antioxidant enzymes data based dendrogram, the genotypes were separated into two clusters *i.e.,* major and minor cluster. The major cluster contained 49 genotypes while the minor cluster had only 4 genotypes *namely*., RVS-24, JS20-84, RVS-14 and JS97-52. The major cluster was further divided into two groups. The major group is consisted 47 genotypes while minor group had only two genotypes *viz.,* NRC-76 and RVS2001-4. The major group was further divided into two sub-groups. Major sub-group contained 38 genotypes and minor sub-group consisted nine genotypes including AMS2014-1, PS-1613, VLS-94, PS-10-92, RSC-10-70, NRC-SL-1, NRC-134, NRC-132 and NRC-125. Among these nine genotypes utmost resemblance was documented between AMS2014-1 and PS-1613 and both genotypes grouped together (Fig.4).

3.6 Protein Profiling Through SDS-PAGE

In this experimentation, we analyzed immature seed protein pattern for fifty-three soybean genotypes using SDS-PAGE (Fig. 5). Dendrogram was generated on the basis of banding pattern and studied genotypes were divided into two clusters (Fig. 6). The major cluster is consisted 50 genotypes while minor cluster had only three genotypes, *viz.,* SL-1068, AGS-111 and NRC-132. Among these three genotypes, NRC-132 was found to be diversed and grouped distantly from rest of the two genotypes. The data were also analyzed on the basis of band intensity. The variations in the intensity of bands with similar molecular weight indicated the expression capability of genotypes of same protein in different genotypes.

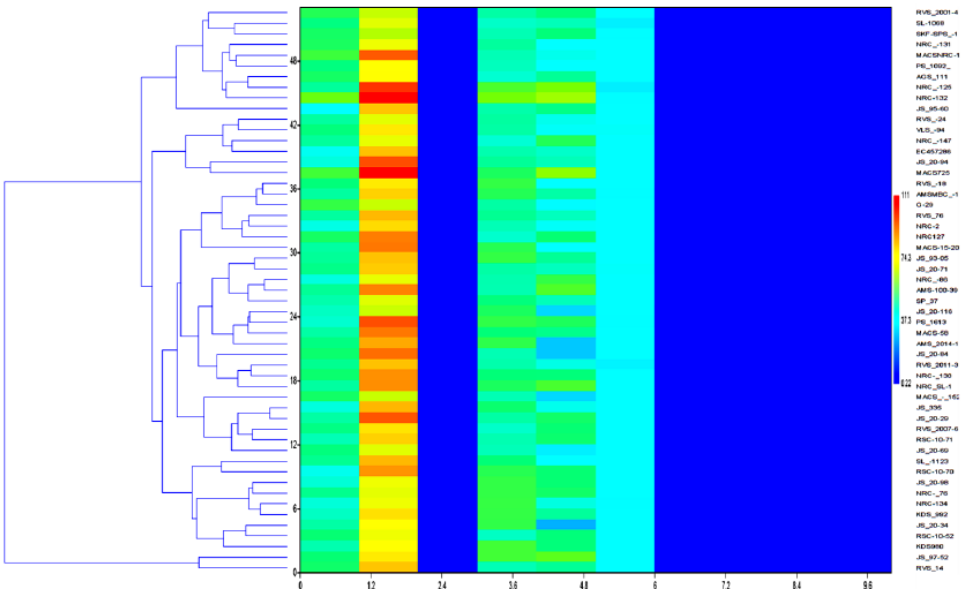


Fig. 1. Biochemical based hierarchical cluster analysis of soybean genotypes based on different biochemical parameters (total chlorophyll content, proline, total sugar, MDA, MSI and protein %)

Table 2. Mean performance of different biochemical parameters and anti-oxidant enzymatic activities of soybean genotypes

S. No.	Parameter Genotypes	Total Chlorophyll Content (mg/ml)	Proline (µg/g)	Total Sugar (mg/g)	MDA (n mole/gm)	MSI (%)	Protein (%)	Ascorbate peroxidase (unit/mg protein/min)	Catalase (unit/mg protein/min)	Glutathione reductase (unit/mg protein/min	Guaiacol peroxidase (unit/mg protein/min)
1.	JS 20-29	53.68	67.90	3.15	46.70	50.50	36.2	0.57	0.74	0.43	0.32
2.	JS 20-69	49.90	70.35	2.70	43.57	45.00	34.5	0.40	0.72	0.30	0.51
3.	JS 335	52.30	66.25	2.75	45.39	50.5	35.6	0.58	0.40	0.29	0.62
4.	JS 20-98	52.79	71.45	3.00	47.35	37.00	37.2	0.67	0.53	0.36	0.24
5.	JS 20-94	55.88	90.40	3.90	45.17	40.50	38.1	0.23	0.44	0.41	0.42
6.	JS 93-05	50.06	75.65	3.00	44.67	36.50	37.4	0.45	0.50	0.27	0.65
7.	JS 20-116	52.39	76.05	3.75	42.73	48.00	36.1	0.31	0.64	0.27	0.75
8.	JS 95-60	47.55	93.95	5.15	57.22	61.00	34.4	0.27	0.43	0.80	0.60
9.	JS 97-52	59.87	111.4	5.45	60.80	64.50	35.7	2.21	0.97	0.87	1.91
10.	JS 20-84	38.13	81.05	4.55	46.19	49.50	36.8	1.79	0.80	0.30	0.72
11.	JS 20-34	47.13	70.50	4.30	47.07	40.50	37.1	0.26	0.85	0.34	0.39
12.	JS 20-71	50.25	77.80	3.75	46.66	39.50	39.3	0.29	0.57	0.52	0.45
13.	RVS 2007-6	47.48	70.95	2.80	42.65	53.50	37.2	0.33	0.44	0.26	0.71
14.	RVS 2011-35	40.01	81.35	3.05	45.88	43.50	38.2	0.46	0.47	0.32	0.50
15.	RVS 2001-4	41.74	91.50	2.75	47.78	45.00	36.8	0.59	0.43	0.76	1.65
16.	RVS -14	56.29	103.45	5.15	52.93	63.00	38.1	1.70	0.86	0.80	1.24
17.	RVS -24	50.39	77.65	3.00	55.12	38.50	36.2	2.04	0.64	0.27	0.37
18.	RVS -18	47.10	79.85	3.30	53.67	46.50	35.7	0.50	0.72	0.22	0.45
19.	NRC- 76	55.07	68.05	4.65	46.87	35.50	37.1	0.32	0.96	0.77	1.58
20.	NRC -86	47.20	82.00	2.65	48.20	44.50	37.8	0.47	0.63	0.63	0.57
S. No.	**Parameter Genotypes**	**Total Chlorophyll Content (mg/ml)**	**Proline (µg/g)**	**Total Sugar (mg/g)**	**MDA (n mole/gm)**	**MSI (%)**	**Protein (%)**	**Ascorbate peroxidase (unit/mg protein/min)**	**Catalase (unit/mg protein/min)**	**Glutathione reductase (unit/mg protein/min**	**Guaiacol peroxidase (unit/mg protein/min)**
21.	NRC- 130	41.21	78.95	2.60	45.29	39.50	36.5	0.48	0.64	0.60	0.59
22.	NRC -131	52.46	87.30	3.95	43.20	51.00	37.2	0.40	0.52	0.52	0.40
23.	NRC -147	46.95	87.90	3.70	53.93	36.50	36.2	0.30	0.55	0.46	0.78
24.	AMSMBC -18	47.81	81.25	3.80	55.08	48.00	38.1	0.26	0.76	0.36	0.32
25.	AMS-100-39	49.09	80.50	3.75	46.61	44.50	39.1	0.24	0.40	0.63	0.51
26.	MACS – 1520	41.58	70.95	3.15	45.76	55.00	37.4	0.55	0.61	0.58	0.79
27.	MACSNRC-1575	48.02	86.80	3.50	45.62	57.50	37.9	0.43	0.55	0.49	0.57
28.	RSC-10-52	46.07	70.15	3.05	50.20	45.00	38.1	0.41	0.35	0.40	0.40
29.	SL -1123	44.20	68.30	2.65	52.71	32.50	36.2	0.34	0.64	0.39	0.61

30.	SL-1068	43.08	91.50	4.20	54.80	53.00	35.7	0.28	0.37	0.32	0.57	
31.	AGS 111	46.63	87.80	4.75	50.58	49.00	37.4	0.42	0.46	0.52	0.85	
32.	EC457286	49.68	83.45	4.25	54.56	31.00	37.1	0.38	0.44	0.55	0.71	
33.	MACS725	51.59	88.75	3.90	45.41	31.00	38.6	0.32	0.50	0.45	0.90	
34.	SP 37	48.51	81.30	3.55	46.09	41.00	34.8	0.43	0.69	0.40	0.53	
35.	NRC -125	51.42	85.60	4.05	50.55	50.50	37.6	0.87	0.43	0.46	0.63	
36.	NRC-132	47.08	85.75	3.25	52.92	57.00	37.9	0.77	0.30	0.62	0.60	
37.	NRC-134	52.14	68.15	3.70	45.33	32.50	37.1	0.82	0.36	0.60	0.64	
38.	NRC SL-1	41.69	82.30	2.90	51.42	40.50	38.1	0.80	0.63	0.60	0.63	
39.	PS 1092	46.87	90.85	2.60	46.63	52.00	37.2	0.82	0.39	0.24	0.81	
40.	PS 1613	49.63	78.30	3.00	43.53	51.00	38.4	0.70	0.44	0.49	0.87	

S. No.	**Parameter Genotypes**	**Total Chlorophyll Content (mg/ml)**	**Proline (µg/g)**	**Total Sugar (mg/g)**	**MDA (n mole/gm)**	**MSI (%)**	**Protein (%)**	**Ascorbate peroxidase (unit/mg protein/min)**	**Catalase (unit/mg protein/min)**	**Glutathione reductase (unit/mg protein/min**	**Guaiacol peroxidase (unit/mg protein/min)**
41.	AMS 2014-1	45.19	80.00	2.75	45.60	51.50	37.2	0.76	0.43	0.47	0.92
42.	KDS 992	50.18	70.35	2.35	45.13	34.50	36.5	0.44	0.59	0.34	0.85
43.	VLS -94	48.38	82.00	3.05	50.68	37.00	36.2	0.63	0.39	0.25	0.80
44.	SKF-SPS -11	40.42	85.30	3.35	53.92	51.00	37.4	0.51	0.43	0.31	0.73
45.	RVS 76	42.42	71.65	2.95	54.92	51.00	36.8	0.68	0.52	0.36	0.58
46.	NRC127	48.76	70.65	2.50	54.91	52.00	37.1	0.44	0.50	0.39	0.46
47.	KDS980	42.19	71.45	2.95	55.23	41.50	35.7	0.47	0.57	0.48	0.72
48.	G-29	44.14	78.55	2.60	55.10	47.50	36.2	0.40	0.45	0.36	0.55
49.	RSC-10-70	46.64	76.00	3.00	54.78	29.00	37.1	0.80	0.64	0.54	0.56
50.	RSC-10-71	51.00	71.55	2.45	44.11	50.50	36.4	0.52	0.60	0.59	0.73
51.	NRC-2	45.78	75.55	2.80	56.41	50.50	37.2	0.67	0.42	0.32	0.60
52.	MACS-15-20	50.98	77.70	2.70	56.37	58.50	37.9	0.62	0.49	0.36	0.47
53.	MACS-58	51.90	81.05	2.85	45.21	48.00	36.5	0.45	0.33	0.45	0.33
SE (m)	1.076566	1.130809	0.154004	3.160806	0.845032	0.147046	0.009329	0.017895	0.014916	0.016474	
CD0.05	3.057168	3.211204	0.437332	8.97587	2.399671	0.417574	0.026	0.050817	0.042358	0.046783	
Range	38.13-59.87	66.25- 111.40	2.35-5.45	42.65-60.80	29.00- 64.50	34.4-39.3	0.23 - 2.21	0.30-0.97	0.22-0.87	0.24-1.91	

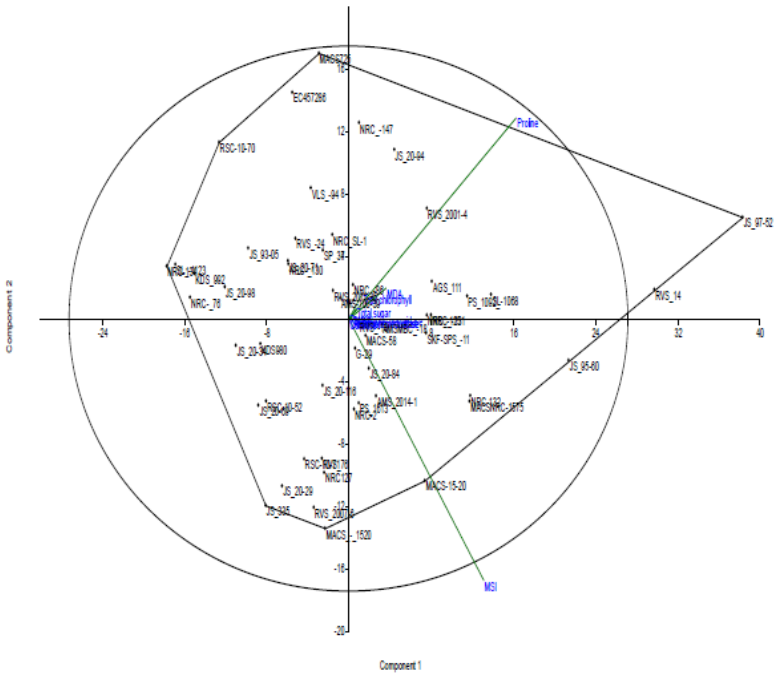


Fig. 2. Principal Component analysis based on different biochemical showing relationship among soybean genotypes

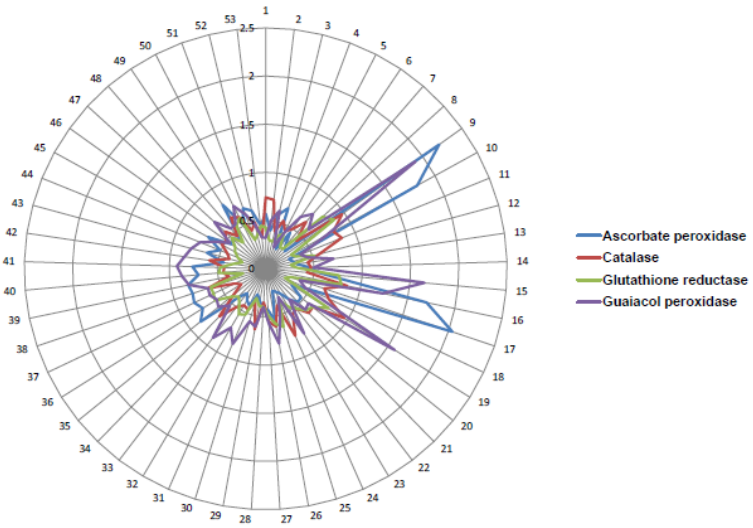


Fig. 3. Anti-oxidative enzymes activities of different genotypes

4. DISCUSSION

4.1 Biochemical Parameters

Generally, the level of chlorophyll content in leaves determines the rate of photosynthesis. In the current study, a reduction in chlorophyll component was found in susceptible genotypes in comparison to tolerant genotypes. Similar results in soybean were found earlier by Hossain et al. [41]. Reduced level of chlorophyll synthesis in susceptible genotypes may be the reason of less activity of the photosynthetic elements. Previously, loss of chloroplast membranes under drought stress has

also been documented by Anjum et al. [42]. Parallel reduction in chlorophyll levels in several other plant species *viz*., soybean, maize, rice, chickpea, peal millet *etc.* have been reported [43] [14] [19] [20]. High reduction in chlorophyll content was found in drought susceptible genotypes in the present study.

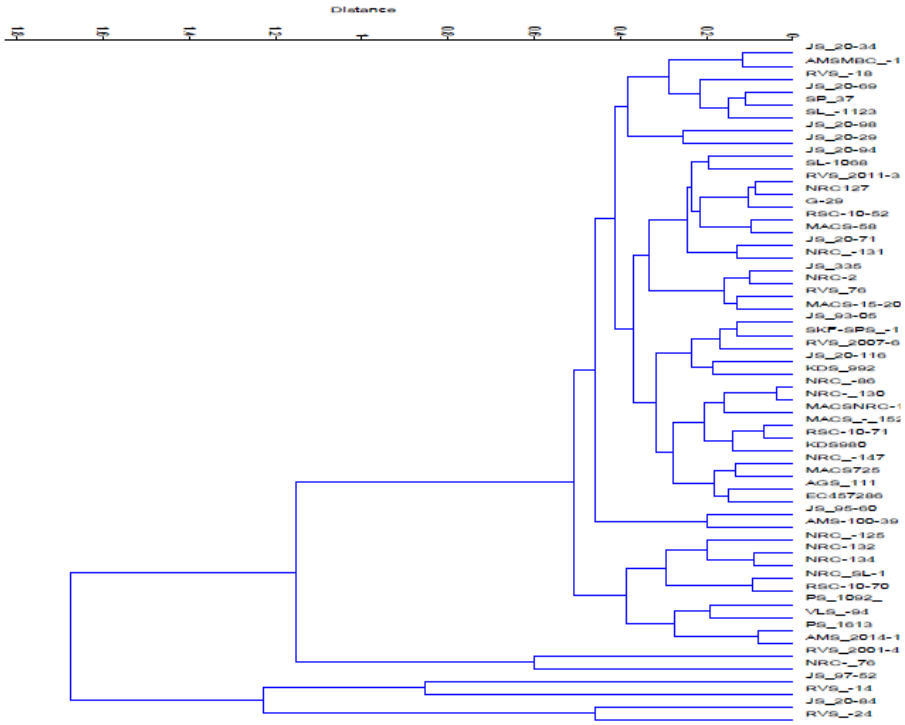


Fig. 4. Dendrogram showing relationship among soybean genotypes based on different antioxidative enzymes (Ascorbate peroxidase, Catalase, Glutathione reductase and Guaiacol peroxidase)

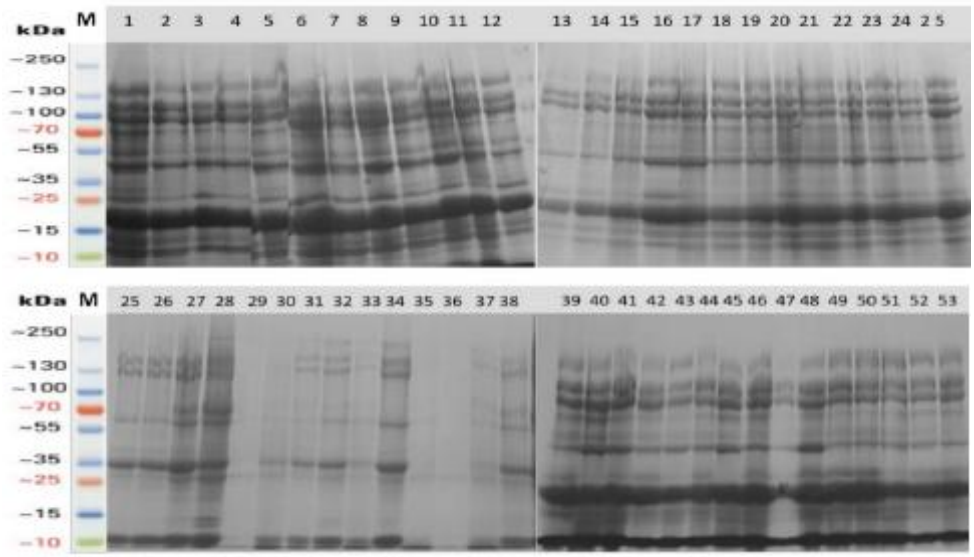


Fig. 5. SDS protein profiling of 53 soybean genotypes

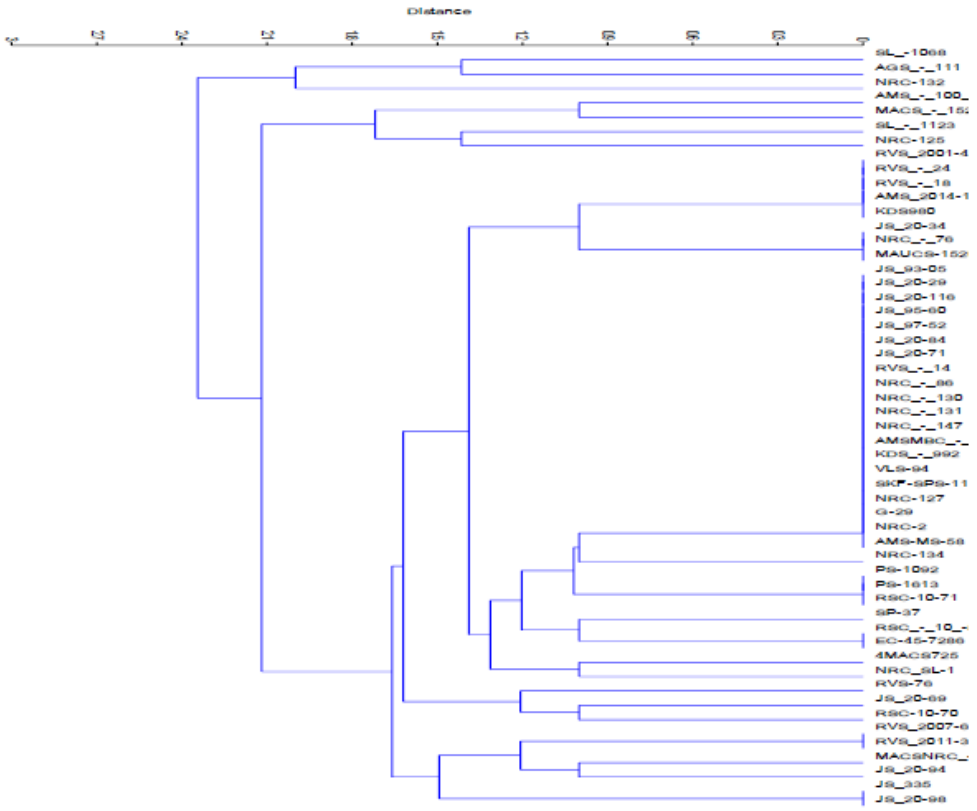


Fig. 6. Dendrogram showing relationship among different soybean genotypes based on SDS-Profiling

Role of proline in osmotic regulation under water stress has been monitored in various plant species [44-45]. Soybean genotypes with significant rise in proline contents have been considered as drought tolerant. Highest proline content increment was found in genotype JS97-52 followed by genotypes RVS2001-4 and JS95-60. Previously, Khan et al. [46], Kachare et al. [11] and Sharma et al. [14] observed comparable trend in the proline content during their study on screening of soybean genotypes tolerant to drought stress. Increased proline content maintains cell water level under drought [47] [20] Further, George et al. [48] suggested that increased proline has osmoprotective functions by preventing separation of enzymes during metabolic activities.

Among osmotic regulating substances, total soluble sugars are noteworthy contributors of drought tolerance [49] 20]. During present investigation, genotype JS97-52 had maximum soluble sugar tracked by genotypes RVS2001-4 and JS95-60. These findings indicate the presence of possible drought tolerance in these genotypes. Previously, Kachare et al. [11] and Sharma et al. [14] also observed momentous enhancement in total soluble sugar content under drought stress while studying on Indian soybean genotypes.

Lipid peroxidation mechanism is a cursor of oxidative stress under water stress. Malondialdehyde (MDA) content is considered as a sign of membrane lipid peroxidation and shows the level of damage in membrane underneath stress [18]. MDA content in nmole gm^{-1} was found greatest in genotype JS 97-52. Genotypes exhibited higher MDA content in leaves might be tolerant against drought [50] due to more synthesis of Reactive Oxygen Species in comparison of the rest of the genotypes and this parameter can be applicable to find out drought tolerance in genotypes [51].

According to Blackman et al. [52] the increments in MSI indicates the reduction of lipid peroxidation with oxidative bursts under water stress conditions. In previous studies, MSI has been used frequently for screening of drought tolerance in crop species [53]. In similar studies, Almeselmani et al. [54]

Kachare et al. [11] and Sharma et al. [14] reported significant genotypic differences in membrane stability as an indirect criterion for selection of drought tolerance in soybean.

4.2 Anti-oxidant Enzymes

Anti-oxidative enzymes have vital role in the protection of plants in water stress due to their contribution in tolerance mechanism [55, 56, 21,22,23,24,]. This may be entire capacity of a particular genotype to combat against these stresses. Previous reports proved the role of antioxidant enzymes in the mechanism of drought and dehydration tolerance in soybean [57] [56]. Major system in plants under abiotic stress for detoxification of hydrogen peroxide is the ascorbate-glutathione cycle in which Ascorbate peroxidase (APX) helps in the conversion of H_2O_2 into H_2O, in chloroplast [58, 59]. Enhanced APX activity was invented in genotypes JS97-52 followed by RV-24 as compared to rest of the genotypes possibly due to appearance of prominent H_2O_2 detoxification with prevention of H_2O_2-mediated cell damage [60]. Increased APX activity in soybean under drought stress has been earlier reported by Kausar et al. [61], Kachare et al. [11] and Sharma et al. [14]. Catalase (CAT) enzymes are capable to alter millions of H_2O_2 molecules into H_2O and O_2 in a second [62]. Enhanced CAT and GR activity was found in soybean genotype JS97-52. In earlier research, conducted by Masoumi et al. [63] a positive correlation between drought tolerance in plant genotypes and enhanced CAT was reported. Similarly, Liu et al. [64], Kachare et al. [11] and Sharma et al. [14]. also observed a significant increase in GR activity in some soybean genotypes. Earlier, Porcel et al. [65] reported increased GR activity in drought tolerant soybean genotypes. This indicates the enhanced ROS scavenging ability of drought tolerant plants as a result negligible injure to plants under stress. Role of GR as an important enzyme to sustain redox condition of plant by converting oxidized glutathione (GSSG) into reduced glutathione (GSH) with the help of NADPH has also been proved by Garg et al. [66]. Enhanced PDX activity was evidenced in genotype JS97-52 while lowest was in genotype JS20-98. This indicates the possible tolerance in these genotypes as earlier stated by Murthy et al. [67]. Similar to the present research, Kachare [11] and Sharma et al. [14] also noticed directly proportional relation between PDX activity and level of water stress in soybean. Akitha-Devi et al. [68] reported enhanced activities of antioxidant enzymes with reduction in osmotic potential. These findings clarify the relation between activity of antioxidant enzyme and defense mechanisms against water stress in soybean.

4.3 Protein Profiling

Soybean seed contains about 35-40% proteins with big contribution of essential amino acids. It is also a rich source of antioxidants and unsaturated fatty acids. Many of these proteins have functions in the management of water deficit [69]. These proteins play major role in uptake of water from environment for the betterment of plant health. So, the proteins help the plants in completion of growth and development cycle normally. On average, 12 bands per genotype were detected in a range of molecular weight from 3.5 to 43.0 kDa in present study correspondingly to Arumingtyas and Savitri [70] and Kachare [11]. Sahu et al. [19] and Gupta et al. [71] also detected range of protein bands in chickpea.

5. CONCLUSION

It is concluded from the present investigation that the different biochemical parameters and anti-oxidant enzymes activities play a significant role in respect to drought tolerance in soybean like other crop species. Genotypes *viz.,* JS97-52, RVS-14 and JS95-60 were found with drought tolerance. The findings of the present study provide a base for the use of these soybean genotypes for further hybridization to develop drought tolerant varieties. These results also open the door for the applications of advanced biotechnological tools for deep analysis of drought tolerance mechanism in soybean crop.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 10
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Morphological and Molecular Characterization of Indian Mustard Germplasm Lines

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ABSTRACT

The purposes of current investigation were to use a set of SSR molecular markers to detect DNA polymorphism among cultivated *Brassica juncea* accessions. The estimation of genetic diversity is not only imperative in crop improvement programmes but also for the competent management and conservation of prevailing genetic variability. Genetic diversity was investigated in forty germplasm lines along with eight cultivars of Indian mustard employing different morphological parameters *viz*., days to 50% flowering and maturity, plant height (cm), length of main raceme (cm), number(s) of primary and secondary branches/plants, number(s) of siliques per plant and seeds per silique, 1000 seed weight (g) and seed yield per plant (g) as well as 50 SSR molecular markers in preliminary investigation, out of which 7 SSR molecular markers were found to be highly polymorphic between all the genotypes of mustard and were employed in an advance research.
An UPGMA phonogram was constructed for all 48 genotypes and the similarity coefficient arrayed between 0.00 to 0.91. Number of alleles ranged from 3 to 4, genetic diversity in between 71% to 65% with a mean worth of 67%, heterozygosity varied between10 to 20 % with an average value of 12% and PIC value for markers ranged between 0.65 to 0.59 with a mean worth of 0.61. All seven SSR primers exhibited PIC value above 0.5 (50%) demonstrating high genetic diversity in the studied genetic stock.

Keywords: Genetic diversity; Brassica juncea L.; morphological traits; polymorphic information content; similarity coefficient.

1. INTRODUCTION

The genus *Brassica* is one of the most vivacious oil seed crops being cultivated in India with high grade of genetic diversity [1-8]. It is mostly self-pollinated crop, nevertheless, up to 30% out-crossing does happen under natural field circumstances contingent to wind and bee actions [9]. The most mustard cultivating nations of the world are India, Canada, China, Pakistan, Poland, Bangladesh and Sweden. In India, it is being cultivated largely in the states *viz*., Uttar Pradesh, Rajasthan, Madhya Pradesh, Haryana, Punjab, Assam, Bihar, Gujarat and West Bengal. In Madhya Pradesh, it is mostly grown in Morena, Bhind, Gwalior and Sheopur districts. Owing to low cost of cultivation and high commercial benefit, the area and productivity of mustard is unceasingly expanding, nevertheless productivity is low.

In almost agricultural crops, the first step in crop improvement is complete assessment of the local genetic stocks, including collection, evaluation and morpho-physiological and molecular characterization of germplasm lines. Consequently, the most applicable tactic to further improve the performance of crop varieties is to judge to large assorted pool of genetic variability. Furthermore,

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information on the genetic diversity of *B. juncea* germplasm collection scan facilitate breeders and geneticists' useful information on the allelic diversity exist in *B. juncea* materials. It may help to identify genetically diverse pools for use in cross combinations to improve important agronomic traits or to better exploit heterosis [10-12]. Conventionally, morphological [13-14], phenological, biochemical [15-20] and agronomical traits have been employed as criteria for the introgression of new variation into breeding lines to breed new cultivar in different crop species including oilseed rape.

In *Brassica*, genome research with the employment of marker-based programme began in the late 1980^{s} when the first RFLP linkage map for *B. oleracea* [21], B. *napus* [22] and *B*. *rapa* [23], was established. For phylogenetic investigations and genetic mapping in *Brassica*, RFLPs and RAPDs have been broadly employed [24]. The number of offered *Brassica* SSRs (microsatellite) primers is increasing gradually. *Brassica* genome integration greatly assisted the release of highly polymorphic map based, vigorous SSR markers of the entire *B*. nigra, *B*. *rapa*, B. *napus* and B. *oleracea* genome into public domain. However, the discovery of the PCR [25], primes the potential to upsurge the variety and density of marker in the already existing genetic maps with ISSR, AFLP and with the microsatellites [26,27], also termed as SSR. SSRs are highly imperative resource of map-based alignment among distinct crosses, owing to their robust, simple analysis and highly polymorphic nature [28]. Molecular markers closely linked to genes of interest are very fruitful for marker-aided selection and pyramiding of two or more genes into a single genotype in *Brassica* breeding programmes [29]. Among diverse types of molecular markers, microsatellite also known as simple sequence repeats (SSRs) have been employed for an array of purposes in plant breeding and genetics including gene mapping, cultivar discrimination and detection of genetic diversity [30-53]. It has been established that SSR markers are beneficial for genetic diversity and structure investigations of *Brassica*. Soengas et al. [54] recognized the genetic relationship among populations and studied the genetic structure by analyzing the polymorphic alleles of SSR markers. The purposes of current investigation were to use a set of SSR molecular markers to detect DNA polymorphism among cultivated *Brassica juncea* accessions. This will facilitate valuable information for *Brassica* breeding programme in the upcoming days.

2. MATERIALS AND METHODS

2.1 Plant Material

Materials for the current investigation comprised 40 germplasm lines and 8 cultivars of mustard obtained from Bastar Jagadalpur, Jaipur, Rajasthan and NBPGR, New Delhi. The field trial on 48 genotypes was carried out in randomized block design (RBD) with two replications Plot size was represented by 2m x 22.5m with a spacing of 30 cm x l0 cm. A fertilizer dose of 80:40:40 kg $NPKha^{-1}$ was applied. Hoeing and hand weeding were done at the time of top dressing and need based plant protection measures were followed.

2.1.1 List of germplasm lines

MRNJ-1, MRNJ-2, MRNJ-3, MRNJ-4, MRNJ-5, MRNJ-6, MRNJ-7 and MRNJ-8 Collected from Bastar Jagadalpur; MRNJ-9, MRNJ-10, MRNJ- 11, MRNJ-12, MRNJ-13, MRNJ-14 MRNJ-15, MRNJ-16, MRNJ-17, MRNJ-18, MRNJ-19,MRNJ-20, MRNJ-21, MRNJ-22 were collected from Rajasthan Jaipur; MRNJ-23, MRNJ-24, MRNJ-25, MRNJ-26, MRNJ-27, MRNJ-28, MRNJ-29 from Rape seed Buster; MRNJ-30, MRNJ-31, MRNJ-33, MRNJ-34, MRNJ-35, MRNJ-36, MRNJ-37, MRNJ-38 from NBPGR, New Delhi; and MRNJ-39, MRNJ-40 and MRNJ- 41 were collected from NRC Bastar.

2.1.2 List of cultivars

Kranti, Maya, Rvm-1, Rvm-2 Rvm-3, Jm-1, Jm-2 and IDM-69.

2.2 Methods

2.2.1 Morphological characterization of mustard germplasm lines and cultivars

Observations for 10 different morphological parameters were taken for morphological characterization including days to 50% flowering, days to maturity, plant height (cm), length of main raceme (cm),

number(s) of primary and secondary branches/plant, number(s) of silique per plant and seeds per silique, 1000-seed weight (g) and seed yield per plant (g). The analysis of variance (ANOVA) of morphological traits for standard error (S.E.), critical difference (CD) and coefficient of variation (CV) was computed employing OP Stat software [55]. The coefficient of correlation among all morphological traits at maturity was calculated employing SPSS ver.19 software. The similarity matrices were employed to construct a dendrogram for all the genotypes using NTSYS-pc as per suggested by Rohlf's et al. [56] based on Unweighted Pair Group Method with Arithmetic Mean UPGMA. The phenotypic and genotypic coefficients of variation in per cent were computed as per formulae prearranged by Burton [57].

2.2.2 Molecular Characterization using SSR Markers

Genomic DNA Isolation: Two to three healthy leaves were collected from each plant of mustered genotypes at seedling stage and leaf samples were grinded to a fine powder with DNA extraction buffer (1M Tris-Cl pH 8.0, 0.5 M EDTA pH 8.0, 4.0 M NaCl, 10% CTAB, β-mercaptoethanol and Milli-Q water) in a mortar and pestle. DNA extraction was done employing method as designated by Murray and Thompson [58], with little modification [59,60]. The DNA was diluted to a final concentration of 10-25 ngµl^{-1} for SSR analysis.

PCR Amplification SSR Markers: A total of 50 microsatellite markers (SSR) were employed to investigate the polymorphism in selected accessions to search out polymorphic markers for diversity analysis among 48 genotypes. Among them, only 8 SSR primers were selected for advance analysis (Table 1). The SSR primers were synthesized by Eurofins Genomics India Pvt Ltd. Polymerase chain reaction was accomplished in 10µl reaction mixture encompassing of 1X PCR buffer, 0.1 U Taq DNA polymerase, 1 µl dNTP (1 mM), 0.5 µl of forward and reverse primers each (10 pM) and 20 ng/µl of genomic DNA in a Thermocycler (Bio-Rad, USA). The Polymerase Chain Reaction protocol embraced of initial denaturation step of 94°C for 5 min tracked by 35 cycles of 94°C for 1 min, annealing at 55°C for 30 sec, elongation at 72°C for 1 min with final extension at 72°C for 7 min. The PCR products were resolved on 3% agarose gel at 100V for 2-3 hrs and documented employing Zenith, Gel Documentation System. The SSR banding patterns were scored based on the size of fragments amplified across all genotypes with known ladder of 100 bp as size standard. The major allele frequency, number(s) of alleles per locus, polymorphism information content (PIC) and gene diversity was analyzed using Power Marker v3.25 software [61], The dendrogram based on unweighted pair group method for arithmetic average (UPGMA) and bootstrap value of 1000 permutations was constructed using MEGA 6.0 software [62].

Table 1. Details of SSR primers selected for mustard germplasm screening

S.	Name of SSR	Forward primer sequence (5'-3')	Ref.
1	BRMS- 240	Forward-CAAGAGTATTTGTGTGGGTTGACTC Reverse-AAATAACGAACGGAGAGAGAGAGAG	He et al. [70]
2	BRMS- 324	Forward-AACTTAACCGAAACCGAGSTAGGTG Reverse-AATCTCGAAATTCATCGACTTCCTC	He et al. [70]
3	SR- 7223	Forward-AGGACCCGACTTTCCTTGTT Reverse-ACCAAACTCGGCGTACAAAT	Wang et al. [71]
4	SR- 9222	Forward-CACCGAACAAAACTGAGGGT Reverse-CGTTTCACTGCGTTCTACCA	Wang et al. [71]
5	SR – 94102	Forward-ATCCCCAAACTACCCTCACC Reverse-AGGATGAGCAAAGGAAAGCA	Wang et al. [71]
6	OI 10-CO 5	Forward-GGCTACAAAATGTTTGATAAGCTCT Reverse-ACCTGAAAGAGAGGCTACACAT	Wang et al. [71]
7	SSR Na10-D09	Forward-AAGAACGTCAAGATCCTCTGC Reverse-ACCACCACGGTAGTAGAGCG	Wang et al. [71]
8	SSR Na10-D11	Forward-GAGACATAGATGAGTGAATCTGGC Reverse-CATTAGTTGTGGACGGTCGG	Wang et al. [71]

3. RESULTS AND DISCUSSION

3.1 Phenotypic and Genotypic Correlation Coefficient

A correlation study was conducted between the different traits investigated and it predicts the germplasm lines that should be given priority while selecting the accessions for further improvement. In general, genotypic correlations were higher than phenotypic ones in magnitude for all the parameters. The character which displayed negative association at genotypic level also exhibited negative association at phenotypic level. Phenotypic correlation coefficient revealed that seed yield per plant was significantly correlated with the length of main raceme at 1% probability level. Among the yield attributing characters, days to 50% flowering displayed significant phenotypic correlation with number (s) of primary and secondary branches per plant and number (s) of silique per plant at p value 0.01 (Table 2). Days to maturity had significant correlation coefficient with plant height, numbers of primary and secondary branches per plant and numbers of siliquae per plant. Length of main raceme also showed significant correlation coefficient with harvest index and days to maturity. Plant height and numbers of siliquae per plant were found highly significantly corelated with numbers of secondary branches. Seed yield per plant was also highly and significantly corelated with harvest index. Mean value of morphological traits was also analyzed for correlation coefficient using SPSS V19 software. Days to 50% flowering was positively and significantly correlated with numbers of primary and secondary branches per plant and numbers of siliques per plant at 1% significance level (Table 3). While numbers of secondary branches were positively and highly corelated with days to 50% flowering (r=0.473) and numbers of primary branches (r=0.589) at 1% significance level. Number of seeds per siliquae are positively and highly corelated with numbers of siliquae per plant (r=0.495**) at 1% level of significance. In glance it is evidenced that these traits are highly influenced by each other and if seed yield per plant is higher, then harvest index will also be increased subsequently. Similarly, if days to 50% flowering is more, it will affect numbers of primary and secondary branches per plant and numbers of siliquae per plant positively. Earlier Ray et al. [63], also reported positive correlations for association of primary branches with plant height. Dawar et al. [64], documented that the phenotypic coefficients of variation were higher than genotypic coefficient of variation for all the characters which support the finding of current investigation.

3.2 Morphological Trait Analysis and Cluster Formation

Cluster analysis exposed those accessions fell into two major groups that further subdivided into 8 sub-groups. Group-'I' contained 2 genotypes *namely* MRNJ-39 and MRNJ-11; while, Group-'II' comprised 46 genotypes. Group - 'II' again classified into two sub-groups 'II-A' and 'II-B'. Sub-group 'II-A' contained 6 genotypes; whereas, sub-group 'II-B' carried 40 genotypes (Fig. 1). Rendering to Jaccard's Similarity Coefficient, highest similarity was documented between genotypes JM-1 and JM-2; MRNJ-12 and MRNJ-19; MRNJ-21 and MRNJ-3; MRNJ-41 and RVM-3; Kranti and RVM-1; MRNJ-11 and MRNJ-39 and MRNJ-8 and MRNJ-22. Consequently, both genotypes were grouped together. On the basis of genetic diversity of morphological traits, highest genetic diversity was experimented between genotypes MRNJ-39 and MRNJ-1 (Table 4).

3.3 Molecular Characterization

Molecular characterization of 48 genotypes was based on electrophoretic banding pattern of all the polymorphic SSR primers is presented in Table 5. Cluster analysis revealed that accessions fell into major group and sub groups. The dendrogram which is designed by molecular characterization formed 8 main groups and 13 subgroups which gives a recognizable result of the characteristics of all the genotypes based on its origin and data interpretation through base pair analysis (Fig, 2). According to Jaccard's similarity coefficient, highest similarity was documented between genotypes MRNJ-4 and MRNJ-8 in group-'I'. RVM-1 and MRNJ-19 in sub group- 8(B), Kranti and MRNJ-22 in sub group- 8 (A). MRNJ-39 and Maya in Sub group- 7 (A), MRNJ35 and IDM-69 in sub group- 6 (B), MRNJ-24 and JM-2 in sub group -6 (B), JM-1 and MRNJ-36 in sub group -3 (A) and genotypes MRNJ-3 and RVM-2 in sub group- 2(A).

Table 2. Genotypic and phenotypic correlation coefficient of grain yield and its attributes in mustard germplasm

	Day of 50% flowering	**Day of maturity**	**Plant height (cm)**	**Length of main raceme**	**No. of pri. branches per plant**	**No. of sec. branches per plant**	**No. of Siliqua per plant**	**No. of seed per siliqua**	**1000 seed weight**	**Seed yield per plant**	**Harve-st Index**
Day of 50% flowering	1	0.246	0.171	-0.154	0.566**	0.455**	0.464**	0.243	0.116	-0.004	0.068
Day of maturity	0.126	1	0.427**	-0.180	0.151	-0.053	-0.160	0.153	-0.029	0.156	-0.125
Plant height (cm)	0.160	0.251	1	0.219	0.319*	0.342*	0.343*	-0.004	-0.109	0.214	0.157
Length of main raceme	-0.137	-0.027	0.168	1	0.150	-0.025	0.148	-0.240	0.093	0.327*	0.461**
No. of pri. branches per plant	0.465**	-0.003	0.256	0.059	1	0.652**	0.634**	0.030	0.140	0.013	0.085
No. of sec. branches per plant	0.415**	-0.016	0.311	-0.018	0.517**	1	0.813**	0.228	0.173	0.071	0.207
No. of Siliqua per plant	0.413**	-0.091	0.337*	0.136	0.506**	0.742**	1	0.216	0.155	0.075	0.228
No. of seed per siliqua	-0.076	0.022	-0.003	-0.206	0.034	0.226	0.186	1	0.288	-0.084	-0.013
1000 seed weight	0.049	0.070	-0.104	0.043	0.168	0.141	0.138	0.238	1	0.008	-0.130
Seed yield per plant	-0.002	0.100	0.214	0.501**	0.015	0.070	0.073	-0.078	0.011	1	0.130
Harvest Index	0.065	-0.075	0.155	0.374**	0.073	0.200	0.221	-0.007	-0.128	-1.231**	1

**, ** significant at 5 & 1 % level of significance, Upper diagonal represent genotypic correlation coefficient and lower diagonal represent phenotypic correlation*

Table 3. Analysis of correlation coefficient of mean value of yield attributing morphological traits in mustard germplasm using SPSS V19 software

Correlations										
	DF	**DM**	**PH**	**LMR**	**NPB**	**NSB**	**NS**	**NSS**	**TKW**	**GYD**
DF	1	0.336*	-0.053	0.014	0.411**	0.473**	0.455**	0.284	0.024	-0.019
DM		1	-0.073	-0.050	0.057	-0.067	-0.047	-0.050	0.070	-0.004
PH			1	0.202	-0.119	-0.076	-0.063	-0.184	-0.204	0.092
LMR				1	.156	0.111	0.203	-0.001	0.161	0.349*
NPB					1	0.589**	0.539**	0.206	0.099	0.012
NSB						1	0.819**	0.495**	0.112	0.041
NS							1	0.396**	0.120	0.077
NSS								1	-0.072	-0.001
TKW									1	0.046
GYD										1

**. Correlation is significant at the 0.05 level (2-tailed)*
***. Correlation is significant at the 0.01 level (2-tailed)*
DF=Day of 50% flowering, DM=Day of maturity, PH=Plant height (cm), LMR=Length of main raceme, NPB=No. of primary branches per plant, NSB=No. of sec. branches per plant, NS=No. of Siliqua per plant, NSS=No. of seed per silique, TKW=1000 seed weight, GYD=Seed yield per plant

Table 4. Important features of clusters formed in analysis of morpho-physiological traits of mustard germplasms using NTSYS software

Cluster/Sub cluster	**No. of Accessions**	**Important features**	**Remark**
Cluster-I	2	MRNJ-11, MRNJ-39	
Sub-Cluster-I(A)	1	MRNJ-39	
Sub-Cluster-I(B)	1	MRNJ-11	
Cluster-II	46		
Sub Cluster-II(A)	6		
Sub Cluster-II(A)-I	2	Kranti, RVM-1	Highest number of secondary branches per plant observed in Kranti (29)
Sub-Cluster-II(A)-II		MRNJ-14, MRNJ-28, MRNJ-41, RVM-3	Highest day of maturity observed in MRNJ-28(124)
Sub-Cluster-II(B)	40		
Sub-Cluster-II(B)-I	26	MRNJ-37, MRNJ-24, MRNJ-15, MRNJ-17, MRNJ-36, MRNJ-34, MRNJ-30, MRNJ-13, MRNJ-26, MRNJ-33, MRNJ-12, MRNJ-29, MRNJ-22, MRNJ-8, MRNJ-6, MRNJ-27, MRNJ-23, MRNJ-21, MRNJ-3, MRNJ-40, MRNJ-35, MRNJ-25, MRNJ-19, MRNJ-7, MRNJ-9, MRNJ-2,	Highest 1000 seed weight observed in MRNJ-24(6.63gm) and highest number of primary branches per plant observed in MRNJ-26(9)
Sub-Cluster-II(B)-II	14	MRNJ-1, RVM-2, JM-1, JM-2, MRNJ-4, MRNJ-31, MRNJ-5, MRNJ-38, MRNJ-20, MAYA, MRNJ-10, MRNJ-16, MRNJ-18, IDM-69	Highest plant height observed in MRNJ-1 (251.4cm), highest number of siliquae per plant observed in RVM-2 (796), highest seed yield per plant observed in MRNJ-10

The summary statistics extracted from Power Marker was observed for Allele number, Major Allele Frequency, Gene Diversity, Heterozygosity and Polymorphism Information Content (PIC). Highest PIC value (0.6888) was detected for marker SSRNa10D09 which has 8 alleles across the 48 genotypes. Markers SR-9222 and BRMS-324 also had higher PIC scores and higher number of alleles (Table 5). Lowest PIC value was documented for marker SR-7223 (0.6231). Mean PIC value was 0.6154, representing high diverse nature of markers, which can be utilized for further characterization. All SSR

primers showed PIC value above 0.5 (50%) indicating high genetic diversity in the studied plant material. Major allele frequency was between 0.33 to 0.44 with an average 0.389. Numbers of alleles ranged between 3 to 4, genetic diversity ranged from 71% to 65% with an average of 67%. Heterozygosity arrayed from 10% to 20% with an average value of 12% (Table 5).

Table 5. Statistical analysis data of polymorphic SSR markers for Polymorphic Information Contents, and other parameters of mustard germplasms

Marker	Major Allele Frequency	Genotype No.	Allele	Gene Diversity	Hetero-zygosity	PIC
BRMS324	0.3750	5.0000	4.0000	0.6866	0.2500	0.6231
SR7223	0.4375	3.0000	3.0000	0.6398	0.0000	0.5642
SSRNa10D11	0.4167	5.0000	4.0000	0.6443	0.0625	0.5731
BRMS224	0.4479	7.0000	4.0000	0.6586	0.1042	0.5966
SSRNa10D09	0.3438	7.0000	4.0000	0.7368	0.2292	0.6888
SR94102	0.3750	4.0000	4.0000	0.6745	0.0000	0.6060
SR9222	0.3333	7.0000	4.0000	0.7118	0.2083	0.6556
Mean	**0.3899**	**5.4286**	**3.8571**	**0.6789**	**0.1220**	**0.6154**

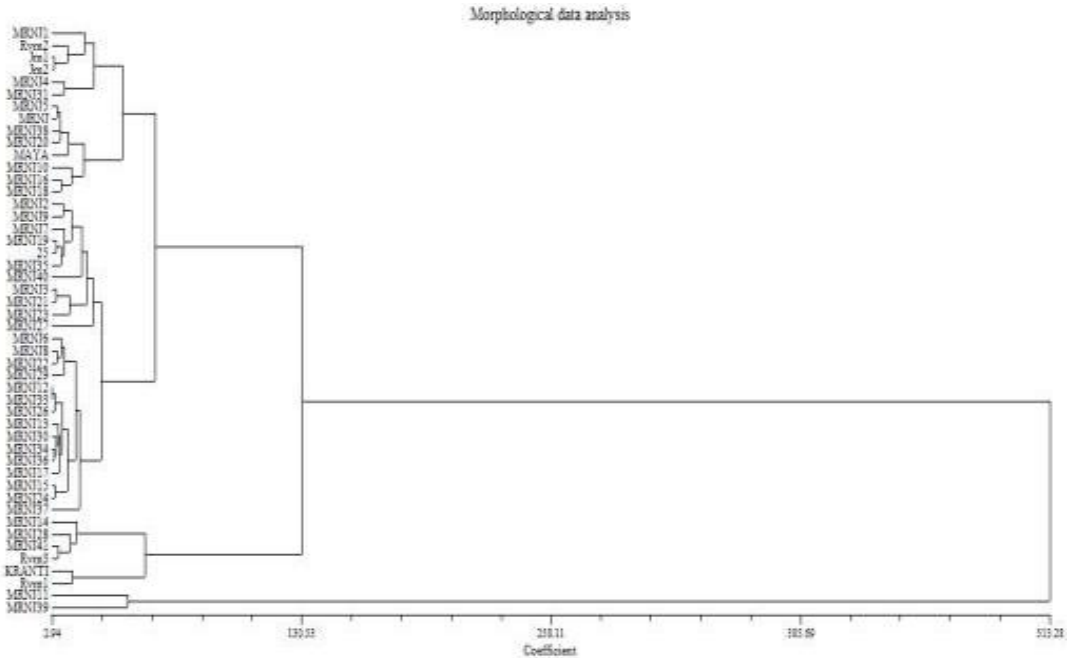


Fig. 1. Dendrogram of *Brassica* germplasm for mean value of yield attributing morphological traits

The results of current investigation displayed somewhat resemblance with the study conducted by Qu et al. [66], as they used 217 genotypes and 37 markers. Recently, Verma et al. [33] and Baghel *et al*. [31], have also done similar kind of work in Indian mustard and employed 11 and 7 markers respectively for diversity analysis. Shyam et al. [30], used 48 *Brassica* genotype (s) with the aim to identify genotypes with low and high erucic acid content on the basis of 23 SSR molecular markers. They have identified a total of 109 alleles with an average of 4.47 alleles per locus for polymorphic SSR markers. Genetic diversity varied from minimum 0.55 for SSR marker Na10- D07 to maximum 0.77 for BRMS-098 with a mean worth of 0.68. Polymorphism information content (PIC) value of the markers speckled from minimum 0.51 for SSR marker Na10-D07 to maximum 0.73for the primer BRMS-098 with an average value of 0.62. In our research, an average PIC values were found to be higher than that of reported by Patel et al. [67], where the PIC value arrayed between 0.427 to 0.730 with an average of 0.555. PIC values experimented by Bharti et al. [68] ranged from 0.34 to 0.49 with an average of 0.41. Gupta et al. [69], also reported low PIC value 0.281. In the present

experimentation; it came out to be 3 to 4 with an average of 3.85 allele which shows that the markers came out with even better results with the present tested genotypes.

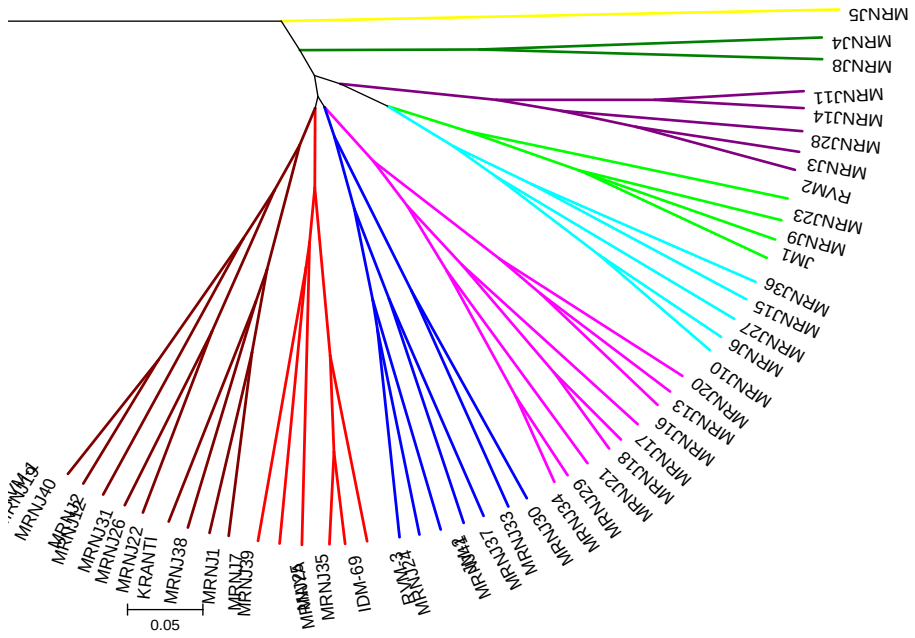


Fig. 2. UPGMA tree based on dissimilarity index for mustard germplasm using polymorphic SSR markers

The estimation of genetic diversity is not only imperative in crop improvement programmes but also for the competent management and conservation of prevailing genetic variability. Molecular profiling has been the ideal choice of breeding being more consistent and faithful and less prejudiced by environmental fluctuations [72,65,30-52]. As such it is important to evaluate newly developed genotypes for their stability in production and screen for variability present which could deliver a source material for further improvement in mustard crop.

4. CONCLUSION

Present investigation accomplished morphological and molecular characterization of Indian mustard germplasm employing yield attributing traits and SSR markers. Phenotypic and genotypic correlation between traits was measured to realize environmental effect. Diverse genotypes formed distinct group owing to morphological and SSR molecular markers data. Diversified parents identified from this investigation may be employed as resource for further mustard improvement programme.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 4
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Plant Tissue Culture Techniques for Conservation of Biodiversity of Some Plants Appropriate for Propgation in Degraded and Temperate Areas

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ABSTRACT

Plant Biotechnology is being employed as a device for conservation of natural habitats and their sustainable utilization for ecological balance, technologies for cultivation of plants in diverse cropping systems and on the problematic soil, improvement of state-of-art technologies for extraction, characterization and utilization of necessary bioactive components, generation of scientific and clinical information to support the health entitlements of botanical medicines, elicitation and improving the production of known and novel metabolites using metabolic engineering technology, DNA barcoding : identification and characterization of plant material, design and discovery of newer molecules for human and plant health, development of post-harvest management including establishment of effective partnerships between different stakeholders. Plant tissue culture can be used to rapidly multiply virus-free planting material in plants suitable for degraded lands and temperate areas by developing micropropagation protocols. Micropropogation is a time and space efficient technique that produces more viral disease-free and elite propagules. Germplasm storage and conservation is possible through the use of *in vitro* gene bank technology, in which vegetatively propagated plants can be conserved in cryogene banks, and recalcitrant seeds, embryos, and pollens can be stored in liquid nitrogen for long periods of time. This chapter discusses micropropagation protocols developed in our lab for various plants suitable for cultivation in undulated lands and temperate regions, as well as detection and production of natural compounds found in plants and ex situ conservation methods. Biotechnology and biodiversity of high value plants can be harnessed together as developmental challenge as well as an economic opportunity in future.

Keywords: Biodiversity; degraded land; plant cell; tissue & organ; culture; medicinal plants; metabolic engineering; bioactive compounds.

1. INTRODUCTION

Plant Biotechnology is being used as a tool for conservation of natural habitats and their sustainable utilization for ecological balance, technologies for cultivation of plants in different cropping systems and on the problematic soil, development of state-of-art technologies for extraction, characterization and utilization of essential bioactive components, generating scientific and clinical data to support the health claims of botanical drugs, elicitation and enhancing the production of known and novel metabolites using metabolic engineering technology, DNA bar coding: identification and characterization of plant material, design and discovery of newer molecules for human and plant

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health, development of post-harvest management and handling technologies including establishment of effective partnerships between different stakeholders. Conservation of biological diversity have assumed substantial significance today as compared to few decades ago owing to increasing human population, depleting natural resource and ruthless exploitation, particularly the plant wealth. Efforts are being directed all over the world to develop strategies to achieve genetic conservation.

2. PROPAGATION AND CONSERVATION STRATAGEMS AND THEIR APPLICATIONS

2.1 Micropropagation and *In vitro* Banking

Micropropagation refers to *in vitro* plant propagation methods. The principal approaches are organogenesis (*i.e*., auxiliary bud proliferation and induction of adventitious buds) and somatic embryogenesis. Somatic embryogenesis has advantages over organogenesis with respect to the yield of plantlets [1-3], especially when a cell suspension culture can be established [4-12]. It is also faster to manipulate than shoot/root organogenesis [13-18]. Various explants can be used to regenerate plantlets including meristem/ shoot tips, nodal and leaf segments, ovular and nucellar tissues and zygotic embryos, protoplast and immature pollen. As an alternative to other vegetative propagation methods, the attraction of tissue culture lies in its aptitude to multiply elite clonal materials very speedily round the year and in short span of time. This is especially desired in commercial production of medicinal and aromatic plants as conservation of rare and endangered medicinal and aromatic plants using tissue culture is highly desirable. It is also an appropriate method for conservation and exchange of medicinal and aromatic plant germplasm for species with recalcitrant seeds or asexually propagated. It also facilitates production of useful secondary metabolites [19-21] and selection of useful mutants by means of *in vitro* selection in conjunction with somaclonal variation against different biotic [22-24] and abiotic [10] stresses together with quality improvement [11].

2.2 *In vitro* Collecting

One other technique that has been found to be increasingly imperative in conservation is *in vitro* collecting. This involves not taking the germplasm to the laboratory but taking the laboratory to the germplasm by using simplified inoculation techniques in the field. Minimal equipment is used and it is easily transported and may sometimes be of dual purpose, *i.e*., a box to hold instruments and cultures in transit which can double as a small work bench or wind shield. These collecting techniques have the advantage of facilitating the movement of germplasm, although collected in such a manner may or may not be pathogen-free [25]. There is definitely potential in the use of *in vitro* conservation alongside other measures for the preservation of plant genetic resources.

2.2.1 Encapsulation of somatic embryo- artificial/synthetic/syn seeds

The production of plant clones multiplied by tissue culture and disseminated as synthetic seeds could be a convenient alternative to the costly crop plants. The conveyance of artificial seeds also enables problems like enterprise numerous traditions for ascending up *in vitro* cultures and acclimatization to *ex vitro* conditions [26-27]. Work done with some endangered plant species indicates the possibility of encapsulating somatic embryos to form artificial seeds which can then be handled like conventional seeds. There are reports on synthetic seed research on cardamon [28], *Santalum album* [29], *Asparagus cooperi* [30] and *V. wallichi* [31]. Thus, with considerable long-term research, developments in this area may enable the direct use of such propagules in the conservation and propagation of important plants.

2.3 Cryopreservation

Recently *in vitro* technology and cryopreservation (freeze preservation) *i.e*., storage in liquid nitrogen (-196°C) have received attention and complement extensively to existing methods such as seed storage in gene bank (-20°C), maintenance under field conditions, orchards, plantation and botanical garden [32]. Propagation and preservation using tissue culture technology can be achieved in the vegetative propagated crop plants using meristem/shoot tip cultures, auxiliary buds, nodal segments or using zygotic and somatic embryos. Slow growth *in vitro* can be accomplished either reduction in

sucrose or the macro and micronutrients, thus restricting the availability of nutrients to culture. Combining it with low temperature depending upon the species can also extend the subcultures cycle. Addition of osmotic agents such as sorbitol and mannitol also help in reduction of growth. Besides, the growth retardants like abscisic acid may also help in slowing the growth rate. Cryopreservation techniques involve preservation of biological material at very low temperatures of cryogenic gases, usually much below (-40°C). It is possible to obtain diverse temperatures down to -196°C using liquefied gases like carbon dioxide, air, oxygen and nitrogen. Liquid nitrogen at the temperature of -196°C is the most preferred choice for cryopreservation. Preservation protocols cannot be generalized, these have to be established before any biological material can be freezed. Therefore, standardization of technique is of prime importance. It is because, the desiccation and chilling sensitivity of a particular species varies [33]. Callus cultures of some species can be stored in slow growth at low temperatures or under mineral oil but there is evidence of poor performance in terms of growth rate and secondary product synthesis in certain cases [33]. Besides low temperature, slow growth rate can be induced by adding osmotic or hormonal inhibitors to the culture medium, or a combination of such inhibiting factors [25,32].

2.3.1 DNA banks and gene libraries

Advances in molecular biology have enabled isolation of intact plant DNA. DNAs from a wide variety of plants have been extracted using CTAB methods [34-36]. Extraction of total DNA and generation of gene libraries provide a mechanism for the preservation of a portion of the existing gene pool of important crop plants. Total DNA extracts can be stored suspended in alcohol for long periods at low temperatures [37]. Gene libraries, which are fragments of DNA housed in host cells using phage, plasmid or cosmid vectors can be stored in lyophilized form in host cells or desiccated as DNA fragments. Hence, establishment of DNA banks and gene libraries for crop plants provided method of choice to conserve a large number of species in a very small facility. However, they should not be used to replace field gene banks as whole plants cannot be regenerated from the total or fragments of DNA. DNA banks and gene libraries assume as an important source for development of diagnostic probes for photochemical screening and for gene transfer experiments. Establishment of DNA banks and gene libraries also facilitates exchange of genetic materials of crop plants for specific purposes between distant countries.

2.3.2 DNA fingerprinting

Powerful techniques of assessing variation at the level of the DNA are through restriction fragment length polymorphisms (RFLPs) and polymerase chain reaction (PCR) based techniques like randomly amplification of minisatellite-region DNA (DAMD) and amplified fragment length polymorphisms (AFLPs). Developed just over 10 years ago, these DNA fingerprinting techniques are potentially unlimited due to the availability or a large number of restriction enzyme and probe combination in the case of RFLP and limitless primers in the case of PCR-based techniques to yield DNA fragments of varying sizes. DNA is robust and able to tolerate desiccation and no stringent requirement for, refrigeration of plant tissues need to be used for such purposes. Exact genotypic characterization can be done when evaluating direct DNA-based variations. Since neither environmental nor epistatic effects influence the interpretation of such DNA based loci, these markers can be scored simultaneously with ease even in single plants. Genotypes at DNA based loci can be scored at any stage of development and any plant part. Genotypic evaluation requires only small amounts of tissue; thus, it is non-invasive to the plant being sampled. DNA fingerprinting is not only useful it is non-destructive to the plant being sampled. DNA fingerprinting is not only useful for genotype characterization or clone identification but also for estimating relatedness and polymorphisms [38]. RFLP and PCR based markers have immediate application in the verification of clonal identity and checking genetic similarities or dissimilarities of *ex situ* including *in vitro* collections of plants.

2.3.3 Estimation of relatedness

DNA markers have important immediate research applications for taxonomic studies of plant species. Taxon specific DNA markers can be developed for confirmation of plant identities in large random, analogue or ethno-botanical screening to yield the first-generation bioactive compounds. Molecular markers *viz*., RAPDs ISSR, SSR including chloroplast and mitochondrial based DNA

markers have been successfully used to clarify phylogenetic relationships [39]. Hence, these DNA markers can be employed rapidly clarify co-evolutionary relationships among diverse plant species. Prior knowledge of phylogenetic relationships among medicinal plants will not only lead to collection of plants with high chemical diversity for conservation but also provide related species with high yielding bioactive compounds [21].

2.3.4 Estimation of polymorphisms

As assessment of the level of genetic variation between and within populations is a prerequisite for conservation, domestication and improvement of wild species. Reasonably well known for established commercial species, patterns of variation are poorly understood for medicinal and aromatic plants of potential commercial value. The use of DNA markers as indicators of genetic diversity has immediate application to these species, in addition to the cheaper usage of isozymes which are still popular among the molecular markers for the estimation of genetic diversity [40-54]. RAPDs are currently most widely applied PCR-based techniques for estimation of genetic diversity of plant species mainly because of their rapidity and ability to handle large sample sizes. New markers such as AFLP, SSR and SNPs are being developed to study polymorphism of crop plant research for improving sampling and selection of genotypes, conservation and production of useful phytochemicals [38].

2.3.5 *In vitro* propagation and conservation strategies of some economically important plants suitable for cultivation in temperate regions and degraded lands

For large-scale cultivation the available methods of propagation are very slow and hence vegetative propagation *in vivo* is difficult. In recent years, tissue culture technique for mass propagation of many rare species, endangered/threatened to extinct have been developed so that their demand can be met. Besides, developing and utilizing *in vitro* techniques as an alternative approach for mass propagation, these will help to reduce their exploitation from its natural habitat. Some of the plants of ravines and temperate regions which needs priority are discussed in this chapter.

2.4 Medicinal & Aromatic Plants

Plant genetic resources are the most essential of biological resources for sustaining life on earth as they donate food, nutritional and health security for the ever-increasing population. Society faces marvelous challenges in the coming decades. Thus, their conservation along with sustainable utilization is of greatest important. Recent developments in plant genomics and biotechnological tools deliver good platform where biotechnology can play an imperative role in initiating climate change effects on crops changes and developing crops adapted to these changes. The changing scenario at global level impacting genetic resources management including the recent international treaties, convention, agreements on global climate change by biotechnological and other technological advances in agriculture.

2.5 Indian Belladonna (*Atropa acuminata*)

Indian Belladonna (*Aropa acuminata* Royle ex Lindl.) belonging to family Solanaceae is distributed over temperate parts of Western Himalaya extending from Kashmir to Shimla and adjoining areas of Himachal Pradesh This is a tall erect, annual herb up to 2 meter in height, woody below, branched above and tillering from the base. The plant is antispasmodic, narcotic, mydriatic, analgesic and antiasthamatic being a source of medicinally important tropane alkaloids - Atropine, Hyoscyamine and Scopolamine. The plants are heterogeneous owing to cross pollination and there is considerable variation in growth and alkaloid content. In India, the drug is still collected from natural sources by uprooting its roots. In view of heavy loss of renewable resources, the natural populations are becoming scarce and species is declared threatened. Realizing the threat of extinction there is a need to develop conservation strategies and quick propagation protocols [55].

Plant material of *A. acuminata* was collected from field grown plants in Field Gene Bank at IIIM (Br.) Srinagar. Plant material was treated with Tween-20 for 10 minutes and later washed thoroughly under tap water for 30 minutes. The shoot tips and nodal segments with auxiliary buds (about 2.5 cm long)

were excised from shoots and used as explants. These explants were treated with 0.1% Bavistin (BASF) for 10 minutes. The explants were surface sterilized with 0.1% (w/v) mercuric chloride for 4 minutes and five times washed with sterile double distilled water. Shoot tip explants (1.5 cm long) were then trimmed from the lower end prior to culture in test tubes (15 cm x 2.5 cm) containing MS [56] medium supplemented with 3% (w/v) sucrose and 0.7% (w/v) Agar. The medium was augmented with 1.0 mg l^{-1} NAA, IBA and 2, 4-D alone and in combination with BAP and Kn. For further development of the multiple shoots, the cultures were transferred to Revised Tobacco Medium amended with 1.0 mgl^{-1} IAA. Primary shoots formed *in vitro* were sectioned into one-node pieces after removing the leaves. The nodal segments containing the dormant auxiliary buds were cultured on MS medium supplemented with 1.0 mg l^{-1} each of IBA and BAP. For subsequent multiplication, callus at base was also subcultured on MS medium amended with 1.0 mg l^{-1} each of IBA and BAP to give multiple shoots. Subcultures were carried out at six-week interval. Rooting of elongated shoots (ca. 5-6 cms) was assessed by subculturing on half and full-strength Revised Tobacco (RT) and B_5 medium containing different levels of IBA. Full strength MS medium without hormonal supplements served as the control. The cultures were incubated at 24±2°C with 16 h (65μm $m^{-2}s^{-1}$) photoperiod provided by cool white-fluorescent tubes. The healthy plantlets with 4-5 nodes and well-developed roots were removed from the culture tubes, washed under running tap water to remove the agar and transferred to sterilized garden soil or soilrite in small polypots. The potted plants were maintained inside an intermittent mist chamber under high humid conditions (80-90% RH) for 3 weeks and irrigated with ¼ strength Knop's nutrient solution. Once acclimatized, the plants were transferred to the Green House (Fig.1 A, H) [57].

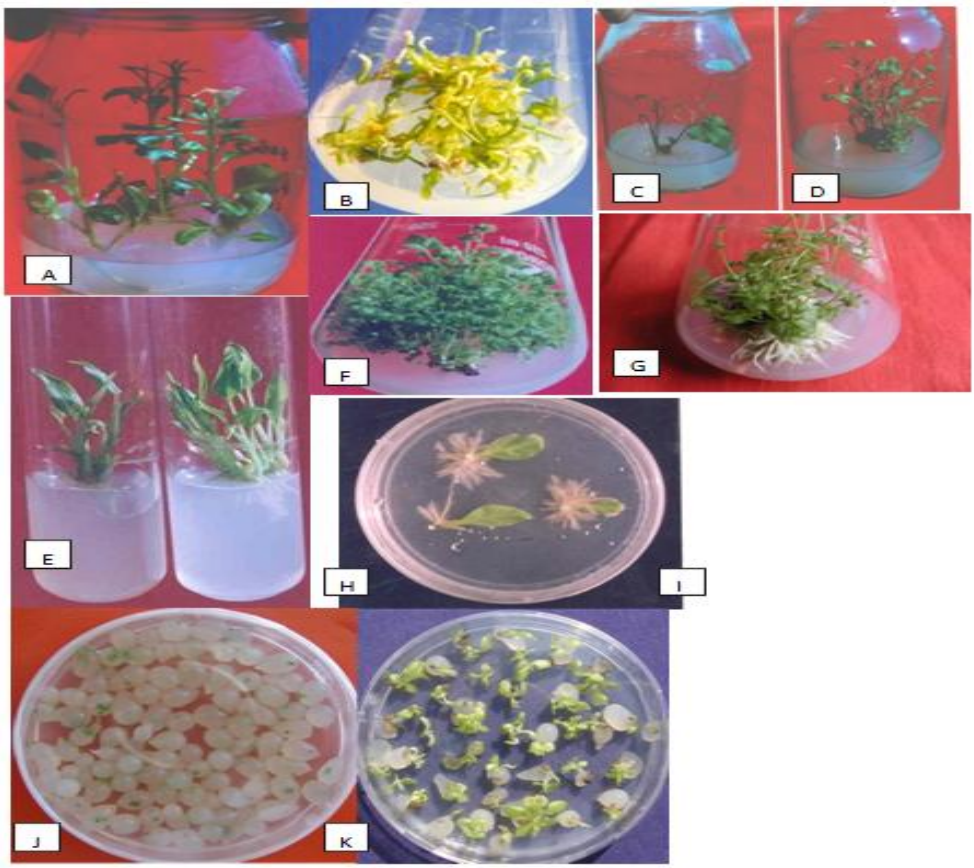


Fig. 1. *In vitro* propagation and conservation of some medicinal plants of Economic value of Himalayan region: (A) *Atropaa acuminata* (B) *Bunium persicum* (C) *Diosco readeltoidea* (D-E) Hedychium spicatum (F) *Potentill fulgens* (G) *Swertia chirata*, Excised root culture as conservation strategy in (H) *Atropaa cuminata* (I) *Picrorhiza kurroa* and (J-K) Encapsulation of shoot tips in *Swertia chirata*

Shoot tip explants showed better response than the nodal segments. Combination of BAP and IBA showed substantial increase in the frequency of responding shoot tip explants. The maximum shoot production (5-6 per culture) was obtained on MS medium supplemented with 1.0 mgl^{-1}BAP in combination with 1.0 mgl^{-1}IBA after 40 days. Shoot cultures were further multiplied by culturing of nodal segments excised from the primary *in vitro* formed shoots on MS medium containing a combination of 1.0 mgl^{-1} BAP and 1.0mgl^{-1}IBA. Callusing also appeared at the base of the shoots during repeated subculturing after every six weeks. Percent shoot development as well as number of shoots per node retained the highest values 90% (5 to 6 shoots per node) during the three culture passages. Shoots induced on MS medium with 1.0 mg l^{-1} BAP and 1.0 mg l^{-1}IBA failed to elongate rapidly. Therefore, young micro-shoots were excised and transferred to RT medium supplemented with IAA alone. Maximum shoot growth in terms of shoot length (ca. 20 cms) and number of nodes/shoots was obtained in RT medium supplemented with 1.0 mg l^{-1} IAA. The excised elongated shoots of 6-8 cm in length showed 80-100% rooting in the various treatments upon transfer to rooting media. The best rooting response in terms of length and number was obtained in full strength RT medium supplemented with 1.0 mg^{-l} IBA. On replacing IBA with NAA or IAA there was less or no rooting on half strength and full-strength MS, RT and B_5 medium. A success rate of 80% was achieved in transplanting of rooted regenerants with 4-5 nodes to small pots. The plantlets attained a height (30-35 cm) within 60 days of transplantation and grow well in the greenhouse at 18±2°C. The observations on various morphological characters such as branching pattern, leaf shape, petiole length and area of leaf lamina did not show any significant variation as compared to parent plant, indicating that multiplication was true-to-type. Shoot cultures of *A. acuminata* were cold stored at 10°C for 8 months with good results. Stored shoot cultures continue to grow slowly and developed etiolated shoots which quickly turned green and resumed normal growth when retrieved from storage. This system can be adopted for clonal propagation of this medicinally important plant species which otherwise figured as threatened (Ahuja et al., 2002). The plant is heterogeneous due to cross pollination and there is considerable variation in growth and alkaloid content. In this context, the presently developed micropropagation system holds promise to maintain clonal uniformity, which is highly desirable from pharmaceutical point of view.

2.6 Brahmi (*Bacopa monnieri* (L.) Wettest.)

Bacopa monnieri (L.) Wettst. Brahmi, an emergent macrophyte, fast proliferating wetland species has attracted considerable attention in recent years owing to its competence of absorbing toxic heavy metals from the aquatic environment. As such plant species having dual importance need to be conserved and propagated on priority basis.

A protocol was developed for regeneration from encapsulated nodal segments collected from *in vitro* propagated shoots of *Bacopa monnieri*. The best gel composition was achieved using 2.5% sodium alginate and 100 mM $CaCl_2.2H_2O$. The maximum percentage response (86.67) for conservation of encapsulated nodal segments into plantlets was achieved on MS medium supplemented with 1.0 mgl^{-1} BAP after 6-8 weeks. Well-developed regenerated plantlets were hardened, acclimatized and established under net house conditions, where they grew well without any detectable variation (Fig. 2 L-M). The regeneration ability of encapsulated nodal segments was affected by the concentration of sodium alginate and calcium chloride [58].

2.7 Malkangni (*Celastrus paniculatus*)

Celastrus paniculatus Willd (Malkangni) belonging to family Celastraceaae is an important medicinal plant indicated primarily in the treatment of mental disorders. The plant is collected from wild sources in the sub-Himalayan regions of India but the increasing demand of the plant is not fully met from the wild sources owing to scarce seed production, poor seed germination because of inherent dormancy, lack of suitable vegetative propagation methods for large scale multiplications and large-scale collections from natural stands has led the depletion of wild stock of this rare and important medicinal plant classified as threatened species. Rapid clonal multiplication and conservation have been achieved through embryo rescue and auxiliary bud proliferation from single node segments in MS medium. The cotyledonary leaves from cultured embryos callused on MS medium supplemented with $5x10^{-6}$ M NAA and $5x10^{-6}$ M Kinetin with a doubling time of 16.2 days. Well-developed multiple shoots

of 5.6 cm length could be obtained *via* organogenesis within 28 days on MS medium supplemented with $2x10^{-5}$ M BA. Bud break and subsequent shoot proliferation was dependent on cytokinin supply. Nodal explants responded most favourably at low BAP concentration and resulted in uniform (4.3 cm) auxiliary shoot proliferation facilitating uniform harvest of shoots for rooting. Repeated subculturing through 3 cycles with auxiliary bud and leaves of shoot cultures enabled consistent proliferation of shoots without any decline even after serial subculture for the period of three years. Shootlets achieved *via* organogenesis and auxiliary proliferation were best rooted in WPM medium amended with IBA resulting > 85% rooting. Seventy percent of the rooted plants were established in earthen pots after acclimatization in Green House conditions [59].

2.8 Yam (*Dioscorea deltoidea*)

The steroidal drugs are some of the costliest and most important medicines used throughout the world. *Dioscorea deltoidea* is the foremost species exploited in India for the steroid industries. It is being felt that the natural stock of this important raw material is being exhausted owing to over-exploitation from the forest regions of the north-west Himalayas. There are apprehensions that this important raw material may be completely lost to the industry if adequate steps to conserve and propagate the stock are not taken up immediately. Vegetative multiplication of *Dioscorea* using rhizomes is exasperatingly slow, as rhizomes of one living plant would give rise to only 8-10 plants. The progeny thus obtained takes at least 3-4 years before it is fit for further multiplication through rhizome cuttings. With the tissue culture method developed one have large number of plants of a selected variety within a short span of time. Callus was initiated by means of hypocotyl tissue of germinating seeds on MS revised tobacco medium containing 2,4-D. The callus on subculture on medium containing 0.5 mgl^{-1}IBA differentiated multiple shoots. Shoot differentiation improved on addition of BA [60]. In another experimentation shoot apices cultured on RT medium supplemented with BA and NAA produced complete plantlets with tubers at the base. Maximum number of shoots recorded were 50 shoots per culture. Light had profound effect on root or callus formation. In addition to light, season of the year in which explants were collected and cultured also affected the frequency of the complete plantlets. Using this procedure selected genotype can be multiplied 10 times over a period of three months. Out of the total plantlets recovered *in vitro* 50 percent survived [61].

2.9 Vajradanti (*Potentilla fulgens Wall*)

Potentilla fulgens Wall (Family: Rosaceae) commonly known as Vajradanti, is a commercially important medicinal plant native to Himalayan alpine region. The plant juice is taken to treat stomach problems, cough and cold. The leaves when masticated are supposed to be beneficial for pyorrhea. Root powder is effective against toothaches and is also used as an antihelmintic and utilized in dental preparations. Besides, antitumor activity and hypoglycemic effects of crude root extract have also been reported. Use of this potent species is limited by very low seed viability and a problem with its propagation as rooting is difficult. Therefore, there is an urgent need for optimizing alternative procedures for mass multiplication of this rare species.

The apical segments (3-5cm) collected from shoots of naturally growing plants in Palampur (32 10^0 N 76 30°E) India during the month of April, were washed under tap water for 30 minutes, tracked by soaking in 1% Teepol (v/v) for 5 minutes. After thorough washing with sterile distilled water the source tissues were surface sterilized with 0.1% (w/v) $HgCl_2$ for 3 minutes. Following repeated washes with sterile distilled water, the shoot apices were excised to 1 cm size and implanted onto culture medium. MS medium with 3% sucrose was used in all the experiments. The pH was adjusted to 5.8 prior to addition of agar (0.8% m/v) and autoclaving at 121°C and 15psi for 20 minutes. The cultures were incubated at 25±2° C under 16 h photoperiod irradiance of 40 micro mol m^{-2} s^{-1}. After profuse shoot proliferation started, the explants were sub cultured in wider culture vessels with more medium (50 ml medium) to meet the increasing requirement of nutrients. The shoots obtained *in vitro* were excised and subcultured at least 4 times at 30 d interval to develop stock cultures. About 2 cm long shoots were used as unit for shoot proliferation experiments. Magnitude of shoot proliferation and their growth was recorded after 4 weeks. Well-rooted plantlets were transferred to plastic pots containing sterile-sand. The plantlets were successfully acclimatized by keeping them in a mist chamber (25±2°C, 80% RH) for three weeks, irrigated with a solution containing half strength MS salts on

alternate days. The plants were transferred to earthen pots containing garden soil after 3 weeks. Following two months of hardening, the growth performance of plants was recorded. Finally, the plants were transferred to the field during the month of April at Srinagar [62].

Explants responded to various media combinations by swelling and development of auxiliary shoots within 6 weeks. The frequency response was greater in treatment with 1.0 mg l^{-1}BAP +1.0mg l^{-1}IAA. However, suppressed shoot growth was noticed in this combination. On MS+IBA along with BAP, rapid extension in growth and increase in number of shoots with small thin roots occurred. These multiple shoots when transferred to MS containing 1.0 mg l^{-1} Kin showed elongation of shoots (10-12 cm) with well-developed roots at the base. Interestingly, when nodal or shoot apices taken from *in vitro* proliferated shoots were cultured on the same media, 12-fold shoot multiplication rate was observed. The increase in shoot production in *in vitro* proliferated shoots might be due to the absence of lag period between explanation and adaptation to *in vitro* conditions. The shoot multiplication rate increased with the increasing number of subcultures till 6-7 subcultures. During subculturing care was taken to remove necrosed or discolored shoots. Root induction was observed within 15 days after transfer of microshoots to the rooting medium. Rooting occurred in all the treatments. The maximum frequency of root formation was achieved in combinations of MS+Kin alone or Kin+NAA. Hardening and acclimatization of all the *in vitro* regenerated plantlets was successful. No phenotypic variation was observed in *in vitro* raised plants. After two months of hardening, the growth performance of plants showed significant improvement although the plant height and other parameters varied significantly. The plants nevertheless grew well and the height increased to 17 cm. The survived (80%) plants were transferred to field in Srinagar (Fig. 1 F).

2.10 Liquorice (*Glychyrriza glabra* L.)

Liquorice (*Glycyrrhiza glabra* L.) is one of the important medicinal plants in Indian system of medicine. In India it is known as “Mulhati”. It is widely distributed in the world from 5°W to 100°E longitudes and 20° to 50°N latitude. The principle constitute of liquorice is sweet tasting triterpenoidal saponin, glycyrrhetic acid and two molecules of D-glucuronic acid. Glycyrrhetic acid has been shown to possess anti-inflammatory activity and used in treatment of gastric ulcer. Although, traditionally *Glycyrrhiza glabra* is propagated through seed and stolon cuttings, commercial cultivation is restricted owing to limited seed set and poor seed viability. Furthermore, the conventional method of propagation is rather very slow and only 40-50% of the cuttings get established in the field. To cater the growing demand for pharmaceutical based industries, it was found essential to multiply this species by adopting tissue culture techniques.

In this context, a reliable and reproducible protocol has been developed by Sharma et al. [63] for plant regeneration from 6-8 mm long leaf discs excised from 4 -5 months-old plants of *Glycyrrhiza glabra* on Gamborg’s medium [64]. Culture medium B_5D.5Kn (B_5 + 1.0 mg l^{-1} 2, 4-D + 0.5 mgl^{-1} kinetin) proved well for callus induction. However, nutrient medium B_5B.5N (B_5 + 1.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA) proved superior for morphogenic calli formation and plantlet regeneration. Higher *in vitro* rooting response (root proliferating efficiency, number of roots and root of higher length) was exhibited by culture medium B_5.5I (B_5 + 0.5 mgl^{-1} IBA). The *in vitro* raised plantlets were acclimatized and established successfully in the field.

In one of the other studies conducted by Sharma et al. [65] a protocol has been established to propagate through indirect and direct *in vitro* organogenesis from cultured nodal segment explants excised from 4-5 months-old plants. Induction medium $B_5$2D (B_5 + 2.0 mgl^{-1} 2, 4 D + 20.0 gl^{-1} sucrose+7.5 g l^{-1} agar) induced callusing in higher frequencies (65.93%). Culture medium B_5.5B.5N (B_5 + 0.5 mg l^{-1} BA + 0.5 mgl^{-1} NAA + 20.0 gl^{-1} sucrose + 7.5 gl^{-1}agar) was found to be more responsive for shoot proliferation (94.12%), shoots per explant (9.32) and mean shoot length (5.20 cm).

Subsequently Gupta et al. [66] have reported direct rhizogenesis from cultured leaf disc explants and establishment of an *in vitro* stolon culture system followed by plant regeneration. MS liquid medium supplemented with 0.01 mgl^{-1} NAA was proved most effective for stolon proliferation. Extensive proliferation of stolon and shoot regeneration was achieved on medium containing 3% sucrose and

0.01 mg l^{-1} NAA. Stolons with nodes showing growth was transferred under light for plantlet regeneration in the same medium. This was the first report in *G. glabra* describing a complete regeneration procedure via *in vitro* stolon proliferation along with quantitative data for glycyrrhizin and genetic fidelity of plant regenerated *in vitro*. *In vitro* stolon proliferation described here would be an efficient way for regeneration of plants for functional genomics studies and better understanding of glycyrrhizin metabolism (Fig. 2 N-O).

2.11 Sarpgandha (*Rauwolfia serpentina (L) Benth*)

Rauwolfia serpentina L. Benth. ex. Kurz (family: Apocynaceae) is a woody perennial shrub, commonly known with different names; sarpgandha, snake root plant, chotachand, chandrika, *etc*. and is an indigenous to India and other tropical countries of Asia. Only a few drugs have attracted such a worldwide attention as the roots of *Rauwolfia serpentina*. The roots contain 50 indole alkaloids including the therapeutically important reserpine, deserpidine, rescinnamine and yohimbine. According to Ayurveda, the roots and the whole plant are used for the treatment of cardiovascular disorder, snake bite, rheumatism, hypertension, insanity, epilepsy and hypochondria. The plant is vegetatively propagated by root cutting because of poor seed viability, low germination percentage, parthenocarpy and somatoplast sterility that may be largely ascribed to the presence of cinnamic acid and derivatives in the seed. Hence improvements in plant tissue culture techniques for the mass propagation of *R. serpentina* are highly desirable.

Nodal segments and leaf discs were cultured by Uikey et al. [67] on MS medium amended with diverse auxins and cytokinins in varying concentrations as sole as well as in various combinations. For nodal segment culture, basal MS media supplemented with 2,4-D/ NAA at the concentration of 2.0 mg l^{-1} as sole or in combination with 0. 5 mgl^{-1} TDZ were proved significantly superior for callus initiation. Higher shoot proliferating efficiency, number of shoots per explant and mean shoot length were documented on culture media fortified with TDZ in the range of 0.2-0.5 mgl^{-1} as alone as well as in combination with 0.5mgl^{-1}NAA (more than 92% nodal segments proliferated shoots, >10.5 shoots/explants and mean shoot length>3.85 cm). However, for cultured leaf disc, maximum callus and morphogenic callus formation as well as plantlet regeneration were documented on nutrient media fortified with 2.0 mgl^{-1}auxins: 2,4-D and/or NAA in combination with 0.5mg $l^{-1}$of a cytokinin BA and/or TDZ (more than 76% leaf discs induced morphogenic calli with >80% regeneration frequencies). Higher *in vitro* rooting response (root proliferating efficiency, number of roots and root of higher length) was exhibited by MS rooting medium amended with 0.1 mgl^{-1}IBA.

In another experiments Uikey et al. [7] established embryogenic cell suspension culture from the embryogenic callus culture of *Rauwolfia serpentina* (L.) by transferring 4-6 weeks-old asynchronous embryogenic calli raised from mature embryo and cotyledon explants cultures in liquid media. The cultures obtained were inundated mostly with clumps of proliferating globular embryos with modest non-embryogenic tissues. The number and size of somatic embryos/clumps was recorded to calculate growth of embryogenic tissues under various conditions. Initiation and proliferation of embryogenic suspension culture was influenced by various exogenous plant growth regulators fortified to the culture medium at variable magnitude. For the establishment of suspension cultures, MS medium fortified with 2.0 mgl^{-1} 2,4-D in combination with 0.5 mgl^{-1} BAP was found to be the most effective. For subsequent subculturing, the reduced level of 2,4-D (1.0 mgl^{-1}) in combination with 0.5 mgl^{-1} BAP promoted somatic embryogenesis at a faster rate. Frequent and efficient plantlet regeneration occurred on MS medium supplemented with 0.5 mgl^{-1}, each of BAP, TDZ and NAA. A combination of 65% relative humidity and 28°C temperature regime exhibited higher survival of regenerated plantlets (~95%) followed by 60% RH and 30°C (~90%). Later approximately 85% plants survived after transplantation in the field (Fig. 2 B).

2.12 Safed musli *(Chlorophytum borivilianum)*

Chlorophytum borivilianum commonly known as “Safed Musli” was declared endangered according to the Red Data Book of India in 1988. *C. borivilianum* naturally propagated through its fleshy roots and rarely by seed. Propagation through conventional method is generally time consuming, expensive and many a times unsuccessful which lead the *species* on the verge of extinction. In addition, plants

propagated through conventional method are very weak and sensitive to pathogenic attack. Micropropagation facilitates conservation and multiplication of medicinal plants in a big way to switch from occurrence in wild form to the arable lands. It allows genetically uniform and large-scale multiplication of disease and insect free plants in a very short span of time.

To search out the responsive culture medium combination with higher regeneration potential, stem disc of *Chlorophytum borivilianum* were cultured on MS basal medium fortified with 12 different combinations of BAP in varying concentrations, bacto or purified agar and certified or purified agar. Higher shoot formation and shoot length was exhibited by MS culture medium supplemented with 3.0mgl^{-1}BAP + 30g l^{-1} certified sucrose + 8.0 gl^{-1} bacto agar. Regenerated plants developed with normal phenotypic appearance after hardening in green house. Non-significant differences were observed for response of cultured stem discs on culture media supplemented with either purified or commercial grade sucrose as well as purified or commercial grade bacto agar. Present results revealed that *in vitro* mass propagation can be made cost effective substantially by supplementing commercial grade sucrose and bacto agar instead of certified sucrose and agar [68].

2.13 Ashwagandha *(Withania somnifera* (L.) Dunal)

Withania somnifera (L.) Dunal*]*, generally known as Indian ginseng belongs to the family Solanaceae, having vast medicinal and aromatic properties and has been included in ancient text of Ayurveda. It is used as an abortifacient, amoebocide, anodyne, bactericide, contraceptive, diuretic and spasmolytic. Biological assays label the plant as having the properties against different diseases like leprosy, nervous disorders, diseases of respiratory and reproductory tract, venereal disorders, rheumatism, inflammation, psoriasis, bronchitis, asthma, consumption, ulcers, scabies, marasmus of children, insomnia, senile debility, alexipharmic, carbuncles, cancer, epilepsy, diabetes *etc*. It can be propagated both by sexual and asexual method. Seed propagation, however is not always satisfactory, since the heterogenetically strain produces a great deal of variation. Again, multiplication through cuttings give rise to less ramified plants and is consequently less productive than plants obtained from seeds. Tissue culture techniques can play an imperative role in the propagation and qualitative improvement of this medicinally important plant.

For higher regeneration potential, mature cotyledon, embryo and hypocotyls explants of two genotypes of *Withania somnifera namely*: JA-20 and MWS-100 were cultured by Jhankare et al. [69] on MS basal media fortified with different concentrations and combinations of an array of auxins and cytokinins. Considering higher *in vitro* response, culture medium MS2D.5B (MS + 2.0 mgl^{-1} 2,4-D + 0.5 mgl^{-1} BA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) initiated calli in higher frequencies from cultured mature cotyledon and embryo explants, while for hypocotyl explant, induction medium MS2N.5Kn (MS + 2.0 mgl^{-1} NAA + 0.5 mgl^{-1} Kn + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) was proved superior for callus initiation. For formation of morphogenic calli and plantlet regeneration nutrient medium MS2B.5N (MS + 2.0 mgl^{-1} BA + 0.5 mgl^{-1} NAA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) was investigated strangely superior. Higher *in vitro* rooting response was achieved on rooting medium (MS+ 2.0 mgl^{-1} IBA + 15.0 gl^{-1} sucrose + 7.5 gl^{-1} agar). Higher *in vitro* morphogenic response was exhibited by explant mature embryo tracked by explants mature cotyledon and hypocotyl. In terms of *in vitro* genotypic response, genotype JA-20 was found significantly superior to MWS-100 for the most of the attributes investigated. Regenerated plantlets were established successfully in the field after hardening

In another experiment, nodal segment explants of two genotypes of *Withania somnifera viz*: JA-20 and MWS-100 were cultured on MS basal media fortified with different concentrations and combinations of diverse auxins and cytokinins as sole as well as in combinations. Considering higher *in vitro* response, culture medium MS2D (MS+2.0 mgl^{-1} 2,4-D) initiated calli in higher frequencies (80.36%). Induction medium MS3B (MS + 3.0 mgl^{-1} BA) initiated shoots in higher proportion (81.74%), while shoot(s) in higher numbers (25.69) and higher length (5.88 cm) were recovered from nutrient medium MS3B.5N (MS + 3.0 mgl^{-1} BA + 0.5 mgl^{-1} NAA [70].

The embryogenic cell suspension culture also was established by Jhankare et al. [5] from the embryogenic callus culture of *Withania somnifera* (L.) by transferring embryogenic callus cultures obtained from mature embryo and hypocotyl cultures in liquid media. The cultures obtained were

swamped with clumps of proliferating globular embryos with modest non-embryogenic tissues. The number and size of somatic embryos/clumps was measured to compute growth of embryogenic tissues under various conditions. The suspensions were subcultured every 15 days by adding 10 ml of the old suspension to 40 ml of fresh medium. Initiation and proliferation of such embryogenic suspension culture depended upon the genotypes and various exogenous plant growth regulators supplemented to the culture medium at variable magnitude. Culture medium MS2D (MS + 2. 0mg.l^{-1} 2, 4-D) was proved superior for callus induction from cultured mature cotyledons and hypocotyl explants. For the establishment of suspension cultures, the liquid MS medium fortified with 1.0 mgl^{-1} 2, 4-D in combination with 0.5mgl^{-1}BAP was found to be the most effective. For subsequent subculturing, the reduced level of 2, 4-D (0.5 mg. l^{-1}) in combination with 0.5mgl^{-1}BAP promoted faster development of somatic embryos. Frequent and efficient plantlet regeneration occurred on MS medium supplemented with 0. 5mg.l^{-1} each of NAA, BAP and kinetin. In terms of genotypic effect, genotype JA-20 responded well as compared to MWS-100. Regenerated plants were found to be phenotypically normal and true to the type (Fig. 2 F, H-K).

2.14 Chitrak *(Plumbago zeylanica)*

Plumbago zeylanica L., belongs to family Plumbaginaceae, is a rambling subscandent perennial herb or under shrub. Phytochemical screening of extracts of *Plumbago zeylanica* revealed the presence of several constituents, including plumbagin, linoleic acid, palmitic acid, nonylnonanoate, stigmasterol acetate, lupeol acetate, friedelinol, lupeol, lupanone, sitosterone and stigmasterol. Propagation of chitrak through seed is very difficult due to poor seed setting, erratic germination and seedling mortality as under natural field conditions. Commercial exploration of wild grown plants has led to the rapid decline of this plant in its natural environment necessitating alternate strategies for cultivation and propagation.

Patidar et al. [71] cultured nodal segment and leaf explants of *Plumbago zeylanica* on three basal media fortified with different concentrations and combinations of diverse auxins and cytokinins as sole as well as in combinations. For cultured nodal segment, culture medium MS2N.5B (MS+2.0mgl^{-1} NAA+ 0.5mgl^{-1}BA) proved well for higher degree of callus induction (91.69%), while nutrient medium MS2B (MS+2.0 mgl^{-1} BA) exhibited higher shoot proliferating efficiency (84.14%). Culture medium MS2B.5N (MS+2.0 mgl^{-1} BA+0.5 mgl^{-1}NAA) produced shoot(s) in higher numbers (11.12) as well as shoot of higher length (7.11 cm). Inoculation medium MS2N (MS+ 2.0mgl^{-1}NAA) initiated callus in higher proportion (91.12%), however enhanced morphogenic calli formation (45.58%) and plantlets regeneration were achieved on culture medium MSN.5Td (MS+1.0mgl^{-1}NAA + 0.5 mgl^{-1} TDZ) from cultured leaf disc. In present study, full strength MS medium supplemented with either of IBA or NAA at the concentration of 0.1 $mgl^{-1}$was found to be optimum for exhibiting higher *in vitro* rooting response *i.e*., root proliferation, number of roots and root length.

Subsequently, Patidar et al. [8] developed a procedure for establishment of embryogenic cell suspension culture from the friable embryogenic callus by transferring 6-8 weeks-old embryogenic calli achieved from nodal segment and leaf disc explants in liquid media. The cultures obtained were swamped with clumps of proliferating embryos of different developmental stages with modest non-embryogenic tissues. The number and size of somatic embryos/cell clumps was recorded to calculate growth rate of embryogenic tissues under different conditions. Initiation and proliferation of embryogenic cell suspension culture was manipulated by supplementation of diverse exogenous plant growth regulators to culture medium at variable concentration. For the establishment of cell suspension cultures, MS medium fortified with 2.0-3.0 mgl^{-1} 2,4-D with 0.5 mgl^{-1} BAP was found to be the most effective. For subsequent subculturing, the media containing with 2.0 mgl^{-1} 2,4-D or the reduced level of 2,4-D (1.0 mgl^{-1}) in combination with 0.5 mgl^{-1} BAP supported somatic embryogenesis at a faster rate. Frequent and efficient plantlet regeneration occurred on MS medium amended with 0.5 mgl^{-1}, each of BAP, TDZ and NAA. Full strength MS medium added with either of IBA or NAA at the concentration of 0.1 mgl^{-1} was found to be optimum for exhibiting higher *in vitro* rooting response *i.e*., root proliferation, number of root(s) and root of higher length. Regenerated plantlets showed normal development and established successfully in the field after hardening with normal phenotypic appearance.

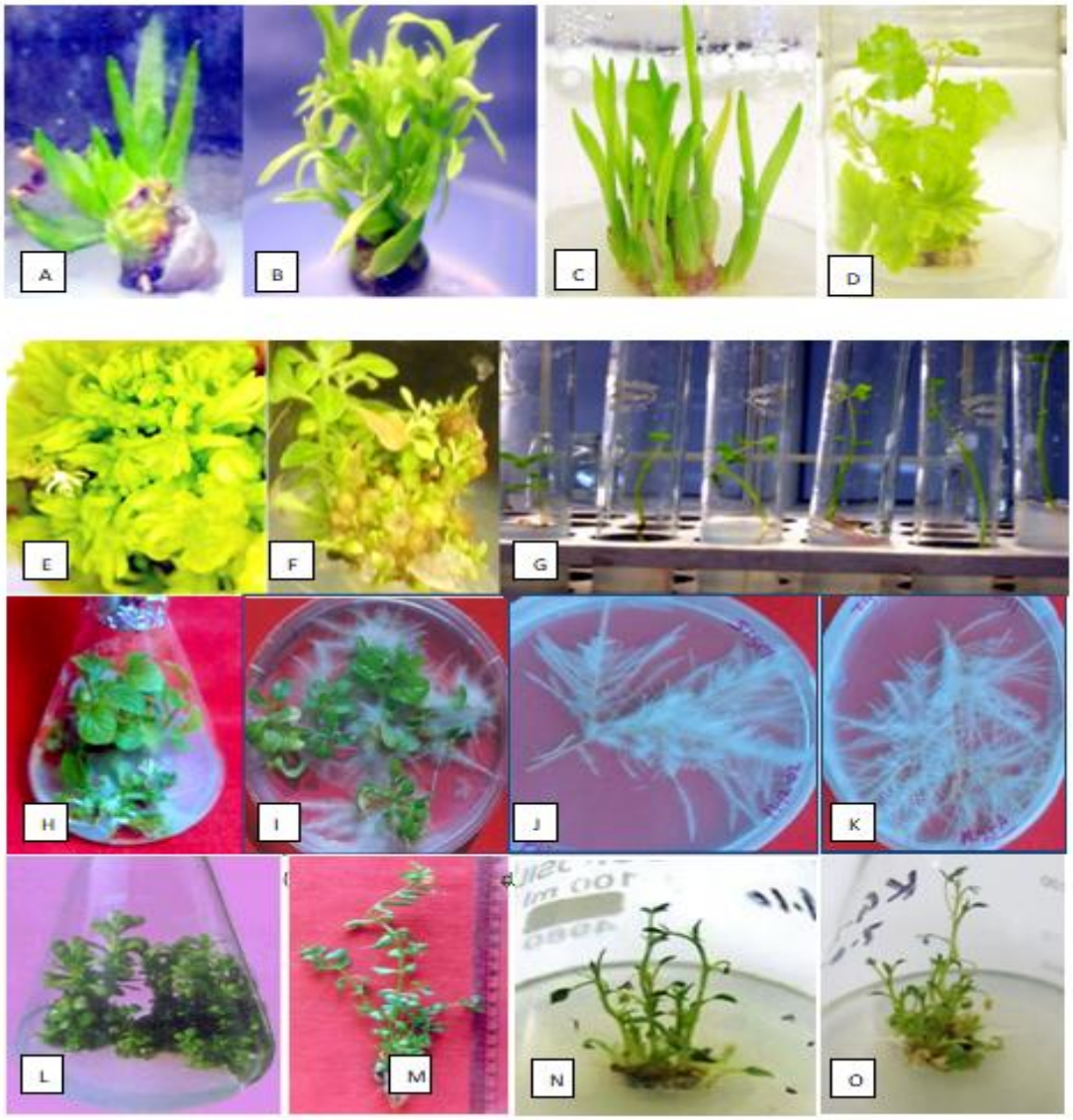


Fig. 2. *In vitro* propagation and conservation of some plants of economic value: (A) Aloe vera; (B) Sarpgandha; (C) Tuberose; (D) Grape; (E) Sandalwood; (F) Ashwagandha; (G) Anola; (H-K) Ashwagandha Root Cultures; (L-M) Brahmi and (N-I) Glycyrrhiza glabra

2.15 Aloe vera *(Aloe barbadensis)*

Aloe vera syn *barbadensis* belongs to the Liliaceae family and is an important medicinal plant. Aloe has been used for pharmaceutical, food, and cosmetic industries. Some of the most important pharmacological activities of *A. vera* are antiseptic anti- tumor anti-inflammatory wound and burn healing effect anti diabetic and as an adjunct to current AIDS therapy. It is xerophytes and can be grown even in dry lands under rain fed conditions. Key constituents present in leaves are Anthraquinones (aloin, *Aloe* - emodin), Resins, Tannins, Polysaccharides *etc*. These qualities have prompted industrial and commercial increase in the production of *Aloe vera* worldwide. The main constraint in this production chain is to obtain healthy primary culture for a sustainable supply of the plant materials. *Aloe vera* is mainly propagated by means of suckers or off shoots. There are two drawbacks in the traditional propagation of this plant; which are, slow propagation rate of auxiliary shoots and male sterility. Furthermore, propagation is too slow for commercial plant production. To encounter these problems, several groups have used *in vitro* conditions and have had varying success. Therefore, it is an urgent need to develop protocol for fast multiplication of plantlets through

micropropagation method to fulfill the increasing demand of planting material for cultivation. The technique of tissue and organ culture is used for rapid multiplication, genetic improvement, obtaining disease-free clones and preserving valuable germplasm.

Uikey et al. [72] cultured stem disc explant of *Aloe* on MS basal medium containing different auxins, cytokinins as sole as well as in combinations. Culture medium MS2D (MS+2.0 mgl^{-1} 2,4-D) initiated callus in higher proportion. Proliferation medium MS3B (MS+3.0 mgl^{-1} 2,4-D) demonstrated higher shoot proliferation efficiency (90.41%) as well as number of shoot (s) / responding explant (9.67), while nutrient medium MS3B.5N (MS+3.0 mgl^{-1} NAA+ 0.5 mgl^{-1}NAA) produced shoot of higher length (7.04 cm). Higher *in vitro* rooting response viz: root proliferating efficiency (92.88%), number of root (s) (9.75) and mean root length (9.10 cm) was shown by rooting medium MS2IB (MS+ 2.0 mgl^{-1} IBA). Regenerated plantlets were established successfully in the field after hardening. Phenotypically normal plants were regenerated (Fig. 2 A).

2.16 Fruit Crops

2.16.1 Aonla *(Emblica officinalis* syn. *Phyllanthus emblica*)

Aonla *(Emblica officinalis* syn. *Phyllanthus emblica*) is an important fruit crop of commercial significance due to its high medicinal and nutritional value. Aonla is the richest source of vitamin 'C' among all fruits after Barbados Cherry. The non-availability of desired number of shoots for grafting or budding is bottleneck problem for multiplication, which is labour expensive and economically not viable due to slow rate of multiplication and field survivability. The use of biotechnological approaches especially micropropagation offers a unique alternative for massive multiplication of true to type plantlets.

Mature cotyledons, cotyledonary node and hypocotyls of NA-7 cultivars of *Emblica officinalis* were cultured on MS basal medium with 20 different combinations of auxins and cytokinins in varying concentrations and combinations. Considering higher *in vitro* response, culture medium MS5DB (MS + 5.0 mgl^{-1} 2,4-D + 0.5 mgl^{-1} BA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) initiated calli in higher frequency (52.44%), morphogenic calli (34.75%) was higher on medium MS4BN (MS + 4.0 mgl^{-1} BA + 0.5 mgl^{-1} NAA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) and higher plantlet regeneration frequency (38.62%) was recorded on culture medium MS4B.5N.5G (MS + 4.0 mgl^{-1} BA + 0.5 mgl^{-1} NAA + 0.5 mgl^{-1} GA_3 + 20.0gl^{-1} sucrose + 7.5 gl^{-1} agar) among twenty-four different tested culture media combinations. Higher *in vitro* rooting response was achieved on MS basal medium supplemented with 2.0 mgl^{-1}IBA and 0.5 mgl^{-1}BA (17.20%). In respect to different explants, higher *in vitro* morphogenic response (callus initiation and morphogenic calli formation) was exhibited by explant cotyledonary nodes followed by mature cotyledons and hypocotyls [73]. Subsequently Patidar et al. [74] achieved the best results on basal MS medium fortified with 4.0 mgl^{-1}BAP in combination with 0.5 mgl^{-1}NAA for shoot proliferation efficiency (47.65%), number of shoot(s) per explant (3.20) and average shoot length (1.43) from cultured nodal segments (Fig. 2 G).

2.16.2 Grape (*Vitis vinifera* L.)

Grape (*Vitis vinifera* L.) is one of the most important fruit crops of the world. Genetic improvement of grapevine is exacerbated by polyploidy, high level of heterozygosity and inbreeding depression; it is also a time-consuming process due to 2-3 years generation cycle. Plant biotechnology could be an attractive means for improving grape however, this requires, as a first step, an efficient and reproducible regeneration system from somatic tissues of mature plants that permits both transformation and regeneration into plantlets. *In vitro* techniques could be employed for vine breeding to overcome the difficulties in traditional breeding studies and obtaining new cultivars.

A protocol has been standardized to regenerate three cultivars *namely*: Thompson seedless, Karnet and Local cultivars of *Vitis vinifera* (L.) through indirect and direct *in vitro* organogenesis from nodal segment explants excised from *in vitro* grown regenerants as well as one-year-old plants by Kurmi et al. [75] Induction medium MS2D (MS + 2.0 mgl^{-1} 2, 4 -D + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) induced callusing in higher frequencies (84.22%). Culture medium MS3B (MS+3.0 mgl^{-1} BA + 30.0 gl^{-1} sucrose

+ 7.5 gl^{-1} agar) was found to be more responsive for proliferation of shoots in higher frequencies (80.67%). Shoot (s) per explant in higher numbers (14.08) was attained with culture medium MS.2Td (MS+ 0.2 mgl^{-1} TDZ + 30.0 gl^{-1}sucrose + 7.5 gl^{-1} agar), while nutrient medium MS.2Td.5N (MS+ 0.2 mgl^{-1} TDZ + 0.5 mgl^{-1}NAA+ 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) produced shoot of higher length (8.78cm). Higher *in vitro* rooting response *i.e*., root proliferation ability (89.94%), number (s) of roots (5.78) and root length (7.75 cm) was exhibited by MS rooting medium fortified with 1.0 mgl^{-1} IBA, 10.0 gl^{-1} sucrose and 7.5 gl^{-1} agar. Genotype Thompson Seedless responded better than Karnet and Local for the most of the attributes investigated (Fig. 2 D).

Subsequently, an *in vitro* regeneration protocol has also been developed from 5-8 mm long leaf discs excised from *in vitro* grown regenerants as well as one-year-old plants of three different cultivars of *Vitis vinifera* L *viz*., Thompson seedless, Karnet and Local to find out the best *in vitro* responsive cultivar, culture media combination (especially appropriate auxin/cytokinin ratio) with their interactive effects on frequency of callus induction, formation of morphogenic calli and plantlet regeneration. MS medium fortified with 2.0-3.0 mgl^{-1} 2, 4-D and 0.5 mgl^{-1} Kinetin or BA proved more responsive for callus induction, while morphogenic calli as well as plantlets in higher frequencies were investigated with culture medium amended with 2.0 mgl^{-1} BA in conjunction with 0.5 mgl^{-1} NAA. In term of genotypic response, genotype Thompson Seedless responded better than Karnet and Local. Higher *in vitro* rooting response *i.e*., root proliferation ability, number (s) of roots and root length was exhibited by MS rooting medium fortified with 2.0 mgl^{-1} IBA and 15.0 gl^{-1} sucrose [76].

An efficient plantlet regeneration also achieved by Sharma et al. [9] from cell clumps/embryoids obtained from embryogenic cell suspension culture. Embryogenic cell suspension cultures were initiated by transferring 6-8 weeks-old embryogenic calli achieved from nodal segment and leaf disc explants in liquid media. The asynchronous embryogenic cultures were swamped with clumps of proliferating embryos of various developmental stages along with modest non-embryogenic tissues. The frequency of embryo proliferation was recorded to determine growth rate of embryogenic tissues under diverse conditions. Modulation of initiation and proliferation of embryogenic cell suspension culture was under regulation of the influence of exogenous plant growth regulators added in culture medium at different concentrations and combinations. Higher relative growth of embryogenic cell suspension cultures was recorded with combination of culture medium MS+2.0 mgl^{-1} 2,4-D+0.5mgl^{-1} Kn+30.0gl^{-1} sucrose, whereas, cell clumps/embryoids in higher numbers were attained on culture medium (MS+2.0 mgl^{-1} 2,4-D+0.5 mgl^{-1} BA+30.0 gl^{-1} sucrose). For subsequent subculturing, reduced level of 2,4-D (1.0 mgl^{-1}) in combination with 0.5 mgl^{-1} BAP accelerated somatic embryogenesis. High frequency plantlet regeneration was recorded on regeneration medium supplemented with 0.5 mgl^{-1}, each of BAP, Kn and NAA. In terms of root proliferating ability and root of higher length, rooting medium MS.5IB.5Kn (MS+0.5 mgl^{-1} IBA+0.5mgl^{-1} Kn+15.0gl^{-1} sucrose+7.5gl^{-1} agar) was found superior among the tested media combinations. However, root formation in higher frequency was attained with media supplemented with 2.0mgl^{-1} IBA. Among two genotypes. Thompson seedless was found consistently superior to Karnet for all attributes investigated. Plantlets regenerated showed normal development and were established successfully in the field after hardening.

2.16.3 Citrus species

Citrus is a common woody subtropical fruit belonging to the family Rutaceae. *Citrus* fruits are not only appetizing and invigorating but also, they provide vitamins, minerals and many other essences. Fruits of this family are very admired all over the world due to the taste, aromatic flavor and medicinal properties. Citrus fruits are also used for patients susceptible to health problems such as gastritis, fever and arterial sclerosis. The juice of this species is used in the pharmaceutical industry since it contains a high magnitude of citric acid. There are also reports about positive effects of citrus fruits against cancer of gastrointestinal and upper respiratory tracts. *Citrus* is being propagated through seed and “shield” / “T” budding, but from seed it does not ensure true to type plants because of cross-pollinating nature. Besides, such plants take more time to bear fruits. The less-availability of authentic and disease-free planting material and shoots for grafting and budding makes field multiplication difficult to the most of citrus species. Given the immense importance of the *Rutaceae* family in various branches of industry, the micropropagation of *Citrus* has always stimulated great concern among scientists.

Hypocotyls of three species of citrus were cultured on different fortifications of MS media to assess their *in vitro* response. Higher *in vitro* morphogenesis (somatic embryogenesis and/or embryogenesis) leading to plantlet regeneration was varied considerably due to species and inoculation medium. Induction medium MS4D.5B (MS+ 4.0 mgl^{-1} 2, 4-D + 0. 5 mg l^{-1} BAP) proved well for callus initiation. Nutrient medium MS4B.5N (MS + 4.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA) performed commandingly for formation of morphogenic calli and plantlet regeneration. Higher root proliferating efficiency was recorded on rooting medium MS.5I (MS + 0.5 mgl^{-1} IBA). Roots in higher numbers were attained from culture medium MS2I.5Kn (MS + 2. 0 mg l^{-1} IBA + 0. 5 mg l^{-1} Kn), while culture media MS.5I.5B (MS + 0.5 mg l^{-1} IBA + 0. 5 mgl^{-1} BAP) supported for enhancing mean root length [77].

Consequently, Vibhute et al. [78] cultured mature cotyledons of three species of citrus on different fortifications of MS media to assess their *in vitro* response. Induction medium MS5D.5B (MS+ 5.0 mgl^{-1} 2, 4-D + 0. 5 mg l^{-1} BAP) proved well for callus initiation (84.18%). Nutrient medium MS4B.5N (MS + 4.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA) performed convincingly for formation of morphogenic calli (31.89%) as well as regeneration of plantlets (79.09%). Higher root proliferating efficiency (77.64%) was recorded on rooting medium MS.5I (MS + 0.5 mgl^{-1} IBA). Roots in higher numbers (3.53) were documented on culture medium MS2I.5Kn (MS + 2. 0 mg l^{-1} IBA + 0. 5 mgl^{-1} Kn), while culture media MS.5I.5B (MS + 0.5 mg l^{-1} IBA + 0. 5 mg l^{-1} BAP) enhanced mean root length (3.55 cm).

In recent study, Vibhute et al. [79] cultured nodal segments excised from *in vitro* germinated seedlings of three citrus species *viz*., *Citrus aurantiafolia, Citrus reticulata* and *Citrus sinensis* on different fortifications of basal MS medium for multiple shoot proliferation*. In vitro* morphogenesis leading to plantlet regeneration varied considerably among species and with medium composition. Plantlets were regenerated directly *via* auxiliary bud proliferation as well as from callus culture. Induction medium MS5N.5B/MS5N.Kn (MS + 5.0 mgl^{-1} NAA + 0.5 mgl-^{1}BA/Kn) induced callusing in higher frequencies. Culture medium MS.1Td.5N/MS.5B.5N (MS + 0.1 mgl^{-1} TDZ/0.5 BA + 0.5 mgl^{-1} NAA) enhanced shoot proliferating efficiency. Shoot (s) per explant in higher number (s) of higher length were documented on medium MS.1Td or MS.2Td (MS + 0.1/0.2 mg l^{-1} TDZ). Higher root proliferating efficiency was recorded on rooting medium MS.5IB (MS + 0.5 mgl^{-1} IBA). Roots in higher frequency were observed on culture medium MS2IB.5Kn (MS + 2.0 mgl^{-1} IBA + 0.5 mgl^{-1} Kn), while culture media MS.5IB.5B (MS + 0.5 mgl^{-1} IBA + 0. 5 mgl^{-1} BAP) enhanced mean root length. In terms of interspecific *in vitro* response, in general, Acid lime followed by Mandarin and Sweet orange responded decisively for the most of the culture phases. The *in vitro* raised plantlets were successfully acclimatized and transferred to the field.

2.17 Flowering and Ornamental Plants

2.17.1 Tuberose (*Polianthes tuberosa* Linn.)

Tuberose (*Polianthes tuberosa* Linn.) originated in Mexico belongs to family Amaryllidaceae is one of the important bulbous ornamental crops in India and being grown in tropical and subtropical areas. Commercially it is being grown in West Bengal, Karnataka, Tamil Nādu, Maharashtra and Madhya Pradesh. Among the commercially grown flowers in India, tuberose occupies a leading position owing its popularity as a cut flower, loose flower, to flower loving people because of its prettiness, elegance and sweet pleasant fragrance, for perfumery as well as its potential as source of secondary metabolites. Tuberose can be propagated by seed as well as vegetatively by bulbs and bulblets. However, the requirement of planting material is more in this method because only one plant can be obtained from a bulb and this method is rather slow. Moreover, only single cultivars set seed and seeds are difficult to germinate. To meet the growing demand, massive *in vitro* propagation through tissue culture is the only option.

Gajbhiye et al. [80] developed a protocol to propagate four cultivars of tuberose *viz*: Phule Rajni, Shringar, Prajwal, and Mexican Single through direct *in vitro* organogenesis from stem disc explants on White's medium [81]. Culture medium Wh.3Td.5N (Wh+ 0.3 mg l^{-1} TDZ + 0.5 mgl^{-1} NAA + 20.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) was found to be more responsive for shoot proliferating ability (98.90%) and shoot (s) per explant (11.48). Induction medium WH3N.5Td (Wh+ 3.0 mgl^{-1} NAA + 0.5 mgl^{-1} TDZ + 20.0 gl^{-1} sucrose + 7.5 g l^{-1} agar) was proved well for enhancing mean shoot length (5.56 cm). In terms of *in vitro* rooting response, culture medium Wh2I (WH+ 2.0 mgl^{-1} IBA + 10.0 gl^{-1} sucrose + 7.5

gl^{-1} agar) was found consistently superior for all culture phases, *i.e*., root proliferating efficiency (96.24%), number of root (s) per responding shootlet (14.20) and average root length (7.56 cm). Among the four cultivars Mexican Single was found significantly superior followed by Prajwal, Phule Rajni and Shringar for the most of the attributes. Regenerated plantlets were established successfully in the field after hardening (Fig. 2 C).

In order to regiment low-cost effective mass *in vitro* propagation protocol, it was evident in experiment of Raghuvanshi et al. [82] that there is no influential difference in response of cultured stem discs on different culture media, supplemented either with purified or commercial grade sucrose as well as with purified or commercial grade bacto agar. Thus, the cost of *in vitro* mass propagation can be reduced significantly by supplementing commercial grade sucrose and bacto agar instead of purified sucrose and agar respectively in culture medium. To search out the responsive culture medium combination with higher regeneration potential, stem disc of *Polianthes tuberosa* was cultured on MS basal medium fortified with different auxins and cytokinins in varying concentrations and combinations. Culture medium Wh4D (Wh + 4.0 mgl^{-1} 2,4-D) proved well for callus initiation. Inoculation medium WhTd.5N (WH + 1.0 mgl^{-1}TDZ+ 0.5 mgl^{-1}NAA) exhibited higher shoot proliferating efficiency, while number of shoot (s) per explant in higher umber (s) was attained on culture medium Wh2Td.5N (Wh + 2.0 mgl^{-1}TDZ + 0.5 mgl^{-1}NAA). Nutrient medium WH.5B.5N (Wh + 0.5 mgl^{-1} BA+ 0.5 mgl^{-1} NAA) produced shootlet of higher length. Higher *in vitro* rooting response (root proliferating efficiency, number of root (s) and root of higher length) was displayed by rooting medium WhI (Wh + 1.0 mgl^{-1}IBA).

2.17.2 Amaryllis (*Amaryllis belladonna* L.)

Amaryllis belladonna L. is a perennial bulbous plant that belong to the family Amaryllidaceae. Worldwide the plants have greatest economic value as ornamentals. Plants of amaryllis are suitable for planting in the bed, pot, rockery, shrubbery and greenhouse. In addition, huge number of plants are traded for conventional medicines. Africans use bulbs and leaves as poultices and decoctions for treating sores and digestive disorders. A number of species of the genus *Amaryllis* have been used in folk medicine, including *Amaryllis belladonna* L. (also named *Hippeastrum equestre*). Several pharmacological activities are attributed to several typical alkaloids they synthesize. It's *in vivo* propagation can be accomplished by using seeds, offsets and bulblets. However, multiplication of plant from seed showed wide variation in flower color, plant shape and time of flowering. Under such circumstances, the use of biotechnological advances particularly micropropagation proffers an exclusive substitute for mass multiplication of true type plantlets.

An efficient method for rapid propagation of *Amaryllis belladonna* L. has been developed by Sarathe et al. [83]) by culturing bulb scales and leaf discs explants on MS medium amended with diverse auxins and cytokinins in varying concentrations as sole as well as in different combinations. For bulb scale culture, culture media MS3N.5B (MS+ 3.0 mgl^{-1}NAA + 0.5 mgl^{-1} BA) proved well for callus initiation. Culture media MS3B (MS+ 3.0mgl^{-1} BA) exhibited higher shoot proliferating efficiency, while number of shoot (s) per explant and shoot of higher length were documented on nutrient medium MSB.5N (MS+ 1.0 mgl^{-1}BA + 0.5 mgl^{-1} NAA). For cultured leaf disc, inoculation medium MS2D.5B (MS+ 2.0mgl^{-1}2,4-D + 0.5 mgl^{-1} BA) initiated higher degree of callus induction. Elevated morphogenic calli formation was displayed by culture medium MS2N.5B (MS+ 2. 0mgl^{-1}NAA + 0.5 mgl^{-1} BA) and plantlets in higher number (s) were regenerated on regeneration medium MS.5Td (MS+ 0.5 mgl^{-1}TDZ). Higher *in vitro* rooting responses *i.e*., root proliferating efficiency, number of root(s) and root of higher length was recorded on culture medium MS2IB (MS+ 2. 0mgl^{-1}IBA).

Subsequently Veeraballi et al. [84] cultured three explants *viz*., bulb scale, leaf disc and mature embryos on different modifications of MS medium to achieve the best *in vitro* response. For bulb scale culture, nutrient medium MS2N.5B proved well for callus initiation. Culture medium MS.4Td exhibited higher shoot proliferating efficiency, while number of shoot (s) per explants and shoot of higher length were documented on media MS2N.5B/ MS3N.5B. For cultured leaf disc, inoculation medium MS2D.5B/MS3D.5B promoted higher degree of callus induction. Higher morphogenic calli formation was exhibited by culture medium MS2N.5B/MSN.5B. However, plantlets in higher number were recovered from regeneration medium MS.5Td/MSB. For mature embryo culture, culture medium

MS2D.5B proved well for callus initiation. Inoculation medium MSN.5B exhibited higher number of shoot proliferating explants. Number of shoots per explant in higher frequencies were obtained on medium MSN.5B/MSB. However, shoot of higher length was recorded on medium MSB. Higher *in vitro* rooting response (root proliferating efficiency, number of roots and mean root length) was exhibited by culture medium MS2IB.

2.17.3 Gerbera (*Gerbera jamesonii*)

The Barberton/Transvaal daisy or gerbera which belongs to the family Asteraceae is one of the leading cut flowers and ranks among the top ten cut flowers in the world. The species, a perennial herb and native to South Africa and Asia is grown all over world in a wide range of climatic conditions. The flowers are hardy and stand the rigours of transportation for long. The genus *Gerbera* was named in honour of a German naturalist Traugott Gerber, who travelled Russia in 1743. The genus consists of about forty species. Out of the recorded species, only one species *Gerbera jamesonii* is under cultivation. The flower makes an excellent choice for any gift basket intended to brighten someone's day, or celebrate joyous occasions. The gerbera daisy has long been a symbol of beauty, purity and innocence. Gerbera can be propagated by both sexual and asexual methods. Most of the commercially grown cultivars are propagated through vegetative means to maintain uniformity and genetic purity. Among the vegetative means, multiplication through division of clumps is the most common method used for several decades. Gerbera can also be propagated through cuttings. The plant multiplication by these methods is too slow to be commercially practicable. For commercialization of this crop, planting material is required on large scale which requires the development of an easier, quicker and economically viable method of propagation. The use of biotechnological approaches especially micropropagation offers a unique alternative for mass multiplication of true to type plantlets.

Somatic tissues and seeds were tried for raising reproducible cultures in *Gerbera jamesonii* by Patidar et al. [85]. Mature embryos and leaf sections were used as explants sources and cultured on MS medium supplemented with various auxins and cytokinins in varying concentrations as sole as well as in different combinations. Callusing was observed in both explants however shoot regenerations was obtained only from mature embryo explant. For mature embryo culture, culture media MS3D.5B (MS+3.0 mgl^{-1}2,4-D+0.5 mgl^{-1} BA) initiated calli in higher frequencies, while nutrient media MS2N.5B (MS+2.0 mgl^{-1}NAA+0.5 mgl^{-1} BA) promoted higher number of shoot proliferating efficiency, number of shoot (s) per responding explant and shoot of higher length. For cultured leaf disc, MS basal medium amended with 2.0-4.0 mgl^{-1} auxins (2,4-D and/or NAA) promoted higher degree of callus induction, however, regeneration was not attained. Higher *in vitro* rooting response (root proliferating efficiency, number of root (s) and mean root length) was exhibited by rooting medium MS.1IB (MS+0.1mgl^{-1} IBA).

The highest percentage of aseptic culture (83.54%) of explants were recorded with 0.5% Bavistin in combination with 0.2% $HgCl_2$ when diverse explants were exposed for 30 minutes. followed by application of 0.5% Bavistin in combination with 0.1% $HgCl_2$ (78.76%), while higher survival percentage (74.32%) of explants were evidenced with 0.5% Bavistin in combination with 0.1% $HgCl_2$ with exposure time 30 minutes. Combination of 0.5% Bavistin with 0.1% $HgCl_2$ exposed for the 30 minutes supported higher percentage of aseptic culture (78.76%) as well as survival of explants (74.13%) so that, this concentration and combination was used for surface sterilization of explants of gerbera by Bhatt et al. (2017b)). The maximum callus was recorded on culture medium MS3D (35.09%), MS3N (33.83%) and MS2D (33.02%) with at par performance. Remaining culture media had low callus forming ability including MS5T (18.87%) that has significantly minimum counts. Maximum callus induction was recorded from cultured mature embryo on nutrients media MS3ip (35.06%) closely followed by a group of four nutrients media *viz.,* MS2ip (29.95%), MSip (29.75%), MS.5ip (29.93%) and MS.2ip (29.12%). different culture media combination, inoculation media MS2N.5ip (55.22%), MS3N.5ip (53.17%) and MSN5ip (45.01%) were proved remarkably superior for shoots proliferating efficiency. Nutrient's media MS5D.5Kn (3.05%) and MS4D.5KN (4.81%) was found low responding in this regard. Nutrient media MS2N (2.14cm), MSN (2.03cm) and MS2D (1.95) had produced shoot of higher length [86].

Afterward, Bhatt et al. [87] cultured mature embryos on MS medium amended with diverse auxins and cytokinins in varying concentrations as sole as well as in different combinations. Culture media MS3D.5B (MS + 3.0 mgl^{-1} 2, 4 D + 0.5 mgl^{-1}BA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar powder) proved well for callus initiation. Culture media MS2N.5iP/MS3N.5ip (MS + 2.0/3.0mg l^{-1} NAA + 0.5 mgl^{-1}2-ip + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) exhibited higher *in vitro* response *i.e.,* number of shoot proliferating explants and number of shoot (s) per explant. However, shoot of higher length was recorded on nutrient medium MSB/MS2B (MS + 2.0/3.0 mgl^{-1}BA + 30.0 gl^{-1} sucrose + 7.5 g l^{-1} agar). Higher *in vitro* rooting response (root proliferating efficiency, number of roots and mean root length) was exhibited by rooting medium MS.1IB (MS + 0.1 mgl^{-1}IBA + 15.0gl^{-1} sucrose + 7.5 g l^{-1} agar).

In another investigation of Bhatt et al. [88] were cultured leaf disc on MS medium amended with different auxins and cytokinins in varying concentrations as sole as well as in diverse combinations. Induction medium containing 2.0-3.0 mgl^{-1} auxins (2,4-D or NAA) promoted higher degree of callus induction. Nutrient medium supplemented with 2.0-3.0 mgl^{-1} NAA proved well for formation of morphogenic calli and regeneration medium MSB proved remarkably superior for plantlet regeneration in higher frequency. Higher *in vitro* rooting response was exhibited by rooting medium MS.1IB (MS + 0.1 mgl^{-1}IBA + 15.0gl^{-1} sucrose + 7.5 g l^{-1} agar).

2.17.4 Gladiolus (*Gladiolus hybridus* Hort.)

Gladiolus (*Gladiolus hybridus* Hort.) belongs to the family Iridaceae is one of the most important bulbous commercial ornamental plants grown for cut flowers. Due to its magnificent inflorescence with a variety of colors makes it attractive for use in herbaceous borders, beddings, pots and for cut flowers. It has a great economic value and wide market in the country. It propagates either by seed, corm formation or by cormel differentiation. Although, seed is an effective means of gladiolus propagation but seed-raised plants may not produce true type population. Additionally, it takes four seasons for blooming. The propagation by corm and cormel formation is another conventional method of multiplication but it may transmit several viral, fungal and bacterial diseases i.e. *Fusarium* corm rot, *Botrytis* blight, bacterial leaf spot *etc*., thus causing a heavy loss. These low propagation rates hinder the introduction of new varieties or virus-free plants. However, in gladiolus there is a clear scope for further refinement in *in vitro* culture methodology to acquire a higher number of shoots to complement traditional nursery methods. Plant tissue culture offers a potential to deliver large quantities of disease-free, true type healthy stock within a short span of time. The method guaranteed the identical reproduction of the parents tested and selected, and prevented genotypic alteration which would occur after the generative multiplication.

Corm slice explant of gladiolus (*Gladiolus hybridus*) was cultured by Tripathi et al. [89] on MS medium amended with diverse auxins and cytokinins in varying concentrations as sole as well as in different combinations. Regarding *in vitro* response, induction medium MS2B.5D (MS + 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} 2,4-D + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) proved well for callus initiation. MS medium supplemented with BA in range of 2.0-3.0 mgl^{-1} in combination with 0.5 mgl^{-1} NAA, 30.0 gl^{-1} sucrose and 7.5 gl^{-1} agar exhibited higher shoot proliferating efficiency, number of shoot (s) per explant and shoot of higher length. In respect to *in vitro* rooting response, higher root proliferating efficiency was documented on rooting medium MS.5IB.5Kn (MS + 0.5 mgl^{-1} IBA + 0.5 mgl^{-1} Kinetin + 15.0 gl^{-1} sucrose + 7.5 gl^{-1}agar), while number of root (s) with higher length were recovered on rooting medium MS.5IB (MS+0.5 mgl^{-1} IBA+15.0 gl^{-1} sucrose+ 7.5 gl^{-1} agar).

Cormel explant of gladiolus (*Gladiolus hybridus*) was cultured by Malviya et al*.* [90] on different fortifications of MS medium amended with different auxins and cytokinins in diverge concentrations as alone as well as in different combinations. Regarding *in vitro* response, induction medium MS2D.5B/MS2N.5B 5D (MS + 2.0 mgl^{-1} 2,4-D + 0.5 mgl^{-1} BA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar/ MS + 2.0 mgl^{-1} NAA + 0.5 mgl^{-1} BA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) promoted higher degree of callus induction. Nutrient medium MS2N.5B/MSN.5B(MS + 2.0 mgl^{-1} NAA+ 0.5 mgl^{-1} BA+ 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar/ MS + 1.0 mgl^{-1} NAA+ 0.5 mgl^{-1} BA+ 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) exhibited higher shoot proliferating efficiency and shoot of higher length, However, higher number of shoot (s) per explant exhibited by regeneration medium MS3B/MS2B (MS + 3.0 mgl^{-1} BA+ 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar/ MS + 2.0 mgl^{-1} BA+ 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar). In terms of *in vitro* rooting, higher root

proliferating competence was documented on rooting medium MS.5IB.5Kn (MS + 0.5 mgl^{-1} IBA + 0.5 mgl^{-1} Kinetin + 15.0 gl^{-1} sucrose + 7.5 gl^{-1}agar), while number of root (s) with higher length were recuperated on rooting medium MS.5IB (MS+0.5 mgl^{-1} IBA+15.0 gl^{-1} sucrose+ 7.5 gl^{-1} agar).

2.18 Tree Species

2.18.1 Sandalwood *(Santalum album* Linn.)

Santalum album L. belongs to the santalaceae family, a medium-sized evergreen hemi root parasitic tree, highly valued for its fragrant heartwood, which contains sandal oil that is used in perfumes, cosmetics, and also in Agarbathi (incense sticks) industries. Conventional breeding of sandalwood for introgression of new genetic information can be an expensive and difficult task because of its long generation time, sexual incompatibility and heterozygous nature. *In vitro* regeneration techniques can be used to encounter difficulties of traditional propagation methods by microcloning of superior lines.

Leaf discs of sandalwood were cultured on different fortifications of MS medium to judge their *in vitro* response. *In vitro* morphogenesis, (somatic embryogenesis and/or organogenesis) leading to plantlet regeneration was influenced considerably by plant growth regulators. Among various medium experimented, culture medium MSD.5Td (MS + 1.0 mg l^{-1} 2,4-D + 0.5mg l^{-1} TDZ) supported maximum direct somatic embryogenesis (11.44%), indirect somatic embryogenesis (54.23%) and mean numbers of somatic embryo(s) per explant (160.08), whereas culture medium MS2D.5Td (MS + 2.0mgl^{-1} 2,4-D + 0.5mgl^{-1} TDZ) promoted indirect organogenesis (20.38%). Inoculation medium MS2Td.5N (MS + 2.0mgl^{-1} TDZ + 0.5 mgl^{-1} NAA) proved superior for direct organogenesis (9.48%) and regeneration of plantlets *via* direct organogenesis (36.69%). MS medium fortified with 2.0 mgl^{-1} TDZ and 1.0 mgl^{-1} GA_3 proved superior for plantlet regeneration *via* somatic embryogenesis (163.63%) while regeneration medium MSTd.5GA.5N (MS +1.0 mgl^{-1} TDZ+ 0.5 mgl^{-1} GA_3 +0.5 mg l^{-1} NAA) regenerated plantlets *via* indirect organogenesis (141.25%). The *in vitro* raised plantlets were acclimatized and established successfully in the field [91].

Subsequently Tripathi et al. [92] cultured three explants *viz*., mature cotyledons, hypocotyls and mature embryos on different fortifications of MS medium to judge *in vitro* response. *In vitro* morphogenesis (somatic embryogenesis and/or organogenesis) leading to plantlet regeneration was influenced significantly due to different plant growth regulators. Induction media (MS + 2.0 mg l^{-1} 2,4-D) proved adequate for callus induction. Culture media (MS + 0.5 mg l^{-1} 2,4-D+0.5 mg l^{-1} BA/MS + 1.0 mg l^{-1} 2,4-D+0.5 mg l^{-1}BA) induced direct somatic embryogenesis and average number of somatic embryos per explant in higher frequencies. While, Induction media (MS + 1.0 mg l^{-1} 2,4-D+0.5 mg l^{-1} BA/MS + 2.0 mg l^{-1} 2,4-D+0.5 mg l^{-1} BA) enhanced the frequency of indirect somatic embryogenesis. Nutrient media (MS + 2.0 mg l^{-1} NAA+0.5 mg l^{-1} TDZ) promotes direct organogenesis and plantlet regeneration via direct organogenesis and inoculation medium (MS + 1.0 mg l^{-1} NAA+0.5 mg l^{-1} TDZ) supports indirect organogenesis. Regeneration medium (MS + 2.0 mg l^{-1} TDZ+1.0 mg l^{-1} GA3) regenerated plantlets in higher frequencies obtained from somatic embryos, while plantlet regeneration *via* indirect organogenic mode was attained in higher ratio on regeneration medium (MS +1.0 mg l^{-1} TDZ+1.0 mg l^{-1} GA3+0.5 NAA) for the most of the explant's cultures (Fig. 2 E).

Later on, nodal segments of sandalwood were cultured by Bele et al. [93] on MS medium amended with different plant growth regulators in varying concentrations to search out higher *in vitro* response leading to plantlet regeneration *via* somatic embryogenesis and/or organogenesis. Higher proportion of direct somatic embryogenesis, number(s) of somatic embryo per explant and plantlet regeneration via direct organogenesis were recorded on MS medium supplemented with a moderate concentration of TDZ (1.0 mgl^{-1}) in combination with comparatively a lower concentration of NAA (0.5 mgl-1). A relative higher concentration of BAP (1.0-2.0 mgl-1) in combination with a lower concentration of NAA (0.5 mgl^{-1}) promoted frequency of indirect somatic embryogenesis. Ratio of organ formation directly from surface of cultured explants was recovered from culture medium fortified with a higher concentration of BA at the concentration of 4.0 mgl^{-1} in combination with a lower concentration of NAA (0.5 mgl^{-1}). Maximum plantlets regenerated via somatic embryogenesis (direct and/or indirect) on

regeneration medium supplemented with 2.0 mgl^{-1}TDZ in combination with 1.0 mg $l^{-1}GA_3$, while plantlets in higher frequencies via indirect organogenesis was attained with regeneration medium amended with comparatively lower concentration of TDZ (1.0 mg l^{-1}) in combination with 0.5 mgl^{-1} GA_3 and 0.5 mgl^{-1} NAA.

Subsequently an easy protocol for establishing cell suspension culture initiated from the loose embryogenic callus mass was realized by shifting 6–8-week-old morphogenic calli acquired from the mature embryonic axis and cotyledon explant cultures in fluid media. The asynchronous embryogenic cultures were sloughed with clumps of flourishing cell clumps and embryos of various progressive phases along with diffident non-embryogenic tissues. The frequency of embryo proliferation was evidenced to determine the expansion pace of embryogenic masses under diverse conditions. The intonation of initiation and creation of cell suspension was under the directive of the influence of exogenous plant growth regulators amended in the nutrient medium at different concentrations and combinations. Maximum relative growth rate (386%) and clumps/embryoids in elevated integers (321.44) were accomplished on MS nutrient medium fortified with 2.0 mg l^{-1} 2,4-D in association with 0.5 mg l^{-1} BA and 30.0 g l^{-1} sucrose raised from mature embryonic axis-derived calli. Plantlet regeneration in higher frequency (84.43%) was evidenced on MS medium amended with 1.0 mg l^{-1} each of TDZ and GA_3 in conjunction with 0.5 mg l^{-1} NAA and 20.0 g l^{-1} sucrose. Mature embryonic axis-derived calli were found to be constantly better than mature cotyledon-derived calli for raising profitable and reproducible cell suspension cultures. Regenerants displayed normal growth and morphology and were founded successfully in the external environment after hardening [12].

2.18.2 Teak (*Tectona grandis* Linn. F.)

Teak (*Tectona grandis* Linn. F.) is an economically important, large deciduous, and semi evergreen tree. Teak enjoys a worldwide reputation as paragon among the most prized tropical timbers and is predominantly distributed in tropical and sub-tropical regions of Southeast Asia including India. Its extraordinarily wood working qualities and durability make it ideal for variety of purposes such as deckhouses, bridge building, rails, latches, weather doors, and other construction in contact with water such as docks, quays, piers and floodgates. Teak is a slow growing tree with low germination rate (usually less than 50%) that make difficult to propagate naturally and does not meet the demand of timber at present. The new gears of biotechnology will to realm us to the production of disease free true-to-type planting material and tailor teak, especially by transferring transgene having higher growth rate.

Higher regeneration potential-was achieved using mature cotyledons, cotyledonary nodes and epicotyls of teak cultured on MS basal medium with different types of auxins and cytokinins in varying concentrations and combinations. Mature cotyledon explants exhibited higher *in vitro* morphogenesis closely followed by cotyledonary node and epicotyls on medium fortified with 4.0 mgl^{-1} BA and 2.0 mgl^{-1} NAA. However, maximum plantlets were recovered from cotyledonary nodes on same medium. Phenotypically normal plants were obtained after hardening in green house [94].

3. CONCLUSION

The rising commercial demand of plants of economic value crossing beyond their sustainable limits of extraction from the wild has alarmingly reduced their natural status and even extirpated habitats. Conservation of biodiversity of crop plants and keenness to conserve these through biotechnological applications have received considerable attention. Progress in *in vitro* conservation methods, *i.e.,* tissue culture, artificial seeds and cryopreservation, and DNA banks have facilitated conservation of rare and endangered plants of medicinal value suitable for cultivation in ravine and temperate areas. Biotechnology and biodiversity of high value plants can be harnessed together as developmental challenge as well as an economic opportunity in future.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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developed methods for genotype identification based on simple sequence repeats marker data in 2017, which is under review. He Revealed DNA barcode in various medicinal plants with universal markers A patent "DNA barcode for species identification of sedge plants and methods thereof" was granted earlier this year. Another patent on DNA barcoding coupled high resolution melting analysis is under review. As a breeder, he developed 2 varieties of rice and collaborator in 2 varieties of soybean (JS 20-94 and JS 20-116) and one variety of chickpea. He has Teaching experience of more than 40 years. He Guided 59 post-graduate and 14 doctoral students in Agriculture Biotechnology and Plant Breeding & Genetics. He is a Supervisor/Mentor of five National Post-doctoral Fellow from DBT, DST and CSIR. He published more than 110 Scientific Papers (out of which 29 are on soybean) in refereed journals, more than 60 papers presented in conferences, 01 book and 12 Book chapters.

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Chapter 7
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Plant Genomic DNA Isolation: An Important Technology for Marker Assisted Selection

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ABSTRACT

Plant biotechnology is necessary to maximize the probability of success in crop improvement programs. One area of biotechnology is DNA marker technology, derived from research in molecular genetics and genomics, offers great promise for plant breeding. Marker assisted selection in plant breeding, increase efficiency and precision. Mapping and marker assisted selection of targeted trait require high quality of DNA from a large number of plants in short time. However, many protocols have been developed and reported for extraction of plant DNA with the primary aim of development of a relatively quick, inexpensive and consistent to extract high quality DNA. The general principle of all these DNA extraction protocols remains the same involving disruption of the cell wall, cell membrane and nuclear membrane to release the highly intact DNA into solution ensuring removal of the contaminating biomolecules such as the proteins, polysaccharides, lipids, phenols and other secondary metabolites by enzymatic or chemical methods. High quality DNA is prime requirement for marker assisted selection applications in crop improvement as well as sequencing-based applications. Current article compiled Plant Genomic DNA isolation and quantification protocols for different crops.

Keywords: Agarose gel electrophoresis; MAS; plant genomic DNA isolation; purification; spectrophotometer; quantification.

1. INTRODUCTION

Plant Genomic DNA isolation is one of the prime requirements for marker assisted selection for precise breeding programmes [1,2]. The plant DNA isolation and purification procedures can be grouped into three categories, viz., (1) Cetyl Trimethyl Ammonium Bromide (CTAB) method [3,4], (2) Rapid DNA extraction method [5], and (3) DNA isolation by commercial kits such as DNeasy Mini and Maxi kits from QIAGEN, NucleoSpin Plant kits from Clontech, PureLink® Genomic Plant DNA Purification Kit from Life Technologies, PowerPlant® DNA Isolation Kit from MO BIO Laboratories, MasterPure™ Plant Leaf DNA Purification Kit from Epicentre, etc. These procedures usually consist of three steps: (1) rupture and lysis of cells to obtain cell extract (tissue maceration), using DNA extraction buffer (2) purification of DNA, and (3) quantification of DNA. Generally, for plant tissues, fresh leaves of 15 to 20 days are preferred (fresh, freeze-dried, or frozen in liquid nitrogen) and usually ruptured by mechanical force in pestle and motor or tissue lyzer. The main objective of various DNA isolation methods is development of relatively quick, inexpensive and consistent protocol to extract high quality DNA with better yield. Leaf samples contain large quantities of polyphenols, tannins and polysaccharides, so purification of DNA is equally important to get high quality DNA. The basic principle of DNA isolation is disruption of the cell wall, cell membrane and nuclear membrane to release the highly intact DNA into solution followed by precipitation of DNA and removal of the contaminating biomolecules such as the proteins, polysaccharides, lipids, phenols and other secondary metabolites by enzymatic or chemical methods. In present review, we have described two modified methods for plant genomic DNA isolation from leaf tissue.

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2. MATERIALS AND METHODS

2.1 Plant Material

The leaf tissue collected from either 7-10 days glasshouse grown seedlings or 21-28 days old plants from field for DNA isolation. It is always better to collect the samples in morning hours to avoid more impurities.

Method 1:

Wheat genomic DNA isolation

DNA isolation using modified CTAB method [3] for wheat is given below

- Pulverized about 2 gm of fresh young leaves in pre-chilled mortar and pestle using liquid Nitrogen.
- Transfer the powdered samples to a 50 ml polypropylene centrifuge tubes containing 10 ml of pre-warmed (65^0C) CTAB extraction buffer (2% w/v CTAB, 1.4M NaCl, 20mM EDTA, 100mM Tris-Cl, pH8.0) and 0.2% of β-mercaptoethanol
- Incubated samples at 65^0C for 1 h with occasional mixing by gentle swirling.
- Allow tubes to cool to room temperature.
- Emulsify the mixture of tubes with an equal volume (10 ml) of chloroform-isoamyl alcohol (24:1) for 5 min by gentle inversion and centrifuged at 15,000 rpm (Sorval RC5) for 10 min at room temperature.
- Remove the separated aqueous phase with a wide-bore pipette to a clean oakridge tube and to it add 2/3rd volume of chilled isopropanol and mixed by quick gentle inversion for five to six times and incubated at -20^0C for 30 mins for coagulation of DNA.
- Spool the DNA using a bent Pasteur pipette and transferred into micro centrifuge tube and centrifuged at 10000 rpm for 5 min at room temperature
- Discard the supernatant
- Wash the DNA pellet with 70% ethanol by brief centrifugation, air dried and dissolve in TE (100 mM Tris and 50 mM EDTA, pH 8.0) buffer or nuclease free water according to DNA pellet size and store at 4°C for complete dissolution.

2.2 Purification of DNA

Major contaminants of crude DNA preparation are RNA, protein and polysaccharides. Inclusion of CTAB in DNA extraction buffer helps in elimination of polysaccharides to a large extent. The RNA is removed by treating the sample with DNase free RNase (10 mg/ml) denatured at 70^0C. Proteins including RNase can be removed by treatment with Phenol: Chloroform: Isoamyl alcohol (25:24:1).

Protocol

- Add RNase @ 5µl RNaseA/gm of leaf tissue to each DNA sample followed by incubation at 37^0C for one hour.
- After RNase treatment to the samples add equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) mix gently by inversion for about 5 mins and centrifuge for 10 mins at 10,000 rpm.
- Transfer the aqueous phase to 1.5ml Eppendorf tube. Equal volume of chloroform-isoamyl alcohol (24:1) was added to it, mix gently for 5 mins and centrifuge at 10,000 rpm for 5 mins.
- Transfer the aqueous phase to fresh Eppendorf tube and 0.1 volume of 3M Sodium Acetate (pH 5.2) and add 2 volumes of chilled absolute Ethanol, mix properly, incubate at -20°C for 30 mins and centrifuge at 10,000 rpm for 5 mins.
- Discard the supernatant and wash the DNA pellet
- Add 70% ethanol and spin at 10,000 rpm for 5 mins and discard the supernatant. Repeat the step twice
- Air dried the pellet properly till even traces of ethanol were removed completely and dissolve finally in TE buffer or nuclease free water based on DNA pellet size.

Method 2:

Rapid Isolation of Total Genomic DNA from Leaf Samples of Rice Using TissueLyer

- Put 100-150 mg of fresh young cut rice leaves on 2ml of safe-lock adapter tubes (Eppendorf, USA) along with one 6.35 mm beads at bottom of the tubes.
- Arrange the safe-lock tubes on 2 X 24 Adapter set holders of Tissue Lyser (QIAGEN, USA), wrap the adapter sets with aluminium foil or rubber band, freeze in liquid Nitrogen for 1-2 min, fit into the Tissue Lyser properly on both side and pulverize at 22 Hz/Sec for 1-2 min.
- Make pre-warmed (65°C) CTAB extraction buffer and add freshly 0.2% β-mercaptoethanol
- Pipette out 700µl of CTAB premixed buffer to the powdered samples containing tubes, mix the content gently and incubate at 65°C for 45-60 mins with occasional mixing by gentle swirling.
- Allow the tubes to cool to room temperature and emulsify the mixture with an equal volume (700µl) of phenol: chloroform: isoamyl alcohol (25: 24: 1) for 1-2 min by gentle inversion and centrifuge at 11,000 rpm for 10 mins at room temperature.
- Transfer the separate aqueous phase from the contents to fresh clean 2 ml 96 well deep well plate.
- Add 3.5 µl of RNaseA (20 mg/ml) and mix the contents and incubate at 37°C for 30-45 mins.
- Add $1/10^{th}$ volume of 3M Sodium Acetate (pH 5.2) and 2 volumes of absolute ethanol to the aqueous solution, mix by quick gentle inversion for five to six times and incubate at -20°C for 30-40 mins (or can leave for overnight at 4°C)
- Pellet the DNA by centrifugation of deep well plate at 4000-5000 rpm for 10 min and discard the supernatant.
- Wash the DNA pellet with 500 µl of 70% ethanol by centrifugation at 4000-5000 rpm for 5 min, air dry properly till ethanol smell goes and dissolve finally in TE (100 mM Tris and 50 mM EDTA, pH 8.0) buffer (100-200 µl) based on DNA pellet size and store at 4°C for complete dissolution.

Note: If Liquid nitrogen is not available crushing of leaf tissue can be done in DNA Extraction Buffer. All other steps after crushing would remain same.

Role of chemicals used for Plant genomic DNA isolation

- The most commonly used detergents such as Cetyl Trimethyl Ammonium Bromide (CTAB) or SDS (Sodium Dodecyl Sulfate) disrupts the membranes (cationic surface detergent) whereas β-mercaptoethanol, a reducing agent, helps in denaturing proteins by breaking the disulfide bonds between the cysteine residues and removes tannins and polyphenols present in the crude extract.
- EDTA (Ethylene Diamine Tetra Acetic Acid) chelates the Mg^{+2} ions required for DNase activity, thereby keeping DNA intact.
- Buffer such as Tris is used to maintain the pH at 8 and a salt such as NaCl aid in precipitation of DNA by neutralizing the negative charges and aggregating the DNA molecules.
- Further, nucleic acid solutions are commonly contaminated with molecules made of proteins. These contaminants are removed by successively washing with phenol: chloroform: isoamyl alcohol (25: 24: 1) and chloroform: isoamyl alcohol (24:1).
- **Phenol:** Phenol is used to denature the proteins. Phenol helps to remove non polar proteins and lipids from the solution.
- **Chloroform:** Prevent shearing of DNA during isolation. It solubilises lipids and a lot of proteins to remove them from the DNA.
- **Isoamylalchohol** or Isopropanol: Used to prevent phosgene, Carbonyl chloride) from reaction of chloroform with air.
- Finally, alcohol is used for nucleic acid precipitation. This requires diluting the nucleic acid with monovalent salts, such as sodium acetate ($C_2H_3NaO_2$), sodium chloride (NaCl), ammonium acetate ($C_2H_7NO_2$), lithium chloride (LiCl) and potassium chloride (KCl).
- In the presence of cations, ethanol induces structural change in DNA molecules that causes them to aggregate and precipitate out of solution.

- The nucleic acid precipitates spontaneously and can be pelleted by centrifugation. The remaining salts and alcohol are removed by washing with 70% alcohol because some impurities are soluble in water and some in ethanol.
- The clean DNA is dissolve in 1X TE (Tris EDTA) or nuclease free water. The purpose of TE buffer is to protect DNA or RNA from degradation. It is a buffer for storage of DNA & RNA. An overview of DNA isolation from plant sample is given in Fig. 1.

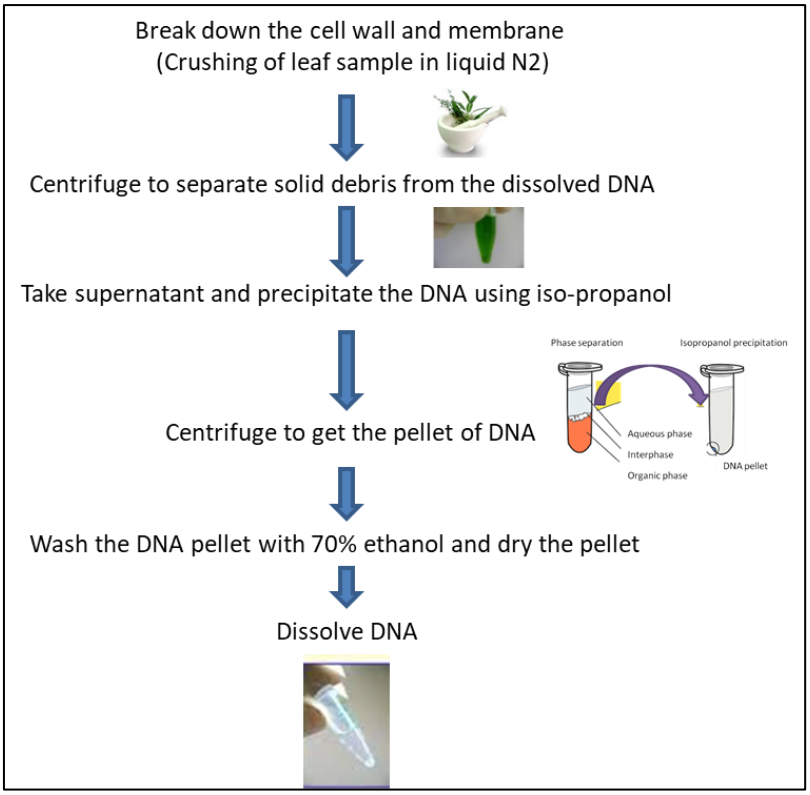


Fig. 1. Overview of Plant genomic DNA isolation from leaf tissue [6]

Reagents for plant total genomic DNA isolation

0.5 M EDTA (pH 8.0) 186.1 g of sodium salt of EDTA was dissolved in 800 ml of MQ water, pH was adjusted to 8.0 with NaOH pellets. The final volume was adjusted to one liter with MQ water and sterilized by autoclaving.

4 M NaCl 233.8 g of NaCl was dissolved in 800 ml of MQ water. The final volume was adjusted to one liter with MQ water and sterilized by autoclaving.

1M Tris-Cl (pH 8.0)121.1 g of Tris-Cl salt was dissolved in 800 ml of sterile MQ water. pH was adjusted to 8.0 with concentrated 1N HCl. The final volume was adjusted to one liter with MQ water and sterilized by autoclaving.

10% CTAB 100 gm of CTAB powder was dissolved in sterile MQ water and the volume was adjusted to one liter.

Phenol: Chloroform: Isoamyl alcohol

Buffer saturated phenol, chloroform and isoamyl alcohol were mixed in the ratio of 25: 24: 1. The equilibrated mixture was stored under a layer of 0.01 M Tris-HCl (pH 7.6) at 4^0C in dark glass bottle.

Table 1. DNA Extraction buffer compositions

Chemical	Concentration	Volume (ml)
1 M Tris-CL (pH 8.0)	100mM	10.0
0.5 M EDTA (pH 8.0)	20mM	4.0
4 M NaCl	1.4M	35
10% CTAB	2% (w/v)	20.0
B-mercaptoethanol	0.2% (W/v)	0.2
Milli-Q water	-	30.8
Total	**-**	**100**

B. Quantification

1. Spectrophotometric: For pure solutions of DNA, the simplest method of quantification is reading the absorbance at 260 nm where an OD of 1 in a 1 cm path length = 50 µg/ml for dsDNA, 40 µg/ml for ssRNA and 33 µg/ml for oligonucleotides and ssDNA in NanoDrop 2000 spectrophotometer (Fig. 2). An absorbance ratio of 260 nm and 280 nm gives an estimate of the purity of the solution. Pure DNA and RNA solutions have OD 260/OD 280 values of 1.8 and 2.0, respectively (Table 2). This method is not useful for small quantities of DNA or RNA (<1 µ g/ml). DNA and RNA absorb maximally at 260 nm, proteins absorb at 280 nm and background scatter absorbs at 320 nm. The Nanodrop spectrophotometer allows a sample to be pipetted directly onto an optical measurement surface. Once the measurement is complete, the surfaces are simply wiped with a lint-free lab wipe. The NanoDrop 2000c integrates the sample retention system with cuvette capability.

A_{260}/A_{280} = measure of purity

$(A_{260} – A_{320})/(A_{280} – A_{320})$

1.7 – 2.0 = good DNA or RNA

<1.7 = too much protein or other contaminant

>2.0 = RNA present (RNase treatment required)

- The A_{260}/A_{280} ratio is ~1.8 for dsDNA, and ~2.0 for ssRNA. Ratios lower than 1.7 usually indicates significant protein contamination.
- The A_{260}/A_{230} ratio of DNA and RNA should be roughly equal to its A_{260}/A_{280} ratio (and therefore ≥ 1.8). Lower ratios may indicate contamination by organic compounds (e.g. phenol, alcohol, or carbohydrates).
- Turbidity can lead to erroneous readings due to light interference. Nucleic acids do not absorb light at the 320 nm wavelength. Thus, one can correct for the effects of turbidity by subtracting the A_{320} from readings at A_{230}, A_{260} and A_{280}.

Table 2. Reading obtained for eight DNA samples on Nanodrop spectrophotometer

No.	A260	A280	A230	A260/A280	A260/A230	Con(ng/ul)	Detect time
1	39.802	19.207	15.941	2.072	2.496	1990.14	01-01 00:31:02
2	25.871	12.301	10.381	2.103	2.492	1293.59	01-01 00:32:06
3	6.187	3.093	3.067	1.999	2.017	309.384	01-01 00:32:51
4	5.827	2.84	2.685	2.051	2.17	291.368	01-01 00:34:56
5	6.539	3.155	3.062	2.072	2.135	326.963	01-01 00:35:52
6	4.701	2.465	2.348	1.906	2.002	235.081	01-01 00:36:37
7	4.787	2.388	2.535	2.004	1.887	239.377	01-01 00:37:28
8	6.714	3.291	3.149	2.04	2.131	335.724	01-01 00:38:02
9	25.839	12.54	12.695	2.06	2.035	1291.97	01-01 00:38:46
10	22.37	11.786	11.591	2.067	2.102	1218.54	01-01 00:39:30

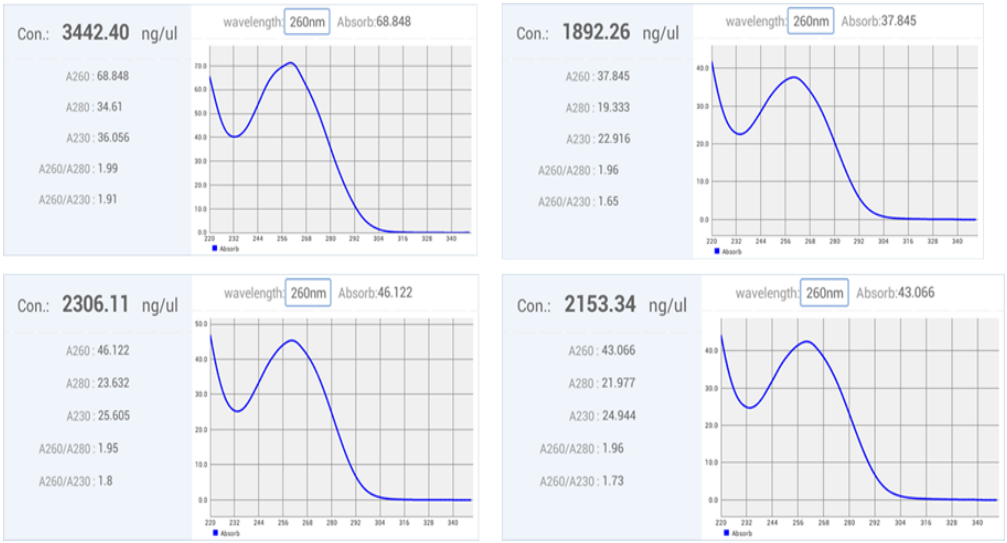


Fig. 2. Quantification result of eight DNA samples using Nanodrop spectrophotometer

2. Agarose Gel Electrophoresis: The amount of DNA in a solution is proportional to the fluorescence emitted by ethidium bromide in that solution. For quantification, load 1µl of DNA samples mixed with loading dye (bromophenol blue), along with known quantity of λ uncut DNA (100ng, 200 ng) on 0.8% agarose gel Stained with ethidium bromide (Fig. 3). Carry out the horizontal gel electrophoresis at 70-80 volts for 45-90 min and observed under gel documentation system. Determine the quantity of DNA by comparing the florescence of sample with that of standards after quantification, dilute the DNA with TE buffer such that the final concentration of DNA to ~ 25 ng/µl. High molecular weight band (>48.5 kb) smearing indicates DNA degradation (or too much DNA loaded). If the thick band is present in lower side the gel, it indicates presence of RNA and needed RNase treatment.

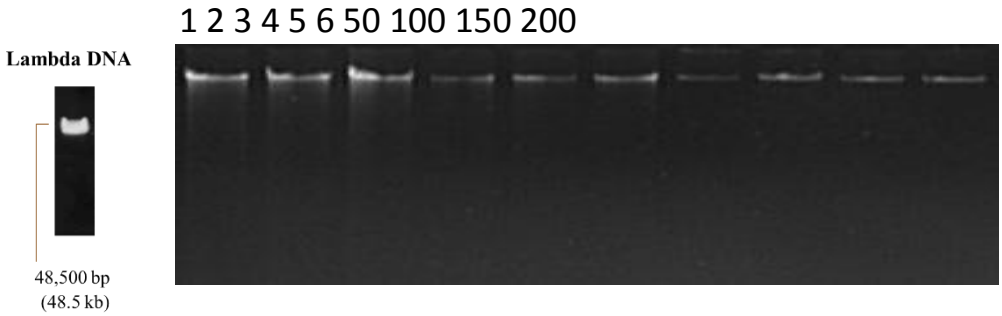


Fig. 3. Lambda DNA and Quantification picture of six DNA samples (1, 2, 3, 4, 5 and 6) and Lambda DNA of four different concentrations (50, 100, 150 and 200ng/µl) on 0.8% agarose gel

3. CONCLUSION

We have discussed about modified protocols for rapid, environment friendly and cheaper method for whole genomic DNA isolation, purification and quantification from fresh and young rice and wheat plant leaves [7,8,6,9]. The same protocol of method 2 was applied to get pure and rapid DNA isolation from young leaves of groundnut, mustard, pearl millet, soybean and chickpea also [10-29]. The quantity and quality of DNA obtained from these methods is enough for all PCR-based genomic analysis. However, various existing genomic DNA extraction protocols available may need modification depending upon stage and plant species. High quality DNA gives better result in PCR amplification and sequencing based applications.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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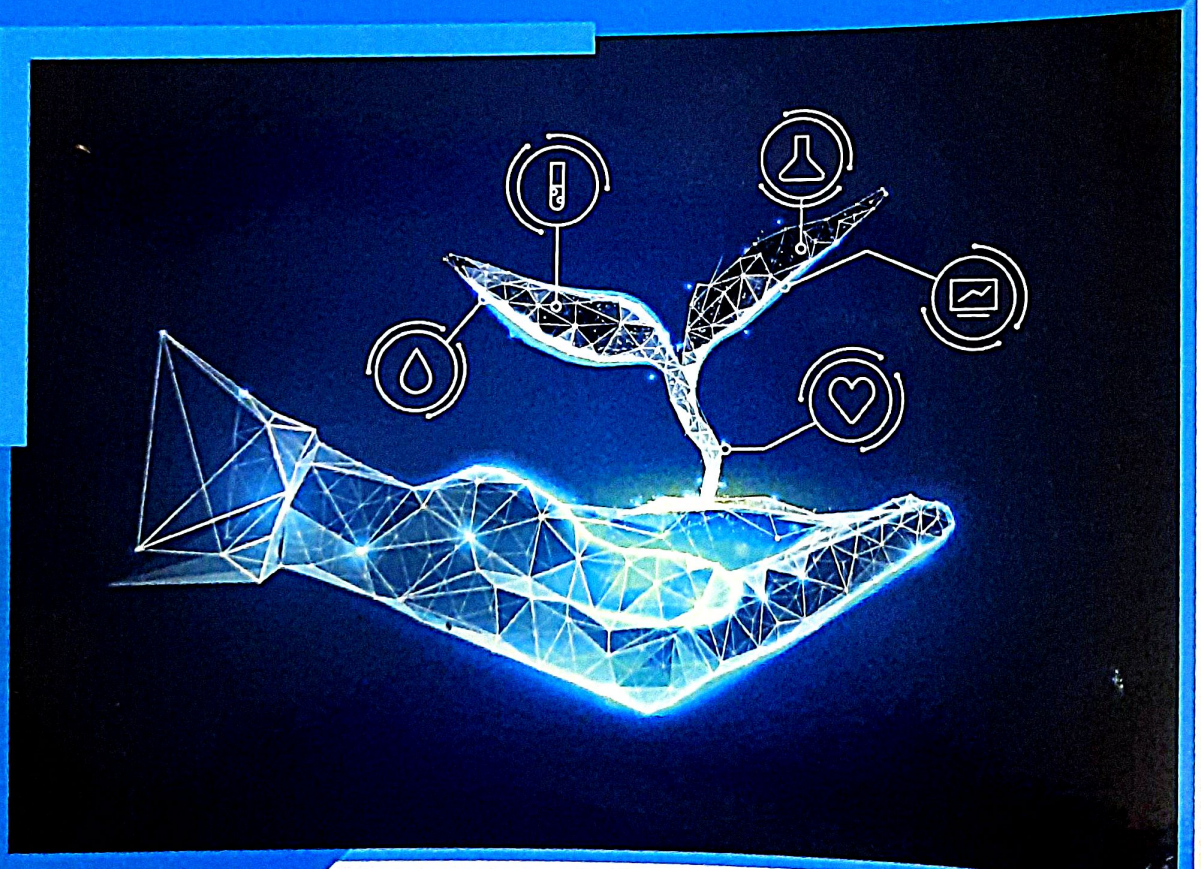
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Chapter - 6
Dragon Fruit: A Super Food in India

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Chapter - 6

Dragon Fruit: A Super Food in India

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Abstract

Dragon fruit (*Hylocereus undatus*) Family *Cactaceae*, Most Hylocereus species principally is originated in Mexico and Central and South America. It is a recently introduced super fruit in India, is considered to be a promising, remunerative fruit crop. It is a climbing vine cactus species which has received worldwide recognition first as ornamental plant and then as a fruit crop. Fruit has very attractive colour and mellow mouth melting pulp with black colour edible seed embedded in the pulp along with tremendous nutritive property which attracts the growers from different part of India to cultivate this fruit crop. It also contains phytonutrients, antioxidants, vitamin C, Protein, Carbohydrate, Vitamin B, Carotene and polyunsaturated fatty acids. Dragon fruit can be processed into a wide range of industrial products such as juice, sherbet, jam, syrup, ice cream, jelly, preserve, candy, pastries and Wine making. The tropical weather conditions are better for the dragon fruit cultivation. The dragon fruit plant gets the flowering in May to June month and fruits from Aug to Dec month.

Keywords: dragon fruit, nutritional properties, medicinal properties, fruit types etc.

Introduction

Dragon fruit (*Hylocereus undatus*) Family *Cactaceae*, Most Hylocereus species principally is originated in Mexico and Central and South America. It is a recently introduced super fruit in India, is considered to be a promising, remunerative fruit crop. It is a climbing vine cactus species which has received worldwide recognition first as ornamental plant and then as a fruit crop. Fruit has very attractive colour and mellow mouth melting pulp with black colour edible seed embedded in the pulp along with tremendous nutritive property which attracts the growers from different part of India to cultivate this fruit crop. The flower is so beautiful that it is nicknamed as 'Noble Women' or 'Queen of the Night'. The biggest advantage of this crop

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ON
DETECTION AND DIAGNOSIS OF DISEASES OF HORTICULTURAL CROPS







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MCQs *for* JRF/SRF/NET/OTHER COMPETITIVE EXAMINATIONS

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Dr. Jyoti Kanwar

KNK College of Horticulture, Mandsaur (M.P.)
RAJMATA VIJAYARAJE SCINDIA KRISHI VISHWAVIDYALAYA
GWALIOR (M.P.)

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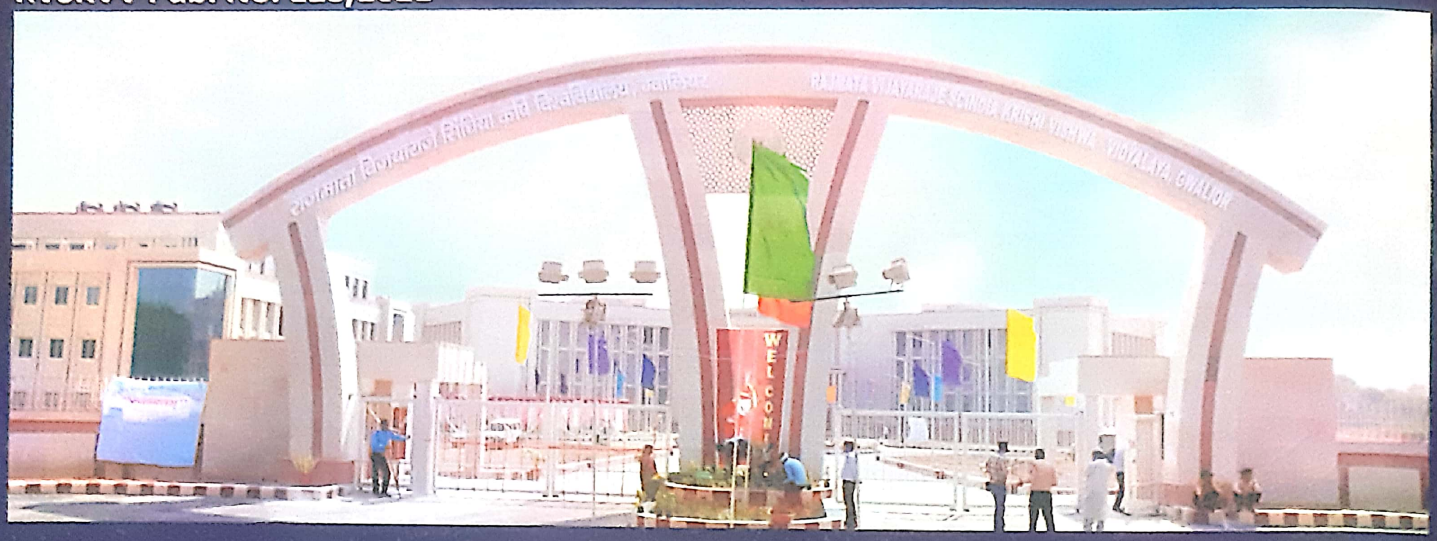
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Due to constant decrement in soil fertility status, the production and productivity of groundnut in India is decreasing. Although, the chemical fertilizers are known to increase production but our complete reliance on chemicals has led to poor soil health and pollution problems which in turn affects crop yield. There is an urgent need to explore an alternative strategy for sustainable production of crops. Liquid biofertlilizers, one of the best modern tools for agriculture, when integrated with chemical fertilizers boost up the crop yield as well as improve soil fertility status. They aid in meeting the nutrient demands of crop by enhancing their availability and absorption by the plants. Taking into account these facts, this book represents the results obtained from the M.Sc. thesis study about Integrated Nutrient Management in kharif groundnut. It also emphasizes on studying the effect of integration of chemicals and liquid biofertilizers on soil health and various quality parameters of groundnut crop concerning economics of INM practices in groundnut cropping.



Neelam Singh pursuing Ph.D. in Agronomy at RVSKVV. The author holds M.Sc. on INM in groundnut & has articles and book chapters about the theme published in several journals. She graduated the B.Sc. in Agriculture at MGCGVV in 2016. She is recipient of INSPIRE fellowship by DST, New Delhi & a gold medalist in both her B.Sc. & M.Sc. degree program.

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Nutrient Management in kharif groundnut

FOR AUTHOR U



Neelam Singh
Ekta Joshi

Nutrient Management in *kharif* groundnut

Integrating chemical fertilizers with biofertilizers: An approach towards sustained crop productivity and soil health

LAP LAMBERT Academic Publishing

Singh, Joshi

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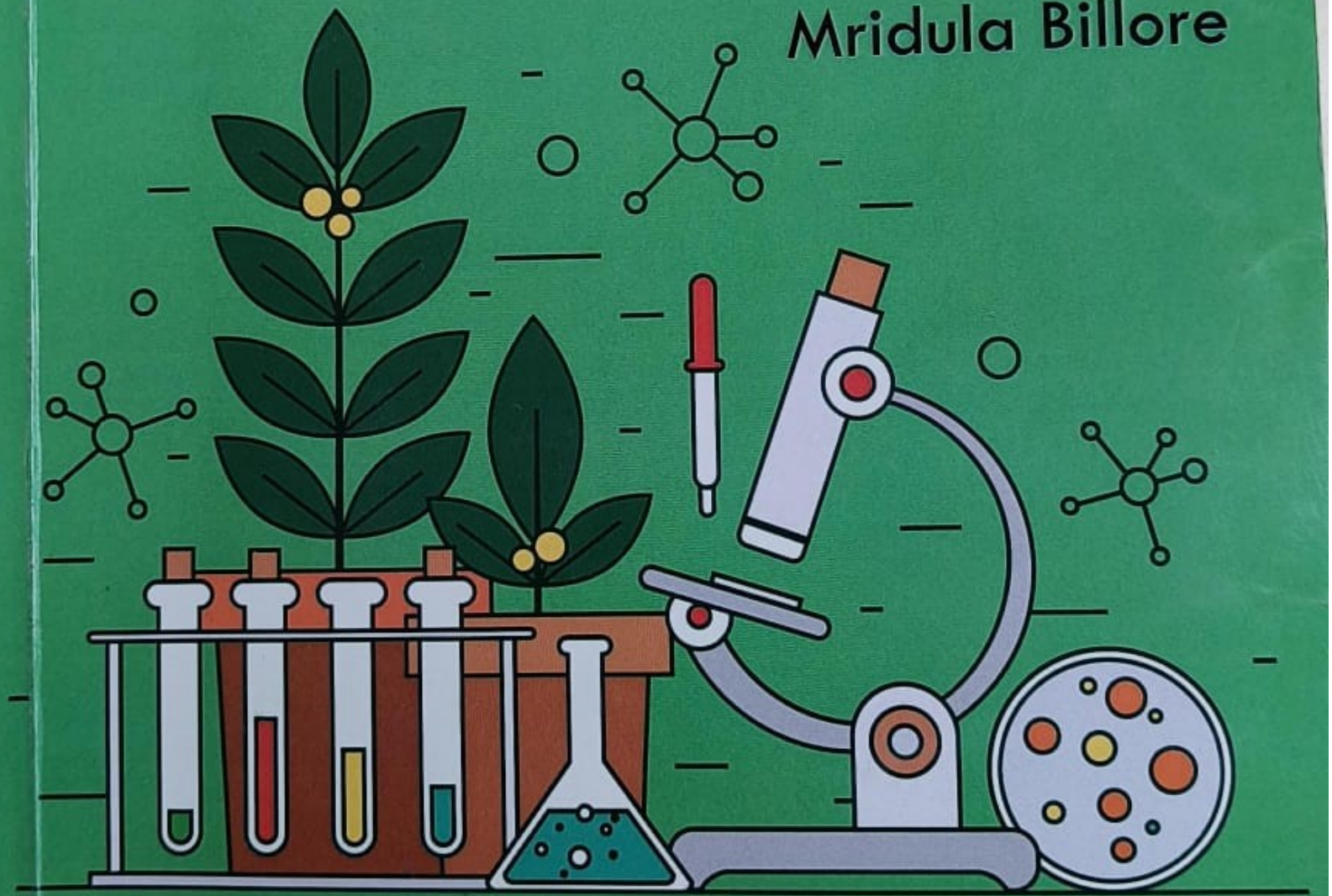
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MOLECULAR BIOLOGY AND PLANT BIOTECHNOLOGY

Phundan Singh
Sushma Tiwari
Mridula Billore



RESEARCH TRENDS AND INNOVATIONS IN PLANT PATHOLOGY

Volume - 1

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Foliar Diseases of Cluster Bean: A Consortium Approach for Disease Management to Improve Productivity

Dr. Rajni Singh Sasode and Dr. Pramod Kumar Fatehpuria

Abstract

Arid legume in India comprises four annual legumes *viz.,* Cluster bean or guar, Moth bean, Cowpea and Horse gram. These crops could not considered as major crops due to their low acreage at the national level. These crops are drought hardy can grow in soils with poor fertility and have deep mat type root system which is of great adaptive significance. Therefore these legumes are specifically important for sustaining production under arid and semi-arid ecosystem. However, these crops are important at the regional level especially, in the traditional farming system. These minor pulses may not substantially influence the productivity figures of pulses as a whole at the country level, the same may; however spectacularly contribute through specific climate zones and ecological niches. The pulses are known for their unique and specific adaptation towards fragile ecosystem where they encounter with degree of adversities composed of moisture scarcity, extremes of temperature and poor agronomic inputs with less care. Besides having versatile uses with low cultivation cost, these crops from important component of complex cropping systems, varying from one climatic zone to other. Suited to nutritionally poor and texturally degraded soils, pulses are recognized as essential segment of traditionally and indigenous and technologies with assured production in such area. The poor productivity of Cluster bean is of major concern. Several biotic and abiotic reasons can be assigned for stagnation in poor productivity of these legumes. Since, arid legumes are grown basically without plant protection and agronomic inputs. In recent year concerted efforts at various levels have led to the development of amenable technological advancements which are quite useful in raising the productivity of crops. In context of diseases the fungal foliar diseases among these legumes play an important role in reducing production and productivity of the crops. Fungal leaf spots are caused by a large number of fungi many belonging to fungi imperfecti. Fungal leaf spot diseases of crop



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D.H. Ranade • M.L. Jadav • Indus Swarup
O.P. Girothia • D.V. Bhagat • Akhilesh Singh • Sharad Choudhary



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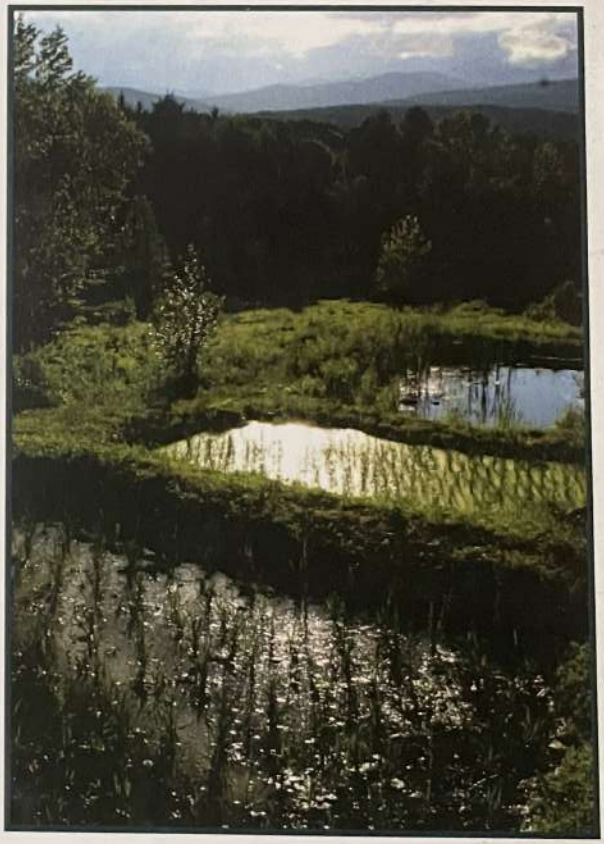
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व
संचित जल का उपयोग









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लोक जैवविविधता पंजी

(People's Biodiversity Register-PBR)

जैवविविधता प्रबंधन समिति सजेली मालजीसाथ
जनपद पंचायत–मेघनगर
जिला – झाबुआ



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–तकनीकी सहयोग–

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जनपद पंचायत–थांदला
जिला – झाबुआ



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A

Text Book

Diseases of Field and Horticultural Crops & Their Management-I

Useful for:

B.Sc. (Agri), B.Sc. (Horti) & M.Sc. courses

"As Per the 5th Dean's Committee of ICAR & New Education Policy (NEP)"

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Diseases of Field and Horticultural Crops
&
Their Management-II

Useful for:

B.Sc. (Agri), B.Sc. (Horti) & M.Sc. courses

"As Per the 5th Dean's Committee of ICAR & New Education Policy (NEP)"

By

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Current Research and Innovations in Plant Pathology

Volume - 8

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Chapter - 2

Management Prospects against Stem Canker of Mustard

Pramod Kumar Fatehpuria, Rajni Singh Sasode, Ajay Singh Kaurav, Hemant Trivedi and R.K. Pandya

Abstract

Sclerotinia sclerotiorum (Lib.) de Bary, the causal organism of stem rot of *Brassica*, with range over 500 host plants distributed worldwide. Sclerotinia rot is menace to cultivation of oilseed *Brassica* crops in the world. Infection occurs on leaves, stems and pods at different developmental stages, causing seed yield losses of up to 80%, as well as significant reductions in oil content and quality. The initial mycelia infection at the base of the stem is an appearance of elongated water-soaked lesions that expand rapidly. Ascosporic infection is quite general and occurs on the leaves or leaf axil. Effective pathogenesis by *S. sclerotiorum* requires the secretion of pathogenicity factors including oxalic acid and extracellular lytic enzymes. Germination of over wintered sclerotia and survival and germination of ascospores are important factors for the development of disease. Isolates of *S. sclerotiorum* show a high level of morphological and molecular diversity. Partial resistance has been identified in some *Brassica napus* and *B. juncea* genotypes, though wild *Brassica* show better resistant reactions. Management of *S. sclerotiorum* still remains as a challenging task worldwide. To reduce the chemical load on the environment, the integration of biocontrol agents (BCAs) and botanical which are able to combat pathogens, as well as the proper combination of appropriate cultural practices, nutrients and water used for the management of stem rot of mustard.

1. Introduction

Sclerotinia rot or white stem rot is caused by *Sclerotinia sclerotiorum* (Libert) de Bary [Syn. *S. libertina* Fuckel; *Whetzelinia sclerotiorum* (Lib.) Korf and Dumont]. The pathogen was first described from Belgium by Madame M. A. Libert (1837) as *Peziza sclerotiorum* Libert (Libert, 1837). *Sclerotinia sclerotiorum* is a plant pathogenic fungus and can cause a disease called white mold if conditions are conducive for *S. sclerotiorum* can also be

ADVANCES IN HORTICULTURE

Volume - 13

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Chapter - 3

Production Technology and Disease Management of Chilli Crop

Pragya Singh, Pramod Kumar Fatehpuria, Hemant Trivedi and Rajni Singh Sasode

Abstract

Chilli is a wonder crop because it has been used as food, spice and household medicine for several common problems such as high cholesterol, high blood pressure, pain to joint, skin problem, and used as carminative, appetizer, stomachic, beverages, relief of pain in neuropathy and counter irritant in treatment of rheumatism. The therapeutic effect of chillies is due to capsaicin, protein, fixed oil, thiamine and ascorbic acid. Among various cultivation practices, judicious use of plant nutrition and efficient plant protection practices enhances yield and quality in most of the crops. It becomes popular among consumers owing to its high food value and among the vegetable growers because of its wide adaptability to various agro climatic conditions prevailing in different part of the country. The disease causes severe damage to both mature fruits in the field as well as during their storage under favourable conditions, which amplifies the loss in yield and overall production of the crop. Though being an important spice crop grown worldwide, many constraints decrease production, causing significant reduction in yield and seed production. Plant diseases have been a major reason for the crop losses worldwide. Diseases caused by fungi, bacteria, viruses or nematodes have adversely affected chilli production in almost all parts of the world. Among the large number of diseases affecting chilli cultivation, anthracnose disease caused by *Colletotrichum* species, bacterial wilt by *Pseudomonas solanacearum* and viral diseases like chilli veinal mottle virus (CVMV) infection and cucumber mosaic virus (CMV) infection have been most detrimental to chilli production. This review gives an account of the loss in production and yield procured in chilli cultivation due to various diseases in Indian sub-continent, with emphasis given to the sustainable management strategies against the conventionally recommended control for the diseases. Also, the review highlights the various pathogenic species of the disease, associated with the host crop in the country. The

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POSTHARVEST MANAGEMENT AND VALUE ADDITION OF FRUITS AND VEGETABLES

(As per Vth Dean's Committee's Recommendations)

M.K. Kureel
D.S. Mandloi
K.V. Singh
Rajesh Lekhi



BIOTECH

Postharvest Management and Value Addition of Fruits and Vegetables

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The practical manual on Postharvest Management and Value Addition of Fruits and Vegetables has been designed strictly according to Vth Deans' Committees' Recommendations. The book contains information on important postharvest technologies such as maturity indices, suitable harvesting timing, pre-harvest and post-harvest handling of crops, fast cooling, preparation of facilities, packing, packaging, transportation and storage. This training manual includes 13 chapters and covers the complete syllabus of UG and by and large a greater portion of the PhD Course.

This practical manual will contribute to the enrichment of the technical library from one side and to be considered as a tool for students, farmers, handlers, sellers and exporters to implement new methods in the production of high quality crops, methods and timing for harvesting, handling, storage, necessary handling both before and after harvesting and finally to prepare the crops for local marketing and for export.

The information in the manual is applicable worldwide. Machines and equipments required in Postharvest Technology of Horticultural Crops is illustrated with color photos, graphs and illustrations. Information related to permitted and banned colours, Additives in unspecified food products, thermally processed fruits and vegetables, microbial requirements of food products etc. have been appended.

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TOXICOLOGY OF INSECTICIDES





Dr S.B. Singh • Dr A.K. Badaya • Dr S.N. Upadhayay



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Toxicology of Insecticides

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India is making headway in her developing industry. Synthesis, production, formulation, distribution and application of the insecticides are on the increase due to the increasing market potential and their great utility in augmenting production by conserving existing resources. Out of the whole chain of agricultural chemicals are one of the major chemicals.

These insecticides are widely used in the country for fighting pests, stored foods and farm animals and insect vectors of diseases in public health. There is no doubt about the benefit of derived by the proper use insecticides and it is one of the great achievements; a tribute to man's ingenuity. Many of the insecticidal chemicals exhibit varying degrees of toxicity towards mammals and therefore, it is essential to get complete information about traditional and new and novel insecticides are being introduced day by day of different groups but their toxicology is somewhat differed in comparison to traditional insecticides.

In writing this book authors have drawn from various published articles and books on the subject in India and abroad [illegible] formulated their own method of presentation.

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Editor-in-Chief
Ashutosh Singh

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Role of Secondary Metabolites in Stress and Plant Defence Mechanism

Rumana Khan, A. Nishant Bhanu, Sharwan Kumar Shukla, Ashutosh Srivastava, A.R.S.S.H. Gupta, A.D.S.S. Ajay Nikhil, H. Sirisha and N. Aneesha

Abstract

Organic substances known as secondary metabolites carry out specific tasks under a specific set of conditions but are not directly engaged in growth and development. There are numerous secondary plant products in the plant kingdom. The plant's ability to survive over the long term is compromised by the lack of secondary metabolites. Typical examples of such substances include phenolic pigments and others. Plant phenolic compounds, including flavonoids and lignin precursors, are thought to be important defence chemicals that can scavenge damaging ROS. They have been documented to include many biotic and abiotic stressors. Brassinosteroids, an important source of plant metabolites categorized as phytohormones suitable for highly promising environment-friendly plant protection and crop yield promotion.

Keywords: Secondary metabolites, stress, defence, abiotic stress, biotic stress, plant pigments.

1. Introduction

Secondary plant products or natural products are secondary plant metabolites. Most secondary plant metabolites are organic substances produced by the members of microbial, fungal and plant kingdoms. They do not actively participate in the physiological activities of the plant, including its growth and development. Secondary metabolites play a major role in plant defence mechanisms against herbivores and other creatures. Plant secondary metabolites are used by humans as recreational drugs, colouring agents and medications. Plants create a remarkably wide range of low-molecular-weight compounds. 25% of all prescriptions written in the US are for natural products and a further quarter is for natural products with structural changes. According to Fransworth (1990), 74 percent of the 119 described medications were discovered using ethnobotanical information. These compounds are still commercially obtained from higher plants (Kumar & Singh, 2021).

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Crop Improvement Strategies for Climate Resilient Agriculture

Rumana Khan, A. Nishant Bhanu, Sharwan Kumar Shukla, Ashutosh Srivastava, R.V. Charitha Reddy, Ajay Nikhil A.D.S.S., Gupta A.R.S.S.H., Sirisha H. and Aneesha N.

Abstract

Globally farming is dependent upon climatic conditions and the impact of weather is tremendous on crop production. In the present era of climate change, majority of cultivable land is prone to extreme climatic conditions of biotic and abiotic factors including drought, flood, heat, cold, diseases, insect, pest and nematodes etc. Therefore, crop improvement strategies should be in accordance to develop novel genotypes for stress resistance. The present chapter covers all aspects of crop improvement including crop diversification, crop wild relatives, pre breeding, high throughput phenotyping, smart breeding and next generation breeding strategies to make our crops climate resilient.

Keywords: Climate change, climate resilience, crop improvement, breeding, genome, QTLs.

1. Introduction

Modern agricultural practices struggle to achieve the level of primary productivity needed to feed roughly 10 billion people by 2050 since land is declining and population is growing rapidly. Causes of climate change are global warming, green-house gases, burning coal, burning oil and burning gas, cutting down forests, increased livestock farming, fertilisers containing nitrogen, Fluorinated gases. Climate change leads to increased frequency of various abiotic (salinity, heat and drought) and biotic stresses (pest and diseases). Additionally, it is anticipated that climate change would result in biodiversity losses, primarily in more marginal environments. It has been estimated that with every degree Celsius increase in the world mean temperature, the yields of the four most important economic crops *i.e.*, wheat (6.0 percent), rice (3.2 percent), maize (7.4 percent), and soybean (3.1 percent) will be lowered globally.

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Floral Biology, Pollination, Genetics, Origin, and Diversity in Barnyard Millet

23

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Abstract

Barnyard millet (*Echinochloa* spp.) has emerged as one of the earliest indigenous millets throughout the semi-arid tropical regions of Asia and Africa. Communities belonging to hilly and tribal regions in Asia, particularly in India, China, and Japan, cultivate two main species for food and fodder: *Echinochloa esculenta* (A. Braun) H. Scholz; syn. *E. utilis* Ohwi Yabuno (Japanese barnyard millet) and *Echinochloa frumentacea* Link; syn. *E. colona* var. *frumentacea* (Link) Ridl. Due to its short life cycle, the crop has an extensive range of adaptations and occupies a specialized niche in marginally humid regions. Despite the crop's endurance to withstand harsh conditions, its importance had been denied substantially over the last 50 years. It serves as a superior alternative during famine periods. In the Indian Himalayan region, the crop appeared to be utilized as a substitute for rice. It has been established to be a great choice for climate-resilient agriculture. It is a functional food crop because of its high nutrient content and antioxidant properties. Because the crop's grains are so incredibly nutritious, demand for it has recently surged. Therefore, it has the potential to ensure both nutritional as well as food security, particularly in high-terrain regions where deficiencies in nutrients are frequent. Despite its enormous potential, the crop has not achieved widespread acceptance and remains visible only as sustenance for the underprivileged. In order to make the crop competitive and modernize its production, this effort aims to consolidate the scant knowledge on agricultural history, domestication, phylogeny, plant architecture, and floral biology of the crop that will be of use to the readers and researchers working on this crop.

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Chapter - 61

Extensions and their Various Forms in Agricultural Programs and their Implications

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Introduction

The word 'extension' is derived from the Latin roots 'ex' which means 'out' and 'tensio' which means ‘stretching.' It literally means "stretching out." This extends beyond the boundaries of universities and research facilities to reach out to rural residents in order to educate them on a variety of topics. Agricultural extension (also known as agricultural advisory services) plays a crucial role in boosting agricultural productivity, increasing food security, improving rural livelihoods, and promoting agriculture as an engine of pro-poor economic growth. More than half of India's cultivable land is dedicated to field crops, primarily cereals (paddy/wheat), cotton, and sugarcane. Extension is interdisciplinary in nature.

Public Sector Agricultural Extension:

It blends educational approaches, communication, and group techniques to promote agricultural and rural development. Technology transfer, facilitation, and advisory services, as well as information and adult education, are all included. Other agricultural development operations, such as marketing and credit services, as well as economic policy and physical infrastructure, are critical to its success. In other words, it is a function that is reliant on other services and institutions for its success. Its ability to shift programme direction and development to stakeholders and programme users is critical in many circumstances. The adoption of a paddy/wheat cropping system has resulted in a number of issues, including environmental, natural resource degradation, and economic sustainability. India is the world's second largest producer of fruits and vegetables. There is a great need and opportunity in India to diversify the existing cropping pattern, and extension education can play a critical role in this. Extension education is inextricably linked with human resource development (HRD), which is the foundation of any development program's success. In India, extension encourages farmers to pursue horticulture as a business. The Directorate of Extension

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Chapter 14

Utilization of Plant Genetic Resources for Food, Feed and Nutritional Security

Bhavna Tomar
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INTRODUCTION

The most valuable and necessary basic raw materials for crop improvement programs that seek to create new plant varieties to boost crop output are plant genetic resources. Represent the full range of genetic diversity or variability found in a crop species. Genetic resources found in plants forms the biological foundation of food stability. The variety of seeds and planting materials from traditional varieties and contemporary cultivars, crop wild relatives, and other wild plant species comprise the plant genetic resources for sustenance and agriculture. In order to adapt food and agricultural production to changing requirements, local communities, researchers, and breeders use living material called germplasm, specifically plant genetic resources for food and agriculture. The cornerstone of managing both the present food crisis and climate change will be preserving and utilizing the global pool of genetic diversity.

Plant Genetic Resources-

The International Board for Plant Genetic Resources defined the term PGR as the genetic variability in crop plants and their wild relatives that is potentially useful in agronomy.

The Convention on Biological Diversity simply says that they are genetic materials of actual or potential value. The sum total of genes present in a crop species is referred to as genetic resources and Gene pool refers to a whole library of different alleles of a species. The sum total of the genetic material, or alleles of the different genes found in a crop species and its wild

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Chapter 15

Resource Conservation Technologies

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INTRODUCTION

Inexperienced revolution has changed the scenario of Indian agriculture from food shortage to meals protection, but the productiveness of the maximum critical staple meals flowers. Rice and wheat are maximum vital staple meal flowers of large loads which decide the photo of meals sufficiency at national in addition to a worldwide diploma. However because of the impact of green revolution and the sick effects of worldwide warming, the productivity of these flora has declined at an alarming fee mainly in Indo Gangetic plains. It's miles because of over exploitation of natural assets in particular water, excessive use of chemical fertilizers and insecticides effects in deterioration of bodily state of affairs of soil. The ones bring about yield stagnation causing the task to approximately the future functionality for productiveness and sustainability. As a end result the vital stressful situations earlier than us to innovate suitable era to supply greater food from faded land and water property for the burgeoning population strain and to improve similarly to hold herbal sources wisely.

Resources maintaining technology (RCTs) preserve the resources and produce more output with less input. It pursuits at reversing the method of deterioration inherent to the conventional agricultural practices like in depth cultivation, burning or removal of crop residues, competitive seed bed education with heavy machineries cause declining the fertility, biodiversity and erosion aid conservation technogies (RCTs) aim to utilize all of the herbal sources accurately to maintain the production and hold ecological balance which is key for a success sustainable agriculture production. There are distinct demanding situations in crop manufacturing like declining floor water desk, deterioration in soil fertility and soil bodily environment, high strength requirements and increasing air and ground water pollutants, so there's need

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Chapter 25

Concept, Importance and Principles of Organic Farming

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INTRODUCTION

Organic farming, sustainable agricultural system that uses ecologically based totally pest controls and organic fertilizers derived in large part from animal and plant wastes and nitrogen-fixing cover vegetation. Current organic farming changed into developed as a reaction to the environmental damage caused by the usage of chemical insecticides and synthetic fertilizers in conventional agriculture.

In comparison with conventional agriculture, organic farming uses insecticides, decrease soil erosion, reduced nitrate leaching into groundwater and surface water, and recycles animal wastes returned into the farm. Those blessings are counterbalanced by higher food fees for consumers and generally lower yields. Certainly, yields of natural crops have been observed to be approximately 25 percentage decrease overall than conventionally grown vegetation, although this may range extensively depending upon the form of crop. The assignment for destiny natural agriculture will be to preserve its environmental advantages, boom yields, and reduce prices at the same time as meeting the demanding situations of weather exchange and an increasing global populace.

Concept of Organic Farming

Organic farming may be described as an agricultural technique that uses biological fertilizers and pest control obtained from animal or plant waste. Organic farming changed into surely initiated as an answer to the environmental sufferings caused by using chemical pesticides and synthetic fertilizers. In other phrases, organic farming is a new system of farming or agriculture that maintenance, continues, and improves the ecological stability.

About the Book

The book "Novel Technologies in Modern Agriculture" is designed to cover all topics of agriculture. Numerous chapters on entomology, agronomy, extension, biotechnology, and other topics are included in the book. The book focuses on the elements that will help students, researchers, and faculty members obtain various types of agricultural information. This book also covered topics that are crucial from the standpoint of the agricultural sector in exams. This book has been edited by the professionals of the S.V.P. University of Agriculture and Technology, Meerut.



Dr. Sushant Kumar is working as Agronomist in Technico agri Sciences ltd, A subsidery of ITC. He has completed his PhD and post-graduation in Entomology from S.V.P. University of Agriculture and Technology, Meerut. Author had been obtained his B.Sc. (Ag.) degree from N.D. University of Agriculture and Technology, Faizabad. He has published more than 10 research paper in National and International journal and 5 book chapters in different publications. He also published many articles/abstract in different magazines and conferences including 1 review paper and 1 practical manual.



Dr. D. V. Singh serves as a professor of Entomology, Co-ordinator of Experiential Learning Programme and Officer in Charge, F.D.R.U. at S.V.P.U.A. & T., Meerut. Dr. Singh received Ph.D. in Zoology in 1998 from Lucknow University, Lucknow. He has been involved with I.P.M.in vegetables development for about 15 years. Dr. Singh has helped secure funding of over 1.0 crore at S.V.P.U, A&T, Meerut through projects. Dr. Singh has published over 60 research papers in peer reviewed journals, one Introductory Entomology laboratory manual eight training manuals and several book chapters. Dr. Singh has made over 42 scientific presentations at scientific meetings and



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Chapter 26

Importance, Components and Principles of Farming System

Bhavna Tomar and Shobhana Tomar
Assistant Professor, College of Agriculture, Indore

INTRODUCTION

Farming System - The farming system refers to the combination of various components such as crops, livestock, and other farming practices that are used by farmers to achieve their production goals.

Developing plans are enormous for dinners presentation and to be expected occupations. They offer the essential assets, tendency, and capacities with respect to ranchers to encourage vegetation, further cultivate trained animals, and adjust neighborhood property fiscally. Here are a piece of the huge perspective significance of creating frameworks:

Dinners security: creating plans are imperative for feasts affirmation considering the way that they offer the fundamental effects and abilities for ranchers to develop feasts crops. Through making a substitute sort of harvests and dairy cows, developing plans can ensure that organizations have help legitimate of stage to nutritious dinners over the a year.

Home grown Positions: developing plans are a type of pay and work for a broad wide assortment of people around the field, surprisingly in like manner locales. With the associate of giving one of a kind benefit conveying sorts out, creating plans can assist with letting desperation and work on the positions free from ordinary organizations.

Conceivable associate make due: creating plans are significant for feasible gigantic asset oversee considering the truth they help to allot and oversee ordinary sources along with locale, water, and biodiversity. Through removing up plausible chips at which incorporate yield flip, agroforestry, and covered problem control, developing plans can redesign soil flourishing, decline water use, and sell biodiversity shielding.

About the Book

The book "Novel Technologies in Modern Agriculture" is designed to cover all topics of agriculture. Numerous chapters on entomology, agronomy, extension, biotechnology, and other topics are included in the book. The book focuses on the elements that will help students, researchers, and faculty members obtain various types of agricultural information. This book also covered topics that are crucial from the standpoint of the agricultural sector in exams. This book has been edited by the professionals of the S.V.P. University of Agriculture and Technology, Meerut.



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Dr. D. V. Singh serves as a professor of Entomology, Co-ordinator of Experiential Learning Programme and Officer in Charge, F.D.R.U. at S.V.P.U.A. & T., Meerut. Dr. Singh received Ph.D. in Zoology in 1998 from Lucknow University, Lucknow. He has been involved with I.P.M.in vegetables development for about 15 years. Dr. Singh has helped secure funding of over 1.0 crore at S.V.P.U, A&T, Meerut through projects. Dr. Singh has published over 60 research papers in peer reviewed journals, one Introductory Entomology laboratory manual eight training manuals and several book chapters. Dr. Singh has made over 42 scientific presentations at scientific meetings and



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3

Food Safety and quality assurance in Processing operations

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Assistant Professor, College of Agriculture,Indore.
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Abstract

This part gives a characterize of the necessities and practices connected with somewhere protected and agreeable and best inside the food business.Overview of food quality and food handling, extent of food security and wellbeing operations.this content will tell about framework program of sanitation, food regulation, guideline and food standard .food business has embraced nonexclusiveFirst-rate frameworks and nice tasks. The standards of cost affirmation (QA) and the method vital to lay out and implement a QA program are dissected in this e book. The food enterprise is the ideal place to learn about food management.These substances incorporate weighty metals (lead, mercury, arsenic), organo-chlorinated mixtures, for example, polychlorinated biphenyls (PCBs), and are considered as modern or natural pollutants..In the U.S., the CFR Title 21 considers PCBs as undeniable ecological toxins due to their boundless event in the climate, and gives resiliences to PCB buildups in a few food varieties (e.g., milk, dairy items, poultry, eggs, and so on.)..Substance depositsIn food handling activities, a few synthetic mixtures that are not allowed substances in food are utilized during specific tasks and mind should be taken to forestall unexpected defilement..These substances incorporate synthetic mixtures utilized for cleaning and disinfecting food contact surfaces of handling, dealing with, and

ROLE OF MODERN TECHNOLOGY IN AGRICULTURE

Dheerendra Singh
Er. Bilal Ahmad Langoo
Er. Dhananjay M. Kadam
Shrankhla Mishra

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Chapter 3

Monitor and Control Crop Irrigation System through Smart Sensors

Ayushi Trivedi[1], Nirjharnee Nandeha[2], J.Himanshu Rao[3], Smita Agrawal[4], Amit Kumar[5],R.S.Dangi[6]

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Abstract

The developing world, where agriculture and climatic factors dominate the economy, served as the inspiration for this chapter. Making the proper operational decision at the right time is essential for production farming profitability based on the circumstances at hand and past performance. Precision farming is a methodical approach created to increase agricultural productivity by precisely adjusting crop and soil management to each field's unique needs while maintaining environmental quality. In order to remotely measure the environmental factors in an agricultural field, this study emphasizes the creation of an automated irrigation system with portable wireless sensor networks and decision support techniques. Ecological characteristics like soil moisture, temperature, humidity, and light intensity are recorded via radio satellites, mobile phones, sensors, internet-based connectivity, and microcontrollers. Using IoT technology, the data gathered from the sensors is sent straight to

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Chapter 6

Application of Remote Sensing and Geographic Information System in Modern Agriculture Technology

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Abstract

Remote Sensing (RS), Geographic Information System (GIS) and Global Positioning System (GPS) are some of the modern technologies that has paved ways for increasing agricultural productivity. These are some of the promising techniques that are being extensively used nowadays to solve complex problems, improve agricultural operations and reduces overall production cost. Satellite based remote sensing have proved itself to be an indispensable tool in understanding the dynamics of earth and atmosphere over the last five decades. Satellite based sensors have the capacity of acquiring information at a much lower cost when compared with ground or aerial sensors. The availability of freely available satellite remote sensing packages have gained due attention from the scientific community in the recent times for management and optimization of farm resources. Remote sensing techniques are helpful in crop health assessment and crop yield estimation leading towards

Land and Water Management Engineering

Vinnakota Yesubabu
Aravind P
Neelendra singh verma
Vikram Parmar

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Watershed Analysis Using Remote Sensing and GIS Techniques

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Abstract

This chapter delves into the critical field of watershed analysis, offering a comprehensive overview of its fundamental principles and the transformative role of remote sensing and Geographic Information Systems (GIS). Watersheds, also known as catchments or drainage basins, are geographical regions where all surface water converges to a common outlet, such as a river, lake, or ocean. The chapter elucidates the core concepts of watersheds, encompassing watershed boundaries, outlets, the hydrological cycle, topography, and land use/land cover, underscoring their significance in managing water resources and making informed decisions for sustainable development. In recent times, remote sensing and GIS technologies have revolutionized watershed analysis, equipping environmental researchers and decision-makers with powerful tools for gathering, processing, and interpreting data. Remote sensing provides diverse data types, including optical and infrared imagery, thermal data, and radar observations, enabling a real-time, multi-spectral perspective on watershed dynamics. This data-rich approach extends to temporal and spatial data, allowing the observation of changes over time and precise spatial analyses. The chapter highlights a range of watershed applications, such as flood modeling and management, water quality monitoring, erosion control, urban planning, natural resource management, and watershed health assessment. It emphasizes how remote sensing and GIS enable critical tasks, from identifying flood-prone areas to tracking pollution sources and assessing the impact of land use changes. The chapter outlines a comprehensive watershed analysis workflow

PRACTICAL MANUAL *on* PROTECTED CULTIVATION *and* SECONDARY AGRICULTURE



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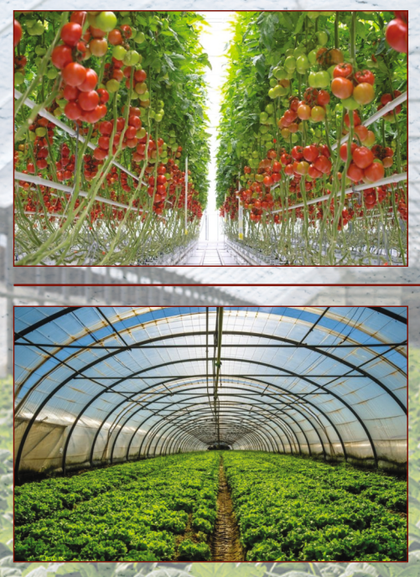
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Practical Manual on Protected Cultivation and Secondary Agriculture

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Advances in SOIL AND WATER CONSERVATION ENGINEERING



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J. Himanshu Rao • Shrankhla Mishra

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PLANT PROTECTION INSIGHTS-DIVERSE PERSPECTIVE ON PLANT PATHOLOGY AND ENTOMOLOGY



Plant Protection Insights

Diverse Perspective on

PLANT PATHOLOGY AND ENTOMOLOGY

Editors : -
Dr. Rudra Pratap Singh
Vaishnavi Rameshrao Tathode
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INTEGRATED PEST MANAGEMENT STRATEGIES

Sitaram Seervi[1], Dr. Pradyumn Singh[2], Dr. Rajni Singh Sasode[3] and Sandeep Patidar[4]

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Abstract

The definition and description of Integrated Pest Management (IPM) and its constituent parts cultural, biological, host resistance, regulatory, and chemical controls have been reviewed. From an economic, social, environmental and biotechnological standpoint, the potential future and problems of integrated pest management have been discussed.

Keywords: Biological, Host Resistance, Control, Pest, Environmental and Biotechnological.

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CHAPTER -18

METHODS OF COLLECTION AND PRESERVATION OF INSECTS INCLUDING IMMATURE STAGES

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INTRODUCTION

For many entomologists, questions regarding how and what to collect and preserve are determined by the research project. The choice of methods may be influenced by the target taxa, life-history stage, geographical scope, type of host plant or host animal, disease vector status, and, most importantly, sampling design and cost-effectiveness. A common factor in all such studies is the need to communicate the information unambiguously to others, particularly concerning the identity of the study organism(s). Identification of specimens will undoubtedly be required to provide names, which are essential not only for communicating the work to others but also for accessing previously published studies on the same or related insects. For identification purposes, material must be preserved appropriately to allow recognition of morphological features that vary among taxa and life-history stages, or to permit the later extraction and amplification of DNA if a barcoding approach is used. After identifications have been made, the specimens retain their importance and even gain added value, making it essential to preserve some material for future reference. As knowledge advances, specimens may need to be revisited to confirm their identity or to compare them with material collected later. In this chapter, a range of collecting methods, mounting and preservation techniques, and specimen curation will be reviewed. Methods and principles of identification, including DNA-based methods, will also be discussed (1).

COLLECTION OF INSECTS

Researchers in vertebrate and plant biology can often study their organisms directly in the field, identify them, and in the case of larger animals, capture, mark, and release them with minimal harm. However, for most insects, these methods are generally applicable only to butterflies, dragonflies, and larger beetles and bugs. In most cases, insects can only be reliably identified after they are collected and preserved. This necessity raises important ethical considerations, including:

1. Collecting responsibly

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Chapter 23

Seed Societies and New Seed Scenario in Madhya Pradesh

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[1] Former RM NSC and Former Advisor, MP Seed Federation
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Good quality seeds availability is behind the development of agriculture production potential on which the efficacy of other applied inputs response dependent. The ideal varieties seed with appropriate characteristics are required to meet the demand of diverse agro-climatic conditions and intensive cropping system. Sustained increase in agriculture production and productivity is dependent to a large extent on new improved varieties and an efficient system for timely supply of quality seeds to the farmers including even in a remote areas. All the seed supply system put together in India accounted for only 25% of total seed required by the country. The unorganized farmer saved / self help group/community seed bank, seed is being sown in about 75% of area is not being addressed by agriculture policies in India. Hence, there is still a need to enhance the availability of quality seed specially in pulses and also in oilseeds to the farmers through informal seed supply system as the farmers have very well recognized the fact that the good quality seed of improved varieties is only had an impact increasing the productivity level of crop.

Gradually seed industry in the state of Madhya Pradesh progressed involvement of public and private sector and quantum of certified seed production initially increased 2500 quintal in 1980 as of 11 lakh quintals in 2004, which later a speedy progress achieved the quantum of more than 35 lakh quintals upto 2012-13. But later years because of subsidy limits, carry over losses, demand fluctuation, poor market network, varietal mismatch, climatic factors and production variations seed quantum has fallen down to the extent of about 20%. About two decades in early years of 2000-01 in the state of MP, the seed

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Seed

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S.K. Rao
S.K. Sharma
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Seed Technological Innovations *for* Self-Sufficiency in Oilseeds and Pulses

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Chapter 30

Sustainable Cotton Production is a Better Alternate of Decreasing Yield and Increasing Cost of Bt Cotton in India

Sanjay Sharma[1], D.K. Shrivastava[2]

1 Director research Services, RVSKVV, Gwalior and 2 Incharge AICRP on Cotton, B.M.College of Agriculture, Khandwa

Cotton is an important cash crop in India. It is grown on 12 million hectares, making India the second largest producer of cotton in the world, behind China. Insect-resistant Bt cotton is the only GM crop currently grown in India.

Facts about Bt Cotton

- Bt introduced in India in 2002.
- 2002-2005 Bt cover 5% of area under cotton production.
- 2005-2012 Bt cover 90% of area under cotton production.
- The production of cotton in India during 2021-22 was 5.8 million metric tons, which was 21 % of the total production of the world i.e. 24.65 million metric tons.
- India has the distinction of having the largest area under cotton cultivation, which is about 38% of the world area under cotton cultivation between 11.9 million hectares to 13.5 million hectares.
- In India, about 6 million farmers are engaged in the cultivation of cotton and the majority of the cotton is produced under rain-fed conditions.
- In India, majority of the cotton production comes from ten major cotton growing states.

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Chapter 38

New Hope of Safflower Crop in Madhya Pradesh

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Safflower,(*Carthamustinctorius L.*), a member of the Compositae family, cultivated mainly for its seeds which is a source of oil and bird feed.Carthamus is the Latinised version of the Arabic word "quartum" which alludes the colour of dyes obtained from the florets. It is grown for centuries in India. Seeds of safflower were found in 4000 years old Egyptian tombs and its use was recorded in China approximately 2200 years ago.

Popularization of new crops in non-traditional areas is an up-hill task in general and for a crop like safflower in particular because of its spiny nature. A well founded massive agricultural extension programme with quality seed production, backed by strong marketing support is needed to fulfill this task. In contrast to traditional areas, where the extension activities seek the transfer of the latest technology concerning an already established crop, the rate of new crops in non-traditional areas hinges on the initial success of the extension programme, and a proper follow-up action. A well-knit, efficient extension approach, which could yield maximum returns in a shortest possible time, is therefore needed.

Introducton

The state of Madhya Pradesh is a non-traditional region with regards to safflower cultivation. In 1971 safflower research work was initiated at Indore (M.P.). The research picked up momentum with the setting up of the IDRC (Canada) centre of Safflower in 1979. Improvement of spiny safflower started with drastic improvement in plant type. The earlier safflower varieties were tall, bushy, late maturing, lanky stature with terminal branching posing enormous handling

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