D. N. BHARADWAJ

BREEDING OF FIELD CROPS

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Editor

Dr. D.N. Bharadwaj

Associate Professor,
Department of Genetics & Plant Breeding,
Chandra Shekhar Azad University of Agriculture & Technology
Kanpur-208002. India

Ex-Visiting Professor of Genetics & Plant Breeding,
Department of Plant Sciences, Haramaya University
Ethiopia (Africa) under United Nations Development Program.

Ex-Visiting Professor of Biology, Department of Biology
Eritrean Institute of Technology, Asmara, Eritrea (N.E. Africa) under
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Foreword

The contribution made in the field of crop breeding is as old as human civilization itself which started as domestication of the wild plants for human food purposes and welfare of society since ancient periods. In India, the development of agriculture is also very old but its modernization started in 1905 after establishment of 'Imperial Agriculture Institute' in PUSA (Bihar), which was later shifted to New Delhi and renamed as Indian Agricultural Research Institute (IARI) in 1936. The further development of Indian agriculture enriched after existence of Indian Council of Agricultural Research (ICAR), New Delhi in 1929 with its 97 Institutes, 47 Agriculture Universities and a number of research centres all over the country. Now ICAR is playing a pioneer role of in ushering Green Revolution in India.

India emerged as a country of agriculture cultivating the entire major and minor crops required for its consumption but the knowledge of many of the crops are not available in the breeding field crops books. The 'Breeding Asian Field Crops' by Poehlman and Borthakur (1959) was the first available book to the students and researchers and other book 'Breeding Field Crops – theory and practices' was by Dr. V.L. Chopra (2000). Both of these books are not providing complete information on all the major crops, therefore, students, researchers as well as teachers of colleges, institutes and universities were facing difficulties to get exact breeding procedures and strategies to be followed on many of the crops included in their syllabi.

The present text book **'Breeding of Field Crops'** edited by Dr. D.N. Bharadwaj, Associate Professor, Department of Genetics and Plant Breeding, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur will be a milestone in the area of field crops providing knowledge on most of the crops which were not earlier available in any of the field crop books. This book covering more than 35 major and minor crops cultivated all over the globe includes cereals, legumes, oilseeds, fiber, cash, forage and narcotic crops with other miscellaneous knowledge. Individual chapters are written by Project Directors, Project Coordinators, Professors of universities and their team working with these specific crops. Chapters are well designed with traditional as well as recent innovative approaches like biotechnological and molecular aspects of breeding and recent achievements available till date.

I personally congratulate Dr. Bharadwaj for such a nice compilation from various specific sources and edited in the form of a text book **'Breeding of Field Crops'**. I hope this book will be widely accepted by students and teachers of various colleges/ universities and researchers of several institutes working on these crops in India as well as abroad.

S. Rajaram
President, Resource Seeds International, Toluca, Mexico
Former Director, Biodiversity and Integrated Gene Management Program, ICARDA, Aleppo, Syria.
Former Director, CIMMYT Wheat Program, El. Batan , Mexico.

Preface

Crop improvement has been started since ancient era of human history of development. Initially domestication and selection were an art rather science, later hybridization also got involved in this programme and created better genotypes by creating variability. In modern time, it is a science of domestication, variation and acclimatization of wild races. Several efforts have also been made during civilization and are still continued by plant breeders, biotechnologists and molecular biologists to improve the crop production in terms of quantity, quality, disease resistance, resistance to herbicides, insects-pests and viruses to minimise yield losses.

The green revolution was started with the aims to feed and save the ever increasing large population from starvation by quantum increase which has now switched to quality improvement. Most modern and sophisticated techniques of molecular breeding, biotechnology, tissue culture and recombinant DNA technology are employed to design transgenic plants that has opened the new era to create genetically modified crops that are not prevailing in the nature like Bt cotton, Bt brinjal, tomato, soybean, maize and several other crops. These genetically modified plants are now producing several new products that are designed for industrial and pharmaceuticals purposes suitable for different agroclimatic regions of the world.

At global level number of Institutes, Organizations and Agricultural Universities are involved in crop improvement programmes for human welfare. Similarly in India, several researchers and plant breeders are now engaged in crop improvements and technology developments, besides producing quality seeds with improved and enhanced quality proteins and other metabolites. The knowledge of conventional, biotechnological and molecular breeding can be enhanced by educating the students and researchers with upto date knowledge to be made available to them.

During my thirty seven years of research and teaching experience in five Universities of India, Asia and Africa, I always felt insufficient teaching information in crop breeding books meant for UG and PG level students. These books are not including all the field crops and even the chapters are not contributed by specific crop breeders, being taught to students. Therefore, I started to make my efforts to cope up with all these shortcomings. As a result the book '*Breeding of Field crops*', is in your hands covering all the major crops and some most economically important minor crops being cultivated in all parts of the India and in most countries of the world. Moreover the chapters in this book are contributed by the Project Directors, Project Coordinators of Indian Council of Agricultural Research (ICAR) and Professors of several Agriculture Universities of India. I hope that this book will fill-up the big gap and prove an asset for UG, PG students; specific crop researchers and also solve the requirements of teaching community engaged in crop breeding programmes. Emphasis has been given to include most recent knowledge of conventional, biotechnological and molecular advancements till date available in every crop breeding programme.

I am highly thankful to all the contributors of chapters on specific crops who voluntarily agreed to support my efforts to bring out this book, '*Breeding of Field Crops*', in the honour of premier Agriculture and educational institute of India i.e. **Chandra Shekhar Azad University of Agriculture & Technology, Kanpur** which is recognized today at the global map by producing world class scientists/ teachers and reputed crop varieties at national level as well as in neighbouring countries. Its foundation was laid down in 1893 when a small school was started by Britishers to impart training to revenue officers. In 1906 it arose into full-fledged Government Agriculture College (Patthar College), then the U.P. Institute of Agricultural Sciences in 1969 and finally to the Chandra Shekhar Azad University of Agriculture & Technology, Kanpur, in 1975. I am dedicating this book to my historical and beautiful university on occasion of completion of its 105 years which is now one of the oldest premier institutes of Asia.

I shall be highly thankful to esteemed readers for their suggestions in improving the quality of this book and to make it more informative and purposeful to them.

Kanpur

D.N. Bharadwaj
Editor
(bharadwajdncsau@gmail.com)

Acknowledgment

This book is the comprehensive product of many individual Scientists of ICAR Institutes, Research Organizations and Professors of State Agriculture Universities of India. I especially thankful to all the authors and appreciate their generous acceptance to contribute the chapters as well as their willingness to respond to my requests and queries throughout the period of this book preparation. In addition to the authors of the individual chapters, I also acknowledge thanks to many unnamed assistances by supporting staff who meticulously collected and processed the manuscript of the individual chapters.

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D.N. Bharadwaj
Editor
bharadwajdncsau@gmail.com

CHAPTER 15

Soybean (*Glycine max* L. Merr.)

S. M. Husain[1] and S. K. Srivastava[2]

[1] Principal Scientist (Plant Breeding), [2] Director
Directorate of Soybean Research, Khandwa Road, Indore-452001 (MP)

Soybean is the most important legume crop of the world and India's number one oilseed crop. Originally a crop of temperate region, it is now grown widely in tropical climate owing to extensive breeding. Being highly self pollinated crop, pedigree, single seed descent and single pod descent methods have been the most favoured techniques of breeding. Currently hybridization followed by rapid generation advance to achieve homozygosity and single plant selections in F_4/F_5 are widely used. Recurrent selection using male sterility for population improvement offers new approach. The traditional breeding techniques have had a significant effect on genetic improvement for yield and other important traits. The yields have increased by 60% in the last 60 years and 3900 varieties of soybean have been released world wide. The advent of molecular techniques has greatly benefited soybean breeding. The use of functional genomics, gene mapping, QTL analysis and transgenic development has accelerated soybean improvement. Glyphosate tolerant Roundup Ready (RR) soybean is the most widely grown Genetically Engineered (GE) crop in the world. These techniques with increased refinement and improvement will find great use in future breeding programmes.

1. INTRODUCTION

Soybean ranks first among the oilseeds in the world. The seeds are rich in both oil (16-21%) and protein (36-42%). The crop contributes for nearly 25% of the world's total oil and fats production. Currently the area under soybean in the world is 96.87 m ha with a production of 230.95 mt and productivity of 2384 kg/ha. The USA is number one in terms of area and production of soybean. The USA, Argentina, Brazil, China and India are the major producer of soybean accounting for 90% of world production. The advent of commercial exploitation of soybean in India is only four decades old. In this short spell of time, the crop has shown unparalleled growth in area and production. India now ranks fourth in area and fifth in production in the world. The area under the soybean has increased from a meager 0.03 m ha in 1970 to 9.21 m ha in 2010. The production in 2010-11 is estimated to be 10.4 million tonnes. Soybean plays a pivotal role in edible oil economy of the country. It contributes 25% to edible oil production in India. Besides oil soybean is a rich source of high quality protein. With nearly 40% protein in seed it has a great potential to alleviate protein malnutrition in the country. India also exports soy meal worth 5000 crore rupees every year thus earning valuable foreign exchange. Soybean seeds containing very high levels of protein that can undergo desiccation yet survive and revive after water absorption. Beans are classed as pulses whereas soybeans are classed as

oilseeds. It is a versatile bean, having a diverse range of uses. The nutritional value of soy consists of following composition per 100 g as given in Table 1.

TABLE 1: Nutritional composition of soybean seeds per 100 g

Source	Unit
Energy	30 kcal
Carbohydrates Sugar	5.94 g
Dietary fiber	4.13 g
	1.8 g
Fat (Total)	0.18 g
saturated	0.046 g
monosaturated	0.022
polyunsaturated	0.058 g
Protein	3.04 g
Water	90.4 g
Vitamin A	Equiv. 1 μg 0%
Vitamin B6	0.088 mg 7%
Vitamin B12	–
Vitamin C	13.2 mg 22%
Vitamin K	33 μg 31%
Calcium	13 mg 1%
Iron	0.91 mg 7%
Magnesium	21 mg 6%
Phosphorus	54 mg 8%
Potassium	149 mg 3%
Sodium	6 mg 0%
Zinc	0.41 mg 4%

2. CENTERS OF ORIGIN AND DOMESTICATION

According to Goldblatt (1981) the base number of Phaseolae is almost certainly x=11. The aneuploid reduction (x=10) is also common among the tribe. Based on this a basic chromosome number of x = 10 is proposed for cultivated soybean. It is hypothesized that a putative ancestor of genus Glycine with 2n = 2x = 20 arose in South East Asia (Singh *et al.*, 2001), however, such a progenitor has either become extinct or is yet to be collected. Tetraploidization (2n = 4x = 40) through auto or allopolyploidy occurred in the progenitor species either prior to or after dissemination from the ancestral region. The progenitor of wild perennial species spread southward to Australian continent and northward to China adapting to ecological niches. The wild perennial species, which evolved on Australian continent, were not domesticated. The path of the migration northwards towards China from a common progenitor is assumed by Singh *et al.* (2001) as: wild perennial (2n = 4x = 40, uncommon or extinct) - wild annual (2n = 4x = 40; G. soja) – soybean (2n = 4x = 40; *G. max* cultigen).

The place of origin of the cultivated form of soybean is eastern Asia. *Glycine soja*, the progenitor of *G. max* is known to occur in China, Manchuria and Korea. Fukuda (1933) argued for Manchuria as the centre of origin as (i) *G. gracilis*, a closely related species is distributed widely in Manchuria, (ii) numerous soybean varieties are grown in Manchuria, and (iii) many of the varieties have primitive characteristics. Vavilov (1949/50) considered Central Western China as the centre of origin for soybean. Nagata (1959, 1960) indicated that the centre of origin was most probably China proper, especially in North and Central China. The Yellow River region in

China is generally considered as center of origin of soybean based on the existence of a great number of wild soybeans and the earliest record of soybean in China (Hymowitz and Kaizuma, 1981; Wang and Wang, 1992).

Linguistic, geographical and historical evidence suggests that the soybean emerged as a domesticated around the eleventh century BC in the eastern half of north China. Domestication is a process of trial and error and not an event. In the case of the soybean, this process probably took place during the Shang dynasty (ca. 1700-1100 BC) or perhaps earlier. By the first century AD the soybean probably reached central and south China, as well as peninsular Korea. The movement of the soybean within the primary gene center is associated with the development, consolidation of territories, and degeneration of Chinese dynasties (Hymowitz, 1997).

3. CROP SYSTEMATIC

The genus Glycine Willd is a member of family Leguminosae, sub-family Papilinoideae and tribe Phaseoleae. The Phaseoleae is the most economically important tribe of Leguminoseae. Glycine has a confused taxonomic history from its inception. The name Glycine was first introduced by Linnaeus in the first edition of his Genera Planetarium. Glycine is derived from the Greek Glykus and probably refers to sweet tubers produced by *G. apios*, which he classified in this genus. Linnaeus listed eight Glycine species in the Species Planterum of 1759. All of these were subsequently moved to other genera.

Systematic classification

Kingdom	:	Plantae
Phylum	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Faboideae
Genus	:	*Glycine*
Species	:	*Glycine max*

The genus Glycine Willd as currently delimited is divided into two sub-genera *Glycine* and *Soja* (Moench) F. J. Herm. According to Hymowitz (2004) the sub-genus Glycine comprises 22 wild perennial species (Table 2). The sub-genus Soja includes *G. max* L. Merrill and its wild annual counterpart *G. soja* Sieb and Zucc. The sub-genus Soja contains in addition, a form known as *G. gracilis*, which is somewhat intermediate in morphology between *G. max* and *G. soja*. Herman (1962) considered *G. gracilis* a form of *G. max* with some *G. soja* genes.

TABLE 2: List of species relationship in the genus of *Glycine* (wild and distribution)

	Sub-genus *Glycine*	**Distribution**
1.	*G. albicans* Tind. and Craven	Australia
2.	*G. aphyonota* B. Pfeil	Australia
3.	*G. arenaria* Tind.	Australia
4.	*G. argyrea* Tind.	Australia
5.	*G. canescens* F. J. Herm	Australia
6.	*G. clandestina* Wendl.	Australia
7.	*G. curvata* Tind.	Australia
8.	*G. cyrtoloba* Tind.	Australia

	Sub-genus *Glycine*	Distribution
9.	*G. dolichocarpa* Tateishi and Ohashi	Taiwan
10.	*G. falcata* Benth.	Australia
11.	*G. hirticaulis* Tind. and Craven	Australia
12.	*G. lactovirens* Tind. and Craven	Australia
13.	*G. latifolia* (Benth.) Newell and Hymowitz.	Australia
14.	*G. latrobeana* (Meissn.) Benth.	Australia
15.	*G. microphylla* (Benth.) Tind.	Australia
16.	*G. peratosa* B. Pfeil and Tind.	Australia
17.	*G. pindinica* Tind. and Craven	Australia
18.	*G. pullenii* B. Pfeil, Tind. and Craven	Australia
19.	*G. rubiginosa* Tind. and B. Pfeil	Australia
20.	*G. stenophita* B. Pfeil and Tind.	Australia, West Central and South Pacific Islands
21.	*G. tabacina* (Labill.) Benth.	Australia, Papua New Guinea, Indonesia, Philippines, Taiwan
22.	*G. tomentella* Hayata.	
	Subgenus Soja (Moench) F. J. Herm.	
23.	*G. soja* Sieb. and Zucc.	China, Russia, Taiwan, Japan, Korea
24.	*G. max* (L.) Merrill.	Cultigen (soybean)

Source: Hymowitz (2004)

4. SPECIES RELATIONSHIP

Both species *G. max* and *G. Soja* are annual. The soybean (*G. max*) grows only under cultivation while *G. soja* grows wild in China, Japan, Korea, Taiwan and Russia. Glycine soja is the wild ancestor of the soybean: the wild progenitor. At present, the subgenus Glycine consists of at least 16 wild perennial species: for example, Glycine canescens, and *G. tomentella* Hayata found in Australia, Europe, and Papua New Guinea (Table 3.).

TABLE 3: Chromosome number and geographical distribution of Soybean (hymowitz and Newell 1981)

Species	Chromosome number	Geographical distribution
Subgenus *Glycine*		
G. cladestine var. *sericea*	40	Australia; South Pacific Islands
G. falcata	40	Australia
G. latrobeana	40	Australia
G. conescene	40	Australia
G. tabacina	40-80	Australia; South China; Taiwan; South Pacific Islands
G. tomentella	30;40;78;80	Australia; South China; Taiwan; Phillipines; Papua New Gunea
Subgenus *Soja*		
G. soja	40	China; Taiwan; Japan; Korea; USSR
G. max	40	Cultigen

5. PLANT MORPHOLOGY AND FLORAL BIOLOGY

The cultivated soybean plant is an annual, erect and sparsely branched. The stem is round, often hairy with three distinct types- determinate, semi-determinate and indeterminate. It has pinnately trifoliate leaves. The leaves are alternate, in general ovate or lanceolate in shape. Leaves are normally shed as the seeds ripen. Flowers are purple to white, borne on short axillary raceme or peduncle. Flower is typical leguminous and pollination occurs before flowers open. The root system consists of a tap root with many lateral branches. Roots carry nodules containing species-

specific strains of Rhizobium japonicum. Out crossing is less than 1%. The fruit is a short, hairy pod usually brown or black at maturity. Pods usually contain two, three or sometimes four seeds that normally shatter when ripe. The soybean seed is small, hard, round or ovoid in shape and varies 5-10 mm in diameter. Testa is smooth and shiny with a small distinct hilum. The seed coat colour is predominantly yellow (commercially acceptable) or could be black, brown or green.

Glycine max- is a species of legume native to East Asia. It is an annual plant that may vary in growth, habit, and height. The pods, stems, and leaves are covered with pubescence. The leaves are trifoliolate, having 3 leaflets per leaf that may be 6–15 cm long and 2–7 cm broad. Soybean is a self pollinated crop; flowers are borne in the axil of the leaf and are of white, pink or purple coloured. The leaves fall at seed's maturity. The fruits are found in clusters of 3-5 pods that are covered with hairs. Pods are usually 3-8 cm long consists of 2-4 seeds/ pod and seeds may be of 5-11 mm in diameter.

The genus *Glycine* can be divided into two subgenera (species), *Glycine* and *Soja*. The subgenus *Soja* (Moench) includes the cultivated Soybean, *G. max* (L.) Merrill, and the wild soybean, *G. soja* Sieb. and Zucc.

6. INHERITANCE OF ECONOMICALLY IMPORTANT GENES/ CHARACTERS

The various genes have been identified for inherence characters, some of them are described below in Table 4.

TABLE 4: Some important gene responsible for inheritance of characters

Genes	Phenotypes
Rpg 1	Resistance to Bacterial blight
rpg 1	Succeptible to Bacterial blight
Rpm	Resistance to Downy mildew
rpm	Sicceptible to Downy mildew
Rmd	Resistance to Powdery mildew
rmd	Succeptible to Powdery mildew
Rpp 1	Resistance to Soybean rust
rpp 1	Succeptible to Soybean rust
rym 1 and rym 2	Resistant to Yellow mosaic virus
Hb	Tolerant to herbicide bentazon
hb	Sensitive to bentazon
E 1 and E 2	For Lateness
e 1 and e 2	For earliness
Dt 1	Indeterminate growth habit
dt 1	Determinate growth habit
Df 2 and Df 3	Dwarfness
Ft	Fertility
I	Seed light hilum
i-i	Seed dark hilum
R	Black seed coat
r	Brown seed coat

7. BREEDING OBJECTIVES

The breeding objectives are determined by the problems encountered by the crop. Soybean was a crop confined to temperate zone between 35°-45°N. Its introduction in sub tropical climate

caused many problems like poor seed longevity, less growth due to short day conditions, disease and pest etc. The main breeding objectives for the crop are enumerated below.

Yield

The amount of genetic improvement in yield through hybridization and selection has been substantial. Recent estimates indicate that soybean yields are improving at a rate of 23 Kg ha-1 (Specht *et al.*, 1999). Wilcox (2001) estimated that public breeders in northern soybean production area have increased seed yields about 60% over the past 60 years. The world average production stands at 2.6 tones/ha. In India soybean yields have improved from 700 Kg ha-1 to 1000 Kg ha-1. The increase has come mostly through improvement in harvest index, increased biomass, high number of pods/plant and increased seed filling duration. The annual genetic gain in seed yield between 1969 and 1993 has been approximately 22 Kg/ha. (Karmakar and Bhatnagar 1996).

Genetic enhancement of yield under rainfed condition has been of utmost importance. This can be achieved through a combination of desired features in a plant. The ideal soybean plant for high yield should have determinate or semi-determinate growth habit (suited to short season of 100-105 days) erect and non lodging. It should have a long juvenile period and long seed filling duration with broad leaves for maximum light interception. Most of Indian soybean varieties have yield potential of 2-3 tones/ha while some can yield up to 4 tones/ha. Further improvement in yield will depend on genetic diversity of parents, plugging the yield loss due to stress and improving the genetic architecture of the plant.

Improved seed longevity

Soybean is categorized as one of least storable crops. It is highly sensitive to mechanical injury due to vulnerable position of its embryo. The deterioration in seed quality is very fast particularly under tropical climate during storage. Many small seeded varieties have better germination than bold seeded ones (Paschall and Ellis 1978). The association between the two traits can be broken through breeding. Karmakar *et al.* (1999) identified bold seeded lines with high seed longevity.

Genotypic differences in seed quality have been reported (Green and Pinnel 1966). Soybean breeders aim to develop varieties which are resistant to mechanical damage and retain a germination of more than 70 % after 8-9 months of ambient storage. Several sources of better seed quality like Kalitur, T-49 have been identified. The incorporation of seed longevity is a prime breeding objective. To identify promising lines, segregating progenies are screened using accelerated aging, electrical conductivity and vigor test.

Early maturity, photosensitivity and long juvenility

Soybean is a short day plant which flowers when the day length becomes shorter than its critical day length. This limits the wider adaptability of soybean cultivars. The maturity and flowering in Soybean are reported to be affected by 7 major gene pairs designated E1 to E7 (Palmer *et al.*, 2004.). The earliness is recessive to lateness; early types are also insensitive to day length. A useful trait 'long juvenility' was reported by Hartwig and Kihl (1979) which delays flowering under short day conditions. This gene has been introduced in determinate background to allow for an optimum vegetative period before onset of flowering.

The soybean varieties in USA are classified in maturity groups according to their maturity and response to photoperiod. There are 13 maturity groups 000 to X, 000 being adapted to higher latitudes of USA and Canada and X to Southern USA. Indian soybean varieties are classified as early (< 90 days) medium (90-105 days) and late (> 105 days). Short duration varieties are

desirable under rainfed conditions for avoiding terminal drought and fit in cropping system. A combination of early maturity, photo insensitivity or long juvenility will impart stability and adaptability to performance. Bhatia (2006) identified MACS 330, EC325097 and EC34101 as being photo insensitive under long days.

Determinate and indeterminate types

The stem termination is affected by two loci Dt1 and Dt 2. Dt1 controls indeterminate habit while Dt 2 is determinate. Semi-determinate character is imparted by Dt-2 allele (Thompson, 1997). The determinate and semi determinate varieties are ideal for providing lodging resistance and synchronous maturity and better yield under high fertility conditions. Under short growing season in India determinate varieties are desirable. Most of the popular Indian varieties like NRC 7, NRC 12, JS 71-05, PK 472 etc. are determinate. Semi determinate varieties provide stability under late sown conditions.

Disease resistance

Soybean is attacked by number of fungal, bacterial, viral pathogens and nematodes. The most important being soybean rust, Phytopthora root rot, stem canker, bacterial blight, soybean mosaic virus and soybean cyst nematode. The genetics of resistance for many of these has been worked out and summarized by Palmer *et al.* (2004). Resistance breeding is a major part of every soybean improvement program.

In India the prevalent diseases are rust, yellow mosaic virus, charcoal rot and Rhizoctonia root rot. Rust is prevalent in southern India. The resistance is governed by a dominant gene *Rpp*. Four alleles *Rpp1- Rpp4* at different loci have been reported (Hartwig and Broomfield 1983; Hartwig 1986). Indian varieties PS 1024, PS 1029 and JS 80-21 are tolerant to rust. In order to search for resistance sources the germplasm collection is being screened at hot spots. Two lines EC 241778 and EC 241780 have shown resistance (Basavraj *et al.*, 2006). Development of YMV resistant varieties like PK 416, PS 564 was made possible by a source of resistance PI 171443 (UPSM 534) and has made cultivation of soybean possible in north India (Singh *et al.*, 1974a). Charcoal rot caused by Macrophomina occurs during moisture stress phases. The active breeding for evolving varieties with multiple disease resistance is very much needed for sustainability in soybean production.

Insect resistance

The most destructive insect pests of soybean include a variety of foliage feeders, stem borers, gram pod borer and stink bug. Although considerable efforts have gone into breeding insect resistant varieties limited success has been achieved (Boethel, 1999). Van Dyun *et al.* (1971) screened USDA germplasm of MG VII and VIII and found three plant introduction PI 171451, PI 227587 and PI 229358 resistance to Mexican bean beetle. In India germplasm lines and elite breeding lines are scored for resistance to major insect pests *e.g.* stem fly, girdle beetle and defoliator. Sharma and Shukla (1993) identified germplasm lines TGX 855-53D and DS 396 as resistant to defoliators. Tawre *et al.* (2004) found 18 advance lines highly resistant to stem fly. The wild progenitor of soybean, Glycine soja is a promising source of resistance to Bihar hairy caterpillar (Ram *et al.*, 1989).

Drought tolerance

Moisture stress is the significant limitation of soybean yield and various trait have been identified that impart tolerance to drought in soybean. These include slow wilting, water use efficiency and nitrogen fixing ability (Sinclair, 2004). Sloane *et al.* (1990) identified a plant introduction PI 416937 as being slow wilting which is being extensively used in breeding program. There is genotypic variability in nitrogen fixation sensitivity to soil drying and ureide accumulation. The cultivar Jackson was found to have higher nitrogen fixation in drought (Sall and Sinclair 1991). Sinclair (2004) suggested combining both these trait to ameliorate yield losses from drought stress.

Quality breeding

Soybean has a unique combination of 40% protein and 20% oil content, however, the two are negatively associated. The oil content is influenced by maternal parent (Brim *et al.*, 1968). Fehr *et al.* (1968) suggested seed density and specific gravity as criteria for breeding for higher oil content. Beside quantity, oil quality is important and therefore, soybean lines with high oleic acid (>40%) and low linoleic acid (<4%) are desirable for oxidative stability (Vineet Kumar *et al.*, 2005). Variability for both characters has been reported. The protein content in soybean varies from 35-50% however soybean protein is deficient in sulphur containing amino acids. Howel *et al.* (1972) attempted to increase methionine content in soy protein.

The soybean food uses in India are limited because of a beany flavour caused by lipoxygenase enzyme. Soybean lines free from lipoxygenase have been bred through mutation breeding (Kitamura, 1985). Another anti-nutritional factor Trypsin inhibitor protein SBTi A2 is present in raw soybean. The lines lacking in Ti were by Hymowitz and Hadley (1972) reported lines low in TI.

8. BREEDING METHODS

Conventional breeding strategies have been highly successful in improving productivity and quality of soybean. Besides yield, progress has also been made in selecting for resistance to diseases, insects, nematodes and abiotic stresses (Specht *et al.*, 1999). The genetic improvement program starts with selection of parents to create a segregating population. Generally elite parents of diverse origin are likely to produce superior progenies (Burton 1997). The identification of germplasm lines with favorable alleles for use as parents is often done through test cross evaluation (St. Martin *et al.*, 1996). The selection of parents for improving yield using best linear unbiased prediction (Henderson, 1995) is an effective approach. The effectiveness of selection depends upon the heritability of the trait and environment where lines are grown. The breeding populations are developed from 2-3 parent hybridizations. The procedures for advancing crosses to homozygosity include pedigree method, bulk method, single seed descent and pod bulk. The latter two methods help to preserve original genetic variation in the population. Other procedures used in soybean breeding are early generation testing where yield testing is initiated in F_2 or F_3 generation; backcrossing; and population improvement via recurrent selection which may involve a genetic male sterility system (Specht and Graef, 1990).

Genetic Diversity in Breeding

The genetic diversity from the past is best applied to future when germplasm collection and breeding programme operate in concert soybean has great diversity, its global germplasm collection being 1,70,000, out of which as many as 45,000 accessions may be unique. Despite

such diversity fewer than 1000 accessions have been used in breeding. The reasons for this are is agronomic inferiority of germplasm, difficulty in assessing the value of alleles for yield and other complexity inherited traits (Carter et al, 2004). When a breeding programme can not sustain long term genetic progress for yield the introduction of new alleles from exotic germplasm into commercial gene pool offers an alternative. Kisha *et al.* (1998) found that biparental crosses with distant parents had high genetic variance for yield. The selection of exotic parents is very critical, they can be selected based on phenotype per se or test cross evaluation (Kenworthy 1980). The proportion of exotic parentage have been of particular interest, Khalaf *et al.* (1984) observed that progenies with 75% exotic parentage were higher yielder and more variable than progenies with 50% exotic germplasm.

Genetic diversity has been very useful in resistance breeding. All major genes for resistance to soybean cyst nematode, bacterial pustule and brown stain rot were derived from exotic sources. Soybean breeders in India have used exotic germplasm to breed varieties like Durga, Gaurav, PS 564 etc. The use of PI 17443 as source of resistance to YMV has led to development of many YMV resistant varieties.

Wide hybridization

The genus Glycine is divided into two sub genera Glycine (perennials) and Soja (annuals). Cultivated soybean and its wild progenitor Glycine soja belong to latter and comprise the primary gene pool while 22 species belonging to sub genus Glycine form the tertiary gene pool. *G. soja* is reported to have many desirable traits like high protein (Sebolt *et al.*, 2000) and resistance to soybean cyst nematode (Wang *et al.*, 2001). Despite the appeal of *G. soja* as a source of diversity and easy crossability with *G. max*, there has been little success in breeding due to many undesirable phenotypic traits *e.g.* extreme twining, shattering and lodging associated with it. Polygenic nature of these traits makes it difficult to recover *G. max* phenotype in F_2. Carpenter and Fehr (1986) reported that at least 2-3 backcrosses with *G. max* were needed to recover agronomically acceptable genotypes. The species belonging to sub genus Glycine do not cross easily with cultigen.

Inter-sub generic hybrids have been obtained through embryo rescue technique. Crosses between perennial and annual *Glycine* results in sterile progenies (Broué *et al.*, 1982). Doubling the chromosome number of *G. max* x *G. tomentolla* hybrid produced seed (Newell *et al.*, 1987). Singh *et al.* (1993) reported fully fertile progeny by subsequent backcrossing in this hybrid. Because of these difficulties, wild perennial have not been exploited in soybean breeding programmes.

Hybrid soybean cultivars

Palmer *et al.* (2001) reported that on the basis of many requirement for successful development of hybrid namely heterosis levels better than pure line; a stable male sterile- female fertile system; an efficient pollen transfer mechanism and economic level of seed increase, soybean appears to be a promising crop for future hybrid development. Some hybrids show 10-20% high parent heterosis, enough to make hybrids attractive. A number of nuclear and cytoplasmic nuclear mutants have been reported, some of them being temperature / photoperiod sensitive. An efficient pollen transfer mechanism is a major obstacle till date. Insect mediated pollen transfer coupled with co-segregation method or cytoplasmic nuclear system may generate large quantities of seed for testing of F_1 hybrids. The commercial success of hybrid will depend upon degree of heterosis and cost of F_1 seed.

Comparison of breeding methods

In a comparison of family and line breeding methods for elevated palmitate, Bravo *et al.* (1999) concluded that former despite being more laborious was at par with later. Byron and Orf (1991) found single seed descent most efficient for developing early maturing cultivars. Degago and Caviness (1987) evaluating four bulks concluded that bulk method is effective in improving yield at locations where there is a consistent natural selection pressure from disease. Early generation testing can effectively reduce the number of progenies to be carried forward. Cooper (1990) described a method where single location, single replication data are used in selecting F_2 : 3 through F_2: 4: 6. Cober and Voldeng (2000) showed that average yield and protein content of single cross and back cross lines were not significantly different. Back cross was successfully used by Le Roy *et al.* (1991 b) to introgress genes for small seed size from *G. soja* to *G. max*. The recurrent selection is cumbersome in soybean due to difficulty in intermating. Lewers and Palmer (1997) devised a method by using linkage of male sterile gene MS6 to flower color W1 and its pleiotropic hypocotyl pigmentation. White flower green hypocotyl male sterile plants ms6ms6w1w1 are identifiable at an early stage. Male sterile facilitated breeding has been successfully used to release nine high yielding cultivars.

Biotechnological or innovative approaches

Soybean is highly amenable to genetic manipulation using molecular techniques. Soybean genomics has been well studied and most of the molecular genetic tools are available in the crop. Soybean genome is of average size 1.1 Mb/c of which 40-60% is repetitive (Gold berg 1978). The detailed physical contigs have been developed using bacterial artificial chromosome (BAC) libraries (Marek and Shoemaker 1997). Gene and Gene sequences have been identified using more than 1.2 lakh expressed sequence tags (EST). The two major approaches in application of molecular technique in soybean improvement are (i) use of marker assisted selection, (ii) development of transgenic. The success in these areas was possible due to availability of a densely saturated genetic map and a well develop transformation system.

DNA markers and molecular map in soybean breeding

The first RFLP based map of soybean was published by Keim *et al.*, in 1990. This map was constructed using a population from cross of cultivated and wild species. Rafalskai and Tinkley (1993) developed an extensive RFLP map with more than 600 loci. Due to low polymorphism of RFLP, SSR markers with high level of allelic variation have been preferred. Akkaya *et al.* (1992) reported eight SSR alleles at one locus in a set of 38 G max and 5 *G. soja* genotypes. Large collection of EST data can be used to develop SSR markers for expressed genes. Cregan *et al.* (1999) developed SSR map based on 600 loci in three populations.

Using these markers an integrated genetic linkage map of soybean was developed by Cregan *et al.* (1999). This map was developed by using three F_2 population of *G max* X *G soja*, RILs of Minsoy X Noir and F_2 derived NILs of Clark X Harsoy. This map consists of 2610 markers comprising 1237 SSR, 805 RFLP, 67 RAPD, 11 AFLP and 27 Classical markers.

Molecular markers have been used to map the genomic location of both major genes and QTL for many agronomic physiological pest resistance and seed composition traits (Table 5). There are approximately 319 QTL reported for various quantitative traits. The proportion of QTL conditioning 10% or more phenotypic variation was 51%. Soybean scientists have selected traits of relatively high heritability and high economic importance. Although seed yield is of highest priority, its low heritability, requirement of extensive data collection, expectant large number of

QTL with small effect have limited the number of QTL identified for this traits.

TABLE 5: Molecular markers for economically important traits in soybean

Characters	Marker	Trait
Disease resistance	RAPD	Frog eye leaf spot, Soybean cyst nematode, Rust, Seedling rot, Sclerotium root rot, Sudden death syndrome
	RFLP	Brown stem rot, Soybean cyst nematode, Southern root knot nematode, Sclerotium root rot
	SSR	Fusarioum leaf spot, Soybean cyst nematode and Sclerotium root rot
Resistance to abiotic stresses	RAPD	Water logging
	AFLP	Salt tolerance water logging
	RFLP	Water use efficiency, Water logging, Aluminum tolerance, Iron deficiency
	SSR	Drought tolerance, Water use efficiency, Iron deficiency
Agronomic Traits	RAPD	Seed size yield
	AFLP	Yield
	RFLP	Seed size logging, Plant height, Leaf size, Maturity, Flowering, Photo period insensitivity
	SSR	Seed size, Yield, Logging, Plant Height, Flowering, Pod shattering, Photo period insensitivity
Quality traits	RFLP	Protein content, Oil content, Fatty acids
	SSR	Oil content, Lipoxygenase
Seed characteristics	AFLP	Seed longevity
	RFLP	Hard seededness, Seed longevity
	SSR	Hard seededness, Seed longevity

Markers have been used in selection of parents (Kisha *et al.*, 1997); back crossing (Frisch *et al.*, 1999) and recovery of recurrent parent (Hospital *et al.*, 1992) and selection in segregating populations. A large number of studies have concluded that Marker Assisted Selection (MAS) could be more effective when the populations are large and traits have low heritability (Zhang and Smith 1993). The first trait in soybean to be selected was resistance to Soybean Cyst Nematode (SCN). Although resistance to SCN is quantitative, few major QTL control a large proportion, for example rhg1 controls 36-86% of variability. Mudge *et al.* (1997) using 2 SSR markers flanking rhg1 identified 98% lines resistance to SCN. Meksem *et al.* (2001a) found additional markers linked to rhg4. These markers are now routinely used in screening against SCN. SSR markers have been used to map the location of QTL controlling resistance to corn ear worm in soybean. Narvel *et al.* (2001) observed that a region on LG-M contains a major QTL which is retained in 13 out of 15 populations selected for resistance phenotypically. Similarly this technique will be useful for other traits in coming years.

Soybean regeneration

Pre-requisite for transformation is regeneration of fertile plants from cultured cells or tissues. *In vitro* regeneration either by organogenesis or somatic embryogenesis is possible in soybean depending on source tissue and growth regulator regime.

***Organogenesis*:** Cheng *et al.* (1980) first reported organogenesis from cotyledonary explants derived from soybean seedlings. Subsequently immature leaves (Kim *et al.*, 1990), hypocotyls sections (Dan and Reichart 1998) and embryonic axes (McCabe *et al.*, 1988) have been reported to undergo organogenesis. Soybean embryonic axes have the advantage of needing minimal tissue in culturing and providing readily available ex-plants source.

***Somatic Embryogenesis*:** Soybean somatic embryogenesis was first reported in suspension cultures obtained from hypocotyl derived callus (Gamborg *et al.*, 1983). The most embryogenic tissue identified was immature cotyledon (Lippmaan and Lippmaan 1984). The other tissues reported to be embryogenic are, embryonic axes, microspores and embryonic leaves. Shoot apices excised from seed and grown *In vitro* using shoot elongation media from organogenesis protocol have let to recovery of transgenic plants (McCabe *et al.*, 1988).

DNA delivery

Both Agrobacterium tumefaciens and DNA coated micro projectile have been used in soybean. Cotyledonary node and to some extent somatic embryos have been good targets for Agrobacterium (Yan *et al.*, 2000). While somatic embryos and shoot apices are used as targets for microprojectile. Agrobacterium mediated transformation combined with organogenesis have been successfully used in various studies. Di *et al.* (1996) used the cotyledonary node transformation system to introduce bean pod mottle virus coat protein gene for viral resistant. Somatic embryo cultures undergoing repetitive proliferation are partially amenable to transformation via micro projectile (Trick *et al.*, 1997). The problem associated with both the process is high number of transgene copies which can be controlled by decreasing amount of DNA.

Transgenics

The most widely adopted transgenic in agriculture namely Roundup Ready (RR) soybean has been developed in soybean. Presently 80-90% area in USA and Argentina is under RR soybean. Glyphosate, the active ingredient in Round up is a non-selective herbicide. It acts on plastid located enzyme EPSP synthase and inhibits aromatic amino acid production. A Glyphosate tolerant EPSP synthase gene isolated from Agrobacterium CP4, aro A was used to transform variety Asgrow via micro projectile bombardment. RR trait has been incorporated in majority of breeding programmes and more than one thousand RR line have been developed. Initial reports indicated no negative impact on agronomic performance however later, contradictory reports came in. The lack of true NILs with and without RR gene has made it impossible to determine whether observed effects are due to linkage drag or pleiotropic effect of transgene.

Another commercially available transgenic soybean is a high oleic acid line developed by Du Pont Company. Oleic acid which normally constitutes 22% of total fatty acids was increased up to 80% with insertion of FAD 2-1 gene (Buhr *et al.*, 2002). Soybean has been transformed with Bt genes. Stewart *et al.* (1996) developed a line by transforming cv. Jack expressing Cry1AC gene showing resistance to corn ear worm, soybean looper and velvet bean caterpillar. Several Bt lines have been evaluated in USA, Argentina and Brazil and found resistant to Lepidopteron defoliators. In addition to these, several other traits have been engineered which include disease and insect resistance, protein/oil quality and pharmaceutical traits. The GE soybeans with neutraceutical and quality traits will be used as specialty soybean.

9. IMPORTANT ACHIEVEMENTS

The introduction of soybean as modern cultivated crop started in 1963-64 with feasibility trials conducted at Pantnagar and Jabalpur using American varieties like Bragg, Clark-63, Davis and Lee etc. The All India Coordinated Research Project on Soybean improvement started in 1967 at Pantnagar. Subsequently a number of introductions from U.S.A. like Bragg, Hardee, Lee etc were released for cultivation. Till 1980 most of the varieties were either introductions or [illegible] These have been called as varieties of 'Selection cycle-1". During

1981-90 exotic varieties were used as parents to generate new variability for selection. The varieties developed since 1990 have been grouped in 'Selection cycle-2'. The varieties of selection cycle-1 have produced 4 times higher yield than indigenous variety Kalitur by virtue of high number of pods per plant and seed weight, short duration and increased biomass. The varieties in selection cycle-2 showed 19% higher yield than selection cycle-1 varieties. This was due to improvement in harvest index and seed filling duration.

The number of soybean varieties released so far is 98. This includes central as well as state releases. A majority of Indian varieties have been developed using exotic parents. Depending on their breeding history, the Indian varieties can be grouped into two. The first group comprises varieties *viz.* Bragg, Lee, Improved Pelican, Hardee, Monetta, Shilajeet, Co 1, Gujarat Soy 1, Gujarat Soy 2, VL Soy 2 and JS 71-05 which owe their evolution to direct selection from exotic and indigenous material. The second group comprises a bulk of the Indian varieties which were developed through hybridization and mutation in/among the varieties of the first group.

10. FUTURE PROSPECTS AND THRUST AREAS

The challenge before the soybean breeders is to integrate conventional and molecular techniques in existing cultivar development programmes. The fast pace of developments in molecular techniques will enhance the genetic improvement of soybean. The use of PCR markers with high throughput genotyping instruments will increase the efficiency of MAS. Single nucleotide polymorphism (SNP) markers are the next generation marker to be used in MAS. Expressed Sequence Tags (EST) are vital in study of gene function, soybean scientists plan to generate 3 lakh EST and deposit them into public data base. The plant genome project aims to identify 30000 unique genes in soybean. The global warming and rising temperate have made breeding for drought tolerance a high priority. Much of the transgenic and molecular research in breeding for drought tolerance has focused on osmotic adjustment. The use of QTL mapping for pyramiding the drought tolerant traits will receive increased attention. Soybean had a head start in transgenic development with RR soybean being the most important GE crop. The genes for other economic traits will be increasingly used for engineering the desired varieties. The pyramiding of transgenes and QTLs identified for the same trait will enhance the expression of the trait. Besides these approaches, new conventional techniques like hybrid soybean, population improvement may receive greater attention.

QUESTIONS

1. Describe the breeding objective of soybean for developing disease resistance and drought resistance. Discuss the source of resistance?
2. What Molecular markers are used to improve the economic traits in soybean?
3. Elaborate the difference between determinate and indeterminate flowering in soybean?
4. Discuss the developing technology for transgenic plants in soya bean and what sources are available for its development?
5. What is relationship between wild and cultivated progenitor and how we can utilize these progenitor for developing superior variety with resistant to insect, disease, drought and improving quality of the crop?
6. What is the reason for popularity of transgenic crops and which genes are using for developing the transgenic variety?

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ABOUT THE AUTHOR

Dr. D.N. Bharadwaj was born in 1952, graduated from Agra University in 1971 and post-graduated from Kanpur University in 1973 with the specialization in Cytogenetics and Crop Breeding. Since 1974 he started working as lecturer in Kanpur University and taught UG and PG students alongwith his research work. In 1978 author was awarded USSR Government Higher Education Fellowship and in 1983 he acquired his Ph.D. degree in Biological Sciences from Academy of Sciences USSR. During his stay in USSR he visited several reputed Institutes and Universities of USSR, Asia and European countries. He has published more than forty good quality research papers in Indian and International journals and presented papers in conferences, symposium and seminars.

In 1983 Dr. Bharadwaj was selected as 'Pool Officer' of CSIR and placed in the Department of Genetics & Plant Breeding of C.S. Azad University of Agriculture & Technology, Kanpur. Further he was appointed as Asst. Professor and started research and teaching of UG and PG students in the area of Genetics, Plant breeding, Cytology, Cytogenetics, Biotechnology and Molecular Genetics etc. Besides this about ten years he worked as Seed Production Officer and has been engaged in production, processing, packing and marketing of seeds of various crops with all categories i.e. nucleus, breeder, foundation and certified seeds.

During the period of 2004-2006 Dr. Bharadwaj worked as visiting Professor of Genetics in the Department of Plant Sciences, Haramaya University, Ethiopia (Africa) under United Nations Development Program. Further during 2008-2010 he was again selected as visiting Professor of Biology in the Department of Biology, Eritrean Institute of Technology, Asmara, Eritrea (N.E. Africa) under World Bank Educational Development Program.

During his 38 years of teaching career Dr. Bharadwaj taught in five universities of three continents and supervised more than 30 M.Sc. (Ag) theses and five Ph.D. theses. He is also author of 'A text book on molecular genetics' (Published by Kalyani Publishers, Ludhiana).

ABOUT THE BOOK

The present book 'Breeding Field Crops' is a very tedious and comprehensive work of more than a hundred renowned Agriculture Scientists of ICAR (Project Directors, Project Coordinators and Plant Breeders of specific crops) and Professors of State Agricultural Universities of India who are engaged in teaching and research of specific crops. This book covers all the major field crops and several minor crops of economic importance growing all over the world. In case of some of the very important crops the literature is not available in any of the 'Field Crop' books. This book 'Breeding Field Crops' will be a milestone and useful for the UG, PG students, Researchers of various Institutes and Professors of Colleges and Universities of India and abroad. In this book chapters are well designed to cover all traditional and most recent innovative (molecular and biotechnological) approaches that are recently employed to the breeding of field crops.

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Behind Nasrani Cinema, Chopasani Road, Jodhpur - 342 002
Ph.: +91-291-2643993, 2643994, Fax: 2642319
E.Mail: agrobios@sify.com, Website: agrobiosindia.com

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