ISSN 0973-1830

Volume 1: 2003

SOYBEAN RESEARCH

Society for Soybean Research and Development
Directorate of Soybean Research
Khandwa Road, Indore 452 017
Madhya Pradesh, India

Society for Soybean Research and Development

(Founded in 2003)
(Registration No. 03/27/03/07918/04)

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Qualitative Genetics of Soybean - A Review

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Soybean [*Glycine max* (L.) Merr.] has been considered as an oilseed crop next to groundnut rapeseed mustard in India. It contains 17-19 per cent oil 38-43 per cent proteins thus; it is an oilseed crop as also a pulse crop. Before introduction of soybean germplasm, black soybean has been under cultivation since ages under different names, viz., *Kalitur, Bhat, Bhatmash, Ramkulthi, Kalikulthi,* etc. in the foot hills of Himalayas scattered pockets of Madhya Pradesh, Maharashtra, and and Karnataka. Spread of soybean in India appeared to be in the last two decades, particularly since the edible oil crisis in the country was experienced. Expansion of soybean cultivation in India is due to its importance as a source of oil, deoiled cake (which is mostly exported), low priced source of high quality proteins, other by-products, and establishment of market and extraction plants. With the result there has been gradual increase in area (6.0 million ha) and production (5.6 million tones) of soybean. Madhya Pradesh state, which has the lions share in soybean production, is often referred as 'Soybean State'. It was followed by Maharashtra, Rajasthan and Karnataka. Since 1985, there was steady increase in area, production and productivity in the state of Maharashtra. Though, India is the fifth country in the world as regards area, the average productivity is very low i.e. 9 to 10 quintals/ha. This is 40 per cent of the world productivity (23.38 quintals/ha), 75 per cent of the Asian average (13 quintals/ha). However, many farmers from Maharashtra state have harvested 45-50 quintals/ha, suggesting the high potential yield that can be obtained. This considerable difference in yield appears to be due to recommended crop management practices may not be followed.

Centre of Origin

Piper and Morse (1923) considered soybean to be native of Eastern Asia. According to them, *Glycine ussuriensis*, the progenitor of *G. max* was known to occur in China, Manchuria and Korea. However, 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.

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Central Western China is considered as the centre of origin for soybean (Vavilov 1949/50). Nagata (1959, 1960) suggested North Central regions of China to be the centre of origin. He based his conclusions on the distribution of *G. ussuriensis,* which is considered as progenitor of *G. max*.

According to Hymowitz (1970) *G. ussuriensis* grows wild in Korea, Japan, Taiwan, throughout the Yangtze River valley, the North-eastern Provinces of China and the adjacent areas of USSR. Hence, it is generally agreed that soybean as native of eastern Asia.

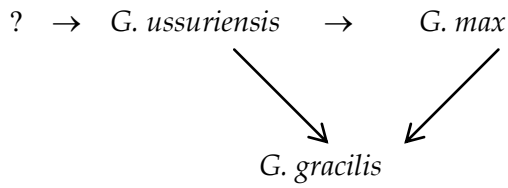
Origin of *Glycine max*

G. max is suggested to be a polyploid derived from one or more diploid ancestors with 2n=20. However, no such form has been found in *G. soja. G max* appears to favour a polyploid origin due to its chromosome number. Many species in the subfamily Papilionoideae having basic numbers of x = 8, 10, 11, 12 (Darlington and Wylie, 1955). Even inheritance of several characters found to be controlled by duplicate factors suggesting the polyploid nature of *G. max.*

Cultivated soybean appears to have originated from *G. ussuriensis* (now it is *G. soja*). Fukuda (1933) suggested the differentiations between wild and cultivated forms involved *G. gracilis* in the following manner.

? → *G. ussuriensis* → *G. gracilis* → *G. max.*

However, Hymowitz (1970) is of the opinion that *G. gracilis* is a result of introgressive hybridisation between *G. ussuriensis* and *G. max,* rather than being an intermediate step between *G. ussuriensis* and *G. max*. This process can be expressed as:



General Review of Taxonomy and Phylogeny

The name *Glycine* was originally introduced by Linnaeus in the first edition of his `*Genera Plantarum*' (Linnaeus 1737). He has described the soybean plant in his '*species plantarum*' (Linnaeus 1753) and *Phaseolus max*. Later he changed it to *Dolichos soja*. However, other botanists did not agree with this nomenclature. Several alternative names have been suggested: *Soja hispida* (Moench 1794), Soja *japonica*, *Soja*

angustifolia, *Glycine ussuriensis* (Regel and Maack 1861), *Glycine hispida*, *Glycine soja* (Siebold and Zuccarini 1846), *Soja max* (Piper 1914). After considerable controversy, the botanical name *Glycine max* proposed by Merrill in 1917 has been accepted for the cultivated soybean species.

Qualitative characters are considered as marker characters in the identification of soybean species and varieties, which are less, influenced by environmental variations. During the last two decades a large number of varieties have been developed with the result it is most essential to have the marker characters for them. Thus, the genetic constitutions of such marker characters have obvious importance. Earlier work on inheritance and linkage studies of qualitative characters have been periodically reviewed by Owen (1928), Woodworth (1932), Bernard and Weiss (1973), Palmer and Kilen (1987) and Halvankar *et al.* (1990).

Growth habit

Monogenic control with indeterminate growth habit dominant over determinate growth habit	Woodworth (1923) Woodworth (1933) Ting (1946) Bernard (1972) Halvankar and Patil (1994) Raut *et al.* (1994)
Digenic control.	Bernard (1972)
Viny and lodging habit in cultivated soybean was genetically controlled by two independent recessive loci	Peng *et al*. (1999)
Growth period	
Monogenic, with late maturity dominant over early maturity	Woodworth (1923)
Monogenic, with early flowering partially dominant over late flowering	Gilioli *et al.* (1980)
Maturity controlled by few major and several minor genes. In some cases earliness dominant, in others it is recessive	Singh and Anderson (1949)
No dominance for days to flowering in most of the crosses	Moro *et al.* (1993)
Earliness was dominant	Moro *et al.* (1993)
Earliness in days to maturity was dominant	Moro *et al.* (1993)
Late flowering (long juvenility) controlled by single recessive gene	Ray *et al*. (1995)

Hypocotyl colour	
Monogenic, with purple colour dominant over green	Probst (1950) Bhatt (1966) Miku (1970) Khadilkar and Ingole (1974) Raut *et al*. (1982) Raut *et al*. (1984) Halvankar (1988) Halvankar and Patil (1994) Raut *et al*. (1994)
Digenic, with complementary gene action	Chaudhari *et al*. (1972)
Monogenic, wide range of pigment intensities suggest presence of several minor genes	Peters (1982)
Short petiole	
Monogenic, short-petiole dominant over longer one	Soare and Denceseu (1994)
Monogenic, short petiole incompletely dominant over normal	Cary and Nickell (1999)
Controlled by one or more recessive genes	You *et al*. (1995)
Two duplicate recessive genes responsible for short petiole	Gai *et al*. (1996)
Branching type	
High branching is dominant over low a branching with two complementary genes (9:7)	Nelson (1996)
Zig-zag stem is monogenic recessive to normal	Gai and Zhao (1996)
Petal colour	
Monogenic, with purple colour dominant over white	Piper and Morse (1923) Woodworth (1923) Probst (1950) Mohmud and Probst (1953) Bhatt (1966) Miku (1970) Khadilkar and Ingole (1974) Raut *et al*. (1982) Ruat *et al*. (1984) Ala A. YA (1989) Halvankar and Patil (1994) Raut *et al*. (1994)
Pink flower colour controlled by a single recessive gene	Stephens and Nickell (1992)

Digenic control with complementary gene action, purple colour dominant over white	Chaudhari *et al*. (1972)
Digenic, with supplementary gene action	Takahashi Fukuyama (1919) Nagai (1926)
Governed by three pairs of major genes and purple colour being dominant over white	Hartwig and Hinson (1962)
Nodulation	
Single dominant for nodulation	Raut *et al.* (1999)
Single recessive gene responsible for super nodulation (hyper-nodulation)	Pracht *et al*. (1993) Kokubum and Kaos (1994) Buttery and Buzzell (1998)
Non-nodulation is epistatic over super nodulation	Mathews *et al*. (1990)
Leaflet shape	
Monogenic, with broad leaves dominant over narrow leaves	Takahashi (1934) Sawada (1988)
Monogenic, with normal leaves dominant over narrow leaves	Probst (1950)
Monogenic, broad leaves incompletely dominant over narrow leaves	Leshchenko and Sichkar (1979)
Monogenic, with lanceolate leaves dominant over ovoid leaves	Halvankar and Patil (1994)
Monogenic, recessive in case of sinuate leaflet	Tharp *et al.* (1997)
Monogenic, with normal (ovate) leaves dominant over oval and narrow, digenic with supplementary gene action, normal being dominant	Domingo (1945)
Leaflet number (Heterophylly)	
Controlled by single gene difference with variation in dominance	Fehr (1972)
Observed digenic control	Takahashi and Fukuyama (1919)
Heterophyllus condition governed by three recessive duplicate genes (63:1)	Raut *et al*. (1986)

Leaf abscission	
Monogenic, normal abscission being dominant over delayed one	Probst (1950)
Yellow foliar mutant	
Uniparental, maternal cytoplasmic inheritance	Cianzio and Palmer (1992)
Pod colour	
Monogenic, with dark brown pod colour being dominant over straw colour	Piper and Morse (1910)
Monogenic, with dark brown pod colour being dominant over light brown colour	Takahashi and Fukuyama (1919) Woodwarth (1923) Kawahara (1963)
Monogenic, with brown pod colour dominant over tan	Raut *et al.* (1994)
Monogenic, with black colour dominant over light brown colour	Woodworth and Veatch (1929) Tang and Chen (1959)
Monogenic, with black colour dominant over yellow colour	Ting (1946)
Monogenic, with brown dominant over tan and black dominant over brown	Chaudhari *et al.* (1972) Chaudhari *et al.* (1973)
Monogenic, with black pod colour dominant over brown colour, brown dominant over tan and black colour dominant over tan	Bernard (1967) Halvankar and Patil (1994)
Monogenic, yellow dominant over ash colour	Khadilkar and Ingole (1974)
Digenic, with duplicate factors, brown dominant over yellow	Raut *et al.* (1984)
Digenic, with black epistatic to brown and tan	Bernard (1967) Halvankar and Patil (1994)
Pod shape	
Monogenic, bloated pod shape being dominant over flat pods	Chaudhari *et al.* (1973)
Pubescence colour	
Monogenic with tawny pubescence dominant over gray pubescence	Owen (1927 b) Mohmud and Probst (1953)

	Bhatt (1966) Chaudhari *et al*. (1972) Chaudhari *et al*. (1973) Halvankar (1988) Ala A Ya (1989) Schori and Gass (1994) Raut *et al.* (1994)
Monogenic, with brown dominant over gray pubescence	Ting (1946)
Monogenic, with yellow dominant over white	Miku (1970)
Monogenic, with orange brown dominant over gray	Khadilkar and Ingole (1974)
Monogenic and digenic, with inhibitory action	Probst (1950)
Digenic, with inhibitory action	Williams (1938)
Digenic, with duplicate factors, brown being dominant over yellow	Raut *et al*. (1984)
Pubescence on pods/pubescence type	
Monogenic, with glabrous pods dominant over pubescent ones	Nagai and Saito (1923) Owen (1927 b) Raut *et al.* (1994)
Monogenic, with pubescent type dominant over glabrous	Stewart and Wentz (1926) Halvankar and Patil (1994)
Monogenic, with glabrous, dense and sparse pubescence dominant over normal pubescence and normal pubescence completely dominant over puberulent, incompletely dominant over curly	Bernard and Singh (1969)
Digenic, with inhibitory action	Woodworth and Veatch (1929) Halvankar *et al.* (1999)
Pod shattering	
Pod shattering dominant over non-shattering	Ting (1946)
Incomplete dominance of pod shattering	Tsuchiya and Sunada (1979)
Monogenic and digenic, susceptibility to shattering incompletely dominant	Tsuchiya (1986)
Monogenic, with non-shattering dominant over shattering.	Halvankar (1988)

Duplicate dominance, trigenic, with any two of the three genes complementary, in both the cases shattering dominant	Halvankar (1988)
Two genes for pod shattering. Pod shattering dominant over non-shattering	Karsakov and Bulakh (1978)
Susceptibility is dominant or partially dominant to resistance.	Tiwari and Bhatnagar (1991)

Seed coat colour

Monogenic, with black dominant over brown First report	Piper and Morse (1910)
Black dominant over brown and brown over reddish-brown	Nagai (1921)
Monogenic, with black dominant over brown	Owen (1927 b)
Monogenic, with black dominant over brown, red-brown and imperfect black; brown over red-brown and reddish brown over buff	William (1952) Seo *et al.* (1993)
Monogenic, black dominant over brown and yellow dominant over black brown	Miku (1970) Mikhailov (1985)
Monogenic, black dominant over green yellow, reddish brown dominant over yellow	Chaudhari *et al.*(1972) Chaudhari *et al.* (1973)
Monogenic, with green being dominant over yellow	Terao (1918) Woodworth (1921) Piper and Morse (1923) Owen (1928) Williams (1938) Probst (1950)
Monogenic, green dominant over yellow and brown dominant over reddish-brown	Bhatt and Torrie (1968)
Monogenic, yellow being dominant over black mottled	Raut *et al.* (1984)
Monogenic, yellow dominant over black colour	Mannur *et al.* (1992) Raut *et al.* (1994)
Monogenic, yellow dominant over brown	Halvankar (1988)
Digenic, yellow dominant over black brown	Mikhailov (1985)
Digenic, with epistatic gene action, black seed coat is epistatic to imperfect black and buff	Probst (1950)
Digenic, with complementary gene action where imperfect black being dominant over buff	Williams (1952)

and brown; epistatic gene action where black is epistatic to imperfect black and buff	
Digenic, with epistatic gene action where yellow epistatic to brown and buff; supplementary gene action where yellow supplements the action of reddish-brown to produce brown seed coat	Bhatt and Torrie (1968)
Digenic, with supplementary gene action where yellow supplements the action of black to produce green seed coat	Halvankar and Patil (1994)
Digenic, with epistatic gene action, yellow epistatic to black and brown	Khadilkar and Ingole (1974) Halvankar (1988) Raut *et al.* (1994)
Trigenic, black dominant over imperfect black, brown and buff	Williams (1952)
Trigenic, yellow dominant over black and brown	Mikhailov (1985)
Quadrigenic, with green dominant over yellow, black and brown	Ting (1946)
Hilum colour	
Monogenic, black being dominant over imperfect black	Probst (1950)
Monogenic, yellow dominant over buff	Bhatt and Torrie (1968)
Monogenic, black dominant over brown	Raut *et al.* (1984) Halvankar (1988) Raut *et al.* (1994)
Digenic, with complementary gene action, black being dominant over light brown	Ting (1946)
Digenic, with complementary gene action, black being dominant over brown	Raut *et al.* (1994)
Digenic, with black dominant with epistatic gene action, yellow being epistatic to brown and buff	Bhatt and Torrie (1968)
Monogenic, digenic and trigenic control, gray colour dominant over all other colours, black dominant over brown, yellow dominant over brown and black and state gray dominant over light brown	Mikhailov (1987)
Trigenic, black being dominant over brown, imperfect black and buff	Bhatt and Torrie (1968)

Trigenic with complementary gene action, black being dominant over brown	Raut *et al.* (1994)
Quadrigenic, gray being dominant over black, brown, imperfect black and buff.	Mohmud and Probst (1953)
Defective seed coat	
Monogenic, normal seed coat being dominant over defective one	Ting (1946) Liu (1949)
Digenic, with complementary gene action, normal being dominant over defective	Nagai (1926)
Digenic, with duplicate factors, normal seed coat dominant over defective one	Stewart and Wentz (1930) Probst (1950) Halvankar and Patil (1994)
Digenic, with complementary gene action with duplicate factors	Liu (1949)
Trigenic, with three duplicate factors, normal seed coat dominant over defective one	Liu (1949)
Trigenic, with one basic and two complementary genes	Halvankar (1988)
Seed permeability	
Monogenic, with both impermeability and permeability dominant in different crosses	Shahi and Pandey (1982)
At least three major genes control permeability, impermeability dominant over permeability	Kilen and Hertwig (1978)
Seed size	
Large seed dominant over small one	Miku (1966)
Monogenic control with bold seeds dominant over small	Raut *et al.* (1994)
Monogenic, flat shape of seed dominant over round one	Halvankar (1988)
Cotyledon Colour	
Monogenic inheritance of yellow colour	Owen (1927 a)
Monogenic, yellow cotyledon colour dominant over green	Woodworth (1928)
Monogenic, either yellow or green being dominant over whitish cotyledon	Chaudhari *et al.* (1973)

Digenic, with duplicate factors yellow being dominant over green	Woodworth (1921) Owen (1927 a) Woodworth (1928) Veatch and Woodworth (1930)
Maternal inheritance of yellow colour	Owen (1927 a)
Maternal inheritance for both green and yellow cotyledon colour	Terao (1918) Piper and Morse (1923)
Male-sterility	
Male and female sterile mutant is a single recessive gene	Ilarslan *et al.* (1999) Stelly and Palmer (1980) Palmer *et al.* (1980) Delannay and Palmer (1982)
Oil content	
Digenic, complementary for high oil content	Raut *et al.* (1994)
Linolenic acid	
Monogenic control with normal dominant over high linolenic acid	Rahman *et al.* (1994) Rahman and Takagi (1997)
Oleic acid	
Monogenic partially dominant with 1 normal : 2 intermediate : 1 high classes	Takagi and Rahman (1996)
Palmitic acid	
At least four independent loci controlling palmitic content	Schnebly *et al.* (1994)
Anomalous flavonol glycosides	
Monogenic, with anomalous dominant	Buzell and Buttery (1988)
Isoenzymes	
Single dominant gene is responsible for the presence of acid phosphatase.	Gorman and Kiang (1977) Hilderbrand *et al.* (1980)
Monogenic, dominant for the presence of alcohol dehydrogenase.	Gorman and Kiang (1978)

Monogenic, dominant for the presence of lipoxygenase.	Hilderbrand and Hymowitz (1981,1982)
Isocitrate dehydrogenase is controlled by single dominant gene	Yong *et al.* (1981,1982) Gorman *et al.* (1983)
Phosphoglucomutase is controlled by single dominant gene	Gorman *et al.* (1983)
Monogenic, dominant for the presence of superoxide dismutase (SOD)	Gorman and Kiang (1978) Gorman *et al.* (1982, 1984) Griffin and Palmer (1984) Zhuang *et al.* (1992)
Kunitz trypsin inhibitor is governed by co-dominant multiple alleles at single locus.	Singh *et al.* (1969) Hymowitz and Hadley (1972) Orf and Hymowitz (1977, 1979)
Lectin	
Single dominant gene is responsible for the presence of lectin	Orf *et al.* (1978) Pull *et al.*(1978) Stahlhut and Hymowitz (1980)
Cold resistance during flowering	
Monogenic, with resistance being dominant over susceptible	Mar'Yushkin and Mikhailov (1988)
Herbicide reaction	
Bentazon-sensitive reaction was controlled by a single recessive allele	Bernard and Wax (1975)
Sensitivity to Metribuzin was controlled by single recessive allele	Edwards *et al.* (1976)
Diseases	
Resistance to purple seed stain (*Cercospora kikuchii*)	
Monogenic resistance, dominant over susceptibility	Srisombun and Supapornhemin (1993)
Resistance to brown stem rot *(Phialophora gregata)*	
Digenic, resistance is dominant	Lohnes and Nickell (1995)

Resistance to powdery mildew	
Controlled by single gene with resistance being dominant	Buzzell and Haas (1978) Lohnes and Bernard (1992)
Resistance to downy mildew	
All races were controlled by a single dominant allele	Bernard and Cremeens (1972)
Resistance to *Peronospora manshuria*	
Monogenic dominant in case of race 2 and 33	Lim and Bernard (1984)
Resistance to stem canker	
Monogenic (3:1), digenic (15:1) and Trigenic (63:1) control with resistance being dominant	Bowers *et al.* (1993)
Frogeye leaf spot	
One dominant allele controlled resistance to race-1 and race-2	Athow and Probst (1952) Probst *et al.* (1965) Bernard and Weiss (1973)
Phytophthora rot	
Monogenic resistance dominant over susceptible	Hartwig *et al.* (1968) Athow *et al.* (1980) Buzzell and Anderson (1981) Kilen and Tyler (1993) Jenks *et al.* (1999)
Controlled by three alleles giving different response to the three races of *Phytophthora*	Bernard and Weiss (1973)
Soybean rust	
Controlled by a single dominant allele giving resistance to an Australian rust isolate	McLean and Byth (1980)
Controlled by a single dominant allele for resistance	Singh and Thapliyal (1977) Hartwig and Bromfield (1983)
Phomopsis seed decay	
Digenic, with complementary action, resistance being dominant (9:7).	Zimmermann and Minor (1999)

Bacterial pustule	
Resistance to bacterial pustule was controlled by a single recessive allele	Hartwig and Lehman (1951) Feaster (1951) Bernard and Weiss (1973)
Bacterial blight	
A dominant allele was reported to control resistance to race-1	Mukherjee *et al.* (1966)
Resistance to soybean mosaic virus	
Single dominant gene is responsible for resistance. Two additional independent resistance gene may be also be involved	Shigemori (1988)
Monogenic dominant gene control the stem tip necrosis caused by SMV	Buzzell and Tu (1989)
Monogenic control with resistant being dominant	Koshimizu and Iizuka (1963) Kiihl and Hartwig (1979) Roane *et al.* (1983) Buzzell and Tu (1984)
Digenic control with two complementary genes with susceptibility being dominant	Koshimizu and Iizuka (1963)
Peanut mottle virus	
Resistance controlled by a single dominant allele	Boerma and Kuhn (1976)
Resistance controlled by a single recessive allele	Shipe *et al.* (1979)
Cowpea chlorotic mottle virus	
Resistance was controlled by a single dominant allele	Boerma *et al.* (1975)
Foliar feeding insects	
Resistance was quantitatively inherited with additive gene action of two or three major alleles	Sission *et al.* (1976)
Partial dominance for susceptibility with the action of only a few major alleles controlling resistance	Kilen *et al.* (1977)
Incomplete dominance of resistance to *Spodoptera littoralis* is at single locus	Ojo and Ariyo (1999)

Nematodes

A recessive allele controlled resistance to race-2	Caldwell *et al.*(1960) Hartwig and Epps (1970)
Resistance to race-4 was controlled by one dominant two recessive alleles	Thomas *et al.* (1975)
Resistance to a specific race was controlled by one major allele with at least one modifying allele	Boquet *et al.* (1975)
Resistance is controlled by a single major allele but one or more minor alleles may contribute to the reaction giving intermediate infection	Williams *et al.*(1981)
Cyst nematode is controlled by two dominant gene	Anand and Myers (1992)

Earlier data on linkage studies in soybean have been periodically reviewed and reported by Woodworth (1932), Weiss (1970*a*, *b, c, d* and *e*), Bernard and Weiss (1973), Palmer and Kilen (1987) and Halvankar *et al.* (1990). Available literature on linkage relationships among qualitative characters is briefly given below.

Linkage

Sr. No.	Characters linked	Recombination value (%)	Author/s
1.	Purple hypocotyl colour and purple flower colour	Pleiotrophy	Takahashi and Fukuyama (1919) Woodworth (1923) Bhatt (1966) Chaudhari *et al*. (1972) Raut *et al*. (1982)
		2.04 to 12.87	Raut *et al*. (1982, 1984)
		1.08 to 2.79	Halvankar (1988)
		15.19-34.06	Raut *et al*. (1994)
2.	Purple hypocotyl colour and pubescent pods	15.82 to 42.5	Halvankar (1988)
3.	Purple petal colour and pubescent pods	14.77 to 43.07	Halvankar (1988)
4.	Purple flower colour and magenta flower colour	2.2	Buzzel *et al*. (1977)
5.	Flower colour and alcohol dehydrogenase	23.7	Burzlaff and Palmer (1999)

6.	Broad leaves and 2 seeded pods	10.0	Takahashi (1934)
7.	Lanceolate leaflet and variegated leaves	35.6	Weiss (1970 c)
8.	Lanceolate leaflet and puberulence (minute pubescence)	26.4	Weiss (1970c)
9.	Lanceolate leaflet and interminate growth habit	29.53 to 31.26	Halvankar (1988)
10.	Lanceolate leaflet and non-shattering pods	29.43 to 38.00	Halvankar (1988)
11.	Determinate growth habit and yellow pod colour	36.0	Ting (1946)
12.	Determinate growth habit and brown pod colour	39.4	Weiss (1970d)
13.	Growth habit and pod colour	35.74 to 41.46	Halvankar (1988)
14.	Determinate growth habit and tawny pubescence	33.60 to 40.09	Halvankar (1988)
15.	Determinate growth habit and pod shattering	32.49 to 41.46	Halvankar (1988)
16.	Determinate growth habit and defective seed coats	34.07 to 35.25	Halvankar (1988)
17.	Chlorophyll deficiency (Y11) and dwarfness (df2)	12.1	Weiss (1970d)
18.	Chlorophyll deficiency (Y12) and earliness	20.2	Weiss (1970a)
19.	Chlorophyll deficiency and red brown seed coat	31.3	Weiss (1970e)
20.	Chlorotic seedling trait with ineffective nodulation	46.0	Devine (1992)
	Chlorophyll deficiency and multiple alleles determining the seed coat pattern of black and brown colour	41.1	Weiss (1970c)
21.	Male sterile female fertile mutant allelic to MS6 with flower colour locus	4.3	Ilarslan *et al*. (1999)

22.	Pod colour and pubescence colour	6.0	Owen (1927b)
23.	Brown pod colour and tawny pubescence	22.67 and 27.07 39.22 to 39.69	Chaudhari *et al*. (1972) Halvankar (1988)
24.	Brown pod colour and brown pubescence colour	8.08	Raut *et al*. (1984)
25.	Brown pod colour and reddish seed coat colour	22.66	Chaudhari *et al*. (1972)
26.	Brown pod colour and brown seed coat colour	44.82	Chaudhari *et al*. (1972)
27.	Brown pod colour *Phytophthora megasperma* reaction	27.0	Kilen and Tyler (1993)
28.	Black pod colour and green seed coat colour	22.40 to 38.63	Halvankar (1988)
29.	Black pod colour and green cotyledon colour	25.51	Chaudhari *et al*. (1973)
30.	Glabrous pod and mottling seed coat	18.0	Nagai and Saito (1923)
31.	Glabrous pod and black seed coat colour	12.0 20.0	Owen (1927b) Bernard and Singh (1969)
32.	Glabrous pod and black seed coat, black concentric stripping on brown seed coat	20.9	Weiss (1970b)
33.	Glabrous pod and defective seed coat pleiotrophy	22.0	Woodworth and Williams (1938)
34.	Tawny pubescence and early maturity	18.0	Owen (1927b)
35.	Tawny pubescence and lateness	Linked	Bernard (1971)
36.	Tawny pubescence and reddish seed coat	22.66	Chaudhari *et al*. (1972)
37.	Tawny pubescence and brown seed coat	44.82	Chaudhari *et al*. (1972)
38.	Tawny pubescence and yellow seed coat	32.65	Raut *et al*. (1994)
39.	Tawny pubescence and yellow cotyledon	13.0	Woodworth and William (1938)

40.	Tawny pubescence and black hilum	Pleiotrophy	Woodworth (1921) Owen (1928) Stewart (1930)
		25.91 to 29.64	Halvankar (1988)
		28.50 to 39.05	Raut *et al*. (1994)
41.	Tawny pubescence and pod shattering	24.40 to 31.63	Halvankar (1988)
42.	Gray pubescence and seedling chlorophyll deficiency (Y_{12})	21.6	Weiss (1970a)
43.	Gray pubescence and earliness	3.9	Weiss (1970a)
44.	Gray pubescence and defective seed coat	Pleiotrophy	Stewart and Wentz (1930) Woodworth and Williams (1938) Ting (1946)
45.	Gray pubescence and soft seed coat	37.91	Ting (1946)
46.	Gray pubescence and brown hilum	Pleiotrophy	Ting (1946)
47.	Gray pubescence and Am^3 (B-amylase)	31.88	Kiang and Chiang (1985)
48.	Brown seed coat and mottling of seed coat	30.0	Woodworth and Williams (1938)
49.	Red brown seed coat and multiple alleles determining the seed coat pattern of black and brown colours	17.8	Weiss (1970*c*)
50.	Brown seed coat and brown hilum colour	0.01 to 1.89	Halvankar (1988)
51.	Black seed coat and flat seed shape	31.29 to 36.72	Halvankar (1988)
52.	Green seed coat and defectiveness of seed coat	24.36 to 27.02	Halvankar (1988)
53.	Green seed coat and yellow cotyledon	12.5 13.0	Owen (1927a) Woodworth and Williams (1938)
		4.2	Weiss (1970b)
54.	Yellow seed coat and yellow cotyledon	14.07	Chaudhari *et al*. (1973)

55.	Yellow seed coat and seed permeability	Linked	Shahi and Pandey (1982)
56.	Yellow seed coat and black hilum colour	49.37	Raut *et al*. (1994)
57.	Bold seed size with black hilum colour	33.98	Raut *et al*. (1994)
58.	Oil content nodulation	0.49	Raut *et al.* (1999)

The above review of qualitative characters suggested that these characters are governed by monogenic, digenic, trigenic or tetragenic action of genes. Most of the characters showed remarkable constancy in their genetic constitutions e.g. hypocotyl colour, leaflet shape, leaf abscission, pod shape, pubescence colour, seed shape, seed size, seed permeability etc. However, inconstancy in the genetic constitutions observed is as noted below.

i) Shift from monogenic to digenic control
ii) Shift from monogenic to trigenic control
iii) Shift from monogenic to tetragenic control
iv) Shift from digenic to trigenic control
v) Shift from digenic to tetragenic control
vi) Shift from trigenic to tetragenic control

It is interesting to note that majority of the characters of economic importance are found to be governed by one or more pairs of genes. Some of the character combinations showed pleiotropic effects of genes. However, in others the recombination values for linked characters ranged from 0.01 to 49.37 per cent.

The above review on inheritance and linkage will help the plant breeder to understand the inheritance and linkage relationships of various qualitative characters of economic importance and their use as marker characters in the varietal identification as also in formulating a suitable breeding programme to evolve new superior varieties.

ACKNOWLEDGEMENTS

Author is grateful to the Director, A.R.I., Pune for providing facilities. Author is also thankful to Shri. Sanjay Rahangdale, Dr. G.B. Halvankar, Dr. S.P. Taware, and Dr. Philips Varghese for their help in preparing this review. This work has been carried out under 'All India Co-ordinated Research Project on Soybean' sponsored by the I.C.A.R., New Delhi.

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Soybean Research 1: 29-42 (2003)

Future Perspectives of Soybean in India

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The contribution of agriculture to Gross Domestic Product (GDP), at present, is parallel to that of industrial sector in India. After independence, the agricultural sector has exhibited a phenomenal growth in the country. The country has witnessed "Green Revolution" from late sixties on ward that was a landmark in Indian Agriculture resulting not only self-sufficiency in food grains but also in the export surplus produce. Subsequent "Yellow Revolution" was the result of enhanced pace in the development of Indian Agriculture in last two and half decades. This unparallel rapid growth in agriculture sector was possible only through the research back up provided by the scientists aided with positive policy support, concerted efforts from developmental workers involved in transfer of technology, increased contribution of private sector and progressive adoption of research emanated technologies by the farming community of the country. "Yellow Revolution" has a remarkable role of newly introduced crops like soybean and sunflower in the country. Although, the oilseed production, which uses to be 5.16 m t in 1950, is now 4 times, the country still is the largest importer of edible oil in the world. This calls for more concerted efforts to re-fix the priority on oilseeds over cereal grains to meet the enhanced demand on account of increased population and per capita consumption of oils.

Soybean [*Glycine max* (L.) Merrill], for a long time known as one of the principal food crops (Brooks 1966), has recently become of vital importance to the agricultural and oil economy of India. At present, the crop contributes about 10 per cent to the edible oil production in India. The country has been earning foreign exchange to the tune of Rs. 15, 000 million (US $ 320 million) each year by way of export of De-oiled cake (DOC). Soybean farming has revolutionized the rural economy and has resulted in improved socio-economic status of farmers. Globally, the country is placed at rank 5th in area and production of soybean after USA, Brazil, Argentina and China (Table 1).

**Principal Scientist*

Table 1. World situation of soybean

Country	Average 1997/98-2001/02			2002-03			Fore cast 2003-04		
	Area (m ha)	Production (m t)	Yield (kg/ha)	Area (m ha)	Production (m t)	Yield (kg/ha)	Area (m ha)	Production (m t)	Yield (kg/ha)
1. USA	28.93	74.75	2580	29.31	74.83	2550	29.36	67.18	2290
2. Brazil	13.96	36.10	2590	18.40	52.50	2850	21.00	60.00	2860
3. China	8.73	15.00	1720	8.72	16.51	1890	9.40	16.20	1720
4. Argentina	9.10	23.70	2600	12.60	35.50	2820	13.20	37.00	2800
5. India	6.06	5.92	977	5.68	4.97	875	>6.50	>7.00	>1077
6. European Union	0.42	1.33	3190	0.24	0.81	3330	0.25	0.70	2800
7. Paraguay	1.26	3.11	2460	1.45	3.90	2690	1.55	4.25	2740
8. Others	4.83	7.59	1571	4.32	7.35	1701	5.01	7.82	1561

Source: *Modified utilizing data from: Oilseeds- World Market and Trade, USDA, April 2003, www.fao.org and Directorate of Oilseeds, Hyderabad*

2. Growth of soybean in India

Black seeded soybean, known by a variety of different names (*Bhat, Bhatmas, Ramkulthi, Kalitur*), has been grown since early times in the northern and north-eastern hills and in scattered areas in the central part of the country. Although introduced way back in 1000 A.D., the crop appears to be known to Indians since ages. It is highly likely that, as in case of niger, soybean was introduced in India probably as soon as it was domesticated in China (Tiwari and Karmakar 2000). India is also considered as a secondary center of domestication for soybean (Khoshoo 1995; Boyden 1992). Concerted research and development (R&D) efforts initiated simultaneously by the agricultural universities at Pantnagar (Uttaranchal) and Jabalpur (Madhya Pradsesh) in 1960s followed by the establishment of ICAR-financed interdisciplinary multilocational All India Coordinated Research Project on Soybean in 1967 and National Research Centre for Soybean in 1987 and support rendered by State Department of Agriculture and private sector brought the crop in commercial cultivation.

In next three decades from 1970s onward, the crop exhibited a phenomenal growth (Paroda 1999). A steady increase in area and production with the growth rate of 15-20 per cent was experienced in the past except the years (2000-2002) when the major soybean-growing region was in the grip of severe drought. Compound

growth rate exhibited between 1981-1991 for area production and productivity of soybean was 17.89, 20.51 and 2.22, which slowed down to 6.61, 7.72 and 1.04, respectively in the next decade (1991-2001) (Mruthyunjaya and Singh 2003). The production of soybean remained raising till 1999 owing to increase in yield and area and subsequently declined in yield in 2000, on account to first of subsequent three drought years. Soybean continued to show positive growth rates, where as other oilseeds like groundnut, linseed, rapeseed, safflower, sesame and safflower have been exhibiting negative growth rate during 1991-2001. The good monsoon during *kharif* 2003 appears to have given restart to the upward trend. At present, the area under soybean is above 6.5 million hectares, which is likely to produce above 7.0 million tonnes (Table 2).

Table 2. Growth in area, production and productivity of soybean

Year	Area (m ha)	Production (m t)	Productivity (kg/ha)
1970	0.032	0.014	426
1981	0.475	0.352	741
1991	3.185	2.492	782
1995	5.035	5.096	1012
1996	5.446	5.376	987
1997	5.800	6.533	1126
1998	6.493	7.143	1100
1999	6.223	7.081	1138
2000	6.418	5.273	822
2001	6.000	5.600	930
2002*	5.680	4.970	880
2003*	>6.50	>7.000	>1070

Note: Soybean crop has been brought on forecast basis for the first time from 1981-82. As such data for earlier years are based on assessments by state agricultural departments.

Source: Various issues of Area and Production of Principal Crops in India, DES, GOI: Agricultural Statistics at a Glance.

Madhya Pradesh, Maharashtra and Rajasthan are the major soybean growing states in that order. Madhya Pradesh contribute to the extent of 70 per cent with respect to area and 64 per cent to production (1998-99). Madhya Pradesh and Maharashtra together grows soybean largely on Vertisols and associated soils and constitute an area of 86.8 per cent. Addition of Rajasthan makes this figure to be 87.67 per cent. For immediate gains in national production, the concentration of efforts to enhance the productivity are essentially required in these three states, followed by other potential areas.

Although, an expansion of crop to 10 million ha by 2010 and 12 million ha by 2020 has been projected (Holt *et al*. 1997), conservative estimates are that the crop will stabilize around 10 million hectares in India. The contribution of area in future is likely to come from the states of Madhya Pradesh (0.5 m ha), Maharashtra (2 m ha), Rajasthan (0.3 m ha), Karnataka (0.2 m ha), Andhra Pradesh (0.3 m ha) and other states (0.2 m ha).

The comparisons being made for Indian productivity levels with those of other big four are not logical as the maturity duration on an average in India is 96 days as compared to the average figures of 120 to 170 days in the countries under reference. Comparison based on per day productivity places India at 11 kg/day against 14 (USA and China) to 19 kg/ha (Brazil and Argentina) elsewhere. Moreover, Indian farmer goes for better system efficiency as he grows wheat (irrigated) and chickpea (rainfed), and in some cases soybean-potato/garlic-wheat crop sequences, thereby increased per day productivity (15 to 70 kg/day depending upon the cropping system adopted).

3. Soybean varieties

The success story of soybean in India was witnessed from mid seventies onwards. During early years of growth, it was only indigenous variety "*Kalitur*" ruled the cultivation. Subsequently, the farmers used the acclimatized exotic introductions and the varieties developed through the breeding programme in the country. Till date, 80 varieties developed/adopted in the country (Table 3) has been providing strength to soybean cultivation. Apart from the varieties (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 remaining comprises a bulk of the rest of the Indian varieties, which were developed through hybridisation and mutation in/among the varieties of the first group. It has been estimated that between 1969 and 1993, the annual genetic gain in seed yield of soybean varieties has been about 22 kg/ha (Karmakar and Bhatnagar 1996). Breeding efforts have successfully been used to cope up with the problems of seed longevity and pod shattering to a reasonable extent in the varieties developed in last few years. More over, the efforts have also been successful to insulate the resistance towards the biotic stresses. The efforts to insulate long juvenility utilizing some from the germplasm lines with long juvenile trait (PI 159925, PI 240664, Santa Maria, Ocepar 8, Ocepar 9 and Paragonia introduced from Brazil and USA) are likely to result in development of higher yielding varieties. It shall be necessary to identify and use lines with long juvenile period coupled with rapid fill to contain the maturity period and achieve high yields (Tiwari and Karmakar 2000)

Table 3. Soybean Varieties identified/released/notified for cultivation in India

Year	Varieties
1969	Clark-63*, Bragg*, Improved Pelican*
1071	Kalitur
1973	Lee*, Davis*
1975	Punjab-1
1976	Ankur
1978	Type 49, Alankar (PK 71-21), PK 262
1980	Shilajeet
1981	Pusa 40**
1982	JS 2, CO-1, KM 1, Gaurav (JS 72-44), KHSb 2, Durga (JS 72-280)
1983	Gujarat Soybean 1, Gujarat Soybean 2, PK 327, PK 262, Birsa Soy 1, Pusa 22**
1984	SL 4
1985	Monetta* (EC 2587), PK 308, MACS 13, VL Soya 1, Pusa 37**
1986	PK 416, PK 472
1987	Pusa 16, Pusa 24, JS 75-46, SL 96,
1988	Pusa 20, PK 471**
1989	MACS 58, VL Soya 2
1990	Shivalik (HIMSO 333), Jawahar Soybean (JS 76-205), ADT 1 (UGM 33)**
1991	PK 564, JS 71-05, JS 80-21
1992	MACS 124, MACS 57, Hardee
1994	JS 335, JS 79-81**
1995	Aarti (MAUS 1)**
1996	VL Soya 21
1997	Ahilya 1 (NRC 2), Ahilya 2 (NRC 12), Ahilya 3 (NRC 7), Pant Soybean 1024, Pant Soybean 1042, Puja (MAUS 2), Co Soya 2, Sneh (KB 79), PK 1029, SL 295
1999	MACS 450, JS 90-41
2000	VLS 47, Parbhani Sona (MAUS 47), Prasad (MAUS 32), Pant Soya 1092, Palam Soya**
2001	Ahilya 4 (NRC 37), Indira Soy 9, Hara Soya (HIMSO 1563), LSb 1,
2002	JS 93-05, Pratikar (MAUS 61), Pratishtha (MAUS 61-2), Samridhi (MAUS 71), Pratap Soya (RAUS 5)**

Source: Joshi and Sharma (2003)

**Exotic varieties; **Identified varieties*

Many of these varieties have the yielding potentials between 2.5 to 3.0 t/ha. The matter of concern is that in spite of yield levels achieved in front line demonstrations using these varieties under real farm conditions (average 1800 kg/ha), crop harvested by progressive farmers (2000 to 2500 kg/ha), and state average of 1400-1500 kg/ha in Maharashtra, the national productivity still remains around 1000 kg/ha. It is understood that the yield gap II, the productivity difference between maximum yield under real farm conditions and the farmers yield can never be abridged fully, more so in rainfed crop like soybean, even by the best possible management in the specific environment. Even in case of cereals under best production systems, it has been observed that annual improvement in national crop yield shows a decline and ceases once the crop reaches about 80% of the potential productivity established by the nation's very best producers (Cassman 1999). It is anticipated that the national productivity level can be raised to about 1600 kg/ha utilizing the available production technology in future years. The continuing breeding efforts are likely to develop varieties with yield potentials of 4000 kg /ha, which will further pave the way for setting targets higher than 1600 kg/ha.

For further enhancing the productivity, the right approach is to keep on raising the genetic potential for yield along with effective transfer of technology. To raise the genetic yield potential of varieties, the country will have to broaden the existing narrow base of germplasm through directed introductions. The R&D efforts underway covering modern tools of science like molecular breeding using marker assisted selection and quantitative trait loci (QTLs) will go a long way to breed/improve varieties with better yield potentials, resistance to biotic and abiotic stresses, better food grade characters and other useful traits. The research is also needed to be continuously geared up to resolve the newer problems like no-podding syndrome, fungal complex etc.

The potentials of soybeans for use as food/functional food and growing awareness on health benefits is a direction to work on development of varieties for food grade characters, particularly to address the problems of energy-protein malnutrition and provision of overall health to the masses. Further, the research on oil and protein content/quality need to be given pace to meet the future needs. Introduction of a concept to consume soybean at green pod stage (Kumar *et al*. 2003) after making suitable varieties available for the purpose, shall also form a research need for future.

4. Cropping Systems

The farmers grow soybean followed by wheat in case adequate/partial irrigation is available. Under rainfed conditions, the succeeding crop grown is chickpea.

During monsoon, the intercropping of soybean with pigeon pea, particularly when no rabi/winter crop is possible and soybean with corn is getting popular. In north hill/plain zones of India, the soybean is intercropped with finger millet, sorghum and corn. When intercropping is followed, the fertilizer is applied to soybean only. Soybean intercropping with finger millet and cotton is also practiced in central and southern zone. The other cropping systems like soybean-linseed, soybean-sunflower, soybean-safflower and soybean-mustard are agronomically feasible and economically viable have been adapted to a limited extent. A detailed account on the soybean based cropping systems in India is documented (Bhatnagar and Joshi 1999). The varietal and crop diversification and working out favourable long duration cropping systems shall be a step towards sustainability. Moreover, there is a need to specify the efficient cropping systems suitable to different agro-ecological zones along with the variety-specific production package so that desired productivity levels at competitive cost of production could be achieved. Approach like Assam rice revolution of recent years (Swaminathan 2003) is to be thought of in case of soybean also, wherein the areas with irrigation facilities (e.g. Kolhapur and Sangli of Maharashtra, Dharwad region of Karnartaka, Ranchi in Jharkhand) could be tried for the cultivation of commercial crop for higher production in season other than monsoon and for remaining rainfed region a multi-graded system with production packages suiting to the economic conditions of the farmers are recommended and adopted.

5. Management of biotic stresses

Weeds that could lead to yield erosion of 68 per cent (Billore *et al*. 1999) are by and large managed using cultural means. Use of herbicide is low (approximately 10%). The suggested integrated approach involves summer ploughing, use of weed free seed, cultivation of more than one variety, keep land surrounding the crop weed free, use of any one of recommended herbicides in rotation and one interculture (Joshi 2001). Little attention towards weed management is one major stumbling block in soybean cultivation in the country. Adoption of integrated schedule shall not only restore yielding potential of soybean varieties but also avoid nutrient drain and reduce the incidence of pest and diseases.

The yield losses through insect-pests and diseases amount to about 32 per cent, if not managed (Sharma and Shukla 1997). The integrated pest management approach involves summer ploughing, planting of resistant/tolerant varieties, incorporation of phorate before seeding, seed treatment with recommended bio-agent (*Trichodema viride*)/ fungicide, removal of affected plant parts in early stages of infection, use of light traps, and use of bio-pesticide /insecticides/ other pesticides whenever necessary. Fair information on the areas of repeated incidence for specific disease

and pests and stage of crop growth venerable to their incidence is available and region specific schedule can be worked out for adoption. The management schedules for new emerging biotic stresses like soybean rust (caused by *Phakopsora pachyrhizi*), no podding syndrome and defoliators have been recommended. However, further research on such biotic problems is warranted.

6. Balanced nutrition

Constraint analysis done in the past (Tiwari 2001) has indicated that the lack of soil fertility management by provision of balanced nutrition is one of the main reasons of low productivity of soybean in India. The fertilization is not only on lower side, but also skewed (N:P:K consumption ratio is 6.7:2.5:1 against ideal 4:2:1) providing sub optimal and unbalanced nutrition. Balanced fertilization (4.2:2:1) in Maharashtra since last couple of years (Anonymous 2003), has led to higher yields of all the crops including soybean (State average of 1.4 t/ha). Awareness is needed to adopt integrated approach in nutrient management with due emphasis on crop residue/ manure recycling and to dispense with the faulty use of fertilizer. Use of bio-fertilizers in the major soybean command area remarkable. Increased use of other bio-fertilizers needs encouragement to reduce dependence on fertilizers. Creation of awareness and practicing on integrated nutrient management system by way of using all the alternative sources of nutrition and filling up the gap between required and available with supplementation through fertilizers may lead to sustainable production. Moreover, farmers need education on developing the habit of compulsorily recycling organic residues and dispense with the habit of application of fertilizer at crop growth stages when it is not warranted.

7. Development of Soybean Industry

Successful commercial cultivation of soybean in India can be attributed to the development of the soy-oil industry, which has provided a remunerative market for growers. During the late 60's, the lack of market has prompted the phasing out of R&D projects on soybean. It was only when the solvent extraction plants in western Madhya Pradesh were established that soybean cultivation resumed. Subsequently, there was an increase in both soybean hectarage and processing plants. However, the proliferation of solvent extraction plants for processing soybean outnumbered the national hectarage of soybean. Influenced by various factors, these units are concentrated in western part of Madhya Pradesh. Today, there are 166 soybean-processing plants with an installed capacity of about 15 million tonnes in different states of the country (Table 4)

Table 4. State wise installed capacity of soybean processing plants in India (1997-98)

State	Plants (No.)	Capacity (tones per day)	Per annum capacity (300 working days) (In tonnes)
Andhra Pradesh	8	2530	759000
Gujarat	18	4120	1236000
Haryana	2	600	180000
Karnataka	4	510	153000
Maharashtra	40	7790	2337000
Madhya Pradesh	76	31110	9333000
Punjab	1	700	210000
Rajasthan	10	3050	915000
Tamilnadu	1	300	90000
Uttar Pradesh	6	1290	387000
All India	166	52000	15600000

Source: SOPA

8. Domestic utilization and export earnings

Soybean has proved to be of vital importance in agriculture and oil economy of India. By contributing about 10 per cent to the oil produced in the country (0.85 mt), it diminishes the expenditure on import of edible oil. On the other hand, the export of DOC fetches earnings equivalent to about Rs. 15, 000 million (320 US $) each year (Table 5). The total soybean production at present is 6 million tonnes. Of this, leaving one million tonnes for seed, the quantity available for oil extraction is 5 million tonnes. It provides 4 million tones of de-oiled cake for domestic utilization and export. The domestic utilization, which used to be 1 million tonnes (Jain 1999), is steadily increasing and is about 1.30 million tonnes at present. Remaining is exported. Of late the export of DOC is looked upon as drain of valuable protein. The changing trend is to add value to the products and export them in place of DOC. Now there are about 80 processing units that have taken up value addition.

Table 5. Export of DOC from India and export earnings

Year	Quantity exported (m t)	Export earning (m Rupees)	Year	Quantity exported (m t)	Export earning (m Rupees)
1988-89	0.76	2990	1996-97	2.59	23977
1989-90	0.94	3640	1997-98	2.48	24290
1990-91	1.24	4380	1998-99	3.08	18038
1991-92	1.38	6700	1999-00	2.46	16806
1992-93	1.83	11144	2000-01	2.37	18400
1993-94	2.33	14735	2001-02	2.80	24377
1994-95	1.64	10278	2002-03	1.49	13410
1995-96	2.41	17200	2003-04	-	-

Source: SOPA

9. Product Processing and Utilization

In April 1985, an Indo-US collaborative project on Soybean Processing and Utilization (SPU) at Central Institute of Agricultural Engineering (CIAE), Bhopal and Govind Ballabh Pant University of Agriculture and Technology, Pantnagar was launched by the Indian Council of Agricultural Research (ICAR) and United State Agency for International Development (USAID). Considering the success achieved under this programme, the ICAR decided to continue the activity therein at CIAE, Bhopal even after its termination on March 31, 1991. The SPU project has developed 17 soy-products, 20 soybean processing equipments and 5 limited scale pilot scale production facilities for full fat soy-floor, partially defatted soy-flour, soy-milk, soy-*paneer* and soy-fortified bakery products (Ali 2000). At present, there exists capacity of one million tonne to process soybean for food. The Indian soybean industry has capability to process soybean for food, feed, pharmaceutical and industrial applications. The industry has about 330 units for the purpose (Ali 2001).

Table 6. The food industry in India

Oil extraction plants	154
Food manufacturing units	125
Equipment manufacturers	30
Trading housed	15
Government and other agencies	06
Total	330

Source: Ali (2001)

10. Soybean as a functional food

Indians know the virtues of soybean as a food plant since ages (Watt 1890). However, its consumption is confined only to marginal regions of Himalayas. As per 57th round of national sample survey (Report on household consumer expenditure and employment-unemployment situation in India 2001-02) highlights that 16 per 1000 rural household and 3 per 1000 urban household were seasonally hungry- that is they did not get enough food in many months of the year. Moreover, there exists a wide spread energy protein malnutrition in the country, particularly rural India.

Ironically soybean, which contains about 40% protein, is mainly exported in the form of DOC. Though it is fetching foreign earning worth Rs1500 crores annually, however, the valuable and cheapest source of protein is being drained out. Apart from protein and oil, soybean contains basic nutrients, attributed to the presence of a biological compound isoflavones (Wang 1999) for which soybean has been lately endowed with epithet ' functional food of the century'. Functional foods are those, which contain significant levels of biologically active components beyond the traditional nutrients they contain (Drozen and Harrison 1998). These provide specific health benefits beyond traditional nutrients they contain. Soybean isoflavones, being structurally analogues to estrogen hormone, moderate the menopause symptoms (hot flashes, night sweats) that often interfere with vitality. Soy isoflavones also reduce the risk of hazardous diseases like breast cancer, osteoporosis, heart attack and kidney stone formation. (Messina 1997; Browning and Niebrugge 1999)

11. Strength of Soybean Sector

The Indian Soybean Farming and Industry is on sound footings and this assumption is based on the following points.

- The gap between the global consumption and production of edible oils (1993-2002) continues to be positive (Comprehensive Agri-Commodity Intelligence, (www. CommodityIndia.com, December 2003). India is the largest importer of edible oils in the recent years.
- The Indian Soybean Industry with crushing capacity of about 16 million tonnes soybean per annum.
- The renewed thrust and inclination of soybean industry towards value addition to soy processed products.
- Present average export earnings of soybean sector is around Rs. 1500 crores per annum.
- Identity preserved nature of soy-processed products leading to preference by importing countries.

- Adequate support from GOI/ICAR through TMOP on R&D in oilseeds.
- Policy support by the Government, particularly by way of keeping the duty on import of soybean oil at bound rate and for other oils at reasonable level.
- The productivity of merely 426 kg/ha in 1970 is exceeding 1000 kg now. Soybean production is estimated to cross 7 million tonnes during the current year indicates the established agronomic feasibility and economic viability of crop in the traditional cropping systems.
- A total of 80 soybean varieties have been developed till date (Table 3). Of these, about 20 soybean varieties are with high yield potentials (25-35 q/ha) and with specific traits like resistance to insect pests and diseases, pod shattering and with better germinability.
- Existence of about 30 varieties in seed chain reveals a step to-wards varietal cafeteria approach.
- The existing varieties have shown yield potential up to 3.5 t/ha under real farm conditions when grown by progressive farmers.
- Production technology for different agro-climatic zones is recommended and is being progressively adopted by the farmers.
- It has been convincingly established through Front Line Demonstrations on farmers' field on sizeable area during last decade that present national productivity of soybean can be doubled.
- A dedicated band of research workers involved in soybean R&D.
- Adequate staff of State Department of Agriculture and SAUs involved in Transfer of Production Technology.
- Progressive trend in participation of private sector/NGOs in soybean development in the country.
- Advent of new tools of science viz. biotechnology will have a great positive impact on soybean. The country has adequate expertise in this regard.

12. Summary

Good monsoon during *kharif* 2003 has resulted in the enhancement of area (above 6.5 m ha), which is likely to produce a record soybean tonnage (above 7 m t). Though, the production and productivity of soybean was adversely affected in three earlier succeeding drought years, it could provide subsistence to the farmers. With normal monsoon in years to come along with increased adoption of improved production technology by the farmers, it is expected that the target of 10 m ha producing 16 m t is achievable in next 10 years.

The growth of commercial cultivation soybean crop in just three decades in spite of geographical limitations is the result of adequate policy support, research and development efforts made so far. Further R&D support being provided as per the guidelines contained in the National Agricultural Policy 2001 through National Agricultural Research Systems (NARS) for oilseeds and pulses shall further favour soybean farming. The continued policy support from Government of India by extending input subsidy/price support to farmers, maintaining the import duty on edible oils and provision of some incentives to private sector for value addition and export of soy and its processed products will further sustain the soybean farming and industry.

The establishment of soybean crop parallel to groundnut and rapeseed mustard, its contribution in oil and national economy and its potentials not only to mitigate wide spread energy-protein malnutrition in India but also serve as functional food to provide health benefits, makes us to conceive that soybean has come to stay in the country**.**

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Soybean Research 1: 43-57 (2003)

Bacterial Pustule Disease of Soybean-Present Scenario and Future Strategies

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Soybean [*Glycine max* (L.) Merrill], a protein and oil rich crop is named miracle bean, golden bean, cow of the field, meat of the field, gold from the soil, pearl of the orient, Cinderella crop etc, and is grown since ages (Brooks 1966, Bhatnagar and Tiwari 1991). Its origin dates back to 2800BC in China (Hymowitz 1970). In India the crop is grown in 5.68 million hectares with 4.97 million tones production in 2002-2003. The acreage of states under soybean is around 4.5 million ha in Madhya Pradesh, 1.1 million ha in Maharashtra and 0.5 million ha in Rajasthan.

Soybean, along with groundnut, rapeseed and mustard has established itself as third important oilseed crop in the country with 41-45 per cent protein, 18-20 per cent oil, 17-24 per cent carbohydrates, 4-6 per cent minerals, vitamins A, B, C, unsaturated fatty acids, higher content of calcium, phosphorus, iron etc. It contains antioxidants to combat cancer.

The black seeded soybean is being grown in the country from early times under the name "*Bhatt, Bhatmash* or *Kalitur*" in Kumaon and Garhwal hills in Uttar Pradesh and in scattered pockets of Himachal Pradesh.

The yellow seeded improved varieties were tried, about four decades back since then a number of new varieties have been evolved to suit to different agro climatic regions of the country. Due to its importance in farm and oil economy, soybean has rapidly spread in some states of the country. Madhya Pradesh provided the prospects for its growth and eventually became "Soybean State" occupying 70 per cent of the total soybean area of the country.

The soybean crop suffers due to plant pathogens at pre- and post- emergence as well as later stages of crop growth till maturity, At early stages, the crop is at-

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tacked by pathogens, which are soil and/or seed borne including fungi, bacteria, viruses, nematodes and phytoplasma. Among bacterial diseases, bacterial pustule is of great importance.

Occurrence and distribution

The bacterial pustule disease of soybean was first reported by Smith in 1904. Clinton (1916) found it to be caused by *Bacillus* sp. Hedges (1922) described the symptoms of the disease in detail and named the causal organism as *Bacterium phaseoli* var. *sojense* Hedges. It is a common disease in most of the soybean growing countries like Brazil, France, Ukraine, Cuba, China, USSR, Columbia, Japan, Formosa, Myanmar, Lithuania, Sudan, Korea, Alma-Ata etc, (Freire 1953, Signoret 1975, Muras 1964, Albornoz 1978, Han 1959, Bakaeva 1976, Morgan 1963, Limonard 1968, Graham 1953, Dunleavy 1955, Tarr 1952, Cho and Yoo 1977, Bakaeva 1978). In India the disease was first reported from Jalgaon (Maharashtra) by Uppal *et al*. 1938) and later from other parts of the country by various workers.

Yield losses

Bacterial pustule can cause yield loss upto 53 per cent (Shukla 1994). According to him 15, 21, 38 and 53 per cent losses were observed with 25, 50, 75 and more than 75 per cent disease intensity, respectively. Though various workers have claimed losses upto 53 per cent but in general 18-20 per cent losses commonly occur.

Symptoms

Minute, pale green spots with raised centres appear on either or both the leaf surfaces. The spots turn brown and centre becomes corky giving appearance of small lesions and pustules. The pustule size varies in size, based on the host-pathogen interaction. It is of pinpoint size in variety T-49 and quite large in Punjab 1, Monetta and MACS 57. The pustule is surrounded by a yellow halo. Several pustules coalesce making large lesions. Under severe disease intensity, the infected leaves turn yellow and drop off. The symptoms also develop on petioles and pods. The disease appears at all stages of plant growth.

Causal Organism

The causal organism of bacterial pustule disease was first named *Phytomonas glycines* (Nak.). Mag., which was later changed to *Bacterium glycines* (Nak.) Elliott; *Pseudomonas phaseoli* var. *sojense* Hedges; *Phytomonas phaseoli* var. *sojense* (Hedges) Burk; *Xanthomonas campestris* pv. *glycines* (Nakano) Dye, *Xanthomonas axonopodis* pv. *glycines* and finally renamed *Xanthomonas campestris* pv. *glycines* (Nakano 1919, Vauterin *et al*. 1995, Widjaja *et al*.1999, Schaad *et al*. 2000).

Xanthomonas campestris pv. *glycines* (*Xcg*) is a motile, gram –ve unicellular rod (0.5 - 0.9 x 1.4 -2.3 µm) with single polar flagellum. Colonies on beef extract agar are pale yellow. They are small, circular and smooth with entire margin. The bacterium is slow growing in culture. It hydrolyses gelatine, starch and casein. The cardinal temperatures for the growth of the bacterium are 10:30-33:38 ^{o}C. Patel *et al.* (1972) tested the isolates from different locations and found them to be similar in virulence. However, Ansari and Joshi (2002) observed variation in virulence in the isolates collected from different agro-climatic zones.

Jones and Fett (1985) reported two strains, virulent and avirulent. Khare (1994) also observed two strains, one with typical glistening yellow colonies and the other with dull white on nutrient agar. The dull white colonies were avirulent and the other highly virulent. However, Ansari *et al.* (2003) on the basis of pathogenicity on a set of soybean verities grouped the isolates into highly virulent, virulent and less virulent strains/pathotype.

The pathogenic isolate of *Xcg* had two cryptic plasmids of 1.5 and 25 Kb and the SDS-PAGE profiles of the soluble proteins had a low band, whereas nonpathogenic isolates were without plasmids and had a different pattern of bands (Sharma *et al*. 1994).

The cells of the genus *Xanthomonas* is non-spore forming, gram negative, strictly aerobic monotrichous rods on agar medium. The cells produce a yellow, non-water soluble carotenoid pigment having absorption spectrum maxima at 418, 437 and 463 mm in petroleum ether. A diffusible brown colour infrequently occurs in some media. Metabolism of glucose is strictly oxidative. In a weakly buffered medium, acid is produced in small amounts from rhamnose, inulin , adonitol, dulcitol, inositol, salicin and rarely from sorbitol, acetate, citrate, malate, propionate but not from benzoate, oxalate and tortrate. Gluconate may be utilized after a day. No species reduces nitrate or produces acid in milk. Acetone, indole, urease are not formed. Hydrogen sulphide is produced from cystein. Asparagine was not utilized as a sole source of carbon or nitrogen. Growth does not occur in the presence of 6.0 per cent sodium chloride (Dye 1962, 1963).

Khare (1994) observed that *Xcg* utilized more fructose as compared to mannose, lactose and starch but did not utilize dextrin, rhamnose, adonitol and inositol. The bacterium was found to liquefy gelatin and was able to produce hydrogen sulphide but did not produce indole. Acid was not produced during its reaction on milk. The bacterium did not grow on Sx medium.

Saify *et al*. (2003) found that the bacterium was positive to oxidase and negative to catalase tests and produced acid from most of the sugars like xylose, D (-) galactose, D (+) glucose, L (+) arabinose, D (-)

lactose, sucrose, fructose, maltose, salicin, starch and glycerin. It utilized glucose oxidatively and hydrolyzed gelatin, starch and casein. It utilized tryptophan and produced indole in the culture media.

Seed borne nature

X. campestris pv. *glycines* has been reported to be seed borne (Patel and Jindal 1970, Mishra and Thapliyal 1982). Khare (1994) observed very low percentage of association of bacterium with soybean seeds under natural conditions even collected from severely diseased plants. Looking to its low percentage of association with soybean seeds, the detection methods used, have to be very selective, and effective. Several methods for detection of seed borne inoculums were tested by various workers. Mishra (1983) and Khare (1994) reported that modified agar plate method and Trypton's agar medium with 1 per cent carbendazim as bavistin + pentachloronitrobenzene as brassicol was found to be the best for the detection of *X. campestris* pv. *glycines* from seeds. According to Srivastava and Srivastava (1986), out of standard blotter method and agar plate method with nutrient agar or water agar and growing on techniques with moistened sand in culture tube, the best results were obtained in water agar technique.

Studies on the location of the bacterium in soybean seeds revealed that the bacterium is present in all the seed parts, seed coat, cotyledons, plumule bud and redickle (Khare 1984, 1994). This confirms the internally seed borne and intra-embryonal nature of *X. campestris* pv. *glycines.* According to Graham (1953), the bacterium remains viable in soybean seeds for two and half years, whereas Singh and Jain (1988) observed the bacterium to survive in soybean seeds upto 9 months at 10.6 40 ^{o}C and for 13 months at 6.0-10 ^{o}C.

Detection Methods

Several methods are available for the detection of seed borne *X. campestris* pv. *glycines* associated with soybean seeds.

i. **Agar plate method:** Seeds are pre-treated with 1 per cent sodium hypochlorite and kept in petriplates containing nutrient agar or yeast extract glucose chalk agar or any other suitable medium. Five seeds are placed at equal distance in each petriplate and incubated at 30±2 ^{0}C for seven days. Typical yellow shiny, slimy colonies of the bacterium grow within 2-3 days.

ii. **Cotyledon method:** Seeds are placed on sterilized wet blotter in petriplates and incubated at 30±2 ^{0}C for seven days. Cotyledons with black, brown and necrotic lesions are collected for ooze test and isolations are made for further confirmation (Hwang *et al.* 1972).

iii. **Water agar method:** Seeds are pre-treated in Aureofungin 200 ppm for 3-4 hours and placed on water agar. The plates are incubated at 20 ^{0}C in darkness and examined on the eighth and twelfth day of incubation. The infected seedlings can be recognized by their tendency to collapse with flaccid stem and pulpy yellowish cotyledons or by the presence of black lesions on cotyledons and true leaves. By making a cut through the lesions, bacterial ooze from vascular bundle is observed in a drop of water. Isolation can be made for further confirmation (Srinivasan *et al.* 1973).

iv. **Test tube soil test:** Each seed is washed in sterilized water separately and placed in a test tube containing moist autoclaved soil upto one fourth level. The test tubes are plugged and incubated at 30±2 ^{0}C for 10 days. The plugs are removed when seedling touches the plug. Sterilized water is added as per need. Cotyledons are removed and examined for necrotic or brown lesions and for ooze test. Isolations are made for confirmation (Shakya and Vintprer 1986).

v. **Powdered seed suspension method:** Soybean seeds are rinsed with sterilized water and dried on blotters. One seed is powdered and mixed in 5 ml sterilized water in a test tube and incubated at 6 ^{0}C for 24 hours. The suspension is shaken and streaked on YGCA medium (yeast extract 10g, glucose 20g, calcium carbonate 20g, agar 20 g, water 1 lit. at pH 7.00) and incubated at 30±2 ^{0}C for seven days. Yellow shining colonies develop exhibiting the presence of the bacterium, which is confirmed after isolations (Gupta and Pathak 2001).

vi. **MXG selective medium:** Prathaungwong and Choochoa (1989) used this medium to examine the efficacy of chemical seed treatment in controlling the seed borne incidence of the bacterium.

Perpetuation

The bacterial pustule bacterium (*X. campestris* pv. *glycines*) remained viable in dry diseased leaves as well partially decayed leaves from one season to another (Lehman and Poole 1939). It can survive for three months on decaying leaves and nine months on dry leaves (Lehman 1931). Graham (1953) observed that the bacterium persisted in sterile soil for at least nine months and in field soil through winter over-wintering in leaves on soil surface. Cho and Yoo (1977) reported that *X. axonopodis* var. *sojense* survived for four days in sterilized soil and remained pathogenic even after 10 months of storage in refrigerated infected plants. They also found that in uncontrolled temperatures in a warehouse, infectivity was lost in six months, during October to April.

The bacterium over winters on the plant debris in soil and undecomposed plant remains on the soil surface (Bakaeva 1975, 1977). The survival of the bacterium was favoured when the plant debris was kept on the soil surface rather than incorporated in soil. The survival is favoured when infested material was kept under dry conditions. The pure culture of the bacterium did not survive under dry and moist conditions in either sterilized or nonsterilized conditions. John *et al*. (1978) found that this bacterium survived and was pathogenic after storage in 15 per cent glycerol at – 70 ^{0}C for 18 months. According to Fett (1979) *X. axonopodis* var. *sojense* could not be recovered from buried infected leaf debris on the soil surface for seven months.

Phad and Kore (1989) reported that *X. campestris* pv. *glycines* survived for a maximum period of 105, 93, 80 and 55 days in infected leaves, calyx, stem and pods, respectively. Survival time was reduced when the plant parts were buried (33, 25, 20 and 4 days respectively). Groth and Braun (1989) found the bacterium in plant debris placed on the soil surface or buried at a depth of 15 cm, to survive through the winters. Khare and Khare (1995) observed that *X. campestris* pv. *glycines* remained viable for 110 days in infected leaves buried at 15 cm depth but did not survive at all at the depth of 30 cm.

Disease cycle

The bacterium perpetuates in diseased crop debris in and on the soil as well as seed. The seed to plant transmission of the bacterium is systemic as well as local. The spread of the bacterium from plant to plant and field to field is through rain splashes and wind blown rain. The plant to seed infection takes place from vascular system through funiculus systemically as well as through infected pods. Seeds also get contaminated with the bacterium during threshing (Groth and Braun 1986).

Dolichos biflorus, Phaseolus vulgaris, P. lunatus, Macrotyloma uniflorum and red vine (*Brunnichia cirrhosa*) are the collateral hosts of the bacterium (Elliot 1951). Hartman *et al*. (1999) have reported some strains of the bacterium to infect common bean and cowpea also.

Epidemiology

The disease incidence depends upon the susceptible genotype, virulent pathogen and favourable weather conditions. For recurrence of the disease, soil and seed borne inoculum is responsible and for spread, fast multiplication of the bacterium and warm and humid weather. Windy rain and rain splashes help in spread of the disease. Certain times, initial lesions are found without typical symptoms and on isolation, *X. campestris* pv. *glycines* was isolated, exhibiting latent infection for a long time if weather conditions are not favourable (Lehman 1931). Severe infection and

disease development occurs at 30-33^{0}C temperature and high humidity. Very few pustules are formed at 22^{0}C day and 15^{0}C night temperature. During incubation, required temperature is essential for disease development. Young leaves are more susceptible. Several scientists have reported higher disease incidence during humid warm weather prevailing in August and September (Kuzin and Serebrennikova 1978, Khare 1994). Bagatseveska and Vitanov (1979) have reported 10 ^{0}C minimum and 39^{0}C maximum temperature for the disease development.

According to Srivastava and Bais (1986) and Khare (1994) moderately warm conditions (average temperature 27 ^{0}C) and moderate relative humidity (83-84.6 per cent) result in severe disease intensity. Such favourable conditions occur in Uttar Pradesh and Madhya Pradesh in August and September. The disease severity gets reduced in October when the relative humidity is much less.

According to Groth and Braun (1986), the pathogen was equally dispersed from a diseased plant in the field in all directions and roughly at the same rate in plots of either resistant or susceptible soybeans. The size of epiphytotic population did not significantly differ between reistant and susceptible varieties during the first three weeks after inoculation, external population of *X. campestris* pv. *glycines* was 20 to 50 times more on the susceptible but not on resistant plants.

Management

For the management of bacterial pustule disease in soybean it is necessary to follow all the possible measures to keep the pressure of the inoculum at the least level.

1. *Cultural:* The soil plays the most important role in perpetuation of the bacterium hence solarization in summer is necessary. The alternative host should be removed.

2. *Physical:* Hot water seed treatment eradicates the seed borne infection of *X. campestris* pv. *glycines*. Mishra (1983) got success in controlling the seed borne infection by dipping the seed in water at 53 ^{0}C for 25 minutes, whereas Khare (1994) found 25 minutes exposure of seed in water at 52 ^{0}C to be excellent.

3. *Chemical:* Thirmulachar (1969) and Rao and Patel (1973) reported that spraying streptocyclin on the crop could control the bacterial pustule disease. Thapliyal and Mishra (1974) observed that combination of fytolan spray and seed treatment with Ceresan reduced the disease in the field. Bakaeva (1978) reported that phytobacteriomycin dust and benlate (both at 2 % concentration) control this disease. Khare (1984) observed the best emergence of soybean seedlings

by seed treatment with thiram (3 g/kg) and the foliar spray of copper oxychloride (0.3 %) + streptocycline (300 ppm) gave the best control of this disease at later stages of crop growth. Thapliyal and Dubey (1986), Thombre *et al.* (1989) and Thrimurti and Agrawal (1992) also found, the combination of an antibiotic with copper fungicide to give better control of *X. campestris* pv. *glycines*. They also obtained highest yield with streptomycin + copper oxychloride. Singh *et al.* (1990) observed that spray of agrimycin 100 + mancozeb gave effective control of this disease in two years trial. Sood and Kaushal (1994) reported that blitox alone or with streptomycin sulphate gave best results against this disease. Prathuangwong and Choochoa (1989) observed that soybean seeds treated with fungicides had a higher germination percentage, number of plants per area and average yield, whereas Singh and Jain (1989) observed best control of seed borne bacterium by seed treatment with streptocycline at 100 ppm. They further reported that seed treatment with streptocycline (100 ppm) and spray of chloromycetin (500 ppm) or streptocycline (200 ppm) + copper oxychloride (2500 ppm) was most effective in controlling the disease. NRC for Soybean, Indore has recommended copper oxychloride 2 kg + streptocycline 200g/1000 litres water spray at the initiation of bacterial pustule (Hussain *et al.* 2002). Parez *et al.* (1990) reported that extract of *Bacillus* sp. Inhibited the development of *X. campestris* pv. *glycines* under *in vitro* conditions. They also observed that spraying of extract on soybean leaves 24 hours before and 24 hours after inoculation with the inoculum reduced the number of bacterial pustule to 60 per cent against untreated control. The fumigation of soybean seed with ethylene oxide gas under vacuum in a heated $17m^3$ chamber controlled the bacterial infection. The gas is introduced as a 90 per cent ethylene oxide + 10 per cent carbon dioxide mixture. Seeds are kept in polythene bags, which are opened before fumigation and sealed immediately after the treatment (Ralf 1977).

Resistance

It is essential to have varieties resistant to disease and insect pests. Search has been made and as a result of screening in the field, a number of varieties have been found resistant to bacterial pustule disease. Patel *et al.* (1972) reported Bragg, Bossier, Clark 63, Hampton-226, Hill, Hood, Lee, Pickett and Stuart resistant to Indian isolates *X. campestris* pv. *glycines*. They tested 225 soybean varieties/lines, of which 34 were resistant under artificial inoculation. Sources of resistance in soybean to bacterial pustule disease are listed by Tisseli *et al.* (1980) Khare *et al.* (1971) found nine cultivars of soybean resistant to bacterial pustule. Sundresh (1977, 1981) screened 45 varieties, of which DS 74 -73, DS 74 -72, Ds 74-18-2, Jupiter, and PK 258 were im-

mune to the disease. Khare (1994) observed 44 entries immune, 14 resistant and 7 tolerant out of 118 entries under natural field conditions. Luzzi *et al.* (1996) observed cv. G-93-9106 to be resistant against the disease.

In All India Coordinated Research Project on Soybean (AICRPS), following varieties were found to be immune/resistant to this disease.

MACS-871, JS 95-60, DS 9909, MAUS 164, MRSV-342, MACS-752, MACS-869, JS (SH) 96-16, PK 1343, SL-633, ANS 2001-1, PK-1303, PK 1341, MAUS-144, KUV-165, SL-518, SL-599, Himso 1597, ULS-56, DS 98-14, RKS-7, MACS-756, JS 93-05, JS-335, VLS -58, VLS-54, SL-637, JS 96-31, KV-261, PK1337, MAUS-162, PK1347, TS 148-32 etc.

Manjaya and Pawar (1999) have reported the resistance against bacterial pustule to be governed by two recessive genes, whereas according to Rao and Patel (1973) only single recessive gene is responsible for resistance. Clemson Non Shatter (CNS) was used as a resistant parent, responsible for resistance, which confirmed the findings of Hartwig and Lehman (1951). Palmer *et al.* (1992) identified isozyme loci linked to the *Rxp* locus and found soybean lines homozygous for the *rxp* allele to be resistant and the *rxp* allele conditions susceptible to *X. campestris* pv. *glycines.*

Rukayadi *et al.* (2000) made an attempt to construct a non-pathogenic mutant derived from a pathogenic wild type strain YR-2 and to evaluate effectiveness in preventing growth of its parent on the soybean phyllosphere. A mini Tn-5 derived transposon was used to generate non-pathogenic mutants. Southern hybridization and pulsed- field gel electrophoresis confirmed the presence of a single transposon in each of the non-pathogenic mutants. One of the non-pathogenic mutant, M 715, failed to induce a hypersensitive response in tomato leaves. An ice nucleation gene (*ina Z*) carried in pIL 1703 was introduced into strain YR 32 as a reporter gene to demonstrate that the presence of M 715, could reduce colonization of the soybean phyllosphere by YR-32. Epiphytic fitness analysis of YR-32 in the green house indicated that the population dynamics of strain YR-32, YR-32 (PJL 1703) and M175 was similar, although the density of the mutant was slightly less than that of its parents. The M 715 mutant was able to survive for 16 days after inoculation on soybean leaves and maintained population densities of approximately 10^4 to 10^5 cells/g (fresh weight) of leaf. Therefore, M 715 shows promise as an effective biological agent for bacterial pustule disease in soybean.

Bhattacharya and Harihar Ram (1999) studied the linkage for resistance among bean yellow mosaic potyvirus (YMV), Bihar hairy caterpillar (BHC), *Spilosoma oblique* and bacterial pustule (*X. Campestris* pv. *glycines*) in interspecific crosses between

Glycine max and G*lycine soja*, a wild accession recognized as a source of resistance to YMV and BHC in soybean. They studied recombination values by using four methods of estimation namely Square root method, Product ratio method, Emerson method and Maximum likelihood method for the character pair resistance to YMV and susceptibility to bacterial pustule as only these characters were shown to be linked (deviation from 9:3:3:1 was found significant). An average value of recombination in all the methods and crosses was 29.05 per cent, indicating moderate linkage between these two genes. Such a moderate linkage should not be of any hindrance in synthesizing a desirable recombinant type having resistance to both YMV and bacterial pustule.

A macro restriction map of *X. campestris* pv. *glycines* chromosome has been generated by Widjaja *et al.* (1999) by employing pulsed-field gel electrophoresis (PFGE), Restriction endonuclease PacI (5TTAATTAA), PmeI (5-TTAAAC) and SwaI (5'ATTTAAAT) digested the chromosomal DNA into 3,5 and 5 fragments, respectively. In addition, intron-encoded restriction endonuclease 1-Ceu 1 was employed to locate the position of the 23 S rRNA genes (rrIA and rrIB). All of the generated restriction fragments were aligned among the chromosome using multiple restriction enzyme digestion and two-dimensional PFGE (2-DPFGE) in conjugation with southern hybridization analysis. This physical map construction has revealed a single circular chromosome with a size of approximately 5 mb. Two rRNA genes were localized on the chromosome map. Several genes involved in pathogenesis (*xpsD, opsX* and *pat*) as well as genes involved in the biosynthesis of xanthan gum (*Xan AB, rfb CDAB*) were also localized.

Screening methods

It is necessary to have excellent dependable method to screen soybean varieties/lines for their reaction to *X. campestris* pv. *glycines*. Several methods are available for this purpose.

Detached leaf technique with inoculation by a pinprick was found most effective by Surin *et al*. (1993). Sharma *et al.* (1994) used excised leaf inoculation method with high concentration of 10^9 colony forming units (cfu)/ml of the pathogen. Hwang *et al.* (1972) developed a rapid technique "soybean cotyledon assay" for screening. Cotyledons were removed from 10-14 days old seedlings grown in green house, pre-treated with 0.5 per cent sodium hypochlorite for 5 minutes. The centre of the cotyledon was wounded and inoculated with the bacterial suspension by cotton swab. They were placed in a tray and inoculated at 30^0C. Within 2-3 days, the inoculated portions of the cotyledons turned yellowish. Cook *et al.* (1990) inoculated cotyledons of one-week-old seedlings by infiltration, with 1×10^4 cfu/ml bacterial suspension.

The cotyledons of resistant plants remained green and firmly attached, whereas in case of susceptible genotypes, the cotyledons abscised within six days.

Future approaches

It appears essential to undertake the researches on the following points.

1. The wild and collateral hosts should be identified.
2. The variability in the isolates from various agro ecological regions needs to be compared for virulence, as well as biochemical and physiological parameters.
3. The disease cycle needs further exploration to understand the exact role of seed and soil borne inoculum of the pathogen in the disease cycle.
4. The yield loss assessment should be made in different agro ecological regions, based on the time of infection and subsequent disease severity.
5. The reasons for the variability in symptoms in terms of pustule size in different soybean varieties, needs to be worked out.
6. The time of plant to seed transmission of the pathogen need to be explored to know the exact stage when a single chemical or bio-control agent spray may check the plant to seed transmission of the pathogen.
7. Botanicals and microorganisms should be tested to find the best ones for bio-control of the pathogen and the disease.
8. The available biodiversity in soybean should be screened to find resistant ones. Collection of new germplasm must be continued.

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Soybean Research 1: 58-64 (2003)

Soybean as a Food Source

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Soybean is a legume crop originated in China many centuries ago and now it is grown worldwide for its oil and protein. The annual world soybean production is now more than 200 million tonnes. Soybean cultivation in India dates back to 1000 AD and it was probably introduced in India from China through the Himalayas (Bhatnagar 1994). The annual soybean production in India ranges between 5-6 million tonnes with 1000 kg/ha yield. 65-70 of total soybean in India is produced in the State of Madhya Pradesh. The other states, which grow significant amount of soybean, are Maharashtra, Rajasthan and Uttar Pradesh. Soybean produces 2-3 times more protein per hectare than any other legume/pulse crop. It contains 40 per cent good quality protein, 23 per cent carbohydrates, 20 per cent oil and reasonable amounts of minerals and vitamins. Soy-protein is superior to other plant proteins and is the most economical source of dietary protein. Soy foods are health promoting. However, soybean also contains some antinutritional factors necessitating careful processing to make it fit for human consumption.

Nutritional importance and food options

Soybean is rich in both protein and oil but contains little or no starch. Soy-protein is rich in lysine but deficient in sulphur-bearing amino acid methionine. Cereals contain adequate quantity of methionine but are deficient in lysine. When soy protein and cereal-protein are combined in appropriate proportions, the nutritional value of the produce improves considerably (Hulse 1996). Soybean is generally processed for its oil, protein and lecithin. Whole beans or partially/fully defatted cake/meal can be used for making various soy based food items. Normally whole beans are used for making full fat soy flour, dairy analogs (soymilk, soy *paneer*, soy-yogurt, soy ice cream), fermented food (Tempeh, Natto, Sauce and Miso), and snack foods (roasted/sprouted beans). Soy flour can also be made form partially/fully defatted beans (cake/meal) and used in making baked products (*chapatis*, bread, biscuits, bun, rusk and cake), texturized soy-proteins (TSP), protein isolates and concentrates, extruded snack foods and so on (Ali, 1993).

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Flours

Soy flours are screened and graded products obtained after expelling or extracting most of the oil from dehulled soybean, except the full fat soy flour, which contains all of the oil originally present in soybean. Full fat soy flour is essentially dehulled, blanched and ground. Defatted soy flour is produced by nearly complete removal of oil from soybean by the use of hexane. It usually contains about one per cent oil. Low fat soy flour is produced either by partial removal of the oil form soybean or by adding back soybean oil and/or lecithin to defatted soy flour to a specific level (5-6). High fat soy flour contains about 15 per cent oil.

Soy-protein concentrates and isolates

Soy-protein concentrate is prepared form high quality cleaned and dehulled soybean after removing most of the oil and water-soluble non-protein constituents. The concentrate contains not less than 70 per cent protein on a moisture-free basis. Yield of protein concentrate is about 65 per cent of the total weight of the flour. Soy-protein isolate contains more than 90 per cent protein. It is extracted from defatted soy flour using dilute alkali. Yield of isolate is about 30 per cent of the weight of the starting flour.

Texturized soy-protein product

Texturized soy-protein (TSP) can be prepared form flour, concentrates and isolates. These are available in textured form with fibrous and chewy properties like meat. In the process, defatted flour is mixed with water and other additives to form dough, which is then fed to an extruder where the material is subjected to high temperature, pressure and mechanical stress. After emerging through the die, the dough puffs and assumes a fibrous texture simulating certain meat products. The size and shape of the textured dough can be controlled at the extruder die. TSP contains 45-50 per cent protein.

Soybean sprouts

Soybean sprouts have been used as food in the orient since ancient times. They may be used uncooked in salad, boiled in water with seasoning or may be tried in fat and used alone or mixed with other vegetables. Sprouting improves the nutritive value of the beans.

Soy fortified baked, extruded and infant foods

The effect of incorporation of soybean flour and its products have been investigated and studies have shown that 5-25 per cent soy flour can be used in bread

cakes and cookies, doughnuts, confectionaries, snack foods, macaroni products and infant foods without any loss of physical characteristics but substantial improvement in the nutritional value of the products. Soybean proteins are often added to food at low level to utilize their functional properties and the contribution to nutrition is minor. At higher levels (5-25 %), these proteins are an important source of protein; they also contribute the desired functional effect.

Soymilk and milk products

Soymilk is one of the popular traditional products of soybean and has been consumed widely in China as a nutritious and economical food. Soymilk is also a base for the preparation of many other products such as tofu (soy *paneer*), soy-yogurt, soy ice cram etc. In order to reduce or eliminate the undesirable flavour and taste in soymilk, beans are subjected to moist-heat treatment to inactivate *lipoxygenase*, blanching in sodium or ammonium bicarbonate, distilling the lactic fermented milk under reduced pressure, contacting the milk with activated carbon, crushing/grinding the beans in the absence of oxygen, and using appropriate flavourants. Soymilk prepared by the improved technique is highly acceptable. Some acceptable products include blend of soymilk with bovine milk, condensed milk, spray dried milk, infant foods, recipes like ice cream, custard, pudding and cake from milk, and *khurma, sev* and *samosa* from the residue/okara. As a matter of fact, 6-8 litres of soymilk can be obtained form one kilogram of dry soybean. The positive features of soymilk are its low-cost, high nutritive value and suitability to lactose intolerant children/people.

Soy *paneer* (tofu) is an unfermented soymilk-based product, which is now popular worldwide. It is prepared by coagulating soymilk using calcium or magnesium salts. It can substitute the regular milk *paneer*. Nutritionally, it is almost at par with its bovine milk counterpart. At 72 per cent moisture, soy paneer contains about 13.8 per cent protein and 8.8 per cent fat, whereas bovine milk *paneer* contains 14 per cent protein and 9 per cent fat. But the cost of soy *paneer* is about one-third of that of milk *paneer*. Shelf life of soy *paneer* and that of milk *paneer* is almost the same. Soy *paneer* can be used for making c*urry, pakoda, paratha*, etc.

Fermented soy foods

Fermented soy foods re used as flavouring agents to generally bland low protein diets and also as source of protein, and perhaps, vitamins. Soybean fermentation is not strictly for soybean alone, nor it is carried out by one kind of microorganisms. Fermentation involves a substrate consisting of bacteria, yeasts and moulds. The various fermented soy foods are soy-yogurt, tempeh, soy-sauce, miso, natto, etc. Out of these tempeh and soy-sauce are very popular in the Far-East.

Tempeh originated in Indonesia and is made by fermenting dehulled and slightly cooked soybean with a mould *Rhizopus*. Traditionally, soybeans are soaked and then dehulled by hand. Small pieces of tempeh from previous fermentation are used as inoculum. The fermentation process does not significantly affect the composition of soybean. The quality of tempeh protein is improved when it is prepared form mixes of cereals and soybeans. Vitamin contents, particularly niacin and riboflavin, increase significantly.

Soy-fortified traditional Indian foods

Indian snack foods like *paratha, halwa, laddu* and cookies have been prepared by replacing the traditional ingredients with up to 50 per cent soy flour. Full fat soy flour, blended with cereals, millets, or pulses flour, gave good preparations of *chapati*, *puri, pakoda, sev* and *halwa*. The soy flour incorporated into the batter at different levels gave acceptable *idli* and *dosa*. And, while the green beans can be used as vegetable, extruded snack foods form soy cereal blend have been found to be quite acceptable.

Research and Development efforts in India

The Indian Council of Agricultural Research (ICAR) in collaboration with the United State Agency for International Development (USAID) launched the Indo-US Sub-project on Soybean Processing and Utilization (SPU) in April 1985 with two centres. One was at the Central Institute of Agricultural Engineering (CIAE) Bhopal and the other one was at the Gobind Ballabh Pant University of Agriculture and Technology (GBPUAT), Pantangar. CIAE was the Coordinating Centre headed by a Project Director (Ali, 1991). The Sub-project SPU was terminated on 31st March, 1991. However, looking into the high degree of success of SPU and the food potential of soybean, the ICAR decided to continue the SPU activities of Bhopal Centre beyond March 1991 and made it an integral part of CIAE and it is, at present, known as the Soybean Processing and Utilization Centre (SPU).

The SPU has developed 18 soy products, 20 soybean processing equipments and pilot scale production facilities for full fat soy flour (FFSF), partially defatted soy flour (PDSF), soymilk, soy *paneer*, soy ice cream, and soy fortified biscuits (Table 1). There are now four manufacturers of cottage level soy *paneer* plant and five suppliers. Soy fortified biscuits (SFB) technology has been commercialised. 450 upcoming entrepreneurs have been trained in soymilk and soy *paneer* production. At present, there are 125 soy food manufacturers in India, which have created awareness about soy food and its nutritional and economic benefits alongwith Technology Mission on Oilseeds, Pulses and Maize (TMOP) and M. P. State Oilseeds Grower's

Table 1. Soy based food products, machinery and pilot plants developed at SPU, CIAE, Bhopal since April, 1985

Soy-products and Technology (19)	
Soy *dal*	Soymilk
Soy flakes	Soy *paneer*
Soy flour	Soy-yogurt
Soy fortified biscuits (Sweet)	Soy-ice cream
Soy fortified biscuits (Salty)	Tempeh
Soy fortified bread	Soy-*sattu*
Soy fortified muffins	Okara based *Burfi* & *Gulabjamun*
Soy fortified bun	Soy-sauce
Soy-snacks (Roasted/fried)	Soy-*shrikhand*
Soybean Processing Equipment (20)	
Grader	Plate type wet grinder
Manual dehuller	Modified oil expeller
Power operated dehuller	Low-cost steam generator
Blancher	Soybean cake grinder
Natural convection tray dryer	Dough mixture
Forced convection tray dryer	Loaf volumeter
Multipurpose LSU type dryer	Lever-type *paneer* pressing device
Three-roller flaking machine	Screw-type *paneer* pressing device
Two state roller mill for soy flake	Cottage level soy *paneer* plant
Low cost single screw forming extruder	Biscuit-cutter/moulder
Limited Scale Pilot Production Facilities (6)	
Full fat soy flour	Soy *paneer*
Partially defatted soy flour	Soy fortified bakery products
Soymilk	Soy-ice cream

Federation (MP OILFED). Interaction with soybean breeder especially at NRCS, Indore for grain quality and other end use traits (Ali, 1996) is being done.

The present major activities of SPU are related to the development of an integral extrusion-expelling units for soybean, enzyme technology to improve soymilk

quality, soy based *rasogulla* and by-product utilization. SPU/CIAE can give technology, prepare project reports, provide support services including consultancy for production and market search of soy products. Training in soy based food products and machinery can also be arranged for individuals, groups, and entrepreneurs for domestic use and commercial production of soy-products.

Acceptability of soy foods

Acceptance of soy foods in India has been rather slow, even though more and more people are tilting towards soy food because of its economic, nutritional and health benefits. The origin of flavour compounds in soybean is attributed largely to linoleic and linolenic acids. Their oxidation and degradation give rise to many of the compounds that contribute to the off-flavour of processed soybean products. In the seed, the oil is stored in spherosomes or lipid bodies and is presumably inaccessible to lipoxygenase catalysis. However, when the seed is crushed, the enzyme and the substrate can interact especially in presence of moisture, enhancing the flavour. There are lack of awareness among the people about the higher nutritional value of soybean and its products. Lack of processing and paucity of technology and training facilities at home, village and small-scale levels also contribute towards slow adoption of soy foods in India.

Future of soybean

Soybean has a very bright future because it is a low-priced and highly nutritive food source. Soy foods in India are gaining consumer acceptance and the demand for these products is increasing through the world. The present production and consumption trends of soy based foods ensure economic viability of soybean industry in India and promise household food and nutritional security and better health and happiness to the people of India and those living in the other parts of the Globe. World production of soybean has now crossed the 200 million tonnes mark and still rising.

Summary

So far soybean breeding in India has been towards developing soybean varieties for better pest and disease resistance and higher yields and at the same time making it possible to take next *rabi* crop utilizing the residual soil moisture. Now there is a need to breed soybean lines having more protein and/or oil, starch and lesser beany flavours.

Small scale decentralized processing of soybean into traditional products would promote soybean utilization. Generic promotion of soybean and its products for

nutritional and health benefits need to be promoted through all means, print and electronic media. There should be a Soy Food and Machinery Manufacturers Association in India to promote soybean and its value added products for the people of India.

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Soybean Research 1: 65-75 (2010)

Plant Regeneration from Mature Cotyledon Derived Cultures of Soybean (G*lycine max* L. Merrill*)*

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ABSTRACT

Mature cotyledons of eleven genotypes of *Glycine max* were cultured on three fortified MS media to assess their *in vitro* response. Higher *in vitro* morphogenesis leading to plantlet regeneration was varied considerably due to genotypes and inoculation medium. The basal MS medium fortified with 0.5 mg.l^{-1} NAA and 3.0 mg.l^{-1} BAP was found to be more responsive for all culture phases. More than 50 phenotypically normal plants per hundred cultured cotyledons were obtained from genotypes Panjab 1 (108.6), JS 72 44 (78.4), Bragg (56.3) and MACS 13 (52.7).

Key words: Soybean, mature cotyledons, callus, morphogenesis, plant regeneration

Soybean (*Glycine max* L. Merrill) is an important source of high quality protein (~40%) and oil (~20%) for vegetarians and vegans, particularly in the third world. The new tools of biotechnology will to ambit us to speed the development of improved cultivars with higher genetic yield potential, increased resistance to diseases and insects and greater tolerance to drought, heat, cold and soil toxicities of cultivated soybean. Before the transformation of *Glycine* to cater to the regional needs and to address the acclimatization issue, responding indigenous genotypes need to be identified.

In soybean, mature cotyledons have been used extensively to raise *in vitro* cultures (Kim and LaMotte 1987, Lazzeri *et al*. 1988, Ferreira *et al*. 1990, Bodanse-Zenettini *et al*. 1993, Fu *et al*. 1995, Nawracala and Konieczny 1996) including somaclonal variation studies (Graybosch *et al*. 1987, Freytag *et al*. 1989, Hawbaker *et al*. 1993), for the production of transgenics (Simpson and Herrera-Estrella 1989, Liu *et al*. 1996, Tokisky *et al*. 1996, Stewart *et al*. 1996), protoplasts (Kim and Chae 1989,

Hammatt *et al*. 1989) and cell suspension cultures (Christianson *et al*. 1983, Lamseejan *et al*. 1992, Bailey *et al*. 1993) and also for *in vitro* selection (Gray *et al*. 1986, Amer *et al*. 1988). This paper reports the induction of morphogenesis and plantlet regeneration to select *in vitro* responding genotypes and culture medium ensuing in an effectual protocol for regeneration of soybean varieties already under cultivation in Madhya Pradesh and adjoining areas.

MATERIALS AND METHODS

Seeds of eleven commercially established genotypes of *Glycine max* (Table 1) were obtained from Jawaharlal Nehru Agricultural University, Jabalpur centre of All India Coordinated Research Project on Soybean.

Mature cotyledon isolation and culture

Mature cotyledons of soybean were obtained from germinated mature seeds of soybean. For this purpose, healthy seeds were surface sterilized with 70 per cent (v/v) ethanol for 1 min followed by a 15 min treatment with 0.2 per cent (w/v) purified $HgCl_2$ and 3 subsequent rinses in sterilized double distilled water under aseptic conditions. Surface sterilized seeds were inoculated in culture tubes containing agar gelled water (8.0 $g.l^{-1}$ agar) under diffused luminance of 16 μ mol m^{-2} s^{-1} provided with white fluorescent lamps. Mature cotyledons excised from 4 days old germinated seeds were inoculated on different inoculation medium, in 100 mm x 17 mm glass Petridishes for incubation. The media was short listed on the basis of preliminary work conducted in this laboratory. The basal Murashige and Skoog medium (MS medium) (Murashige and Skoog 1962)) was supplemented with 5.0 $mg.l^{-1}$ p-chlorophenoxyacetic acid (PCPA) (MS5P), 3.0 $mg.l^{-1}$ PCPA and 0.5 $mg.l^{-1}$ 6-benzylaminopurine (BAP) (MS3PB) and 3.0 $mg.l^{-1}$ BAP and 0.5 mg. l^{-1} α-napthaleneacetic acid (NAA) (MS3BN) to fortified three different culture media. Unless, specified otherwise, all the media contained MS macro and micro nutrients, vitamins, 30.0 $g.l^{-1}$ sucrose with 8.0 g. l^{-1} agar and were autoclaved at 121° C under 15 psi for 20 min after adjusting the pH to 5.6 ± 0.1 with 1 N KOH. Each 100 mm x 17 mm Petri dish was plated with 6-8 cut portions of mature cotyledons. The cultured petridishes were sealed with Parafilm® and incubated under complete darkness at 25 ± 2°C for 1 week after which incubates were subjected to 12 h photoperiod regime of 30 μ mol m^{-2} s^{-1} luminance provided by white fluorescent lamps. MS basal medium and all other add-ons were procured from HiMedia Laboratories, Mumbai, India.

The experiment was laid out in factorial completely randomized design with three replications and each replication consisted of 120-150 mature cotyledons. The arc-sine transformation was made before the analysis of data.

Callus induction

The performance of each genotype in terms of callus induction was recorded after 5 weeks incubation. Based on the appearance, the 4 main types of calli were formed (a) compact dark green in colour with few or many bead like structures; (b) compact light green in colour, with few or many dark green bead like structures, and some times covered with a thin layer of white loose callus; (c) light green with dense and glossy appearance; and (d) cream in colour and soft friable in texture. Callusing efficiency was defined as the percentage of explants that produced callus. Data on the first three categories was grouped together to constitute morphogenic callus (Fig.1 C-D) for statistical analysis. Morphogenic efficiency was defined as the percentage of explants in which such callus proliferated.

Plantlet regeneration

All calli were transferred to a plantlet regeneration MS medium fortified with 0.4 $mg.l^{-1}$ BAP and 0.4 $mg.l^{-1}$ NAA and 20.0 $g.l^{-1}$ sucrose. Where necessary, the *in vitro* shoots were subsequently transferred to a MS rooting medium supplemented with 1.0 $mg.l^{-1}$ indole-3-butyric acid (IBA) and 15.0 $g.l^{-1}$ sucrose. The competence of genotypes for *in vitro* shoot proliferation in the back ground of the explant inoculation media was evaluated 4 weeks after the subculture in to the plantlet regeneration medium. The number of complete plant(s) produced per 100 mature cotyledons constituted plant regeneration efficiency.

Acclimatization of regenerated plants

The roots of plantlets were rinsed in sterile lukewarm water to wash-off the agar. The plantlets were potted in plastic root trainers containing a 3:1 mixture of autoclaved sand and vermiculite soil. Root trainers with transplanted plants were transferred under 30± 2^{o} C and 60±5 per cent RH for 30 days in a glass house for acclimatization (Fig.1F).

RESULTS

During the first week of mature cotyledons in culture, the explants enlarged but no callus proliferation was evident until the second week started where a majority of the incubated tissues exhibited signs of callusing (Fig.1 A-B). Callus proliferation started usually from the edges. The callus varied characteristically in distinct phenotypes viz. wet, rough, hard, dense and glossy which reflected diversity in the *in vitro* developmental potentials not only from various genotypes but also from the plant growth hormones regimes. Visual selection followed by sub-culture of these pheno-variant calli regenerated plantlets routinely and efficiently.

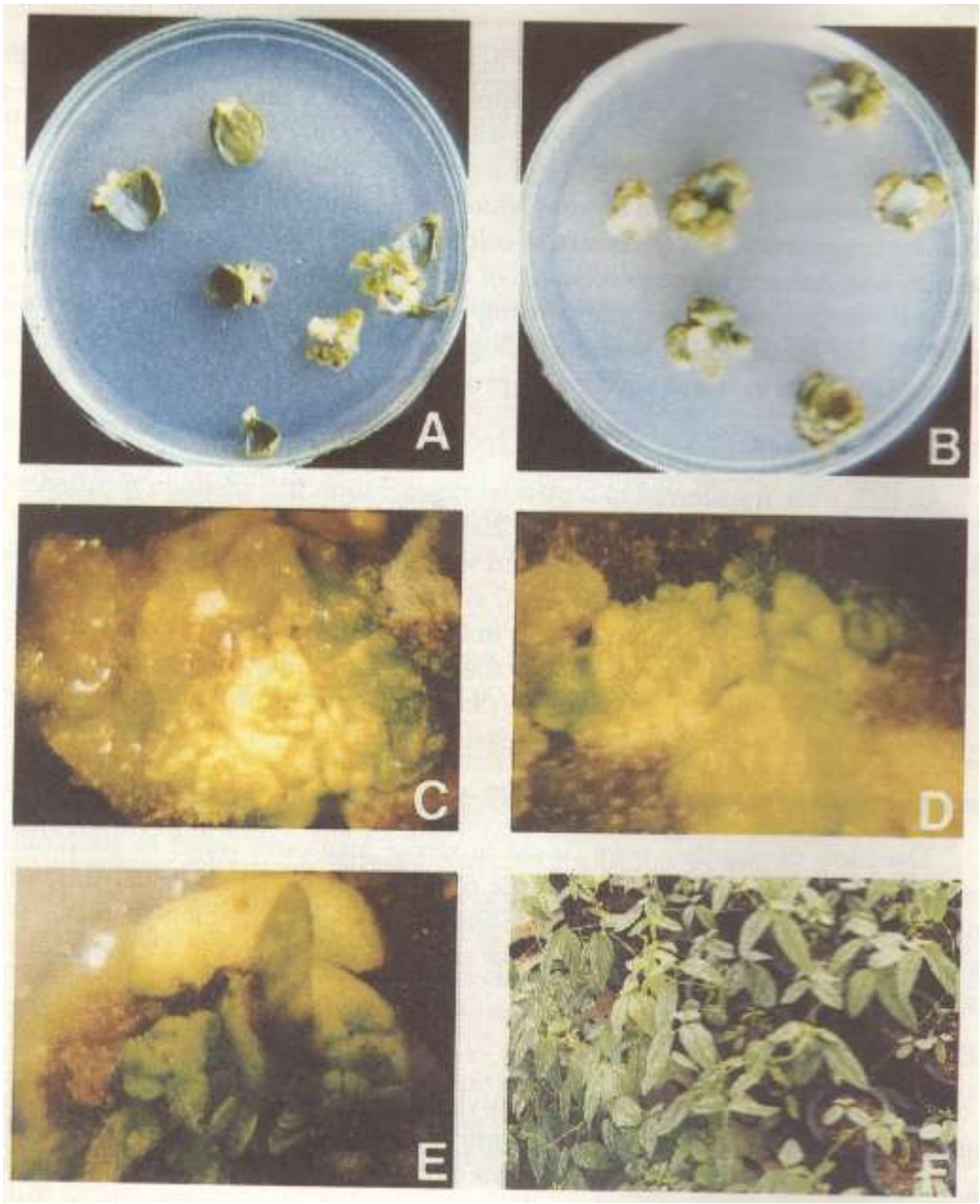

Figure 1. *Plant regeneration from soybean mature cotyledon:* ***A.*** *Cultured mature cotyledons after 15 days in culture;* ***B.*** *Cultured mature cotyledons after 35 days in culture;* ***C-D.*** *Morphogenic calli;* ***E*** *Shoot forming calli; and* ***F.*** *Plantlets transferred in glass house for hardening.*

The data on ‘a’, ‘b’ and ‘c’ type calli was compiled together to form the 'morphogenic callus' (Fig. 1 C-D) group. The bead like structures formed on ‘a’ and ‘b’ type calli were with irregular boundaries, usually appeared in clusters and did not form a bipolar structure. Some beads like structures imitated somatic embryos were not confirmed with a histological study. The study revealed absence of unambiguous bipolar structure. Undifferentiated 'd’ type calli were unable to regenerate plants despite several sub-cultures. In a few cases, different types of growth patterns were observed on the same callus.

Callus morphology varied considerably among three culture media. Although, callus initiation was observed from all the genotypes on different media tested, frequency as well as quality varied considerably. Mature cotyledons cultured on medium MS3BN comprising relatively higher concentration of a cytokinin and lower concentration of an auxin mostly produced embryogenic calli with high regeneration potential. Non-morphogenic creamish friable calli was proliferated with the addition of an auxin (MS5P) alone to the MS medium. Culture medium containing higher proportion of an auxin with lower concentration of a cytokinin (MS3PB) promoted the formation of organogenic calli that in turn preferred rhizogenesis instead of shoot induction.

The analysis of variance presented in Table 1 revealed highly significant ($p<0.01$) differences between the response of genotype, culture medium and genotype x medium interactions in terms of overall callus induction, morphogenic callus formation and shoot forming callus initiation. The medium MS3BN was consistently superior over MS3PB and MS5P (Table 1) for overall callus initiation (89.61%) morphogenic callus formation (56.55%) and shoot induction (35.65%) in all the eleven genotypes. The medium MS5P proved to be significantly ($p<0.05$) inferior to MS3BN and MS3PB for all the parameters recorded for evaluation.

In terms of the varietal response to *in vitro* culture, MACS 13 (92.37%) and Panjab1 (89.75%) were found more responsive than the next responding group of four same capable genotypes included JS 90 41, JS 80 21, JS 75 46 and JS 72 44; for the parameter callus initiation. Remaining genotypes were at par with each other and JS 335 (67.87%) proved to be the lowest performer. Proportion of calli resulting in morphogenesis was highest for the genotypes JS 72 44 (52.52%) followed by Panjab 1 (41.65%). Lowest result was exhibited by the genotype PK 472 (26.30%). Remaining genotypes being almost similar in their performance were in between them.

The data presented in Table 1 suggests that the *in vitro* performance of genotype JS 72 44 (40.04%) was significantly higher than rest of all genotypes for shoot forming calli (Fig. 1 E) initiation. Group of three genotypes MACS 13 (28.90 %),

Table 1. Callus induction, morphogenesis and shoot formation from mature cotyledon cultured on three different fortifications of MS media.

Genotypes▼ *Culture Media* ►	Callus induction (%)				Morphogenic calli (%)				Shoot forming calli (%)			
	MS5P	MS3PB	*MS3BN*	Mean	MS5P	MS3PB	*MS3BN*	Mean	MS5P	MS3PB	*MS3BN*	Mean
Bragg	51.78	66.28	96.42	**71.49**	16.22	23.02	77.34	**38.86**	13.50	13.39	55.71	**27.53**
JS 72-280	58.55	74.45	85.78	**72.92**	30.30	35.41	40.65	**35.45**	13.06	8.71	18.70	**13.49**
JS 72-44	89.49	73.42	88.24	**83.68**	36.14	37.98	83.49	**52.52**	21.66	26.56	71.91	**40.04**
JS 75-46	73.70	87.68	91.55	**84.31**	22.50	26.28	38.33	**29.03**	16.90	24.75	16.02	**19.22**
JS 80-21	78.70	91.77	86.37	**85.61**	32.21	38.51	44.09	**38.27**	14.64	28.41	20.10	**21.05**
JS 90-41	78.78	88.05	92.79	**86.54**	17.73	23.82	60.19	**33.91**	8.11	22.88	34.99	**21.99**
JS 335	35.23	73.21	95.17	**67.87**	20.28	32.74	53.88	**35.63**	10.24	26.40	31.31	**22.65**
MACS 13	91.94	90.68	94.51	**92.37**	23.10	26.79	65.14	**38.34**	27.20	16.71	42.80	**28.90**
NRC 2	77.24	91.58	73.11	**80.64**	32.37	36.57	22.37	**30.43**	16.22	31.06	12.82	**20.03**
Panjab 1	87.65	89.56	92.05	**89.75**	23.03	17.19	84.74	**41.65**	11.25	9.19	65.17	**28.53**
PK 472	63.72	65.88	89.78	**73.17**	18.97	8.01	51.92	**26.30**	10.95	4.46	22.72	**12.71**
Mean	**71.51**	**81.14**	**89.61**		**24.77**	**27.98**	**56.55**		**14.88**	**19.31**	**35.65**	
CD (0.05)												
Genotypes				**3.00**				**1.57**				**1.53**
Media				**1.56**				**0.82**				**0.80**
Genotype x medium				**5.20**				**2.73**				**2.66**

Panjab 1 (28.53%) and Bragg (27.53%) was significantly superior to remaining genotypes. Furthermore, the shoots induced in the genotype Panjab 1 were realized into plantlets at relatively high frequency followed by successful transfer to the soil. The Fig. 2 clearly illustrate that albeit plant regeneration was observed in all the genotypes, the overall response of Panjab 1 was superior to the other exceedingly responsive genotypes such as JS 72 44, Bragg and MACS 13.

The interaction between the genotypes and the media was also evident from the media dependent response of the genotypes for plant regeneration (Fig.2). Whilst the culture medium MS3BN regime promoted the highest rate of plant regeneration in genotypes Panjab 1 followed by JS 72 44 and MACS 13, induction medium MS3PB stimulated the regeneration of maximum number of plants in genotypes Panjab 1 followed by NRC 2, JS 80 21 and JS 72 44. The incorporation of PCPA (MS5P) was best suited for the genotypes JS 72 44 followed by NRC 2 and JS 75 46 respectively in terms of the regeneration leading to entire plant. Results exhibited in Fig. 2 reveal that, all the 11 genotypes studied, produced elevated plants quantity when incubated initially on the induction medium MS3BN as compared to the other two culture media under consideration.

The plants, after survival in the glasshouse conditions, were evaluated visually on the basis of appearance. Although, the traits were not scored quantitatively, the plants appear phenotypically normal and true to the type.

DISCUSSION

The plant growth regulators were compared with each other in combinations as well as alone. The combined effect of a cytokinin and an auxin was found more impressive. During the course of preliminary investigation (data not presented), it was observed that both cytokinin as well as auxin, were essential for increased morphogenesis but they were most effective when used in combination, and that the type and pace of callus growth was dependent on their relative ratio. Earlier similar response was also reported by Lazzari *et al*. (1988) and Li *et al*. (1989).

Callus initiation was observed from all the genotypes in all culture media tested but there were qualitative and quantitative differences. Of the three plant growth regulators combination regimes, culture medium MS3BN with comparatively higher cytokinin and a relatively lower auxin concentration produced calli with high morphogenic potential. This observation suggests that a high auxin concentration may not be imperative for *in vitro* morphogenesis but is in contrast with the reports that favor a high auxin concentration in induction medium enhanced morphogenesis (Nawracala and Konieczny 1996).

Plant regeneration was primarily through organogenesis in this study that was realized through the regeneration of either plantlets (shoot and root) or only shoots. Such isolated shoots were rooted separately. In addition, rhizogenesis alone was also observed but never lead to viable plants. Visual observations revealed that the meristems located at periphery of the callus mass developed into shoots whereas those embedded deep into the bodies lead to root formation. Similar observations have been made in *Nicotiana* (Thorpe and Murashige 1970) and *Linum* (Sarathe and Tiwari 1997, Bunning 1952), which develop into, shoots and roots as observed occasionally during the present work.

The embryogenic and organogenic calli were found to be morphologically distinctly dissimilar at the time of data collection in this study. Despite this, a system of clubbing the data on the two together as 'morphogenic' calli was followed not only for the ease of analysis and interpretation but also in view of the fact that anatomical and biological examinations were not carried out to confirm embryoid like structures as true embryoids. There have been suggestions that the embryoid like structures, speculated as embryoids, may in fact is shoot and root structures initiation from neighboring cells and resembling pseudo-embryos (Swamy and Krishnamurthy 1981).

The observations made during the course of present investigation revealed that organogenesis and embryogenesis coincided spatially as well as temporally, though at varying degrees, between genotypes. Normally, only one event is reportedly observed at a time (Henshaw *et al*. 1982). Not much is known about the mechanism of epigenesis but it has been suggested that the time and venue of embryo development is possibly governed by a complex interaction of biochemical and physical environments with the genetic factors (O'Neill *et al*. 1996). Cell cycle is a dynamic process and it may be hypothesized that whilst cells in a given stage support embryogenesis, those in another may lead to organogenesis. A contrary hypothesis is that the cells are steered towards either embryogenesis or organogenesis. According to this theory, the metabolites of plant growth regulators may produce cytotoxic effects (Bhaskaran and Smith 1990) and said 'switch' that triggers organogenesis or embryogenesis may be associated with the rate and mechanism of detoxification.

For complete plant regeneration from *Glycine* mature cotyledon explants, genotype Panjab1 facilitated the production of highest number of regenerants followed by JS 72 44 and MACS 13 when compared to other genotypes. Within each media treatment, the variation observed for the *in vitro* response resulted from genetic and physiological differences between genotypes as the culture condition were standardized. In addition, a difference between the endogenous hormone levels (Norstog 1970) amongst the genotypes also contributes to variability. Genotypic variations

have been widely recorded in *Glycine* cotyledon cultures (Ferreira *et al*. 1990, Bodanese - Zanettini *et al*.1993, Nawracala and Konieczny 1996, Bailey *et al*. 1993, Parrott *et al*. 1989, Komatsuda 1990, Kothari *et al*. 1991, Komatsuda *et al*. 1991, Thome *et al*. 1995, Li and Grabau 1996).

The present report clearly illustrates the intriguing relationship between initial and final results of *in vitro* morphogenesis studies, not so much with the chemical parameters as with the biological ones that are less predictable. The cultures demonstrating a high organogenesis rate produced multiple shoots though this did not necessarily mean an inflated plant regeneration frequency. The translation and manifestation of plural shoot induction into complete plant regeneration varied from 0 (no plant) to many (28 plants) per callus mass suggesting that not all the shoot developed in to complete plants. This variation may, at least in part, be attributed to the physiological status of the mother plant at the time of excision of the explant. On some instances, shoots ceased to develop after initiation, were deformed, or did not rooted. The relationship between initial and final results is best explained by the performance of genotypes JS 72 44 and Panjab 1. The former exhibited high proliferation of morphogenic and shoot forming calli but the number of complete plants produced was comparatively poor. On the contrary, genotype Panjab1 did not produce very many number of such type calli initially but the total number of complete plantlets recovery was high, due to the multiple shootlet induction and high survival rate of plantlets especially regenerated by calli obtained from culture medium MS3BN, after subsequent sub culturing. The rooting per cent also was comparatively high in genotype Panjab1, which is necessary for complete plants.

Under reported condition, plant regeneration from *Glycine* mature cotyledons took place after an intervening callus phase through embryogenesis as also via organogenesis. Direct somatic embryogenesis with singular shoot initiation was seldom observed. In the light of the above, genetic instability of the callus cells is expected and the possibilities of Somaclonal variations cannot be ruled out (Graybosch *et al*. 1987, Freytag *et al*. 1989, Hawbaker *et al*. 1993).

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Soybean Research 1: 76-86 (2003)

Effect of Tempering and Blanching followed by Microwave Treatment on the Content of Isoflavones, Saponins and Anti-nutritional Factors in Soybean Seeds

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ABSTRACT

Soybean seeds of two varieties, namely PK 416 and PK 472, cleaned and treated with microwave for 0.0, 0.5, 1.0, 1.5 and 2.0 min after tempering as well as blanching were analysed for the content of isoflavones, saponins and anti-nutritional factors. Microwave, heating of 2.0 min reduced phytic phosphorus content from 0.340 to 0.270 percent in variety PK 416 and from 0.215 to 0.140 per cent in variety PK 472 in tempered seeds. Blanching of seeds reduced the content of phytic phosphorus from 0.430 to 0.302 per cent in PK 416 and from 0.215 to 0.186 per cent in variety PK 472, whereas the reduction in trypsin units inhibited was from 36.89 to 15.58 per cent in variety PK 416 and from 36.66 to 14.18 per cent in PK 472. However blanching reduced it to 24.56 and 22.12 per cent in the variety PK 416 and PK 472, respectively. Raffinose content was reduced from 1.204 to 0.856 per cent in variety PK 416 and 0.836 to 0.568 per cent in variety PK 472 by tempering and microwave treatment, whereas blanching followed by microwave treatment showed greater reduction in raffinose content as compared to tempered and microwave treatment. The reduction in stachyose content of tempered seeds as well as blanched seeds was higher in variety PK 416 than PK 472. However, greater reduction in saponin content was observed for tempered and blanched seeds of variety PK 472 than the variety PK 416. Microwave treatment reduced isoflavones content in both the varieties, but tempered seeds showed a greater reduction in the isoflavones content than the blanched seeds.

Key words: Soybean, tempering, blanching, microwave treatment, flatulence factors, anti-nutritional factors.

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Soybean (*Glycine max*) described as "golden bean" or "wonder bean" is known to be a native crop of Eastern Asia where it has served as an important part of diet for centuries. Soybean is an excellent source of quality protein and compare well with animal protein in essential amino acid pattern with the exception of sulphur containing amino acids (Gandhi, 2001). Soybean fat, a highly unsaturated, has been found to be nutritionally desirable (Westcambe 1997). Therefore, it can easily supplement the diet of poor Indians. Research on the potential health benefits of soy foods is particularly intriguing with respect to cancer prevention, cardiovascular disease, osteoporosis, menopausal symptoms and in lowering of cholesterol (Waggle and Potter, 2000). Though soybean were introduced in India during mid sixties, soy based foods are not widely accepted because of development of beany flavour, difficulty in cooking and possess anti-nutritional factors such as trypsin inhibitor, hemagglutines, flatulence factors, phytic acid etc. Several attempts have been made to remove anti-nutrients from soybeans including soaking (Nelson *et al*. 1976), blanching (Tezuka *et al*. 1995), cooking (Rao and Belavady 1978), microwave heating (Fasina *et al*. 2001), germination (Duhan *et al* 2001), enzymes (Maity and Paul 1991) and ultra-filtration (Ang *et al*. 1986). Limited information is available on the effects of tempering and blanching associated with microwave treatments on the content of isoflavones, saponins and anti-nutritional factors such as trypsin inhibitor activity and phytic acid.

MATERIAL AND METHODS

Soybean seeds of two varieties namely, PK 416 and PK 472, were procured from Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar, Uttaranchal, India. The seeds were cleaned and all the foreign particles were removed manually. The seeds of both the varieties were tempered with 2 per cent moisture for 2 hrs and then treated with microwave for 0.0, 0.5, 1.0, 1.5 and 2.0 min. In another set of experiment, the seeds were blanched with 0.5 per cent sodium bicarbonate for 30 min and then treated with microwave for the same period as in the case of tempered seeds. The treated soybean seeds were evaluated for the content of phytic phosphorus, trypsin inhibitor activity, raffinose, stachyose, saponins and isoflavones.

Phytic phosphorus

The phytic acid is precipitated with an acidic iron-III solution of known iron content. The decrease in iron (determined colorimetrically with 2,2- bipyridine) in the supernatant is a measure of the content of phytic acid (Hang and Lantzsch 1983).

Assay: Weighed quantity (0.0 5 g) of sample was extracted with 10 ml of 0.2 N HCl. An aliquot (0.5 ml) of extract was pipetted into a test tube fitted with a ground

glass stopper. Ferric solution (1 ml) was added in the tube and stoppered. Then the tube was heated in a boiling water bath for 30 min followed by immediate cooling in ice water for 15 min. Thereafter, it was allowed to adjust to room temperature. The content of tube was mixed after adding 2 ml of 2,2′-bipyridine solution. The absorbance was measured at 519 mm against distilled water after defined time (0.5-1.0 min). The amount of phytate phosphorus in the sample was calculated by comparing with the standard graph.

Trypsin inhibitor activity

Trypsin inhibitor activity was determined by modified method of Kakade *et al*. (1974). This method is based on the principle that when enzyme trypsin is allowed to act upon substrate, benzoyl-DL-arginine-p-nitroanilide (BAPNA), it releases aniline under specific conditions of pH and temperature. The aniline is yellowish in colour, which is measured colorimetrically at 410 nm, and the intensity of yellow colour is directly proportional to the amount of aniline released.

Flatulence factors

Raffinose and stachyose were determined by the paper chromatography method of Tanaka *et al*. (1975).

Preparation of sample: The seeds (10 g) were ground to 20 mesh and suspended in 100 ml of 80 per cent ethanol. The suspension was refluxed for 1 hr and filtered through Whatman no.1 filter paper. The residue was stirred in 100 ml of distilled water for 30 min and filtered again, then washed with water until the filtrate gave negative triphenyl tetrazolium chloride test. The extracts and washings were pooled and concentrated to 100 ml under vacuum below 50^0C.

Assay: The amount of 20 µl of concentrated extract was applied on chromatogram sheet and developed by descending chromatography using a mixture of n-propanol, ethanol and water (7:1:2, v/v). The modified α- naphthol reagent (1%, in ethanol) was sprayed to locate fructose containing sugars. A sugar spot on the chromatogram was cut off and the sugar was extracted with 2 ml of distilled water in a test tube overnight at room temperature. One ml of eluent (containing 10-70 µg of sugar) was mixed with 1 ml of concentrated HCl. The mixture was heated in a boiling water bath for exactly 6 min, and then cooled under running water. The intensity of yellow colour produced was read at 432.5 nm using Spectronic-20 (Genesys 5). The concentration of sugar was calculated by comparing the absorbance with working standard of raffinose and stachyose (Sigma Chemicals) ranging from 10-100 µg sugar per 20 µl.

Saponins

Saponins were extracted and isolated by the method of Lalitha *et al*. (1987).

Assay: The defatted soybean flour (100) was extracted with 80 per cent (v/v) ethanol (300 ml) in a Soxhlet apparatus and the extract was concentrated under reduced pressure at 40-50^{o} C in to a syrupy liquid. This was extracted several times with diethyl ether in a separating funnel, until the colouring matter was removed. The syrupy mass was diluted to about 50 ml with water and extracted four to five times with butanol in the ratio of 5:1 (v/v). The pooled butanol extracts were evaporated under reduced pressure and dried over anhydrous phosphorus pentaoxide. A solution of the powder thus obtained taken in 5-10 ml of dry methanol, was poured in to excess quantities of ether (50-60 ml), and the precipitated saponins were centrifuged out and dried over anhydrous phosphorus pentaoxide to constant weight.

Isoflavones

Total isoflavone content was determined by the method of Naim *et al*. (1974).

Assay: Finely ground defatted soybean flour (100 g) was exhaustively extracted with 60 per cent ethanol. The ethanol extract was concentrated to a syrupy mass, stirred with two volume acetone for 2 hrs at room temperature, filtered and the filtrate was concentrated to a syrup, which was adsorbed on 1-2 g Kisselgel G. The cake was extracted with diethyl ether for 3 days and then with acetone for another 3 days. The solvents were removed, the acetone residue crystallized from 80 per cent ethanol and washed with chloroform to give a mixture of isoflavones, which was dried to a constant weight.

RESULTS AND DISCUSSION

Phytic phosphorus

Microwave heating for 2.0 min caused a reduction in phytic phosphorus content from 0.340 to 0.270 per cent in the variety PK 416 and from 0.215 to 0.140 per cent in the variety PK 472 in the tempered seeds. The variety PK 472 showed higher reduction in phytic phosphorus content than the variety PK 416 in tempered and microwave treated seeds. Blanching of seeds in 0.5 per cent sodium bicarbonate for 30 min reduced the content of phytic phosphorus from 0.340 to 0.302 per cent in the variety PK 416 and from 0.215 to 0.186 per cent in the variety PK 472. The phytic phosphorus content was further reduced to 0.232 and 0.116 per cent after microwave treatment for 2.0 min in the varieties PK 416 and PK 472, respectively. The variety PK 472 showed much higher reduction in phytic phosphorus content than the variety PK 416 in the blanched and microwave treated seeds (Table 1).

Table 1. Effect of microwave treatment on phytic phosphorus (%) content of soybean seeds

Time of treatment (min)	PK 416		PK 472	
	Tempered	Blanched	Tempered	Blanched
0.0	0.340	0.302	0.215	0.186
0.5	0.325	0.286	0.196	0.162
1.0	0.306	0.264	0.172	0.150
1.5	0.286	0.250	0.156	0.138
2.0	0.270	0.232	0.140	0.116

	F value	CD at 5%	SEM±
a	**	0.0055	0.0019
b	**	0.0035	0.0012
c	**	0.0035	0.0012
a*b	NS	0.0078	0.0027
b*c	**	0.0078	0.0027
a*c	NS	0.0049	0.0017
a*b*c	NS	0.0110	0.0039

a: Time of treatment b: Variety c: Type of treatment

Increase in time of microwave heating significantly ($P \leq 0.05$) reduced the phytic phosphorus content. The phytic phosphorus content also differed significantly ($P \leq 0.05$) among the treatments and between the varieties. The effect of interactions among time of heating, treatment and varieties were significant ($P \leq 0.05$). Agte *et al*. (1998) also reported a decrease in phytic acid content during microwave heating of legume seeds. Hafez *et al*. (1989) observed that phytic acid and phytate phosphorus content decreased form 18.02 mg/g and 5.07 mg/g to 13.86, 11.81, 9.74 mg/g and 3.91, 3.31, 2.74 mg/g, respectively, after 9, 12 and 15 min microwave heating of soybeans.

Trypsin inhibitor activity

Microwave treatment of tempered soybean seeds for 2.0 min caused a reduction in trypsin units inhibited from 36.89 to 15.58 per cent in the variety PK 416 and from 36.66 to 14.18 per cent in the variety PK 472 (Table 2). Blanching reduced the trypsin inhibitor activity to 24.56 and 22.12 per cent in the variety PK 416 and PK 472, respectively and was further reduced to 10.20 and 11.80 per cent, respectively, after

2.0 min of microwave treatment. Time of treatment, type of treatment and varieties differed significantly ($P \leq 0.05$) with respect to the effect on trypsin inhibitor activity. Rahman and Aleem (1998) observed that increasing moisture level of soybeans caused more destruction of trypsin inhibitor activity during microwave heating. Rajko *et al*. (1997) recommended 6.5 to 7.7 min of microwave heating of soybeans for complete removal of trypsin inhibitors.

Table 2. Effect of microwave treatment on trypsin inhibitor activity (TIA/ML)[1] of soybean seeds.

Time of treatment (min)	PK 416		PK 472	
	Tempered	Blanched	Tempered	Blanched
0.0	36.89	24.56	36.66	22.12
0.5	30.76	23.00	28.01	20.64
1.0	24.54	20.32	22.14	17.58
1.5	18.92	15.82	18.60	14.26
2.0	15.58	10.20	14.18	11.80

	F value	CD at 5%	SEM±
a	**	2.0471	0.7161
b	**	1.2946	0.4529
c	**	1.2946	0.4529
a*b	NS	2.8947	1.0128
b*c	NS	2.8947	1.0128
a*c	**	1.8309	0.6405
a*b*c	NS	4.0941	1.4324

a: Time of treatment b: Variety c: Type of treatment

[1]1g sample extracted with 50 ml of 0.01N NaOH, 1ml of extract diluted to 50 ml, 1 ml of dilution taken for assay

Flatulence factors

Microwave treatment given for 2 minutes to tempered soybean seeds reduced the content of raffinose from 1.204 to 0.856 per cent in the variety PK 416 and from 0.836 to 0.568 per cent in the variety PK 472 (Table 3). However, microwave treatment given to blanched soybean seeds caused greater reduction in raffinose content as compared to tempered soybean seeds. The degradation of raffinose content was more in the variety PK 472. Fasina *et al*. (2001) observed similar results in kidney beans, green peas and black beans, which were soaked for 24 hrs and then infrared heated.

Table 3. Effect of microwave treatment of raffinose (%) content of soybean seeds

Time of treatment (min)	PK 416		PK 472	
	Tempered	Blanched	Tempered	Blanched
0.0	1.204	0.726	0.836	0.491
0.5	1.078	0.706	0.744	0.408
1.0	0.954	0.680	0.681	0.390
1.5	0.938	0.638	0.609	0.362
2.0	0.856	0.610	0.568	0.345

	F value	CD at 5%	SEM±
a	**	0.0193	0.0067
b	**	0.0122	0.0043
c	**	0.0122	0.0043
a*b	NS	0.0273	0.0095
b*c	**	0.0273	0.0095
a*c	**	0.0173	0.0060
a*b*c	**	0.0386	0.0135

a: Time of treatment b: Variety c: Type of treatment

Stachyose content was reduced from 3.605 to 2.836 per cent and from 3.082 to 2.430 per cent in the seeds of variety PK 416 and PK 472 and given microwave treatment of 2.0 min after tempering. Blanching reduced the stachyose content to 2.136 and 2.112 per cent in the variety PK 416 and PK 472, respectively (Table 4). It was further reduced to 1.662 per cent in the variety PK 416 and 1.645 per cent in the variety PK 472 after 2.0 min of microwave treatment. The decrease in stachyose content was found to be significant ($P \leq 0.05$) as the function of treatment, varieties and time of heating. Fasina *et al*. (2001) observed 17, 24.3 and 19.3 per cent retention of stachyose in kidney beans, green peas and black beans, respectively after 24 hrs of soaking and then treating with infrared.

Saponins

Soybean seeds tempered and treated with microwave for 2 min showed a decrease in saponin content from 0.308 to 0.260 per cent in the variety PK 416 and from 0.241 to 0.210 per cent in the variety PK 472, whereas, it reduced to 0.255 and 0.180 per cent in seeds that were blanched and treated for 2.0 min with microwave in the variety PK 416 and PK 472, respectively (Table 5). A significant ($P \leq 0.05$) difference was observed in the

Table 4. Effect of microwave treatment of stachyose (%) content of soybean seeds

Time of treatment (min)	PK 416		PK 472	
	Tempered	Blanched	Tempered	Blanched
0.0	3.605	2.136	3.082	2.112
0.5	3.426	2.040	2.915	2.060
1.0	3.160	1.862	2.726	1.948
1.5	2.964	1.790	2.640	1.1788
2.0	2.836	1.662	2.430	1.645

	F value	CD at 5%	SEM±
a	**	0.0595	0.0208
b	**	0.0376	0.0131
c	**	0.0376	0.0131
a*b	**	0.0842	0.0294
b*c	**	0.0842	0.0294
a*c	**	0.0532	0.0186
a*b*c	**	0.1191	0.0416

a: Time of treatment b: Variety c: Type of treatment

saponin content due to time of heating, type of treatment and variety whereas the effect of their interactions were non-significant ($p \leq 0.05$).

Isoflavones

Isoflavone content decreased significantly ($P \leq 0.05$) due to tempering and microwave treatment as well as due to blanching and then microwave treatment of both the varieties. The tempered seeds of the variety PK 416 showed that the isoflavone content reduced to 0.281, 0.266, 0.254 and 0.250 per cent after 0.5, 1.0, 1.5 and 2.0 min of microwave treatment, respectively from initial concentration of 0.309 per cent (Table 6). However, in the variety PK 472 the isoflavone content decreased form 0.266 to 0.230 per cent after 2.0 min of microwave treatment for tempered seeds. The blanched seeds showed a content of 0.226 and 0.155 per cent in the variety PK 416 and PK 472, respectively.

From this investigation it can be concluded that the reduction in the content of isoflavones, saponins, trypsin inhibitor activity and flatulence factor was of greater magnitude in the seeds of variety PK 472. Microwave treatment after blanching caused greater reduction as compared to after tempering.

Table 5. Effect of microwave treatment on saponin (%) content of soybean seeds

Time of treatment (min)	PK 416		PK 472	
	Tempered	Blanched	Tempered	Blanched
0.0	0.308	0.274	0.241	0.214
0.5	0.300	0.270	0.235	0.210
1.0	0.296	0.268	0.226	0.190
1.5	0.274	0.260	0.214	0.186
2.0	0.260	0.255	0.210	0.180

	F value	CD at 5%	SEM±
a	**	0.0194	0.0068
b	**	0.0123	0.0043
c	*	0.0123	0.0043
a*b	NS	0.0275	0.0096
b*c	NS	0.0275	0.0096
a*c	NS	0.0173	0.0061
a*b*c	NS	0.0388	0.0136

a: Time of treatment b: Variety c: Type of treatment

Table 6. Effect of microwave treatment on isoflavone (%) content of soybean seeds

Time of treatment (min)	PK 416		PK 472	
	Tempered	Blanched	Tempered	Blanched
0.0	0.309	0.260	0.266	0.182
0.5	0.281	0.252	0.251	0.180
1.0	0.266	0.245	0.245	0.174
1.5	0.254	0.230	0.237	0.162
2.0	0.250	0.226	0.230	0.155

	F value	CD at 5%	SEM±
a	**	0.0060	0.0021
b	**	0.0038	0.0013
c	**	0.0038	0.0013
a*b	*	0.0085	0.0029
b*c	**	0.0085	0.0029
a*c	**	0.0053	0.0019
a*b*c	NS	0.0120	0.0042

a: Time of treatment b: Variety c: Type of treatment

ACKNOWLEDGEMENT

This work has been carried out under the project entitled "Processing of Soybean for Diversified Uses and its Socio-economic Aspects" funded by NATP MM (ICAR), New Delhi, India. The financial assistance is duly acknowledged.

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