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Soybean Based Intercropping Systems in India- A Review

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ABSTRACT

The work done so far on soybean-based intercropping systems in India involving crops like sorghum, maize, pigeonpea, pearl millet, cotton, sugarcane, minor millets, wheat, rice, oilseeds and plantation crops is reviewed. The compilation brings out the possibilities of rational utilization of natural resources by resorting to diversified cultivation rather than monoculture of crops. Soybean being a short duration leguminous crop with wide agro-climatic adaptability, offers a good opportunity to fit in cropping systems in different regions with aided advantage of better economic returns, risk coverage and utilization of natural resources. The benefits of sustainability can be harnessed by adoption of scientifically evaluated and suggested intercropping systems.

Key words: Aggressivity, competition, intercropping, land equivalent ratio, monetary advantage, nutrient management, productivity, soybean, weed control

Intercropping has been recognized as a potentially beneficial system of sustainable crop production in semi-arid tropics. Subsequent evidences affirm the utility of the concept in realizing substantial yield advantages over their sole cropping. The advantages may especially be important as these are achievable not by means of costly inputs, but by simple expedient of growing crops together (Willey, 1979a). In addition to making optimum use of available resources, the potentials of intercropping have been highlighted as late as 40s (Donald, 1946 and Aiyer, 1949). Various aspects of intercropping systems were

also looked into in-depth by Donald (1961 and 1963) and Francis and Stern (1987). Willey (1979a, 1979b) made a critical analysis of the advantages accrued from the system. Salter *et al.* (1985) further focused on the advantages of intercropping under mechanized farming. Chatterjee and Mandal (1992) reviewed this subject and documented the trends in research on intercropping in early 90s.

The provision of species diversity (Holdridge, 1959 and Igbozurike, 1971) by mixed cropping is considered as major advantage over sole cropping. This diversification tends to promote yield

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stability because all the crops in a mixed culture are not likely to be affected by weather vagaries or pests and diseases. Yield stability and protection against crop failures are the primary reasons prompting a farmer to resort to mixed cropping (Doutt and Nakata, 1965, Dickinson, 1972; Flinn and Lagemann, 1980; Jodha, 1976; Olukosi, 1976). An interfering row strips in mixed cropping can act as physical barrier to insects (Altieri *et al.*, 1978; Gerard, 1976; Suryatra and Harwood, 1976; Satpathy *et al.*, 1977) and decrease in the spread of diseases further confirming the yield stability of the system (Koli, 1975 and Shoyinika, 1976).

As for as soybean (*Glycine max* L. Merrill) is concerned, it offers excellent potentials to get involved in the intercropping systems, as it is a short duration (85 to 130 days depending on genotype and latitude) leguminous crop which can fit in well with traditional cropping pattern (Bhatnagar and Joshi, 1999). It also shows comparative tolerance to drought (Lawn, 1982) and excessive soil moisture conditions (Hunter *et al.*, 1980; Tanaka, 1983; Nathason *et al.*, 1984; Wright *et al.*, 1988). The other advantages offered by the crop are better ability to fix atmospheric dinitrogen, tolerance to low pH and high level of aluminum (Tanaka, 1983), and economic viability (Soni *et al.*, 1990). Besides, the crop imposes no allelopathic effects on companion crop and amicably adjusts with partial shade of companion crop in the system.

Soybean has shown a spectacular growth in last four decades establishing itself as premier oilseed crop in India.

During 2010, it covered an area of 9.21 million hectares with estimated production of 9.81 million tonnes (http://eands.dacnet.nic.in/latest_2006.htm). The crop is predominantly grown during monsoon on Vertisols and associated soils with major niche in Madhya Pradesh, Maharashtra and Rajasthan. Further, it is being fast adapted to non-traditional areas such as Chhatisgarh, Andhra Pradesh, Karnataka, *etc.* The research results on soybean based intercropping in India have been compiled in the present paper to take the message on advantages of intercropping to extension workers and soybean growers so that the practice can be widely adopted to sustain the productivity of crops in addition to other associated advantages.

Soybean with sorghum

Yield and Monetary advantage

As early as 1951, Kaushik reported that intercropping of sorghum [*Sorghum bicolor* (L.) Moench] with soybean, green gram [*Vigna radiata* (L.) R. Wilczek], black gram [*Vigna mungo* (L.) Hepper], and pigeonpea [*Cajanus cajan* (L.) Millsp.] was more remunerative than their pure stands. Shrivastava and Singh (1975) and Trikha (1983) concluded that soybean + sorghum intercropping system proved to be superior to other crop combinations and produced highest net returns. Hosmani *et al.* (1986) reported that sorghum could profitably be intercropped with cowpea [*Vigna unguiculata* (L.) Walp], *Sesbania grandiflora* or soybean or sunhemp (*Crotalaria juncea* L.) in north Karnataka. Several workers recorded an advantage of intercropping soybean in sorghum over

sole cropping under different agro-climatic conditions (Singh *et al.*, 1973; Shrivastava and Singh, 1975; Tarhalkar and Rao, 1975; Chandravanshi, 1975; Singh, 1979; Dusad and Morey, 1979; Ravindran and Palaniappan, 1979; Waghmare and Singh, 1982; Venketeshwarlu, 1982). Compatibility of soybean, green gram and cowpea with sorghum was found comparable under dry land as well under irrigated conditions (Singh, 1980; Waghmare *et al.*, 1982; and Waghmare and Singh, 1982). Pal *et al.* (1991) brought out that the sorghum + soybean in 3:3 or 4:2 row ratio was profitable indicating 1.2 land equivalent ratio (LER). Singh *et al.* (1983) rated soybean as the better intercrop in sorghum than groundnut.

De and Singh (1981) found that sorghum + soybean system could give yield advantage of 22 to 23 per cent. Sorghum was considered to be inherently efficient to fit in intercropping with legumes and invariably results in 10 to 15 per cent higher monetary returns than from sole crops (Willey *et al.*, 1982). Bhargava *et al.* (1987) also stated that intercropping with soybean/black gram/green gram did not influence sorghum yields. Singh (1983) reported 28 per cent increase in grain and fodder yield of oat (*Avena sativa* L.) when grown after sorghum + soybean intercropping as compared to sole sorghum.

Varietal compatibility

Singh and Singh (1991) stipulated that soybean varieties JS 72-44, PK 472 and JS 80-21 sown in alternate or paired row configuration are suitable for intercropping in sorghum. Raghuvanshi

et al. (1991) and reported LER value of 1.36 from sorghum (CHS 5) + soybean (JS 72-44) system planted in 1:1 row geometry.

Planting geometry

Tarhalkar and Rao (1979) opined that low canopy short duration crops like soybean could give 90 to 98 per cent yield recovery of sorghum with 1.60 LER, while the green gram and sunflower (*Carthamus tinctorius*) reduced the sorghum yields to the tune 35 to 75 per cent. Chaudhary and Singh (1982) also recorded that soybean had no adverse effect on the yield of sorghum in intercropping and results in additional yield. In Rajasthan, the yield of sorghum at 60 cm rows or paired rows at 30/90 cm remained unaltered by intercropping soybean as compared to pure stand (Gupta and Singh, 1988). However, the difference in total productivity of the system was narrowed down with increasing N application rates. In red soils of Andhra Pradesh, soybean seemed rather better intercrop than cowpea in alternate row pattern with sorghum as it resulted in extra yield advantage as compared to sole crop (Willey and Rao, 1979). Similarly, growing of one-soybean row in between two rows of sorghum at 90 cm under Delhi conditions gave an LER of 1.35 (Mohta and De, 1980). Intercropping of one row of soybean between normally spaced sorghum at Dharwad, while at Sehore two rows of soybean between paired (30/90 cm) rows of sorghum and 1:1 alternate sowing was found to be remunerative. However, in both the cases increasing row proportion resulted in progressive

decrease in total production (Anonymous 1971-94). Soybean or pigeonpea intercropped with sorghum proved to be more stable systems as compared to groundnut (*Arachis hypogaea* L.) under *Malwa* conditions (Umat, 1985). Singh and Jha (1984), and Sidhu *et al.* (1988) contemplated that such intercropping not only generated higher long-term returns but also was more stable than sole cropping. Billore and Joshi (2005) reported that the planting of soybean and sorghum either in 4:2 row ratio (30 cm) or two rows sorghum between paired rows of soybean (22.5/90 cm) proved to be better over other planting patterns by giving highest total productivity, monetary advantage and LER (1.21/1.19) with better companion indicating low competition interference and high energy output and energy use efficiency and energy productivity.

Nutrient management and improvement in soil environment

Reddy and Chatterjee (1973) recorded enhanced yields of soybean when main crop sorghum was dressed with 80 kg N per ha than that of 20 kg N per ha. Similarly, Ahuja and Singh (1987) and Balyan and Singh (1987) have reported the increased doses of NPK resulting in increased productivity of the system. The benefit of 30-80 kg N per ha to the succeeding wheat (*Triticum aestivum*) crop when grown after sorghum + soybean intercropping than sole sorghum has been reported by Singh and Ahuja (1990). Besides the residual benefits of nitrogen, they also concluded an additional benefit of 40 kg N per ha to the companion sorghum crop. Similarly,

saving of 50 per cent on account of fertilizers was observed for the sorghum + soybean intercropping system (Anonymous, 1990). Raghuwanshi *et al.* (1993) achieved LER values between 1.10 and 1.16 when both the crops were fertilized with 100 per cent NPK. Lomte *et al.* (1993) reported that sorghum + legume intercropping enhanced the bulk density, water stable aggregates, infiltration rate, hydraulic conductivity and organic carbon contents of the soil.

Ghosh *et al.* (2006) opined that yield and LER of both the intercrops increased over sole crops, though based on aggressivity RCC, sorghum is more competitive than soybean. Interaction of yield with different components indicated that three belowground components, i.e., NR activity in root ($r = 0.62$, $r = 0.63$, $P < 0.05$), root length density ($r = 0.36$, $r = 0.33$, $P < 0.05$), and Soil Microbial Biomass Carbon (SMBC) ($r = 0.71$, $r = 0.66$, $P < 0.05$) of both intercrop soybean and intercrop sorghum, respectively, had the greater effect on yield advantage in the intercropping system. Soybean did not benefit from intercropping to the same degree as sorghum under N-P-K. Nutrient application influenced LER, RCC, and monetary advantage and was found in the order of N-P-K plus farmyard manure (FYM) > N-P-K plus poultry manure (PM) > N-P-K plus phosphor-compost (PC) > N-P-K > control. However, based on competition ratio, yield advantage was greater under N-P-K plus PM. The results suggest that sorghum is the major contributor to the mixture yield and that the integrated use of N-P-K plus FYM or N-P-K plus PM is an important nutrient management option for sustaining this

intercropping system, particularly to benefit the legume component.

In general, nodule number and its mass were lower in intercrop soybean than sole soybean. Also there was decrease in the nodule number with higher NPK dose. The FYM treated plots recorded 22.0 and 7.6 per cent higher nodule mass than poultry manure and phospho-compost plots, respectively. Also, the total chlorophyll content was higher in organically treated plots than that in 100 per cent NPK particularly at 30 days after sowing (DAS, pre-flowering). In sorghum the peak nitrate reductase (NR) activity was recorded at 60 DAS while in soybean it was at 30 DAS. The NR activity was higher in intercrop sorghum than that in sole sorghum. Maximum NR activity was observed in 100% NPK. Soybean/sorghum intercropping systems recorded significantly higher root and shoot biomass than sole soybean and sorghum. The crop growth rates were relatively rapid during 30–60 DAS and followed the order; intercropping > sole sorghum > sole soybean. With the increase in NPK dose from 0 to 100 per cent there was significant improvement in the dry matter (DM) production in sole sorghum and soybean/sorghum intercropping system. Soybean as preceding crop recorded the highest DM, chlorophyll content, NR activity in wheat (*Triticum aestivum* L.) while these values were the lowest in sorghum–wheat system (Ghosh *et al.*, 2004).

Ghosh *et al.* (2009) concluded that the Relative Dry matter Yield (RDY) and Relative Nitrogen Yield (RNY) of

sorghum were greater than the values of RDY and RNY of soybean indicating inter-species competition for N between component crops, peak competition being at 80 DAS. Using the concept of RDY and RNY, it was observed that having coincided the maturity period and peak demand for N of both the crops, soil N was exhausted by sorghum because of its strong competitive ability and N was liming for soybean at 80 DAS. Strong competitive ability of sorghum was also evident from higher biomass, root mass, root length density and contribution to the mixture yield. Once sorghum entered its maturity phase, its competitive effect on soybean was greatly reduced. Competition for P between two species is more prominent up to 60 DAS and P was not limiting to any of the species after 60 DAS as the relative phosphorus yield (RPY) values were equal to corresponding RDY values. Based on relative potassium yield (RKY) value, none of the component species suffered from K deficiency at any stage even if it was not applied. This implied that competition exists for soil N and P and not for K up to 60–80 DAS in soybean/sorghum intercropping system. The result showed that competition between two crops measured in terms of RNY, RPY and RKY under organic-fertilizer was less; however, recorded higher soybean equivalent yield and monetary advantage index than inorganic-fertilizer. The study thus suggested that in soybean/sorghum intercropping system to minimize competition between two crops in N and P deficient Vertisol, application of 75 per cent NPK + FYM/PM/phospho-compost is a viable nutrient management option.

Weed management

Although, intercropping of sorghum with soybean had smothering effect on weeds (Balyan and Singh, 1987), herbicidal weed control utilizing either Atrazine @ 1.0 kg a. i. per ha or Alachlor @ 1.5 kg a. i. per ha or Thiobencarb @ 3.0 kg a. i. per ha or Diuron @ 1.0 kg a. i. per ha as pre-emergence is suggested (Tiwari and Khurchania, 1991) for harnessing maximum benefits and checking the nutrient drain from soils through weeds.

Soybean with maize

Yield and monetary advantage

Choudhary and Singh (1982) reported no adverse effect of intercropping soybean, green gram, black gram, cowpea and cluster bean (*Cyanopsis psoroioides* L.) on main crop of maize (*Zea mays* L.). Further, De *et al.* (1978) reported an increased productivity to the extent of 66 per cent for maize + legume intercropping. Reddy and Chatterjee (1975), Mohta and De (1980), Prusty *et al.* (1985) and Jayaraman *et al.* (1988) also noted that maize yields were unaffected by inter/mixed-cropping with soybean. An increase in the productivity was recorded in maize + soybean/black gram/cluster bean/sun hemp (*Crotalaria juncea* L.) (Singh *et al.*, 1988). Maize + soybean system recorded yield advantage (LER 1.71) and profitability under Nagaland hilly conditions (Tripathi and Sharma, 1988). Sidhu *et al.* (1988) contemplated high productivity and greater stability through intercropping soybean with maize and suggested row orientation in north-south direction for achieving maximum productivity.

Hiremath *et al.* (1993) also advocated soybean to be ideal intercrop in maize.

Varietal compatibility

Soybean varieties DS 74-24-2, PK 308, SL 4, Ankur, Pb 1, JS 72-44 JS 71-05, PK 472 and JS-2 were found to be suitable for intercropping in maize (Anonymous, 1971-1994).

Planting geometry

In an intercropping trial consisting of different row configurations of maize + soybean system at different locations revealed the better economic returns from alternate rows (Shrivastava *et al.* 1980; Shah and Modgal, 1986), 2:2 rows (45/30 cm) and 1:2 rows (Arya and Saini, 1989). Maize + soybean planted in 4:1 and 1:1 row ratio orienting in north-south direction could yield 300 kg per ha additional with LER between 1.23 to 1.27 (Angadi and Gumaste, 1989). They further reported that the maize fodder + soybean system is also feasible and profitable. Vyas *et al.* (1995a) concluded that the intercropping of soybean + maize in 2:2 row ratio was most suitable for getting higher yield, LER and monetary advantage. Maize dominated soybean in all the intercropping treatments indicating higher values of aggressivity and competition ratio with lower values of competition index and competition coefficient. The relative crowding coefficient (RCC) showed the greater degree of non-competitive interference in 2:2 row planting patterns. Billore *et al.* (2004) reported that the planting of soybean and maize either in 4:2/ 2:2 row ratio (30 cm) or 100 + 50 per cent seed

mixture (Billore *et al.*, 2004) or in 4:2 row ratio (30 cm) or two rows sorghum between paired rows of soybean (22.5/90 cm) (Billore and Joshi, 2005)-proved to be better over other treatments of seed mixture by giving highest total productivity, monetary advantage and LER indicating low competition interference and high energy output, and energy use efficiency and energy productivity.

Nutrient management

Like in sorghum + soybean, maize + soybean system also exhibit beneficial effects on the succeeding wheat crop and led to nitrogen economy (Nair *et al.*, 1979; Singh and Kaushik, 1987).

Reddy *et al.* (1987) realized additional yield of maize (at 75 cm rows) intercropped with soybean or black gram or groundnut and also noted an increasing trend in yield with increasing levels of N up to 120 kg per ha which had no significant effect on the yield of companion crops. In contrast, Sharma *et al.* (1991) noted reduction in yields of maize/soybean with increasing N levels. Cent per cent application of NPK to maize + soybean is reported to pay handsome remuneration with 262 index of returns (Anonymous, 1990). An increase in yield of maize by 32 per cent and of soybean by 30 per cent in alternate rows at 60 cm when irrigation was superimposed on 125 per cent application of recommended NPK levels was reported by Chakor and Kumar (1988). A significant impact of application of 25 kg N per ha to intercropped soybean in addition to the recommended NPK to maize was also noted in a study carried

out in Tamil Nadu (Jayaraman *et al.*, 1988). An increase in soil organic matter and no adverse effect on the levels of NPK was reported for maize + legume system (Adhikari *et al.*, 1991). In a sequential trial involving soybean + maize succeeding wheat crops, Badiyala and Verma (1991) concluded that the maize and wheat yields increased as the level of N was increased to 120 and 80 kg per ha, respectively. There was consistent and significant reduction in soybean yield with the addition of nitrogen. Venugopal and Shivshankar (1991) reported that the intercropping of maize + soybean under paired row planting was superior to sole maize.

Ramesh *et al.* (2005) concluded that the sole cropping of sorghum and maize recorded higher biomass yield than sole soybean and their intercrops. In contrast, the biomass yield of wheat during post-rainy season was higher when the preceding crops were sole soybean or soybean + maize intercropping. At N₀ level, intercropping of soybean + maize followed by wheat system was more productive and remunerative with a gain of 7.7 and 27.0 kg per ha of soil available P and K, respectively, but resulted in a net loss of 4.0 kg per ha of soil available N over a period of 2 years. Padhi and Panigrahi (2006) reported that the maize with soybean and black gram with maize significantly recorded the highest maize-grain equivalent yield of 2,570 and 1,180 kg per ha at 1:1 row ratio, respectively. Among various intercropping systems, maize + black gram at 1:1 row ratio significantly achieved the maximum maize-grain equivalent yield (37.5 q/ha), LER (1.68), ATER (1.61), MA (9,102), net

return (₹ 10,511/ha), return per rupee invested (1.84) and energy output (144.2, 1000 MJ/ha) compared to sole maize and black gram. Maize + soybean at 1:1 row ratio closely followed this system on the above aspects. However, maize + soybean followed by maize + black gram recorded the highest available soil N at 1:1 row ratio and available soil P and K at 2:1 row ratio among various intercropping systems. Meena *et al.* (2006) concluded that the highest maize equivalent yield was observed with 2:2 maize + soybean intercropping sown at 30 cm distance with each other. Application of 75 per cent of RDF to maize (90 kg N/ha and 40 kg P/ha) and 50 per cent to soybean (60 kg N/ha and 40 kg P/ha) significantly increased their respective yields, maize-equivalent yield, net returns and benefit: cost ratios over 50 per cent RDF in maize and no fertilizer in soybean. The nutrient uptake by maize was highest with 1:1 ratio, while by soybean it was with 3:3 ratios. Increasing levels of fertility to maize and soybean up to 100 per cent RDF increased the total nutrient uptake significantly over 75 and 50 per cent in the both crops in intercropping system.

Weed management

Pre-emergence application of Metolachlor @ 1.25 kg a. i. per ha (Thakur, 1994) and Oxidiazon @ 1.0 kg a. i. per ha or Alachlor @ 2.0 kg a. i. per ha (Tiwari and Khurchaniya, 1991) have been recommended for effective control of weeds and realizing more yields in maize + soybean system.

Soybean with pigeonpea (*Cajanus cajan*) Yield and monetary advantage

Saraf *et al.* (1975) opined that pigeonpea intercropped with soybean, cowpea, black gram, and green gram gave higher yields than when intercropped with maize or sorghum. Similarly Wanjari *et al.* (1993) reported soybean + pigeonpea as more promising than pigeonpea intercropped with sorghum, cotton (*Gossypium* spp.) groundnut and black gram. This could be on account of relatively less competitiveness of the component crops, which utilized the environment most efficiently generating LER value of 1.46 (Anonymous, 1976; Prasad and Srivastava, 1991). It has been reported that the system as a whole offers higher net returns over sole crops (Tiwari and Bisen, 1975; Ray *et al.*, 1981; Tulsidass *et al.*, 1983; Prasad and Gautam, 1987; Joshi *et al.*, 1994). The yield of intercropped soybean was reduced to the extent of 50 per cent (Saxena and Yadav, 1976) and reduced yield of intercropped pigeonpea was also noted by Yadav and Yadav (1981). On the contrary, intercropping of soybean has been reported to have no adverse effects on the yield of pigeonpea with an additional yield (Singh and Singh, 1980; Singh, 1982; Anonymous, 1984). In confirmation to majority of reports on successful companionship of soybean and pigeonpea, Fryman and Venkataswarlu (1977) contemplated that the short duration legumes like soybean escaped successive competitive effect from long duration crop like pigeonpea. Chari (1995) also proposed that growing of pigeonpea as an intercrop in soybean in 5:1 row geometry was feasible and profitable and

can even be adopted by the farmers as an alternative to the traditional cultivation of FCV tobacco in black soils of Andhra Pradesh.

Sarkar and Shit (2008) concluded that the intercropping cereals, pulses and oilseeds with normal planted base crop of pigeonpea increased land use efficiency and gave higher total yields compared to pure cropping of pigeonpea under rainfed conditions on upland Oxisols of Bihar plateau. Cereals like maize, rice (*Oryza sativa*) and finger millet (*Eleusine coracana* Gaertn.) were more aggressive in intercropping system and tended to have depressing effect on red gram. Intercropping of short duration pulses like black gram and green gram and legume-oilseed crops like peanut and soybean with pigeonpea showed balanced competitive abilities and proved more efficient in the system.

Ghosh *et al.* (2006b) further reported that there was a reduction in growth and yield of intercrops; higher soybean equivalent yield and Area Time Equivalent Ratio (ATER) value in soybean/pigeonpea intercropping system as compared to sole soybean had a yield advantage. The average yield advantage in intercropping system was 60 per cent higher than that from sole soybean. The yield advantage of intercropping system in terms of ATER was 7 per cent greater with sub-soiling than conventional tillage. The yield response to sub-soiling was consistent over the period and on an average, sub-soiling increased yield by 20 per cent. The effect was associated with improved water storage and root length density.

However, with respect to energy use efficiency and profit, the effect of sub-soiling was comparable to conventional tillage. The variation in net return and benefit:cost ratio in sub-soiling every year and sub-soiling in alternate years in sole soybean and soybean/pigeonpea intercropping was not significant. However, in sole pigeonpea sub-soiling every year out-yielded sub-soiling in alternate years. The interactive effect of sub-soiling and intercropping increased the yield by 21–25 per cent. Thus, under rainfed cropping where drought of unpredictable intensity and duration is a prevailing feature, soybean/pigeonpea intercropping could be a promising option, especially when combined with sub-soiling in alternate years.

Talnikar *et al.* (2008) reported that weeds caused 79.93 per cent reduction in pigeonpea grain yield if weeds were allowed to grow till harvest; however, grain yield losses were only 38.19 per cent in pigeonpea + soybean intercropping system.

Planting geometry

Short duration legumes which have fast early growth and close canopies are good competitors with weeds (Ali, 1988). Studies in Kanpur, India at the Directorate of Pulses Research (ICAR) indicate that intercropping with these legumes can effectively suppress weed growth in pigeonpea. The studies examined the weed suppressing ability and total productivity of four short duration legumes, namely urdbean [*Vigna mungo* (L.) Hepper], mungbean, cowpea, soybean and one cereal namely sorghum intercropped with pigeonpea under two

planting systems for each crop combination (uniform rows of alternating pigeonpea and an intercrop, rows 30 cm apart; and paired rows of pigeonpea alternating with one row of an intercrop, all rows 40 cm apart). The intercrops suppressed weed growth more effectively (20-45 %) as compared to unweeded sole pigeonpea and paired row plantings. Rows were more closely spaced in uniform row plantings than in paired row plantings; this probably increased the intercrop's ability to compete with weeds. All intercrops of legumes with pigeonpea were more productive than the unweeded pigeonpea monocrop.

The studies carried out by Tomar *et al.* (1984 and 1990) and Dubey *et al.* (1991) pointed out that since soybean grows faster than pigeonpea, good yield advantage could be achieved even by planting the two crops in 2:1 row arrangement which was superior to intercropping black gram, groundnut, green gram and sorghum under Madhya Pradesh climatic conditions. Even reversing the above row arrangement of the two crops yielded 79.9 and 66.7 per cent higher than respective sole crops with LER of 1.43 (Upadhyay *et al.*, 1987). Patra and Chatterjee (1986) stipulated that soybean + pigeonpea in 1:1 ratio at 30 cm apart gave 35 to 40 per cent more yield. However, the combinations offering higher yield advantages in order were two row of pigeonpea between two paired rows of soybean at 22.5/90 cm (LER 1.37) followed by alternate paired rows of soybean and pigeonpea at 30 cm (LER 1.44) and by 4 rows of soybean and two rows of

pigeonpea at 30 cm (LER 1.18). Better performance of two rows of pigeonpea between paired rows of soybean was also reported (Anonymous, 1971-94). Rathore *et al.* (1988) brought out that one row of soybean or sesame (*Sesamum indicum* L.) in between two rows of pigeonpea at 75 cm yielded LER of 1.51. Furthermore, Jain *et al.* (1991) recorded higher production and economic advantage on following skip row pattern as compared to substitution of soybean with green gram or black gram. Row ratios of 2:2 (Bhalla, 1991; Shrimal and Sharma, 1991) or 2:4 (Shrimal and Sharma, 1991) were also found to offer yield advantages.

Joshi *et al.* (1999) reported a significant reduction in yield of soybean and pigeon pea planted at different row arrangements as compared to their sole crops and concluded that the planting of soybean and pigeonpea in alternate paired row (30 cm) gave highest LER, monetary advantage and IER due to minimum competition between the crops

Billore *et al.* (2000) concluded that the maximum biological efficiency of system (LER 1.50 and ATER 1.18) were with soybean + pigeonpea in 2:1 row ratio, which resulted in highest monetary advantage due to non-competitive interference between the two crops (RCC 10.53). Aggressivity values showed their dominance of soybean over mesta and pigeonpea. Vyas *et al.* (1995b) reported that the intercropping with soybean in 2:2 row ratio was the most suitable for getting higher yield, pigeonpea equivalent yield, LER and monetary advantage. Pigeonpea dominated soybean in all the planting patterns indicating positive values of aggressivity. The lowest competition

index and highest competition coefficient were associated with 2:2 row ratio. RCC showed the greater degree of non-competitive interference in 2:2 row planting pattern.

Varietal compatibility

Comparative better suitability of soybean variety JS 71 05 over PK 416 and Amass 21 (Holkar *et al.*, 1991 and 1992) and PK 472 (Joshi *et al.*, 1994) has been reported. A decrease in LAI of seven soybean genotypes throughout the growing period was recorded on intercropping with pigeonpea (Holkar *et al.*, 1992). The pigeonpea varieties found suitable for intercropping were ICPL 83-57, AS 71-37, C-11, ICPL 416 and ICPL 87 in these studies. Billore and Joshi (2004) observed that the NRC 37 (Ahilya 4), PK 1029 and PK 1024 were found most compatible with pigeonpea variety ICPL 871 19 in 4:2 row ratio as adjudged by higher yield levels, soybean equivalent yield, LER, RCC, monetary returns and income equivalent ratio (IER) with low competition ratio. In a study involving six soybean varieties, based on aggressivity Billore and Joshi (2004) reported soybean varieties JS 335, PK 416 and JS 90 41 dominated pigeonpea. This system also produced higher energy, energy use efficiency and energy productivity. PK 1024 and PK 416 with pigeonpea ICPL 871 19 was found the most energy intensive system. Billore *et al.* (2002) concluded that the pigeonpea (ICPL 871 19 and ICPL 940 63) proved their compatibility when planted with soybean (Ahilya 3) in 4:2 row ratio indicating higher yield levels, soybean equivalent yield, LER, RCC, monetary returns and IER with low competition

ratio. Aggressivity values indicated that soybean dominated pigeon pea varieties except ICPL 840 31. This system also produced higher energy, energy use efficiency and energy productivity. ICPL 850 10 + soybean was found the most energy intensive. Sree Rekha and Dhurua (2009) reported that the highest seed yields were recorded by sole crops of pigeonpea (1401 kg/ha) and soybean (1853 kg/ha). Planting of pigeonpea (MRG-66) at 90 cm with 1 row of soybean (Durga) and pigeonpea, 150 cm with five rows of soybean recorded maximum net returns of ₹ 17, 226/ha) and ₹ 22, 035/ha, respectively. Pigeonpea, MRG-66 at 180 cm with six rows of soybean recorded maximum (1.39) LER.

Nutrient management

Some attempts have been made to look into the cultural and nutritional aspects of the system. Thakre *et al.* (1988) noted that yield of component crops and pigeonpea equivalent yield were maximum when seeding of soybean was done at the rate of 75 kg per ha as against 50 or 100 kg per ha. The yield of soybean increased with the NPK application to soybean. The maximum yield was recorded on supplementation of 100 per cent N:P₂O₅ (20:50 kg/ha) to soybean (JS 71 05) and 75 per cent N:P₂O₅:K₂O (15:45:15 kg/ha) to pigeonpea (AS 71-37) with an index of main crop yield equivalent of 90 per cent (Anonymous, 1990; Billore *et al.*, 1993) and offered highest monetary advantage (Billore *et al.*, 1991). In another study, highest net returns were achieved by soybean and pigeonpea intercropping in 2:1 configuration only by fertilization

with recommended rates to pigeonpea than pigeonpea + black gram system. Billore *et al.* (1991) and Billore and Upadhyay (1992) stated that 60 kg P₂O₅ and 40 kg S per ha were adequate for soybean and pigeonpea system. Further increase in fertilizer levels decreased the energy use efficiency and energy productivity from the system. Although these studies indicated that adoption of soybean + pigeonpea intercropping not only resulted in enhanced productivity but also helped in economizing the costly and environment unfriendly inputs like fertilizer.

Ghosh *et al.* (2006a) observed that before soybean harvest, the relative yield (RY) and relative nitrogen yield (RNY) of soybean were greater (1.0) than the corresponding values of RY and RNY of pigeonpea (0.6). This implied that competition exists for soil N between the component crops during the first half of the cropping system. It was observed that soybean harvest did not coincide with peak flowering of pigeonpea, the stage when biological nitrogen fixation (BNF) was the maximum. Thus, BNF dependency of pigeonpea was low before soybean harvest and the plants suffered from N deficiency more when no fertilizer-N was applied and diminished at a high-N level. Pigeonpea attained its peak flowering after the harvest of soybean and increased its dependency on BNF when soil N was exhausted by soybean. Thus, after the harvest of soybean, RY and RNY of pigeonpea gradually increased and approached 1.0 at maturity at all nutrient levels. The RPY values showed that phosphorus was not

the limiting factor to any of the crop in the system even if it was not applied. The study thus suggests that in the soybean/pigeonpea intercropping system, N is a limiting factor for growth of pigeonpea intercrop during the first half of its growth and application of 100 per cent NPK (30 kg N) + 4 t FYM could meet N demand of pigeonpea in N deficient soils as this nutrient management option gave higher yield, root length density and profit under soybean/pigeonpea intercropping system than 100 per cent NPK and control. Jain (2006) while working with Vertisols of Central India, reported the maximum gain of available N, P and K status (103.10 kg /ha, 13.53 kg/ha and 32.26 kg/ha, respectively) in soil with the application of 50 per cent RDF + Vermicompost @ 3 t per ha + bio-fertilizer in pigeonpea occupied soil, while in soybean occupied soil, the maximum gain of available NPK status was 126.10 kg per ha, 15.11 kg per ha and 66.56 kg per ha, respectively. In comparison, the values of overall gain through application of 50 per cent RDF + FYM @ 5 t per ha + bio-fertilizer were 96.8 kg per ha, 13.52 kg per ha and 29.83 kg per ha, respectively in pigeonpea occupied soil, while in soybean occupied soil, these were 106.5 kg per ha, 12.74 kg per ha and 53.86 kg per ha, respectively.

Vyas *et al.* (2006) and Billore *et al.* (2009) reported that the maximum soybean and pigeonpea yield was with FYM @ 5 t per ha + 75 per cent of RDF and Zn @ 5 kg per ha + RDF. Significant reduction in seed yield of soybean (18 to 36 %) and pigeon pea (32 to 47 %) was noticed when planted in intercropping systems at different fertility levels as compared to

their sole plantings. The maximum soybean equivalent yield and LER were recorded with RDF + Zn during both the years. The application of Zn + RDF produced the maximum net returns and remained at par with Zn + 75 per cent of RDF and RDF alone. While the highest B:C ratio was associated with Zn + 75 per cent of RDF.

Weed management

Pre- emergence application of alachlor @ 2 kg a. i. per ha with hand weeding and hoeing at 6 weeks after sowing proved most effective and economical in controlling weeds and enhancing the grain yield in pigeonpea + soybean intercropping system. Nutrient drain and the yield losses on account of weeds in soybean + pigeonpea system could be controlled by the herbicides Metribuzine @ 0.35 kg a. i. per ha or Butachlor @ 2.0 kg a. i. per ha or Oxadiazon @ 1.0 kg a. i. per ha as pre-emergence for controlling the weeds (Tiwari and Khurcharia, 1991).

Water management

Provision of irrigation appears to further enhance the efficiency of system. It was observed that supplementation of 0.75 cm water as post-monsoon irrigation at 0.8 IW/CPE ratio resulted in highest yield of pigeonpea + black or yellow soybean or sorghum system than their pure stands (Tiwari *et al.*, 1988). Extending their studies, Tiwari (1991) reported that the monetary advantage doubled when pigeonpea + yellow soybean in 2:1 ratio was given three irrigations at 0.6 IW/CPE ratio.

Soybean with pearl millet (*Pennisetum glaucum*)

Yield and monetary advantage

Intercropping soybean in pearl millet [*Pennisetum glaucum* (L.)R.Br.] could successfully be done without affecting the yields of later (Chaudhary and Singh, 1982; Palaniappan, 1983). The bonus crop of soybean yielded to the tune of 0.65 tones per ha in Tamil Nadu (Palaniappan, 1983).

Nutrient and weed management

Gautam and Kaushik (1987) brought out that application of phosphorus @ 40 kg per ha is essential and N @ 60 to 90 kg per ha is optimum for meeting the requirement of the two crops and profitable production. Atrazine @ 1.0 kg a. i. per ha or Butachlor @ 2.0 kg a. i. per ha as pre-emergence are effective for control of weeds and reducing the yield losses from the system.

Soybean in cotton

Yield and monetary advantage

Agronomic feasibility and economic viability of intercropping soybean in cotton (*Gossypium Spp.*) has been reported by several workers (Tulsidass *et al.*, 1983; Shanthaveerbhadraian and Patil, 1986; Sankaranarayanan *et al.*, 1989; Anonymous, 1971-94). The marginal yield reduction was observed in both the crops under intercropping system as compared to their sole crops (Muralikrishnaswamy *et al.*, 1990) but the system as a whole was more productive. Since the prolific soybean

varieties have smothering effect on the performance of cotton, the use of short, erect, less leafy and short duration varieties is recommended (Mishra and Mandloi, 1991).

Asewar *et al.* (2008) opined that the all the intercropping systems recorded significantly higher seed cotton equivalent yield over sole cotton. Among intercropping systems, cotton + green gram was significantly superior to cotton + soybean and cotton + black gram. Opening of furrow after every row resulted in higher seed cotton yield and seed cotton equivalent yield over flat bed land layout and it was at par with opening of furrow after alternate row. Sonwane *et al.* (2009) reported that the intercropping system of cotton + soybean (2:4) was significantly superior to cotton + pigeonpea (2:1), cotton + black gram (2:4) and sole cotton, black gram and pigeonpea but it was at par with sole cropping of soybean. Cotton seed yield was not reduced when it was cultivated with soybean. It was, however, reduced when grown with pigeonpea.

Planting geometry

The LER achieved by planting one row of soybean between two rows of cotton was 1.60 (Tulsidass *et al.*, 1983). One row of soybean (Monetta or JS 2) was recommended for intercropping in normally spaced (60 cm) cotton under Dharwad and Parbhani conditions (Anonymous, 1971-94). Wanjari *et al.* (1993) also found cotton + soybean (1:1) in 45/30 cm configuration to be promising. The system is reported to give LER 1.12, ATER 0.85 and Area Harvest Equivalent Ratio (AHER) 0.90

(Anonymous, 1971- 94). The results revealed that the benefit accruing through this system can be realized in cotton growing areas of Madhya Pradesh, Maharashtra, Tamil Nadu and Andhra Pradesh.

Giri *et al.* (2006b) concluded that the highest and significant cotton-equivalent yields were recorded in cotton + black gram intercropping, followed by cotton + soybean than sole crop of cotton. Further, yield of seed cotton, intercrop and cotton equivalent were enhanced significantly with every higher fertilizer level and highest values were recorded with 100 per cent RDF. The uptake of NPK by cotton was significantly more under sole cotton and cotton intercropped with black gram than the cotton intercropped with soybean. Sree Rekha *et al.* (2008) noted that the intercropping of cotton under rainfed conditions with soybean followed by green gram either in 1:1 or 1:2 ratio of each crop was more remunerative than sole crop of cotton. The total productivity of the system increased by 15 and 14 per cent respectively, with legumes intercropped in cotton either in 1:1 and 1:2 row ratios over sole cotton.

Weed management

Giri *et al.* (2006a) reported that in cotton + soybean intercropping, pre-emergence application of oxyfluorfen @ 0.10 kg a. i. per ha supplemented with hand-weeding and hoeing at 6 weeks after sowing proved equally effective in controlling the population and dry weight of weeds, and was as economical as that of cultural practice of 3 hand-weedings and hoeings at 3, 6 and 9 weeks after sowing.

Soybean with sugarcane

Yield and monetary advantage

The soybean with sugarcane (*Saccharum officinarum* L.) system received major attention in Tamil Nadu and Karnataka and was found economically viable. Shakti Soya Ltd, Coimbatore (Personnel communication) reported that the system not only provided bonus crop of soybean (1.25 and 1.75 t/ha from one and two rows of intercropped soybean, respectively) but also improved the cane yield. The system could yield average LER value of 1.44. However, Jayabal *et al.* (1991) observed comparable yield of soybean only when three rows of soybean (Co 1 or UGM 27) were intercropped in paired rows (30/130 cm) which provided maximum net returns. The trials conducted at Sugarcane Breeding Institute, Coimbatore (Personnel communication) indicated approximately 10 per cent reduction in cane yield by inclusion of soybean as first sequential crop intercropped in paired rows of sugarcane, but the same appeared to have been compensated by higher soybean yield (1.1 t/ha) compared to safflower (0.97 t/ha) and black gram (0.53 t/ha). Another study (Anonymous, 1971-94) from Dharwad indicated that planting one row of soybean on one side of ridge at 10 cm apart within the row is more practical and profitable.

Incidence of insect pest

Srikanth *et al.* (2000) reported that with black gram, cowpea, green gram and soybean as intercrops in sugarcane, the incidence of shoot borer *Chilo infuscatellus* Snellen (Lepidoptera) did not

differ significantly amongst different combinations and control. The incidence of top borer *Scirpophaga excerptalis* Wlk. (Lepidoptera) was negligible in all combinations. Counts of predators, comprising spiders and coccinellids, showed marginal differences. In the second study at farmers' fields, shoot borer incidence was significantly higher in 25 days and 65 days old sugarcane-soybean diculture plots than in sugarcane monocrop plots of corresponding age; the differences were not significant in a 30 days old crop. Mean predator numbers did not differ significantly between intercrop and monocrop in these three plots.

Soybean with minor millets

Yield and monetary advantage

Budhar and Gopaldaswami (1987), Purushottam (1987) and Devi *et al.* (1990) reported successful intercropping of finger millet and soybean with recommended fertilizer. Purushottam (1987) although recorded higher returns when both the crops received recommended levels but returns per rupee invested was maximum when expenditure on fertilizer was not incurred on soybean.

Varietal compatibility

Soybean varieties like Monetta, Hardee, KHSb 5 were suitable for intercropping with finger millet at Bangalore (Anonymous, 1971-94)

Nutrient management

Chandel *et al.* (1989) stipulated that beneficial effects in terms of mineral

nitrogen equivalent were 41.1 and 82.4 kg N for finger millet with one or two rows of nodulating soybean, respectively. Finger millet at 30/90 cm + two rows of soybean KHSb 5 gave higher monetary returns. Geeta Kumari and Shivashankar (1991) stated that the application of 4 t/ha organic amendments increased N uptake by finger millet and soybean crops in intercropping. Residual availability of P was higher in intercropping than sole cropping. While Baghel *et al.* (1991) found highest total productivity when kodo millet (*Paspalum scrobiculatum*) intercropped with soybean in alternate rows. Singh and Singh (1968) reported the soybean could be successfully grown as an intercrop with setaria [*Setaria italica* (L.) P. Beauvois] with 30:30:0 N:P:K kg per ha.

Soybean with roselle (*Hibiscus sabdarifa*)

Gupta (1989) concluded that roselle (*Hibiscus sabdariffa* L.) could be successfully intercropped with soybean.

Soybean with wheat

Yield and monetary advantage

The study conducted by Hiremath *et al.* (1989, 1990) at Dharwad on black soils depicted that wheat cv. Kiran could be intercropped with soybean (cv. Monetta) without affecting the grain yield of wheat in 1:1 to 4:3 row combinations. They further reported that the highest LER 1.33 was recorded in 1:2 row arrangements. The preliminary study conducted under *Malwa* conditions on Vertisols indicated no yield advantages with this intercropping

system when tried in 2:6, 4:6 and 6:6 row ratio of soybean and wheat. However, in view of limited information available on the system and looking to the benefits of cereal + pulse combination, the system needs to be given fair trial in major wheat growing area of Central India. It requires mention here that soybean-after soybean is not advocated at present on account of possibility of incidence of soybean rust.

Soybean with rice

Yield and monetary advantage

Mandal *et al.* (1989, 1990, 2008) while attempting intercropping of rice with soybean, green gram, groundnut and black gram in simultaneous and deferred plantings observed that fertile tillers per panicle were higher in rice grown alone while intercropping could increase grains per panicle and 100 grain weight. They further recorded rice + legume intercropping resulted in greater LER, relative net return and monetary advantage under west Bengal conditions. The soybean rice planting in 1:2 row ratios produced significantly higher grain yield than 1:4 ratio. Simultaneous planted crops produced higher yields than deferred planting due to marked differences in the maturity period. They further reported that among legumes, pure crops of soybean and peanut always gave increased number of yield components than the other crops grown in association with rice. While at Kalyani and Jorhat, the 2:2 row combinations gave higher monetary returns and LER 1.16 (Anonymous, 1974). The soybean is likely to form a sound combination with upland paddy.

Soybean with oilseeds

Yield and monetary advantage

Desai and Goyal (1980) opined that the intercropping of sesame with soybean, castor (*Ricinus communis* L.), sunflower (*Helianthus annuus* L.) and groundnut increased the total oilseed production as well as economic returns. Three rows of soybean in between two rows of sesame spaced at 160 cm gave the highest benefit cost ratio of 4.72 at Jorhat (Anonymous 1971-80). Mixed cropping of soybean with groundnut could provide 0.5 t/ha without affecting productivity of two crops in Tamil Nadu (Palaniappan, 1983). However, Senthivel *et al.* (1989) recorded reduction in yield of groundnut by intercropping with soybean. Bhalla (1991) advocated that groundnut + soybean in 4:1 row ratio can be grown successfully. According to Parmeshwaran *et al.* (1988), soybean (Co 1) intercropped with mustard (*Brassica* spp.) in 5:1 ratio was the most economic viable than other combinations. While Patil *et al.* (1991) reported that soybean, sunflower and groundnut could be successfully intercropped with niger [*Guizotia abyssinica* (L.f.) Cass] in Maharashtra. Shivaramu and Shivashankar (1992) and Shivaramu *et al.* (1994) contemplated that in sunflower and soybean intercropping under Bangalore conditions, the yield of sunflower was reduced by 23 per cent and that of soybean by 37 per cent as compared to their sole crops. However, their total productivity in terms of total oil yield, gross income and LER (1.39) was far superior to pure cropping of either of the crop. Uniform row planting of sunflower was conducive to better

growth. Shivaramu *et al.* (1993) further opined that soybean intercropped with sunflower produced higher yield when both the crops were enriched with carbon dioxide.

Weed management

Tiwari and Khurchania (1991) advocated the weedicides like Diuron or Pendamethalin @ 1.0 kg a. i. per ha as pre-emergence or Fluchloralin 1.0 kg a. i. per ha as pre-plant soil incorporation for soybean + sesame, Oxydiazone @ 1.0 kg a. i. per ha or Alachlor @ 1.5 kg a. i. per ha as pre-emergence for soybean + sunflower and soybean + niger intercropping.

Soybean in plantation crops

Yield and monetary advantage

In the southern zone, excellent possibility of intercropping soybean with coconut (*Cocos nucifera* L.) exists since later offers adequate sunlight to infiltrate throughout the age of plantation except between 10 to 20 years (Nelliath, 1979). The coconut crop in Tamil Nadu, Kerala, Karnataka and part of Maharashtra is planted at a distance of 6-8 m and leaves enough inter space for intercropping of field crops. It has been suggested that two soybean crops (May-June and September-October) can be successfully grown (average yield 0.9 and 0.7 t/ha, respectively) with coconut (Palaniappan, 1983). Cultivars Pb 1, Hardee, Davis and Forest were found to be most suitable for intercropping in coconut (Ligance and Martin, 1987). Similar to coconut plantation in southern India, the fruit plantation [mango (*Mangifera indica* L. and

guava (*Psidium guajava* Mill] in Uttar Pradesh and adjoining states offers possibilities of soybean cultivation as intercrop. The work done by Rajput *et al.* (1989) on successful cultivation of cereal-pulse sequence in mango orchards indicates that a possibility to replace any of the rainy season legumes with soybean exists. Preliminary reports from Srinivasan *et al.* (1990) indicated that at least 3 to 6 successive intercropping of soybean, sunflower, cotton, green gram, sesame, sorghum, cowpea, turmeric (*Curcuma domestica*), maize, black gram and groundnut can be taken in agro-forestry with trees like *Eucalyptus tereticornis*, *Casurina equisetifolia* and *Leucaena leucocethala* up to the age of 36 months. Although with each successive cropping the yields of intercrop was observed to be progressively decreasing. Further experimentation in this direction is warranted to generate more details. Possibility of successful cultivation of soybean in oil palm and rubber plantations (Mak and Yap, 1983) and in cassava (Tsay *et al.* 1983) was suggested.

Shanker *et al.* (2005) concluded that the tree growth was affected by both density and intercrop in the initial years of growth. Photosynthetic photon flux density (PPFD) available to the intercrops reduced with increasing densities. Transpiration rate and stomatal conductance in intercrops decreased due to the presence of trees. No significant changes in leaf temperature were observed till the fifth year of the growing season. Yield was significantly higher in pure crop in comparison with all the densities in mustard. Soybean yield

under 200 trees per ha was comparable to that of the pure crop. Trees at the density of 200 trees per ha provided a conducive micro-environment to the intercrops.

Soybean with spices

Yield and monetary advantage

Chaudhry (1988) reported that ginger (*Zingiber officinale*) at 60-cm row spacing intercropped with soybean produced handsome remuneration. As there is meager information on intercropping of soybean with spices and vegetables, the profitability of such systems needs further investigation.

Conclusion

Extensive work done on agricultural feasibility and economic viability of soybean based intercropping systems can be utilized to promote intercropping systems suitable for different agro-climatic regions. It also brings out that irrespective of row ratios, soybean offers better yield advantage than any other crop intercropped with traditionally cultivated crops. There is a possibility of adding to this yield advantage by suitable nutritional and water management. The work compiled above clearly brings out that soybean can be advantageously intercropped with most of the traditional crops grown in different agro-climatic regions of the country. In addition to higher combined yield, taking advantages of crop diversification, fertilizer economy, salutary soil environment for plant growth, smothering effect on weeds, natural check on pests and diseases and risk coverage,

adoption of system is likely to provide sustainability. These findings also suggest that mono-cropping of soybean could be successfully diversified through adoption of soybean based intercropping systems. It is also suggested that the soybean crop may be introduced in the newer areas as intercrop so that the commodity balance can also be ensured. Although agronomic feasibility and economical viability of soybean based intercropping systems has been demonstrated along with generation of limited information on integrated nutrient management, the future research should be directed towards integrated management of nutrient, water and pests, suitability and compatibility of crop varieties for intercropping so as to widen our vision in the direction of sustainable agriculture.

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***Colletotrichum truncatum* [(Schw.) Andrus & W.D. Moore], the
Causal Agent of Anthracnose of Soybean [*Glycine max* (L.)
Merrill] - A Review**

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ABSTRACT

Soybean anthracnose caused by Colletotrichum truncatum is the most important seed-borne fungal pathogen of soybean which also infects seedlings, stems, petioles, leaves and pods. The disease is prevalent in almost all the soybean growing areas of the world and substantial yield losses have been experienced. A lot of work has been done on anthracnose of soybean but it remains scattered. Therefore, it is essential to review the available relevant information on taxonomy, biology, ecology, epidemiology, life cycle, host-pathogen interaction, resistant sources and effective eco-friendly management aspects, to bring them together in a systematic manner. The same is presented herewith.

Key words: Anthracnose, *Colletotrichum truncatum*, disease, management, soybean, yield loss

Soybean being rich in protein (40 %) and moderate in oil (20 %), it is suitable for animal and human consumption (Golbitz, 2003; Olguin et al., 2003; Belewu and Belewu, 2007). Soybean seeds, when carrying pathogens, become a primary source of inoculums for damaging seeds and plants with varying degrees (Maude, 1996). *Colletotrichum truncatum* (Schw.) Andrus & W.D. Moore is prevalent in almost all the soybean growing areas of the world. It produces symptoms on stem, leaf, pods

and seeds. According to Wrather *et al.* (1997), the yield reduction caused by this disease in 1994 in major soybean producing countries was highest (around 77, 500 t) in Brazil, followed by the United States of America (71, 400 t), Argentina (36, 700 t), India (35, 000 t), Paraguay (12, 200 t), Bolivia (3, 000 t), Italy (700 t) and Indonesia (40 t). Warm (temperature around 35°C) coupled with rain, dew or fog, which can provide free moisture for the periods of 12 h or more, favours the disease.

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The fungus grows in seeds externally and internally leading to local and systemic infections. Thus, it causes pre- and post-emergence mortality; resulting in reduced seed germination and final seedling stand of soybean (Manandhar and Hartman, 1999; Begum *et al.*, 2007). Fungicides have been used intensively to control seed-borne fungi of soybean. However, indiscriminate use of fungicides causes several negative effects. Increasing concern among researchers, environmentalists and the public at large regarding possible risks on human health associated with fungicides has given an impetus to search for alternative strategies for disease control programme.

A critical review of the available literature on the pathogen *Colletotrichum truncatum* with special reference to anthracnose of soybean is presented herein.

Taxonomy and nomenclature

The genus *Colletotrichum* was first described as *Vermicularia* in 1790 (Sutton, 1992; Hyde *et al.*, 2009). Thereafter, the genus *Colletotrichum*, characterised by hyaline, straight or falcate conidia and setose acervuli, was established by Corda (1831). Later, Arx von (1957) studied the taxonomy of this genus carefully and reduced the number of described taxa from several hundred to 11 accepted species. However, later on number of accepted species of *Colletotrichum* was increased to 39 (Sutton, 1992), 60 (Kirk *et al.*, 2008) and 66 (Hyde *et al.*, 2009). However, according to Nguyen (2010), the taxonomic position of some species

remained unclear. Several species of *Colletotrichum*, i.e. *C. gloeosporioides*, *C. acutatum*, *C. graminicola* and *C. dematium*, are broadly defined and considered to be species complexes or 'group species'. The currently defined species boundaries are vague and relationships within some of these species complexes are not well-resolved (Sutton, 1992; Cannon *et al.*, 2000). However, DNA sequencing of fungal genomes can be of immense value in assisting species identification (Sreenivasaprasad *et al.*, 1996; Farr *et al.*, 2006; Cannon *et al.*, 2008; Cai *et al.*, 2009; Hyde *et al.*, 2009) and is therefore can be used to complement the morphological data.

Geographical distribution

The first report of anthracnose leaf spot caused by *C. truncatum* was from Korea in the year 1917 from Asian continent (Nakata and Takimoto, 1934). Subsequently from Japan, Hara (1930) gave the descriptive account and Hemmi (1980) provided the full description and the morphology of *C. glycines* (*C. truncatum*) on soybean.

Reports of parasitisation by *Colletotrichum glycines* (= *C. truncatum*) on soybean have been from world over and was documented in Delaware (Manns and Adams, 1928), Java (Goot and Muller, 1932) Jamaica (Martyn, 1942), Canada (Connors and Savile, 1943), Florida (Rhoads, 1944), Georgia (Weimer, 1947), Iowa (Crall, 1951), China (Ling, 1948), Taiwan (Han, 1959), North Borneo (Johnston, 1960), North Carolina (Wolf and Lehman, 1924), Soviet Union (Nelen, 1968), Iran (Zad, 1979), Hungary (Ersek, 1979), Cameroon (Bernaux, 1979), Senagal

(Girard, 1979), Yugoslavia (Jasnic, 1983), Bangladesh (Fakir and Mridha, 1985), Indonesia and Philippines (Kobayashi and Zinno, 1984), Nepal (Manandhar and Sinclair, 1982), Malaysia (Nik and Lim, 1984), Singapore (Ling and Tang, 1984), Pakistan (Mirza and Rehman, 1985), Mozambique (Vermetti et al. 1985), UAE (Burhan, 1986), Italy (Calzolari et al., 1987), Ghana (Twumasi et al., 1989), South Africa (Koch et al., 1989), Gabon (Ndzoumba et al., 1990), Slovakia (Ondrej, 1994), Nigeria (Amusa, 1994), Argentina (Pioli et al., 1997), Brazil (Goulart, 1991), Bulgaria (Kaiser et al., 1998), Korea (Oh Jeung, 1998), Turkey (Eken and Demirci, 2000) and Australia (Parbery and Lee, 1972).

The existence of the fungus *C. truncatum* which causes pod blight of soybean was reported for the first time from India by Nene and Srivastava (1971) and subsequently its occurrence was recorded from Uttar Pradesh (Singh et al., 1973; Saxena and Sinha, 1978; Singh and Shukla, 1987), Andhra Pradesh (Saikia and Phukan, 1983), Himachal Pradesh (Bhardwaj and Thakur, 1991); Karnataka (Banu et al., 1990), Madhya Pradesh (Nicholson and Sinclair, 1973; Singh, 1993) and from Maharashtra (Rao et al., 1989).

Symptoms

Soybean plants are susceptible to anthracnose caused by *C. truncatum* at all the growth stages. Infection during early reproductive stages causes appearance of irregularly-shaped, brown areas on stems, petioles and pods.

Pre-mature defoliation may occur throughout the canopy when cankers

girdle the leaf petiole. The cankers often occur where the leaflets join the petiole, resulting in a shepherd's crook. Morgan and Johnson (1964) gave a descriptive account of the cankers that girdled petioles and caused leaf blades to shed, leaving only the shriveled petioles attached. The petiole lesion resembled target spot but occurred earlier. Many times leaf rolling is also noticed. Melhus (1942) reported that fungus *C. truncatum* occurred on branches of weak soybean plants. Infected plants were stunted at apical region, tip of the stem was curled and brown upper leaves showed small necrotic lesion. The pulvinus of most leaves showed a dark-brown water-soaked appearance. Nelen (1968) and Nelen and Zhukovskaya (1968) observed that the early and severely infected soybean plants lagged in growth developing few or no branches and only few roots, and soon died. Rhoads (1944) and Parbery and Lee (1972) also observed the poor seed germination and cankers on cotyledons due to *C. dematium*.

Infection at early stage may cause no seed formation or, if seed develops it may be smaller and fewer in number. Infected seeds are shriveled or moldy and can develop irregular brown to grey area with black specks or may not show any symptoms. Such seeds may die during germination or if they germinate may produce infected seedlings. Infected seeds as well may lead the pre- and post-emergence damping off and seedling blight. Cotyledons may have dark-brown, sunken lesions which may gradually extend up to epicotyls and radical and may become water-soaked. Infection may also spread from the cotyledons to the young

stem where small, deep-seated cankers (lesion) are formed, which may often kill the seedlings (Hartman *et al.*, 1986). Infected seeds also have symptoms-less establishment of internal mycelium. Mycelium from infected cotyledons usually established in the cortical cells of the stem and remained localized in the immediate stem area until flowering time. It then resumed growth and penetrated the lower stem, petioles, leaves and developing seeds and pods, without the immediate development of disease symptoms. At the host maturity under proper environmental conditions, the fungus may fruit abundantly on stem and pods (Tiffany, 1951), producing black fruiting bodies (acervuli) with minute black spines (setae) uniformly scattered over the surface of affected parts which can be seen by unaided eye. These are the diagnostic characters of the disease (Gupta and Chauhan, 2005). Finally the disease causes premature death of the plant and failure of the pod to fill.

Yield loss

Colletotrichum spp. cause considerable damage in a large number of crops such as cereals, coffee and legumes (Bailey and Jeger, 1992; Lenne, 1992). The disease is widespread in temperate soybean production zones but causes minor losses (Athow, 1987; Tiffany, 1951). Losses are more severe in warm humid regions. In Alabama, yields were reduced by an average of 19.4 per cent for three cultivars compared to plots in which the disease was controlled by fungicide applications (Backman *et al.*, 1982). Yield losses of 30 per cent were

attributed to anthracnose in Nigeria in 1975 (Rheenen, 1975). A survey in two states in Brazil detected the disease in 57 per cent of the fields (Lehman *et al.*, 1976). Anthracnose on maturing plants causes serious losses, particularly during the rainy period when shaded lower branches and leaves are killed. Yield losses up to 50 per cent in Thailand and 100 per cent in India have been reported due to anthracnose (Sinclair and Backman, 1989; Ploper and Backman, 1992; Manandhar and Hartman, 1999). In 2006 yield loss of 2.54 million tons and 1.18 lakh tons was estimated by anthracnose in top 8 soybean producing countries of the world and in India alone, respectively (Wrather *et al.*, 2010).

Survival, epidemiology and disease cycle

The pathogen was recorded from anthracnose lesions on seedlings grown in sterilized soil from naturally infected seeds (Khare and Chacko, 1983) and from inoculated seeds grown in sterilized sand under greenhouse conditions (Dhingra *et al.*, 1978; Roy, 1982). Symptomless seedlings developed in unsterilized soil in the greenhouse from seeds inoculated with a conidial suspension of *C. truncatum* were also reported. The pathogen was later detected in the cotyledons and cortex of the stem and subsequently advanced longitudinally in the pod and cotyledons of developing seeds (Tiffany, 1951).

C. truncatum also survives on soybean stem residues (Lehman and Wolf, 1926). The pathogen was reported to survive for over 10 years when stored at 50°C (Siddique *et al.*, 1983). Fruiting bodies of *Colletotrichum* and a *Glomerella* teleomorph were found on 22 per cent of

5000 stubble samples in Illinois field (Hartman *et al.*, 1986). Inoculum applied to soil in pots in late autumn, provided active inoculums in the following spring (Ling, 1940). Soybean cultivars A.K. (Kansas), Boone, and Williams 82 grown on sand infested with sclerotia-forming isolates of *C. truncatum* in soil tanks at 20, 25, 30 or 35°C and at greenhouse ambient temperature (21-28°C) showed root and hypocotyls infection on all cultivars at all temperatures. Lesion size and number generally increased with an increase in soil temperature up to 30°C and then declined. Isolates of *C. truncatum* from soybean seeds produced microsclerotia in culture and in soybean tissues (Khan and Sinclair, 1992). Though, the ascogenous stage of *Colletotrichum glycines* (= *C. truncatum*) was found on diseased stem of soybean and also in culture (Lehman and Wolf, 1926) but their role in survival is still not clear.

Singh *et al.* (2001) observed that maximum disease incidence was in the second fortnight of September and the first fortnight of October when the average temperature, relative humidity and rainfall were 28.4°C, 76 per cent and 92.5 mm, respectively. They also recorded, minimum disease incidence in the second fortnight of July when higher temperature prevailed with lower relative humidity and rainfall. It was also observed that seed infection with *C. truncatum* was low in areas with low rainfall and high when crop populations were high (Khare and Chacko, 1983). In Puerto Rico, soybean seeds produced in the wet season were more heavily infected than those produced in the dry

season (Sinclair, 1988). The role of light, temperature and relative humidity on the germination of *C. truncatum* and soybean pod infection studied under laboratory conditions in India (Kaushal *et al.*, 1998) projected that the optimum temperature for conidial germination and germ tube elongation was 20°C, and for soybean pod infection was 25°C. Three hours of light followed by 9 hours of dark period was the best for spore germination and germ tube elongation. Twelve hours of light followed by 12 hours dark period was most suitable for pod infection. Pod infection and the development of acervuli took longer time in continuous light. High relative humidity (100 %) was required for pod infection and the development of acervuli. However, in slide germination test at 100 per cent RH, none of the spores germinated.

Disease cycle initiates with the infection from the mycelium of the pathogen in infected seeds or debris. The fungus after germination produces numerous small, deep seated lesions on cotyledons and keeps on multiplying and moves in to developing seedlings which results in causing post-emergence damping off of seedlings under humid condition. Alternatively, mycelium may also establish in infected seedlings without symptom development (latent infection) until plants begin to mature. Conidia produced in acervuli on infected plant parts under favourable conditions may initiate secondary infection by producing appressoria after germination. The disease cycle takes about 60-65 hours to develop symptoms (Kuo *et al.*, 1999).

Seed pathological aspects

Begum *et al.* (2008) recorded maximum infection frequency of *C. truncatum* on seed coat (100 %) that started just after the incubation and remained the same through the whole incubation period. The infection of *C. truncatum* on cotyledon and embryonic axes increased gradually with increasing incubation time without any external symptoms during incubation period. This could be attributed to the latent infection of *C. truncatum* into soybean seed coats (Sinclair, 1991). The highest level of infection on cotyledon and embryonic axes recorded was 43.0 per cent and 30.0 per cent, respectively 4 days after incubation and remained the same till the end of the incubation period. However, Hepperly *et al.* (1983) and Srichuwong (1992) noticed that the *C. truncatum* decreased the seed viability and consequently reduced germination because of its infection in seed tissues and thus damaging the seed coat, cotyledons and embryonic axes. Seed infection levels recorded in tropical and sub-tropical regions were as high as 81 per cent (Verma and Upadhyay, 1973, Fulco *et al.*, 1979; Hepperly *et al.*, 1983). In Florida, the pathogen was detected in 30 per cent of 73 seed samples, with infection level ranging from 5 to 20 per cent (Franca Neto and West, 1989). In temperate regions, such as the northern USA, seed infection is rare despite extensive pod infection (Athow, 1987;

McLean and Roy, 1988). An Illinois report indicated that less than 1 per cent of the seed was infected (Jordan *et al.*, 1986). Goulart (1998) and Picinini and Fernandes (1996) reported 6.8 - 7.5 per cent and 1.0 - 3.75 per cent incidence, respectively of *C. truncatum* in soybean seeds.

The hyphae of *C. truncatum* generally occur in the seed coat and also in the embryo in heavy infections, but never in the embryo alone. The pathogen is transferred from the seed coat to the seedling by colonizing the cotyledons during germination. It is occasionally present in the superficial stem tissue of the cotyledonary node, but has not been observed growing through the stem. Inoculum in later on reaches to the developing pod, where it colonize the cell layers situated outside the sclerified dehiscence layer. In the later stages of pod development (pod filling stage) the sclerified cell layer is not able to resist fungal penetration, consequently the pathogen is able to grow through the pod wall and infect the seed coat of the new seed. If humid conditions prevail, then infection may go up to inner tissues of the seed during the late stages of pod maturation (Neergaard *et al.*, 1999).

Infected seeds are shriveled or moldy, have a brown discolouring, or are asymptomatic (Athow, 1987). Heavily infected seeds are unable to germinate (Neergaard *et al.*, 1999). Seed inoculated with *C.*

truncatum and planted in the greenhouse experienced pre-emergence death, in which embryo and cotyledons were invaded within 3-4 days followed by rapid breakdown of tissues. Severe reductions in seedling emergence have also been reported in tropical areas (Athow, 1987; Khare and Chacko, 1983), and seed infection has been well correlated ($r = 0.64$) with seedling mortality in laboratory germination tests (Franca Neto and West, 1989). Seeds inoculated with *C. truncatum* and planted in the greenhouse experienced pre-emergence death, in which embryo and cotyledons were invaded within 3-4 days followed by rapid breakdown of tissues, and seedling blight in which seedlings developed normally for a short period and then the pathogen invaded the various seedling parts; or symptomless establishment of internal mycelium in normal seedlings (Tiffany, 1951). Pre- or post-emergence blights also were reported in seeds inoculated with other species of *Colletotrichum* and *Glomerella* and planted in sterile sand or on blotter (Ling, 1940; Rodriguez-Marcano and Sinclair, 1978; Roy, 1982).

C. truncatum significantly reduced seed germination by 46.4 per cent, viability by 26.8 per cent, pre-emergence damping-off by 48.0 per cent and post-emergence damping-off by 28.5 per cent within 14 days after sowing (Begum *et al.*, 2008). Significantly higher electrolyte leakage was found in inoculated seeds than those of un-inoculated seeds, which indicated the low seed vigor of soybean.

Histological studies have shown mycelia of the fungus in the seed coat layers, epidermis (palisade), hypodermis (hourglass) and endodermis (parenchyma) (Kunwar *et al.*, 1985; Nik and Lim, 1984). The pathogen has been detected in wounds in the seed coat (Schneider *et al.*, 1974).

Bio-chemical changes

Soluble protein concentration of *C. truncatum* inoculated seeds was significantly higher than those in the uninoculated seeds of soybean (Begum *et al.*, 2008; Srichuwong, 1992). The increase in enzymatic activity following fungus invasion in seeds may result in higher level of total proteins (Farag *et al.*, 1985). Begum *et al.* (2008) reported that the *C. truncatum* infection did not change the amount of extracted oil in inoculated seeds compared to un-inoculated seeds. Reductions in arginine, glutamic acid, histidine, lysine, methionine, phenylalanine, threonine and valine, and increase in cystine and proline were reported by Singh and Shukla (1987) in *Vigna mungo* consequent to infection by *C. truncatum*.

Isolate variability and pathogen population

The etiological and morphological studies indicate that the pathogen of soybean anthracnose is included in the two different species *Colletotrichum truncatum* and *Glomerella glycines*. Isolates of *Colletotrichum* and *Glomerella* species from soybean vary in their cultural characteristics and pathogenicity (Manandhar *et al.*, 1984, 1988; Tiffany and Gilman, 1954). Differences in pathogenicity were noted between Florida and Australian isolates of *C. truncatum* on species of *Stylosanthes* (Lenne and Sonoda, 1978). In the USA, falcate spore isolates of *Colletotrichum* isolated from different parts of soybean plants were separated into two colony types: *C. dematium* f sp. *truncatum* (*C. truncatum*) and *C. capsici*. *C. capsici* was not pathogenic or weakly pathogenic on soybean seedlings, with pathogenicity limited primarily to the cotyledons. In contrast, *C. truncatum* was extremely virulent on soybean seedlings and caused

considerable pre- and post-emergence seedling death in the USA (Roy, 1996). The virulence of 11 isolates of *C. truncatum* was studied on set of 12 arbitrarily selected *Vigna mungo* differentials in India. The isolates were grouped into six pathotypes on the basis of reactions of the test isolates on the differentials. The reaction type of different isolates was also studied on related legumes: azuki bean (*Vigna angularis*), mung bean (*Vigna radiata*), lentil (*Lens culinaris*), faba bean (*Vicia faba*), pigeon pea (*Cajanus cajan*), soybean (*Glycine max*), cowpea (*Vigna unguiculata*), french bean (*Phaseolus vulgaris*) and horsegram (*Macrotyloma uniflorum*). *C. truncatum* had a wide host range among the local edible legumes and infected all of the species tested. However, none of the 11 isolates showed a compatible reaction with pigeon pea cv. *Parbhat*, which was considered immune (Sharma and Kaushal, 1999).

Isolate of *Colletotrichum truncatum* from lima bean (*Phaseolus limensis*) was compared with other falcate *Colletotrichum* species such as *C. truncatum* and *C. capsici* by Kuo *et al.* (1999). No morphological differences were observed. However, *C. truncatum* was easily differentiated from other falcate species such as *C. falcatum* (*Glomerella tucumanensis*), *C. liliacearum* and *C. graminicola*. The *C. truncatum* isolate from lima bean could not attack other leguminous plants. Other falcate *Colletotrichum* such as *C. fematium*, *C. falcatum*, *C. liliacearum*, *C. capsici* and *C. graminicola* also failed to infect lima beans in inoculation tests. Of the falcate *Colletotrichum* tested, only *C. truncatum* was

pathogenic to lima beans. On the basis of morphological characteristics and host range, the fungus was identified as *C. truncatum*.

Colletotrichum spp. are also commonly isolated as endophytes from healthy plants, and have been identified as saprobes on dead plant material (Photita *et al.*, 2001 a; 2004; Promputtha *et al.*, 2002; Toofanee and Dulymamo de, 2002; Kumar and Hyde, 2004). Endophytic, saprobic and many pathogenic strains in the genus have been frequently classified as *Colletotrichum gloeosporioides* or *Colletotrichum* sp. (Brown *et al.*, 1998; Bussaban *et al.*, 2001; Photita *et al.*, 2001b; 2003; Promputtha *et al.*, 2002). *Colletotrichum gloeosporioides* is a commonly isolated endophyte from a range of plant species (Rodrigues, 1994; Brown *et al.*, 1998; Bussaban *et al.*, 2001; Photita *et al.*, 2001b). Therefore, it is important to establish the relationship among strains of various *Colletotrichum* isolates with different like forms and to establish diversity of the species.

Morphological and cultural characters were used by Photita *et al.* (2005) to group 36 *Colletotrichum* isolates into species *C. musae*, *C. gloeosporioides* groups I, II and III and *C. truncatum*. Sequence comparison of ITS (Internal Transcribed Spacer) regions of these isolates from banana, ginger and *E. thymifolia* against published sequences confirmed them as *Colletotrichum* species. They also correlated the sequence data with the morphological grouping of these species. They reported that there were four pathogenic isolates of *C. truncatum* isolated from soybean, which clustered with a GenBank sequence for *C. truncatum* (AF 451899). These isolates had

falcate conidia and clustered at the base of the parsimony tree. They did not cluster with any other isolates for hosts, indicating that this pathogen may be host-specific to soybean. However, Ford *et al.* (2004) recently showed 99.8 per cent identity among rDNA sequences of *C. truncatum* that was isolated from soybean (*Glycine max*), common bean (*Phaseolus vulgaris*) and alfalfa (*Medicago sativa*). This indicated a near complete conservation in ITS sequence between isolates from different host species.

To date only preliminary molecular studies have been done to resolve the phylogeny in genus *Colletotrichum* and therefore, many questions regarding the evolutionary relationships within the genus remain unanswered. According to Cunningham *et al.* (2004) sequence analysis from ITS regions has made some progress towards a better understanding of the taxonomy of *Colletotrichum* yet, further molecular analysis using other genes and more sensitive molecular approaches are required.

Management

Cultural practices

The effect of soil management practices and fungicide spraying timing on the control of late season diseases of soybean including anthracnose was studied in Brazil (Klingelfuss and Yorinori, 2000; Klingelfuss *et al.*, 2001). Treatments included; 3 tillage practices (non-tillage, minimum tillage and conventional tillage); and difenconazole applied at R5.1, R5.2, R5.3, R5.4, R5.5, R6, R5.1 + R5.4, R5.2 + R5.5 and R5.3 + R6.

They observed the significant differences when the fungicide was applied at R5.1 + R5.4. Among the soil management practices, conventional tillage resulted in low severity of diseases and percentage defoliation, and higher crop yield and 1000-seed weight as compared to than non-tillage. Khare and Chacko (1983) also observed more anthracnose infection in early planted soybean. Depending on planting time and row spacing, weeds also sometime increase the infection (Hepperly, 1984). Therefore, weed free cultivation of soybean will result in low anthracnose incidence.

Seed lots were collected in October from five soybean cultivars planted at seven different dates during the rainy season 1971 at Jabalpur, Madhya Pradesh, India. The lots were separated on the basis of cultivars and planting dates and stored at room temperature (15 - 45°C) or in a refrigerator (1 - 5°C). Seeds from the first three planting dates stored in a refrigerator had greater frequency of internally borne fungi *C. truncatum*, lower germination and emergence and more emerging seedlings had *C. truncatum* lesions than those from remaining four planting dates and stored at room temperature (Nicholson and Sinclair, 1973). Thus, Seedling emergence was reduced and plant infection increased when seed harvested from early planting was used.

Chemical control - seed treatment and foliar sprays

In India, several fungicides are reported to reduce infection of soybean seeds by *Colletotrichum truncatum* and

improve emergence, the most effective being thiram, difolatan, and captan (Khare and Chacko, 1983). Benomyl + thiram, thiophanate methyl + thiram, thiabendazole, and phenapronil gave effective control in Korea (Lee, 1984). In Portugal, seed treatment with tolyfluanid, pencycuron + tolygluanid, pencycuron, captan, thiram and benomyl were observed to control the disease, but tolyfluanid was the most effective, followed by benomyl and pencycuron + tolyfluanid (Goulart, 1991).

Ahn and Chung (1970) observed that seed inoculation with arasan, orthocide, phygon-XL, PTAB, and mercuron resulted in significantly less anthracnose infection in soybean seedling than was obtained in control. Growth of *C. truncatum* and *Glomerella glycines* was greatly inhibited by antimucin, gramosan, rioben, ruberon, sankinon and takedo meru by the proper disk plate method (Lin and Wu, 1966). However, the regular rotation and seed treatment with granosan or thiram with heat treatment at 50°C for 6 h are among the controls recommended for *Colletotrichum glycines* (= *C. dematium* f. *truncate*) (Nelen and Zhukovskaya, 1968).

Among the fungicides thiram (75 %), captan (75 %), PCNB (75 %), and a mixture (thiram + captan + PCNB in a 1:1:1 ratio) @ 4.5 g per kg of seed and with aureofungin @ 25 ppm for seed treatment, thiram proved to be the best among all treatments in improving the emergence (Nene *et al.* 1971). Soybean seed treatment with captan, carboxin + thiram (WP and EC), thiram (WP and EC), thiabendazole + thiram and

thiabendazole + quintozone eradicated different levels of *Colletotrichum truncatum* incidence (Picinini and Fernandes, 1996). In Brazil, the most effective control of soybean seed-borne pathogens, including *Colletotrichum truncatum* was obtained with tolyfluanid + thiabendazole, tolyfluanid + thiabendazole, tolyfluanid + carbendazim, thiabendazole + thiram, thiabendazole + captan, carbendazim + thiram, benomyl + captan and benomyl + thiram (Goulart, 1991). Seed treatment with the mixture of euparen with benlate and with cercobin, tegran, tegran + micro, metiltiofan + rhodiauran and the mixture of tecto with captan and with rhodiauran led in best control of the *C. truncatum* along with other seed borne fungi (Goulart, 2001). Yuyama and Henning (1997) evaluated the efficiency of different formulations of thiabendazole and thiram for controlling *C. truncatum* and other pathogens in soybean seeds. They reported thiram as the most efficient treatment for *C. truncatum*.

Lopes and Leonel (2000) observed the efficacy of fungicides and cobalt (Co) and molybdenum (Mo) viz. Derosal TS (30 g carbendazim / 100 kg seed) + thiram (49 g /100 kg seed) + Wuxal Co Mo (4.2 g Co / 100 kg seed + 21 g Mo / 100 kg seed); Derosal TS (30 g carbendazim / 100 kg seeds) + 70 g thiram 500 SC (70 g thiram / 100 kg seed); Derosal 500 SC (30 g carbendazim / 100 kg seed) + thiram 500 SC (70 g thiram / 100 kg seed) + Derosal 500 SC (30g carbendazim / 100 kg seeds) + thiram 50 SC (70 g thiram / 100 kg seed) + Wuxal Co Mo (4.2 g Co / 100 kg seed + 21 g Mo / 100 kg seed); Prelude WS (80 g carbendazim / 100 kg seed + 21.6 g prochloras/ 100 kg seed) and Prelude WS

(100 g carbendazim / 100 kg seed + 27 g prochloras / 100 kg seed) as seed dressing for the control of seed-borne fungi. All treatments reduce the incidence of *Phomopsis* sp., *Fusarium* spp., *Cercospora kikuchii* and *C. truncatum*. The best seedling emergence was obtained with the treatments containing carbendazim + thiram with or without cobalt and molybdenum. Klingelfuss *et al.* (2001) could observe significant differences in disease severity when fungicide difenconazole was applied at R5.1 + R5.4.

Seed treatment with fungicide plus a foliar spray with *Lawsonia inermis* leaf extracts plus alum (0.1 %) decreased leaf anthracnose and pod blight. Seed treatment with fungicide + *L. inermis* (1 %) + *Trichoderma viride* (0.4 %) + alum (0.1 %) also reduced leaf anthracnose and pod blight of soybean (Chandrasekaran *et al.*, 2000a). The polyamine biosynthesis inhibitors DFMO (*alpha*-difluoromethylornithine) and DFMA (*alpha*-difluoromethylarginine) limit production of certain amino acids in the fungus *Colletotrichum truncatum*. The decarboxylase enzymes that aid in formation of putrescine, cadaverine, spermidine, and spermine are inhibited. Since these amino acids are important to the development of the fungus, these inhibitors could be useful in controlling the fungus (Gamarnik *et al.*, 1994).

Colletotrichum truncatum was completely eliminated when soybean seeds were pre-treated with a dye like methylene blue, methyl red or carmine and irradiated with a laser for 10 min. Seed germination was stimulated on exposure of the seed to 1 min. of

irradiation. At this dose, most of the dyes were accelerators where as the higher doses were inhibitory to seed germination (Ouf and Abdel Hady, 1999).

Biological control

Hundred per cent extract of Feykuy-Eaka, ginger (*Zingiber officinale*), garlic (*Allium sativum*) and neem (*Azadirachta indica*) gave excellent control of seed borne *C. truncatum* when seeds were dipped for 30 minutes (Hossain *et al.*, 1999). According to Chandrasekaran *et al.* (2000b), seed treatment with 10 Chacko (1983) leaf extract of *Lawsonia inermis* reduced anthracnose disease incidence significantly compared to the other treatments in vitro and pot culture conditions.

The screening of methanolic extracts of 41 plant species for their fungicidal activity against 3 soybean fungal pathogen i.e. *C. truncatum*, *Fusarium oxysporum* and *Macrophomina phaseolina*, showed that dry hot water extracts of *Berberis aristate*, *Boenninghausenia albiflora* and *Lantana camara* were highly potent, and *Boenninghausenia albiflora*, *Polygonum glabrum*, *Origanum vulgare*, and *Rhododendron arboretum* (*R. arboretum*) also significantly inhibited the growth (Arora and Kaushik, 2003).

The individual and combined effects of plant extracts, biological control agents and chemicals on foliar anthracnose and pod blight caused by *Colletotrichum truncatum* of soybean was studied by Chandrasekaran and Rajappan (2002). They observed that the combined application of leaf extracts of *Lawsonia inermis* (5 %) with alum at 1 and 0.1 per cent

showed cent per cent reduction in pod blight infection under laboratory conditions. They also recorded that seed treatment + foliar spray of *L. inermis* (1 %) + alum (0.1 %) minimizes leaf anthracnose and pod blight incidence of 10 and 7 per cent, respectively and it was at par with anthracnose and pod blight incidence recorded by seed treatment + foliar spray of *L. inermis* (1 %) + *Trichoderma viride* 4 (0.4 %) + alum (0.1 %) and there was no significant difference between seed treatment recorded in those two treatments.

Resistant sources

Under field conditions Chacko and Khare (1978) screened 47 varieties of soybean by artificial inoculation to identify the source of resistance against anthracnose and reported variety Kalitur as resistant and PK 72-92 as moderately resistant. Later on, Khare and Chacko (1983) screened 26 soybean varieties and lines against anthracnose and found five of them *viz.*, Kalitur, EC-14437, Lee, N-67 and EC-2586 completely free from the disease. During 1981-83, only 43 out of 1500 soybean accessions were rated as resistant after second screening cycle (Bowers, 1984). However, Singh (1990) evaluated soybean cultivars to pod blight caused by *C. truncatum* during the rainy season of 1990 and found only two cv. HM-1 and Birsa soybean-2 as resistant. Under severe natural infection, Kaushal and Paul (1991) screened 331 local and exotic soybean cultivars against *C. dematium* in the Kangra valley, Himachal Pradesh and recorded 18 cultivars as moderately resistant (up to 10 % disease incidence), 146 as moderately susceptible,

150 as susceptible and 17 as highly susceptible.

Under controlled conditions Manandhar *et al.* (1985) evaluated 200 soybean cultivars. They found that plants were susceptible at all growth stages and early infection lead to death of the seedlings. Only two varieties (Tarheel and PI 95-860) were found resistant to this disease. Further Manandhar *et al.* (1988) tested 414 germplasm lines, but observed none of the germplasm lines were immune to the disease.

Work carried out at AVRDC, Taiwan for the evaluation of soybean lines against anthracnose revealed that only five lines *viz.*, AGS-18, 128, 138, 139 and 151 recorded severity index below three and hence were classified as resistant (Anonymous, 1992). Nuntapunt *et al.* (1993) selected 64 soybeans anthracnose resistant lines at seedling stage. In later screening of 47 plants from 8 resistant lines which had disease rating grade 1 and grade 0 were selected and grown for seed multiplication. They were from Doikam-3, Doikam-4, UFV 80-84-2, Canapolis-3, Canapolis-10, BR 1-8, 8046-39-5-4 and 8046-39-5-2.

In Thailand, Kaveeta *et al.* (1994) made selections of soybean lines resistant to anthracnose disease in 1993, where 220 F7-lines obtained from pedigree selection of the crosses between SJ.2 and SJ.4 (commercial cultivars, susceptible to anthracnose disease) with 2 resistant varieties (Davis and Bossier) were grown in rainy season under natural infestation, the 22 promising lines were evaluated in the preliminary yield test with 2 standard

cultivars, SJ.4 and NS1. The experiment was conducted in 1994 rainy season in 2 replicates at 1 month after sowing the susceptible rows of SJ.4 in the every 6 rows of the tested lines. Based on the visual evaluation of the disease reaction and other agronomic traits, 4 promising lines (20 per cent selection intensity) were then selected for further yield testing in 1995. These lines were 8403-1-6-2-4, 8403-4-1-1-2 and 8403-4-1-1-9, all from the cross SJ.2 x Davis and 8415-1-1-9-1 from the cross SJ.4 x Davis. Amusa *et al.* (1994) in Ibadan, Nigeria, evaluated 14 cultivars and only two cultivars (PI 17144 and TGM 236) were considered very resistant to soybean anthracnose.

Out of 42 cultivars of soybean, Shirshikar (1995) found none of the cultivars as either immune or highly resistant, only one cultivar i.e., NRC-1 was found moderately resistant and cultivar Durga showed resistant reaction. Ghawde *et al.* (1996) observed varieties JS 22 and PKV 1 as highly resistant and MACS 3 as resistant. Madhusudhan (2002) evaluated 60 soybean genotypes against *C. truncatum* under glass house condition with artificial inoculation. He reported only three genotypes viz., PK 1129, DSb 2 and Cockstaurt as resistant and 27 genotypes as moderately resistant and remaining genotypes as susceptible to highly susceptible. Out of 184 soybean genotypes evaluated under field condition, Gawade *et al.* (2009) found no genotype as immune or highly resistant to the disease. However, 4 genotypes (Kalitur, PKV-1, MAUS 13 and Birsa) were resistant (2-5 per cent pod infection), 109 genotypes were

moderately resistant (6-25 % pod infection), 65 genotypes were susceptible (26-50 % pod infection), and 6 genotypes were highly susceptible (> 51 % pod infection).

Mahesha *et al.* (2009) screened the 204 soybean genotypes and observed Birsa soya - 1, JS (SH) 98-22 and Lee as anthracnose resistant. Out of 48 commercial cultivars of soybean evaluated by Costa *et al.* (2009) in the greenhouse facilities of the Universidade Federal de Santa Maria, only cultivars Tabarana, Cometa and Emgopa 316 showed susceptibility to *C. truncatum*.

Conclusion

Colletotrichum truncatum, as one of the potent pathogens, causing substantial yield losses in many crops including soybean. *Colletotrichum* genus is composed of many species but the currently defined species limits are not clearly distinguishable and relationships within some of these species complexes are not well resolved. Therefore, research on DNA sequencing of fungal genomes and other molecular approaches will be of tremendous value in resolving the phylogeny of the genus as well as in establishing evolutionary relationship with in the genus. Similarly, physiological variability and pathogenicity is still need to be characterized genetically using polymorphism analysis of the ITS region and by other relevant molecular techniques. For a disease to start and establish, environment plays a crucial role and therefore, urgent need is there to work out weather based forecasting models for prediction of disease outbreak. Selection of effective crops for crop rotation, use of composting and biocontrol agents

including fungal antagonists, possible use of arbuscular mycorrhizal fungi, soil solarization coupled with organic amendments could be the other areas of research which can assure the effective containment of the disease in the absence of highly resistant varieties and very effective chemicals. Alternatively, research work is to be strengthened to come out with high yielding and durable anthracnose resistant varieties, and highly effective as well ecofriendly chemicals to get rid off from such a major yield robber. As reaction of genotypes and cultural practices to disease varies with the regions, development of region specific management strategy should be a field of priority and for this location specific factors associated with disease development should be worked out. As such, the disease and pathogen has been researched to a limited extend in the country and therefore, systematic research work on loss assessment, cultural characteristics and variability of the pathogen, epidemiology and integrated management of the disease is required.

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Significance of Four Seeded Pod trait in Soybean Yield Improvement

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ABSTRACT

Soybean breeders focused attention on three major components of yield viz., number of pods per plant, number of seeds per plant, and seed size to enhance yield, so far. However, an increase in yield often results from increase in the number of seeds per pod. In this regard, the four seeded pod trait has shown a good promise. In this study, comparison was made between four seeded pod cultures and common cultures (without four seeded pods). Analysis revealed that among four seeded pod cultures 81.5 per cent (24.5 % four seeded and 57 % three seeded) pods were contributed by pods with higher number of seeds. Whereas, among common cultures only 40.7 per cent pods were shared by three seeded pods and remaining 59.3 per cent by two and one seeded pods. In this way, 16.3 per cent more three seeded pods and 36.6 and 4.2 per cent less two seeded and one seeded pods, respectively were recorded among four seeded pod cultures than common cultures. However, common cultures were found superior for number of pods per plant recording more average (56.5) and maximum (92.6). As four seeded pods and number of pods are independently inherited traits, the hybridization between genotypes of four seeded pods and common cultures with higher number of pods may lead to better recombinants. On that basis, the proposed four seeded plant ideotype may enhance 27 per cent yield potential of future cultivars.

Key words: Breeding, four seeded pod, ideotype, *Glycine max*

Soybean [*Glycine max* (L.) Merrill] occupies coveted place with top rank among the oilseed crops of world as well as India. The phenomenal growth in soybean has been experienced by the development of soybean genotypes congruous to different eco-edaphic situations. The most important goal of many soybean breeding programmes is

the development of stable genotypes with enhanced seed yield. The three major components of soybean yield viz., number of pods per plant, number of seeds per plant, and seed size are easily identifiable traits which have assisted the selection for higher yield. However, an increase in yield often results from the

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increase in the number of seeds per pod (Zhu and Sun, 2006). Soybean pods contain one to five seeds per pod but only few genotypes exhibit five seeded pods with very low frequency. The majority of soybean varieties released so far, have one to three seeded pods. However, the recently released varieties viz., JS 90-41, JS 93-05 and JS 95-60 contain four seeded pods also. The four seeded pod character whose frequency differs in different genotypes has shown promise to realize quantum jump. It has been realized in preliminary observations that among four seeded pod cultures, three seeded and four seeded pods contribute more proportion of total pods. Yet the four seeded pod character is not fully exploited through hybridization. Keeping in view, the present investigation was carried out taking elite lines of common cultures and four seeded pod cultures which include recently isolated mutants to know the extent of variability and to determine the optimum ideotype for a plant having four seeded pod character.

MATERIAL AND METHODS

The experimental material was comprised of four seeded pod cultures and common cultures including elite lines and mutants isolated from JS 335, JS 93-05, and NRC 37 induced by gamma- rays' irradiation. The four seeded pod cultures included JS 93-05, JS 95-60, JS 90-41, JS 92-12, JS 92-22, JS 99-71, JS 99-72, JS 99-76, JS 99-90, (elite lines) and JSM 37, JSM 52, JSM 76, JSM 141, JSM 142, JSM 207, JSM 282, JSM 160, JSM 238, JSM 256, JSM 258 (mutants) whereas, common cultures included JS

335, NRC 37, JS 97-51, JS 97-52, JS 98-63, NRC 7, PK 472 (elite lines) and JSM 5, JSM 13, JSM 120, JSM 121, JSM 191, JSM 203, JSM 136, JSM 138, JSM 149, JSM 152, JSM 283, JSM 284, JSM 246, (mutants). These genotypes were grown in randomized complete block design with three replications. The sowing was performed in *kharif* 2006. In each replication the plot size was maintained as (2 x 0.90) m² and each plot had two rows with 45 cm row to row and 5 cm plant to plant distance. Observations were recorded on ten randomly selected plants from each replication and each genotype for characters viz, height, primary branches, number of pods per plant (four seeded pods + three seeded pods + two seeded pods + one seeded pods), number of seeds per plant, number of seeds per pod, 100 seed weight and yield per plant. The data were subjected to analysis of variance, mean and range for each trait under study. By taking rounded values of different traits of best genotype of common culture and converting their expression recorded as mean performance of four seeded pod cultures, the four seeded soybean plant ideotype was proposed.

RESULTS AND DISCUSSION

Analysis of variance

The analysis of variance revealed that all the characters under study exhibited highly significant mean sum of squares in both the sets of analyses (Table 1a, b). This finding indicated that sufficient variability existed among the genotypes of both groups for all the seven characters.

Table 1a. Analysis of variance of seven traits in four seeded pod cultures and common cultures

Source of variation	DF	Plant height (cm)		Branches (No/plant)		Pods (No/plant)	
		A	B	A	B	A	B
Replication	2	21.26	6.61	0.42	0.21	60.46	10.92
Variety	19	508.78**	128.48**	1.97**	1.09**	22.70**	680.68**
Error	38	15.81	7.22	0.27	0.28	22.35	44.48

A: Four seeded pod cultures; B: Common cultures

Table 1b. Analysis of variance of seven traits in four seeded pod cultures and common cultures

Source of variation	DF	Seeds (No/plant)		Seeds (No/pod)		100 seed weight (g)		Yield (g/plant)	
		A	B	A	B	A	B	A	B
Replication	2	315.97	0.77	0.38	0.24	1.95	1.11	1.68	0.05
Variety	19	1416.58**	3359.27**	1.15**	1.18	9.25**	4.82**	14.64**	29.51**
Error	38	148.12	349.50	0.25	0.26	1.36	1.88	1.88	2.09

A: Four seeded pod cultures; B: Common cultures

Further frequencies of four seeded, three seeded, two seeded and one seeded pods were also analyzed for variance (Table 2). The results revealed that there were highly significant variations for distribution of different types of pods among both the sets of cultures showing sufficient amount of variability.

Table 2. Analysis of variance of 4, 3, 2 and 1 seeded pods frequency distribution among four seeded pod cultures and common cultures

Source	DF	4 seeded pods per plant		3 seeded pods per plant		2 seeded pods per plant		1 seeded pods per plant	
		A	B	A	B	A	B	A	B
Replication	2	15.03	NA	15.68	3.44	14.38	8.34	0.36	6.17
Variety	19	79.46**	NA	139.58**	188.67**	54.05**	761.00**	0.90**	13.85**
Error	38	3.39	NA	15.09	37.28	5.44	55.08	0.31	3.71

NA: Not applicable; A: Four seeded pod cultures; B: Common cultures

Mean and range of seven characters

Estimates of range and mean for all the seven characters have been depicted in table 3.

(a) *Four seeded pod cultures:* Plant height showed a variation ranging from 72.63 (JSM 282) to 33.40 (JS 95 - 60) cm with mean 52.67 cm. Number of branches per plant varied from 4.40 (JSM 238) to 2.60 (JS 92 - 22 and JSM 52) had mean value 3.37. The highest number of pods per plant was recorded by JS 92-12 (58.91) and the lowest by JS (b)

99 -71 (28.53) and mean 41.49. The highest number of seeds per plant (148.66) was recorded by JSM 282 and the lowest (79.96) by JS 95-60 with mean value of 108.66. Number of seeds per pod recorded as the maximum in JS 93-05 (2.95) and the minimum in JS 95-60 (2.00) having mean of 2.62. 100 seed weight ranged from 16.05 g (JS 99-72) to 8.85 g (JSM 207) with mean estimate of 12.41 g. Yield per plant varied from 13.59 (JSM 238) to 6.25 (JS 99-76) with mean value 9.49 g.

Table 3. Mean and range of seven characters in four seeded and common cultures

Characters	Range				Mean	
	Maximum		Minimum		A	B
	A	B	A	B		
Plant height (cm)	72.63 (JSM 282)	60.66 (JS 97-52)	33.40 (JS 95-60)	36.73 (JSM 246)	52.67	48.49
Branches (No/plant)	4.40 (JSM 238)	5.00 (JSM 203)	2.60 (JS 92-22 and JSM 52)	1.93 (JSM 152)	3.37	3.81
Pods (No/plant)	58.91 (JS 92-12)	92.66 (JS 97-52)	28.53 (JS 99-71)	34.96 (JSM 152)	41.49	56.53
Seeds (No/plant)	148.66 (JSM 282)	199.73 (JS 97-52)	79.96 (JS 95-60)	62.63 (JSM 152)	108.66	121.56
Seeds (No/plant)	2.95 (JS 93-05)	2.53 (JSM 138)	2.00 (JS 95-60)	1.71 (NRC 7)	2.62	2.15
100 seed weight (g)	16.05 (JS 99-72)	14.02 (JSM 138)	8.85 (JSM 207)	9.48 (JSM 121)	12.41	11.36
Yield (g/plant)	13.59 (JSM 238)	21.67 (JS 97-52)	6.25 (JS 99-76)	6.93 (JSM 152)	9.49	10.93

A: Four seeded pod cultures; B: Common cultures

(b) Common cultures: JS 97-52 recorded the maximum height (60.66 cm) and JSM 246 the minimum height (36.73 cm) with a mean estimate of 48.49 cm. Number of branches per plant ranged from 5.00 (JSM 203) to 1.93 (JSM 152) having mean value 3.81. Pods per plant varied from 92.66 (JS 97-52) to 34.96 (JSM 152) and with mean value 56.53. The highest number of seeds per plant was observed by JS 97-52 (199.73) and the lowest by JSM 152 (62.63) with mean value of 121.56. Number of seeds per pod ranged from 2.53 (JSM 138) to 1.71 (NRC 7) with mean values as 2.15. 100 seed weight ranged from 14.02 g (JSM 138) to 9.48 g (JSM 121) with mean value of 11.36 g. Yield per plant varied from 21.67 g (JS 97-52) to 6.93 g (JSM 152) having a mean value of 10.93 g.

It is obvious from the above data that plant height, number of seeds per pod and 100 seed weight showed wider range with higher mean values among four seeded pod cultures than common cultures. Whereas, common cultures had wider range for number of branches, number of pods per plant, number of seeds per plant and yield per plant with higher values of mean in comparison to the four seeded pod cultures.

Mean, range and percentage frequencies of different types of pods

The mean, range and percentage frequencies of four, three, two and one seeded pods in both sets of cultures have been presented in table 4.

(a) Four seeded pod cultures: Range of 4 seeded pods per plant varied from 22.46 (JSM 142) to 2.33 (JSM 256) with a mean of 10.15. JSM 141 expressed the highest (47.8 %) frequency of four seeded pods and JSM 256 the minimum (4.3 %) frequency with mean value as 24.5 per cent. Number of 3 seeded pods per plants ranged from 37.73 (JSM 282) to 14.96 (JS 99-71) with a mean of value 23.67. The highest frequency was recorded by JSM 258 (75.7 %) and the lowest by JSM 141 (44.2 %). The overall mean was recorded as 57.0 per cent. Number of 2 seeded pods per plant ranged from 17.20 (JSM 256) to 2.96 (JSM 52) with mean value 7.17. The maximum percentage of two seed pod was recorded by JSM 256 (32.2 %) and the minimum by JSM 141 (6.9 %) with mean percentage of two seeded pod as 17.3 per cent. Number of 1 seeded pods showed variation ranging from 1.63 (JS 95-60) to 0.03 (JSM 52) with mean of 0.50. JS 95.60 recorded the highest percentage (4.0 %) and JSM 52 the lowest (0.1 %) with over all mean as 1.2 per cent.

Table 4. Mean, range and coefficient of variation of 4 seeded, 3 seeded, 2 seeded and 1 seeded pods frequencies among four seeded pod cultures

Character	Types of cultures	Range				Mean	
		Maximum		Minimum		No.	%
		No.	%	No.	%		
4 seeded pods per plant	A	22.46 (JSM 142)	47.80 (JSM 141)	2.33 (JSM 256)	4.30 (JSM 256)	10.15	24.50
	B	NA	NA	NA	NA	NA	NA
3 seeded pods per plant	A	37.73 (JSM 282)	75.70 (JSM 258)	14.96 (JS 99-71)	44.20 (JSM 141)	23.67	57.00
	B	39.60 (JSM 283)	61.10 (JS 335)	2.70 (PK472)	6.30 (PK472)	23.00	40.70
2 seeded pods per plant	A	17.20 (JSM 256)	32.20 (JSM 256)	2.96 (JSM52)	6.90 (JSM 141)	7.17	17.30
	B	68.40 (NRC 37)	77.70 (NRC 37)	16.43 (JSM 5)	35.00 (JSM 5)	30.45	53.90
1 seeded pods per plant	A	1.63 (JS 95-60)	4.00 (JS 95-60)	0.03 (JSM52)	0.10 (JSM52)	0.50	1.20
	B	8.76 (NRC 7)	17.70 (PK472)	1.00 (JS 97-51)	1.70 (JSM284)	3.05	5.40

NA: Not applicable; A: Four seeded pod cultures; B: Common cultures

(b) Common cultures: Number of 3 seeded pods per plant ranged from 39.60 (JSM 283) to 2.70 (PK 472) with a mean of 23.00. JS 335 had the highest percentage frequency (61.1 %) of three seeded pods and PK 472 had the lowest (6.3 %). The over all percentage mean of three seeded pod was 40.7 per cent. Number of 2 seeded pods per plant varied from 68.40 (NRC 37) to 16.43 (JSM 5) with mean value of 30.45. The percentage ranged from 77.7 per cent (NRC 37) to 35.0 per cent (JSM 5) with mean percentage estimate as 53.9 per

cent. Number of 1 seeded pods per plant ranged from 8.76 (NRC 7) to 1.0 (JS 97-51) with mean of 3.05. The maximum percentage as 17.7 was recorded by PK 472 and minimum percentage as 1.7 by JSM 284. The overall percentage means was observed was 5.4 per cent.

Comparison between four seeded pod cultures and common cultures

When comparison was made between four seeded pod cultures and common cultures, a noteworthy trend

among four seeded pod cultures was recorded as the substantial contribution of pods having higher number of seeds 81.5 per cent (24.5 four seeded pods+ 57.0 three seeded pods) as depicted in table 4. Whereas, in case of common cultures only 40.7 per cent pods were shared by three seeded pods and 59.3 per cent pods having lower number of seeds comprising 53.9 per cent two seeded pods and 5.4 per cent one seeded pods. It was also important to note that there was 16.3 per cent increase for three seeded pods among four seeded pod cultures and simultaneously, decrease for two seeded and one seeded pods observed as 36.6 and 4.2 per cent, respectively in comparison to common cultures. However, common cultures were found superior in respect of number of pods per plant recording more average value as 56.5 and maximum value as 92.6. This study indicated that by incorporating four seeded pod trait through breeding programmes, a substantial increase in number of seeds per plant may be achieved through increase in proportion of four and three seeded pods with same number of pods per plant.

Tiwari and Bhatnagar (1994) have also reported the higher frequency of four seeded pod to an extent of 28 per cent in hybrid generations and in later generations, 4 seeded segregants had excelled their parents thus concluded as a transgressive segregation., Chen (1998) reported that among hybrids the average number of seeds/pod was 2.94 in four seeded pod lines and 2.4-2.64 in common lines, Khare *et al.* (1998) found 42 per cent four seeded

pods in *rabi* season in JS 90-41, Gour *et al.* (1992) reported the cultivated soybean *Glycine max* (L.) Merrill was crossed with its proposed wild progenitor, *Glycine soja* (Seib and Zucc.) to create useful genetic variability and reported 45 per cent four seeded pods per plant and Verma *et al.* (1993) found nearly 50 per cent four seeded pods with an average of 2.8 seeds per pod in EC 172665. Oneml (2003) and Banger *et al.* (2004) have reported significant positive association of number of pods per plant with seed yield. Similar association of number of seeds per plant with seed yield has been reported by Wang *et al.* (1996).

As the genes controlling four seededness and number of pods are independent, the crossing between genotypes of two groups in single or double cross manners may produce genotypes having more number of pods combined with four seeded pods will be an improved version of soybean genotype having good yielding potential.

Such approaches have also been suggested by Gai (1999) towards genetic improvement for soybean yield which consist of assembling positive yield genes and to support yield genes with plant architecture genes as their genetic background. Tiwari (2001) felt a need to enhance the genetic potential for yield in soybean by evolution of genetic resources and enhancement of genetic diversity and Zhu and Sun (2006) emphasized high seed per pod value as a crucial component of soybean.

Past success in increasing yield potential has mainly been the result of an empirical selection approach, which is, selecting yield per se. A further increase in

yield potential is difficult to attain using the empirical selection approach since the crop has already reached a high yield potential. There is interest in taking an ideotype approach to design plants for target environments, to take advantage of interactions of plant characteristics and the environment.

Keeping in view the above results of the present study and suggestions of researchers a four seeded pod plant ideotype has been suggested by taking nearer values to the averages of different traits of best genotype (JS 97-52) from

common culture. If we convert the expression as realized as average values of the characters especially types of pods, number of seeds per pods and 100 seed weight of four seeded pod cultures and other characters remaining same as of JS 97-52, the picture of ideotype appears as given in table 5. While converting the number of seeds received from different types of pods natural and physiological compensation has been taken into consideration as experienced in the study.

Table 5. Conversion of best genotype of common culture (JS 97-52) into four seeded genotype on the basis of average values of important traits

Character	Near values of best genotype from common culture (JS 97-52)	Proposed ideotype for four seeded pod culture
Height (cm)	60	60
Branches (No/plant)	4	4
Pods (No/plant)	90	90
a) Four seeded	-	22
b) Three seeded	22	51
c) Two seeded	65	16
d) One seeded	3	1
Realized No. of seeds from 90 pods	190	235
a) From four seeded pods	-	80
b) From three seeded pods	60	135
c) From two seeded pods	127	20
d) From one seeded pod	3	0
Seeds (No/pod)	2.2	2.6
100 seed weight (g)	10	12
Yield (g/plant)	22	28

On the basis of above facts the ideal four seeded soybean plant type is proposed which should have height 60 cm, number of branch 4, number of pods/plant 90, number of seeds/plant 235, number of seeds/ pod 2.6, 100 seed weight 12 g and yield per plant 28 g. It is obvious that with the same number of pods the yield increased from 22 g to 28 g i.e. 27 per cent, just by incorporating four seeded pod character. In this way, at least, 27 per cent increase in seed yield can be achieved by proposed ideotype converting the best common culture into four seeded pod culture having same number of pods per plant.

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Efficient Plant Regeneration System from Half Seed Explant of Soybean [*Glycine max* (L.) Merrill]

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ABSTRACT

A rapid and efficient regeneration protocol for multiple shoot induction from half-seed explant of soybean was established. The effects of plant growth regulators thidiazuron (TDZ), N6-benzyladenine (BA), kinetin (KIN) and indole-3-butyric acid (IBA) were evaluated. TDZ alone was most efficient for high regeneration as well as multiple shoot formation than other cytokinins or combinations of auxins and cytokinins. The optimal TDZ concentration for shoot regeneration was 0.57 μM . Any increase over 0.57 μM led to induction of multiple buds in very high number, which hindered normal elongation process and proper plant development. The regenerated shoots were elongated on MSB5 medium containing 1.5 μM gibberellic acid (GA_3), 2.9 μM zeatin riboside, and 0.6 μM indole-3-acetic acid (IAA) and sufficiently elongated shoots were transferred on half strength B5 medium containing 9.84 μM indole-3-butyric acid (IBA) for development of root. The rooted plantlets were transferred in the pot containing a mixture of soil: pot mix: sand (2:2:1) for two weeks and finally transferred to soil, which showed 95 per cent survival rate. The regenerated plantlets flowered and set seed normally.

Key words: Direct organogenesis, half seed, soybean, thidiazuron

Soybean [*Glycine max* (L.) Merrill] is not only good source of oil and protein, but it contains various nutraceutical compounds that have been implicated in reducing the risk of major killer diseases viz; atherosclerosis, breast cancer, diabetes and osteoporosis (Messina, 1999). A lot of work is being done on modifying

the seed compositional traits for development of specialty soybean (Falco *et al.*, 1995; Dinkins *et al.*, 2001; Kita *et al.*, 2010). Moreover soybean productivity is drastically reduced by various biotic and abiotic factors (Boyer, 1982). The breeders have developed specialty soybean and high yielding soybean varieties resistant to

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biotic and abiotic factors using traditional breeding methods (Dita *et al.*, 2006). The genetic base of soybean is not sufficient to meet up above challenges.

An alternative approach for development of improved soybean varieties is to introduce exogenous gene in soybean genome using gene transfer technique. However, the successful development of transgenic soybean depends upon an efficient plant regeneration protocol and its suitability to transformation techniques. Many researchers have used different parts of the soybean plant as explant for successful regeneration. The explant used in various shoot regeneration protocols are stem node (Saka *et al.*, 1980), hypocotyl segments (Kaneda *et al.*, 1997; Yoshida, 2002), immature cotyledon (Parrott *et al.*, 1989), epicotyl (Wright *et al.*, 1987), young embryonic axes (Liu *et al.*, 2004) primary leaf node (Kim *et al.*, 1990) and cotyledonary node (Barwale *et al.*, 1986; Franklin *et al.*, 2004; Shan *et al.*, 2005). First successful transgenic soybean was developed from cotyledonary node (CN) through *Agrobacterium*-mediated transformation (Hinchee *et al.*, 1988). Since then various workers have tried to improve CN protocol and increase transformation efficiency (Zhang *et al.*, 1999; Zeng *et al.*, 2004; Paz *et al.*, 2006). The CN system requires wounding of explant by making accurate incisions on the adaxial side using surgical blade. But non-reproducibility of CN wounding procedure leads to discrepancies in transformation efficiency via *Agrobacterium* method (Paz *et al.*, 2006). Recently an improved transformation

method using an explant derived from mature seed following an overnight imbibitions termed as 'half-seed' explant for efficient *Agrobacterium*-mediated transformation has been reported by Paz *et al.* (2006). The transformation efficiency using half-seed explant was reported to be 1.5 fold higher than cotyledonary method. But the culture conditions optimized for CN system was not found suitable for efficient regeneration from half-seed explants. The regeneration protocol standardized by Paz *et al.* (2006) is not as efficient as CN protocol and is genotype dependent. However, such type of work was not reported in India. Herein, we report highly efficient and genotypic independent regeneration protocol for Indian cultivars of soybean.

MATERIAL AND METHODS

Plant material and preparation of explants

Mature seeds of soybean cultivars JS 335, NRC 2, NRC 12, NRC 37 and NRC 7 were used in these experiments. Soybean seeds were sterilized by placing seeds into a tightly sealed chamber filled with chlorine gas produced by mixing 3.5 ml of 12 N HCL and 100 ml bleach (4.0 % approx Sodium hypochlorite) for 6 hours (Liu and Wei, 2002). The sterile seeds were soaked in sterile distilled water for overnight at the room temperature. A longitudinal cut along the hilum was made to separate the cotyledons and the seed coat was removed. The embryonic axis found at the junctions of the hypocotyl and the cotyledons were excised to obtain the half-seed explant (Paz *et al.*, 2006).

Culture media and culture conditions

Plant growth regulators (PGRs) were added to B5 (Gamborg *et al.*, 1968) basal medium supplemented with 3 per cent sucrose, 0.7 per cent agar and the pH was adjusted to 5.7 before autoclaving at 121°C (15 psi) for 20 min. The culture was maintained in culture room at $26 \pm 2^\circ\text{C}$ under 18 h/6h light/dark regime, at a photon flux density (PFD) of approximately $150 \mu\text{mol s}^{-1} \text{m}^{-2}$. Explants were sub cultured into the same medium at 12 days intervals.

Concentrations of cytokinins and auxin on plant regeneration

The explants were placed in shoot induction medium consisting of B5 basal medium supplemented with different concentration of thidiazuron (0.28, 0.57, 1.14, 2.27, 4.54 and $9.08 \mu\text{M}$) N6-benzylaminopurine (1.25, 2.5, 5, 7.5, and $10 \mu\text{M}$), kinetin (2.5, 5, 7.5, 10, and $12.5 \mu\text{M}$) and Indole-3-butyric acid (0.12, 0.25, 0.5 and $1.0 \mu\text{M}$) for shoot induction. The base of explant (i.e., the part of the explant from where the embryonic axis was removed) was embedded in the medium. The frequency of shoot regeneration was determined as the percentage of cultures showing shoot formation.

Shoots elongation, rooting and transplantation

After 3 weeks on shoot initiation medium, the explants were transferred on shoot elongation medium [MS salts (Murashige and Skoog, 1962), B5 vitamins, 3% sucrose, $1.5 \mu\text{M}$ GA3, $2.9 \mu\text{M}$ zeatin riboside, $0.6 \mu\text{M}$ IAA and 0.7%

agar pH 5.8] for elongation of shoots. The shoots elongated were cut from the explants and placed on different concentrations of naphthalene acetic acid (NAA 1.35, 2.7, 5.4, and $10.8 \mu\text{M}$) and IBA (1.23, 2.46, 4.92 and $9.84 \mu\text{M}$) for root induction. The frequency of root formation was determined as the percentage of shoots showing root formation. Rooted plantlets were thoroughly washed with sterile distilled water and grown in plastic pots containing pot mix (Kamdhenu, India). The plantlets were then covered with polyethylene bags for hardening and after 12 weeks polyethylene bags were removed and plants were transferred to greenhouse.

Statistical analysis

Each experiment was conducted in a Completely Randomized Block Design and in three replicates. Data were analyzed with the least significance difference (LSD) test at the 1 per cent and 5 per cent level of probability.

RESULTS AND DISCUSSION

Effect of cytokinins and auxin on plant regeneration

Three cytokinins were used in the present study to determine their shoot regeneration capacity. TDZ was found to be the most efficient for high regeneration frequency as well as multiple shoots formation than other cytokinins (Table 1). TDZ is a substituted phenylurea compound with both cytokinin and auxin-like effects (Visser *et al.*, 1992). TDZ is considered to be one of the most active cytokinins for shoot induction in plant tissue culture (Murthy *et al.*, 1998). Little is

known about mechanism of TDZ inducing direct organogenesis in plants. TDZ has been suspected for promoting regulated morphogenesis in plants through the modulation of endogenous cytokinin and auxin (Thomas and Katterman, 1986). The highest frequency of shoot formation from half-seed explants was 89.3 per cent, 44 per cent and 10.7 per cent on regeneration medium with TDZ, BA, and KIN respectively (Table 1). Kaneda *et al.* (1997) also reported that TDZ was responsible for higher regeneration capacity and multiple shoot formation efficiency than BAP. Same trends were also reported in

another study on soybean organogenesis using cotyledons as explant, wherein shoot regeneration frequency was 68 per cent with TDZ and 50 per cent with BA (Franklin *et al.* 2004). Srinivasan *et al.* (2006) reported that TDZ at a concentration of 0.5 mg/l induced highest shoot regeneration frequency (92.0%) with mean number of 7.0 shoots in *alfalfa* where higher concentration of cytokinins was used than other study. All these cytokinins were tested in combination of IBA (0.12, 0.25, 0.5 and 1.0 μM) and found to have no significant difference on efficiency (Data not shown).

Table 1. Effect of cytokinins on *in-vitro* multiple shoot proliferation from half-seed explant (cv. JS 335) after 20 days of culture initiation

Cytokinins	Concentration μM	Percent Response	Mean no. of buds	Mean no. of shoots
Control	0	0	0	0
KIN	2.5	7.3 \pm 2.0 e	0	1.2 \pm 0.1 e
	5	8.7 \pm 1.8 e	0	1.4 \pm 0.2 d
	7.5	10.7 \pm 1.6 e	0	1.2 \pm 0.1 e
	10	9.3 \pm 1.3 e	0	1.5 \pm 0.2 d
	12.5	7.3 \pm 1.4 e	0	1.5 \pm 0.2 d
	BA	1.25	9.3 \pm 1.8 e	0
2.5		19.3 \pm 1.3 d	0	1.5 \pm 0.2 d
5		44 \pm 1.3 c	0	2.1 \pm 0.1 c
7.5		37.3 \pm 1.1 c	0	2.6 \pm 0.2 b
10		20.7 \pm 2.1 d	0	2.0 \pm 0.2 c
TDZ	0.28	60.7 \pm 3.1 b	2.1 \pm 0.2 d	2.1 \pm 0.1 c
	0.57	84 \pm 2.8 a	3.6 \pm 0.4 d	3.8 \pm 0.2 a
	1.14	85.3 \pm 3.0 a	8.9 \pm 0.8 c	2.7 \pm 0.2 b
	2.27	88 \pm 3.2 a	10.8 \pm 0.8 b	1.3 \pm 0.1 d
	4.54	89.3 \pm 3.6 a	11.6 \pm 1.0 b	1.0 \pm 0.1 e
	9.08	66 \pm 2.1 b	14.3 \pm 1.5 a	0.5 \pm 0.1 f

Control without growth regulators, B5 medium; Values represent the means \pm SE; Means followed by the same letter are not significantly different at the 0.05 level of confidence.

Optimal TDZ concentration for shoot organogenesis

In the present investigation the optimal TDZ concentration for shoot regeneration from half-seed explants was found to be 0.57 μM (Table 1). However, shoot regeneration frequency showed an incremental albeit statistically non-significant trend with increasing concentration of TDZ up to 4.54 μM which turned negative at further higher concentrations. Similar results have been reported using hypocotyl segment explants (Kaneda *et al.*, 1997), cotyledonary nodes (Franklin *et al.*, 2004) where use of 4.54 μM gave highest regeneration frequency. Half-seed explants cultured on B5 medium supplemented with TDZ (0.57 μM) began to swell after 6 days, and the first bud formation was observed after 12 days (Fig. 1 a and b). It is noted that shoot regenerated from explants without callus phase. It is known that regenerants from callus that has been maintained for several cycles is always coupled with somaclonal variation (Freytag *et al.*, 1989). Therefore, direct regeneration may minimize somaclonal variation. Kaneda *et al.* (1997) reported the optimal concentration for shoot organogenesis from hypocotyl segments between 2.24 μM and 4.54 μM . In another study Yoshida (2002) reported 2-10 μM TDZ in general, to be optimal for organogenesis while 4 μM TDZ in combination with basipetal hypocotyl end of upper section (BUS) was found to be the most efficient for organogenesis. In present experiment, low level of TDZ (0.57 μM) concentration in the nutrient medium produced shoots most efficiently which slightly varied

from the study by Kaneda *et al.* (1997), where shoot regeneration was less efficient at lower concentration. Increase in TDZ concentration over 0.57 μM led to formation of calli from the base of explants and formed multiple buds in very high number which hindered normal elongation of shoots. Similar results have also been documented by Shan *et al.* (2005). They reported that low concentration of TDZ led to fast shoot development and too high concentration resulted in abundance of compact calli. Thomas and Katterman (1986) have proposed that higher concentrations lead to suppression of cytokinin breakdown and/or the continued biosynthesis of purine cytokinins which results in rooting inhibition and many smaller plantlets being produced.

Shoots elongation, rooting and transplantation

The regenerated shoots from half seed explant after 3 weeks culture were transferred on shoot elongation medium consisting of MS salts, B5 vitamins, 3 per cent sucrose, 1.5 μM GA3, 2.9 μM zeatin riboside, 0.6 μM IAA and 0.7 per cent agar pH 5.8. The shoots grew quickly on medium and reached 2 cm in height within 15 days (Fig. 1 c). Elongated shoots were transferred to various concentrations of NAA and IBA for root induction. NAA and IBA concentrations had significant effects on rooting percentage. Maximum rooting (100 %) was observed with 9.84 μM of IBA (Table 2, Fig. 1 d). However, these treatments showed sufficient callusing at basal part of the stem. The rooted plantlets were transplanted to plastic pots containing soil, pot mix and sand (2:2:1)

and almost all of the plantlets survived in the greenhouse (Fig. 1 e). Regenerated plants were uniform with normal leaf,

flower shape and color. No morphological variation was observed.

Table 2. Effect of different concentrations of auxins on rooting of shoots of soybean after culture for 30 days

Auxin	Concentration (μM)	Rooting Rate (%)
Control	0	11.7 \pm 1.5 f
NAA	1.35	28.3 \pm 2.9 e
	2.70	51.7 \pm 3.5 d
	5.40	68.3 \pm 4.5 c
	10.80	38.3 \pm 2.6 d
	IBA	1.23
2.46		65.0 \pm 4.8 c
4.92		76.7 \pm 6.1 b
9.84		100 \pm 0.0 a

Control without growth regulators, 1/2B₅ medium; Values represent the means \pm SE; Means followed by the same letter are not significantly different at the 0.05 level of confidence

Genotypic response to the shoot regeneration system

Half-seed explants of five genotypes were cultured on B5 medium containing 0.57 μM TDZ or 5.0 μM BA or 7.5 μM KIN to investigate the genotypic response *in-vitro* (Table 3). Result showed that the regeneration frequency was genotype independent and was affected by only plant growth regulator regime in

the medium. Similar observations were reported by Sairam *et al.* (2003) and Franklin *et al.* (2004). Yoshida (2002) reported genotypic differences in the organogenesis of the hypocotyl end using TDZ. However, no genotypic differences were observed for shoot bud formation frequency in acropetal hypocotyl end of upper section (AUS).

Table 3. Effect of cytokinins and genotype on percentage response of shoot regeneration, 4 weeks after culture using half-seed explant

Genotype	Kinetin	Benzylaminopurine	Thidiazuron
JS 335	10.7±0.8	44±1.2	84±1.6
NRC 2	9.3±1.2	43.3±1.3	82.7±1.79
NRC 12	10±1.4	42.3±1.0	83.3±1.78
NRC 37	9.7±1.1	40±1.2	79.3±2.1
NRC 7	10.3±1.2	43.7±0.8	83.3±1.3

*Kinetin (7.5 μM), Benzylaminopurine (5 μM) and thidiazuron (0.57 μM); Values represent the means ± SE. Genotype MS = 6.623; PGR MS = 8304.913**; Genotype X PGR MS = 1.18; ** Significant at 0.01 level of confidence.*



Fig. 1. *In vitro* regeneration of soybean (cv. JS 335): (a) Half seed explant at the time of culture, (b) Multiple shoot formation from explant on TDZ (0.57μM), (c) Elongated shoots on shoot elongation medium after 3 weeks, (d) Root induction of elongated shoots on medium containing 9.84 μM IBA, (e) Plants established in the green house after hardening

Barwale *et al.* (1986) reported significant variation in shoot regeneration from cotyledonary node among 178 genotypes in different maturity group '000'- 'VIII'. Though somatic embryogenesis is an efficient system to produce genetic modified plants, it is genotypic specific and accompanied with a high level of somaclonal variation in the regenerated plants (Parrott *et al.*, 1989)

In conclusion, the half-seed explants derived from the mature dry seeds of soybean can be induced to form

direct shoots in high frequency in almost a single step on a simple medium containing only TDZ. This system is rapid with initiation of tissue culture to transplanting of regenerants to soil completed in 8 weeks. Since the regenerant developed directly without an intervening callus phase, the somaclonal variation can be avoided. The regeneration system developed in this study can be employed in soybean genetic transformation via *Agrobacterium*.

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Parental Polymorphism Survey of Popular Soybean Varieties in Combination with the Source of Null Alleles of Kunitz Trypsin Inhibitor and Lipoxygenase-2 Using Linked SSR Markers

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ABSTRACT

For introgression of a desirable trait into a variety from the donor genotype using simple sequence repeat (SSR) marker linked to it, the polymorphism for polymerase chain reaction products generated is the prerequisite. In the present study, SSR markers Satt228 and Satt409 reported to be linked to kunitz trypsin inhibitor and Sat_074 and Satt522 to lipoxygenase-2 were surveyed for parental polymorphism in 5 popular soybean varieties, the exotic soybean genotype with null allele of kunitz trypsin inhibitor (PI542044) and the exotic genotype with null allele of lipoxygenase-2 (PI596540). Satt409 showed distinct polymorphism between all the 5 varieties and PI542044 while Satt228 also showed clear polymorphism except for JS 335. Sat_074 and Sat522 showed polymorphism between all the 5 soybean varieties and PI 596540. The results showed that Satt409 can be used for development of kunitz trypsin inhibitor free while both Sat_074 and Satt522 for lipoxygenase-2 free varieties through Marker Assisted Backcross (MABC) selection with all the five varieties as the recurrent while PI542044 and PI596540 as the donor parents, respectively.

Key words: Kunitz trypsin inhibitor, lipoxygenase-2, soybean varieties, SSR markers

Soy-food has been acclaimed as the “functional food of the century” owing to its nutraceutical value that reduces the risk of major killer diseases such as atherosclerosis, cancer, and diabetes. However, presence of kunitz trypsin inhibitor (KTI) and off-flavor inducing lipoxygenases in soybean seed deter the wide utilization of the bean in food uses in the country, which stands meager 5 per cent of the

total soybean produced (Anonymous, 2010). Kunitz trypsin inhibitor, a non-glycosylated monomeric protein, constitutes about 80 per cent of the trypsin inhibitor activity and is also known as SBTI-A₂. Five electrophoretic forms of soybean kunitz inhibitor have been reported to be controlled by multiple alleles Ti^a, Ti^b, Ti^c and Ti^d at single locus (Orf and Hymowitz, 1979). The fifth form does

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not exhibit a soybean trypsin inhibitor-A2 band and is inherited as a recessive allele designated as *ti*. The off-flavour has been attributed to the release of hexanal compounds during the catalytic oxidation of polyunsaturated fatty acids by seed 'lipoxygenase, which exists in the three isozymic forms viz. Lox-1, Lox-2, Lox-3 (Axelrod *et al.*, 1981). Lipoxygenase-2 is the principal contributor to the off-flavour (Davies *et al.*, 1987) and its absence is indicated by *lx2* which is recessive to *Lx2*. Though both kunitz trypsin inhibitor and lipoxygenase-2 are heat labile; thermal treatment employed to inactivate these two biological components has its own limitations. A residual activity of kunitz trypsin inhibitor always remains depending upon the level of temperature and time of heating. In addition, thermal treatment employed to inactivate both kunitz trypsin inhibitor and lipoxygenase affect not only the protein solubility (Anderson, 1992) but also cost-ineffective for the soy processing units. Therefore, genetic elimination of these two undesirable biological components from the soybean genotypes is the best approach to boost the utilization of soybean in food uses.

In the plant-breeding programme focusing on development of kunitz inhibitor and lipoxygenase-2 free soybean varieties, SSR (simple sequence repeat) markers linked with these traits can be used for Marker Assisted Selection because the routine polyacrylamide gel electrophoresis employed for identification of kunitz trypsin inhibitor and colorimetric method for lipoxygenase-2 may damage the embryo

while removing a part of seed tissue for the analysis. Kim *et al.* (2006) reported the linkage of two SSR markers Satt228 and Satt409 with *Ti* locus and Satt409 has been validated recently in our laboratory in Indian soybean population (Rani *et al.*, 2011). Sat_074 and Satt522 markers have been reported to be linked with *Lx2* locus (Kim *et al.*, 2004). These SSR markers linked with these two important biological components can be used for Marker-Assisted Backcross (MABC) selection for the introgression of null alleles of kunitz trypsin inhibitor and lipoxygenase-2 in the genetic background of recipient parent. However, this necessitates the confirmation of the polymorphic nature of linked SSR markers for the recipient and the donor parents for both these traits. This would enable the identification of heterozygous plants i.e. *Titi* and *Lx2lx2* in the BC₁F₁, BC₂F₁, and the following backcross generations for development of soybean varieties free from kunitz trypsin inhibitor and lipoxygenase-2. Soybean accessions with null alleles of kunitz trypsin inhibitor and lipoxygenase-2 as donor parents have been procured from USDA at Directorate of Soybean Research for this purpose with the aim of introgressing null alleles into popular soybean varieties. The present investigation was undertaken with the objective of survey on polymorphism for the popular soybean varieties in combination with source of null alleles of kunitz trypsin inhibitor (KTI) and lipoxygenase using SSR markers linked with the *Ti* locus and *Lx2* locus.

MATERIAL AND METHODS

Soybean genotypes PI 542044 and PI 596540 which have null allele of kunitz

trypsin inhibitor and lipoxygenase-2, respectively, were procured from United States Department of Agriculture. Five popular soybean varieties (NRC 7, JS 97-52, JS 335, JS 93-05, JS 95-60), PI 542044 and PI 596540 were raised in the glasshouse. After 10-15 days of planting, the tender young leaves were collected and genomic DNA was extracted from the finely ground leaf tissue by means of CTAB (cetyl trimethyl ammonium bromide) procedure (Doyle and Doyle, 1990). Crude DNA obtained above was purified following phenol extraction and buffer (pH 8.0). The PCR was performed in a MJ Research Thermocycler model PTC100 and the reaction mixture (10 μ l) contained 2 μ l DNA (20 ng/ μ l), PCR 10X buffer (1 μ l), 1.1 μ l MgCl₂ (25 mM), 0.1 μ l dNTPs (25 mM), 0.4 μ l each forward and reverse SSR primers (30 ng/ μ l), 0.068 μ l Taq DNA polymerase (3 units/ μ l), 4.932 μ l distilled water. Initially, DNA was

denatured at 94 $^{\circ}$ C for 2 min. followed by 30 cycles each consisting of denaturation at 94 $^{\circ}$ C for 1 min, primer annealing at 50 $^{\circ}$ C for 2 min., primer elongation at 72 $^{\circ}$ C for 3 min. Finally, elongation was carried out at 72 $^{\circ}$ C for 10 min. Amplified products so obtained were resolved on 3 per cent metaphore agarose gel stained with ethidium bromide using a 96-well horizontal gel electrophoresis unit (Atto Corporation) and analyzed in a gel documentation unit (Syngene). For parental polymorphism survey, SSR markers Satt228 and Satt409 linked with *Ti* locus while Sat_074 and Satt522 linked with *Lx2* locus (Fig. 1) with the oligonucleotide sequences given in Table 1 (USDA 2008) were synthesized by Sigma Aldrich. Polymorphic information content (PIC) of SSR markers were calculated as $PIC_i = 1 - \sum_{j=1}^{n_j} p_{ij}^2$ where *i* denotes the SSR marker while *p_{ij}* is frequency of *jth* allele.

Table 1. List of SSR markers used for the parental polymorphism survey

SSR	LG	Forward Sequence(5'→3')	Reverse Sequence(5'→3')
Satt409	A2	CCTTAGACCATGAATGTCTCGAAGATA	CTTAAGGACACGTGGAAGATGACTAC
Satt228	A2	TCATAACGTAAGAGATGGTAAAAC	CATTATAAGAAAACGTGCTAAAAGAG
Satt522	F	GCGAAACTGCCCTAGGTAAAA	TTAGGCGAAATCAACAAT
Sat_074	F	GGGTGAGAAAATACATGCAACTTACA	GGGCATCAAAAATTGATATTAATGTCTAA

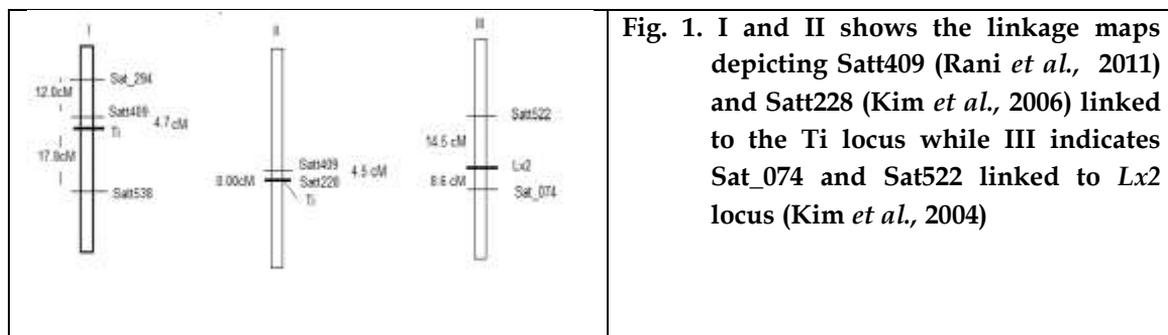


Fig. 1. I and II shows the linkage maps depicting Satt409 (Rani *et al.*, 2011) and Satt228 (Kim *et al.*, 2006) linked to the *Ti* locus while III indicates Sat_074 and Satt522 linked to *Lx2* locus (Kim *et al.*, 2004)

RESULTS AND DISCUSSION

The introgression of a recessive trait into high yielding varieties can be expedited through Marker Assisted Backcross (MABC) selection. SSR markers (tightly linked with the trait), because of their co-dominance property, can help in the identification of the individual plants carrying the recessive allele in the backcross generations. Kunitz trypsin inhibitor, the antinutritional factor and lipoxygenase-2, the principal contributor to off-flavor in soy products, are major reasons for poor utilization of soybean in the country because of their presence in all the major popular varieties of soybean. However, absence of these biological components in soybean is controlled by recessive alleles. This can be exploited to make high yielding soybean varieties of the country free from these two undesirable components by introgression of their null alleles through MABC selection using the tightly linked SSR markers. Figure 2 depicts the resolution of PCR products of all the soybean varieties and PI 542044 generated using SSR markers Satt228 and Satt409 reported to be tightly linked with *Ti* locus. Satt228 generated PCR products

with very good polymorphism between 4 varieties (NRC 7, JS 97-52, JS 93-05, JS 95-60) and PI 542044. The difference between the size of the PCR products observed for PI 542044 and the varieties NRC 7, JS 97-52, JS 93-05, JS 95-60 was at least 50 bp. However, this primer was monomorphic for JS 335 and PI 542044 as the PCR product amplified in case of JS 335 was almost of the same size (200 bp) as observed in PI 542044. Furthermore, the product formed in case of variety JS 93-05 was higher in size than the remaining four varieties, thereby showing three allelic forms of Satt228 in the total 6 genotypes surveyed. Satt409 showed polymorphism in respect of all the 5 popular varieties in combination with PI 542044, exhibiting a difference in size of 15-50 bp between the PCR amplified resulted from varieties and PI 542044 (Figure 3). Maximum product size was observed for varieties JS 97-52 and JS 335 (200bp) while minimum for JS 95-60 and NRC 7 (175bp). The size of PCR product in case of variety JS 93-05 was 185bp. Exotic soybean accession PI 542044 showed a PCR product of 150bp size. Thus, total 4 allelic forms were observed for Satt 409 in the six genotypes surveyed.



Fig 2. Parental polymorphism survey of soybean varieties and PI 542044 (donor of null allele of kunitz trypsin inhibitor) using Satt228. Lanes 1 and 11 depict the 50 bp ladder. Lanes 2, 4, 6, 8 and 10 indicate the DNA finger prints generated in varieties NRC 7, JS 97-52, JS 93-05, JS 335, JS 95-60, respectively and lanes 3,5, 7 and 9 in PI 542044.

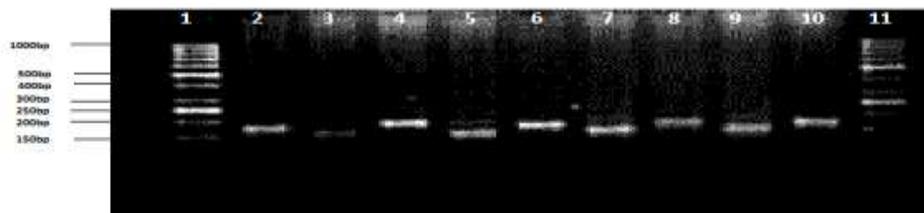


Fig 3. Assessment of polymorphism of soybean varieties and PI 542044 (donor of null allele of kunitz trypsin inhibitor) using Satt409. Lanes 1 and 11 show the 50 bp ladder. Lane 2, 4, 6, 8 and 10 depict the DNA finger prints generated in varieties NRC 7, JS 97-52, JS 93-05, JS 335, JS 95-60, respectively while lanes 3, 5, 7 and 9 all depict the PCR products generated in PI 542044.

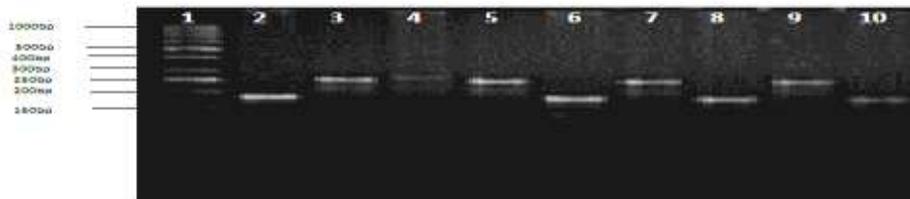


Fig 4. Assessment of polymorphism of soybean varieties and PI596540 (donor of null allele of lipoxygenase-2) using Sat_074. Lane 1 shows the 50 bp ladder. Lanes 2, 4, 6, 8 and 10 depict the finger prints generated in varieties NRC 7, JS 97-52, JS 93-05, JS 335, JS 95-60, respectively while lanes 3, 5, 7 and 9 in PI 596540.

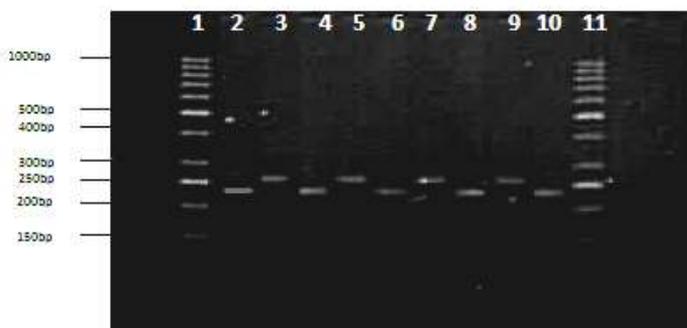


Fig 5. Assessment of polymorphism of soybean varieties and PI596540 (donor of null allele of lipoxygenase-2) using Satt522. Lanes 1 and 11 depict the 50 bp ladder. Lanes 2, 4, 6, 8 and 10 show the DNA finger prints generated in varieties NRC 7, JS 97-52, JS 93-05, JS 335 and JS 95-60, respectively while lanes 3, 5, 7 and 9 in PI 596540.

Figure 4 and 5 depict the resolution of PCR products amplified using *Lx2* linked SSRs Sat_074 and Satt522, respectively. Sat_074 amplified PCR products of size 250 bp for PI596540, 160 bp for 3 varieties (JS 93-05, JS 335, JS 95-60) and 190 bp for NRC 7, thereby showing very good resolution between the donor and 5 recipient popular soybean varieties. However, the resolution of the PCR products formed in case of JS 97-52 and PI 596540 was poor, though the parents were polymorphic. Thus, Sat_074 exhibited 4 allelic forms among the 6 genotypes surveyed. Satt522, the second SSR reported to be linked with *Lx2* locus, showed polymorphism between all the 5 varieties and PI596540. Though, only two types of PCR products were generated, with size 250 bp in PI596540 and 230 bp in all the five varieties.

The polymorphism information content of all the 4 SSR markers used in the investigation ranged from 0.27 for Satt522 to 0.72 for Satt409 (Table 2). The lower PIC value for Satt409 is because of the lesser number of the alleles (2) compared to the other 3 SSR markers. An entirely different size PCR product generated for PI542044

Table 2. Polymorphism information content of SSR markers used for parental polymorphism survey in 6 genotypes

SSR	Number of alleles	PIC
Satt228	3	0.62
Satt409	4	0.72
Sat_074	4	0.66
Satt522	2	0.27

compared to popular soybean varieties by all the 4 SSR markers indicates the genetically different background of the exotic accession from all the 5 Indian soybean varieties. In brief, for introgression of null allele of kunitz trypsin inhibitor through Marker Assisted Backcrossing from PI542044 in 4 popular varieties *viz.* NRC7, JS97-52, JS335, JS93-05, JS95-60 undertaken in the study, both the two reported linked SSR markers Satt228 and Satt409 can be used. For JS335, only Satt409 can be employed for selection of target plants. A very good polymorphism observed using SSR markers Sat_074 and Satt522 in study confirmed that both these reported linked SSRs can be employed for the development of lipoxygenase-2 free soybean genotypes in the genetic background of 5 popular soybean varieties.

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Genetic Studies in Black-seeded Soybean from NW Himalayan Regions of Uttarakhand

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ABSTRACT

Estimates of variability, heritability and genetic advance for yield and its components in Bhat (black-seeded soybean) germplasm explored from NW Himalayan regions of Uttarakhand revealed maximum phenotypic coefficient of variation for seed yield (61.82), followed by pods per plant (48.48) and 100-seed weight (37.47). Heritability estimates of the characters ranged from 0.60 for number of primary branches to 0.94 for 100-seed weight. Expected genetic advance as per cent of mean, was high for seed yield per plant (122.31), pods per plant (92.06) and 100-seed weight (74.90). Significant positive association of days to 50 per cent flowering (0.448), pods per plant (0.802), pod length (0.356) and 100-seed weight (0.597) with grain yield was observed. Days to 50 per cent flowering, pods per plant and 100-seed weight were the major characters influencing seed yield directly and indirectly. The overall results suggested that pods per plant and 100-seed weight may be taken as the most reliable and effective selection criteria for genetic improvement of black-seeded soybean. The existence of wide variation for the traits of agronomic importance, seed yield per plant and pods per plant in particular, in the local germplasm suggested scope for exploitation of naturally existing variation for genetic enhancement of the crop.

Key words: Bhat, black-seeded soybean, correlation, genetic advance, *Glycine max*, heritability, path analysis

Soybean is traditionally grown on a small scale in Himachal Pradesh, the Kumaon Hills of Uttar Pradesh (now Uttarakhand), eastern Bengal, the Khasi Hills, Manipur, the Naga Hills, and parts of central India covering Madhya Pradesh. The bean is referred to locally as *bhat*, *bhatman*, *bhatmas*, *ramkulthi*, *garakalay*, and *kalitur* (Singh, 2006). Black-seeded soybean (*Glycine max* (L.) Merr.), vernacularly known as *bhat*, is an important *kharif* pulse crop of Uttarakhand hills. The use of *bhat*

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as a pulse crop differs from that of the regular cream-seeded soybean, which is cultivated as an oilseed crop. The protein- and oil-rich grains are used whole or after grinding coarsely for preparation of a variety of traditional dishes. The grains and the protein-rich crop residue make nutritious feed for the cattle. A recent study by Suneja *et al.* (2010) on a set of black- and creamish yellow-seeded soybean genotypes has revealed that, in general, black seeds were more in protein and less in oil content in comparison to creamish yellow colored seeds. In the state of Uttarakhand, *bhat* is grown over an area of 5, 428 ha with a total production and productivity of 5, 021 t and 925 kg per ha, respectively, and is the third most important *khariif* pulse after blackgram and horsegram (Anonymous, 2010). Black-seeded soybean occupies an important place in agriculture in far-east Asian countries. In India, however, the crop is yet to receive due attention as a potential crop for the vast rain-fed areas of the country. The yield levels of *bhat* in the hills of Uttarakhand are considerably low as farmers continue to grow traditional cultivars and improved varieties to replace them are yet to arrive, despite existence of ample genetic diversity for yield in the local germplasm (Khulbe *et al.*, 2010). Besides, the information on various genetic aspects of the crop is meagre. A prior knowledge of the nature and magnitude of genetic variability, heritability, expected genetic advance, the gene action conditioning a trait and the relationship between yield and its components is of paramount importance in the selection of appropriate

breeding methodology aimed at bringing about genetic enhancement in a crop. The present study was undertaken to generate information on various genetic parameters in *bhat*, which will serve as a lead for workers interested in initiating crop improvement programmes in black-seeded soybean.

MATERIAL AND METHODS

Forty-seven accessions collected from various parts of Uttarakhand and three checks (PS 1092, VLS 47 and VLS 65) representing wide range of variability were chosen for the study. Of the 47 accessions, 35 were collected from various regions of Uttarakhand and 12 accessions were provided by NBPGR Regional Station, Bhowali, Nainital (Uttarakhand). The genotypes used in the study along with their source are given in table 1. The set comprising of 50 entries was evaluated in a completely randomized design with three replications during *khariif* 2007 at GBPUA&T, Agriculture Research Station, Majhera in district Nainital of Uttarakhand. The material was planted in 3 m long two-row plots spaced at 40 cm and plant to plant distance maintained at 10 cm. The trial was sown on 15th July, 2007 in organic mode (FYM @ 40t/ha) under rainfed conditions. Observations were recorded for nine traits on five randomly selected plants in each plot. The data were subjected to analysis of variance. Phenotypic coefficient of variation (PCV) and genetic coefficient of variation (GCV) were calculated following Burton (1952). Heritability (*h*²) and genetic advance were estimated according to Allard (1960). Path coefficient analysis was done using genotypic correlation coefficients following Dewey and Lu (1957).

Table 1. Black-seeded soybean genotypes used in the study and their source

Genotypes	Source
PCPGR 7857, PCPGR 7858, PCPGR 7860, PCPGR 7861, PCPGR 7862, PCPGR 7863, PCPGR 7864, PCPGR 7865, PCPGR 7866, PCPGR 7867, PCPGR 7868, PCPGR 7870, PCPGR 7871, PCPGR 7872, PCPGR 7873, PCPGR 7875, PCPGR 7876, PCPGR 7877, PCPGR 7878, PCPGR 7879, PCPGR 7880, PCPGR 7883, PCPGR 7884, PCPGR 7886, PCPGR 7888, PCPGR 7890, PCPGR 7891, PCPGR 7893, PCPGR 7894, PCPGR 7895, PCPGR 7897, PCPGR 7898, PCPGR 7899, PCPGR 7901, PCPGR 7902	Pantnagar Centre for Plant Genetic Resources, GBPUA&T, Pantnagar (Uttarakhand)
IC-419815, IC-419823, IC-419842, IC-419847, IC-436967, IC-444239, IC-444241, IC-444248, IC-469767, IC-469833, IC-524256 and IC-538042	NBPGR Regional Research Station, Bhowali, Nainital (Uttarkhand)
Checks : VLS 65 (black-seeded), VLS 47 and PS 1092 (yellow-seeded)	VPKAS, Almora and GBPUA&T, Pantnagar (Uttarakhand)

RESULTS AND DISCUSSION

Analysis of variance revealed wide variation among genotypes for all the nine characters studied (Table 2). High PCV and GCV values for seed yield per plant (61.82 and 64.36), and moderately high values plant height (27.36 and 28.55), primary branches (21.62 and 27.86) and 100-seed weight (37.47 and 38.63) suggested the possibility of improving these traits through selection (Table 3.). Low values of GCV and PCV for days to 50 per cent flowering (2.31 and 2.85), days to maturity (4.30 and 5.02), pod length (10.61 and 12.95) and seed per pod (8.54 and 11.79) suggest the need for augmentation of variability for these traits through hybridization among promising genotypes. The heritability estimates for the characters ranged from 0.53 for seeds per pod to 0.94 for 100-seed

weight. High heritability values for most of the characters indicated effectiveness of early generation selection for these traits. High heritability with high genetic advance with for seed yield per plant (0.92 and 122.31), 100-seed weight (0.94 and 74.90) and pods per plant (0.85 and 92.06) suggested major role of additive gene action in conditioning the traits. Moderate genetic advance for plant height (54.02) and primary branches (34.56) indicated role of both additive and non-additive gene action in the inheritance of these traits. Moderate heritability with low genetic advance for days to 50 per cent flowering (0.66 and 3.86), pod length (0.79 and 19.38) and seed per pod (0.53 and 12.75) suggested that selection would be less effective owing to greater influence of environment on the trait.

Table 2. Analysis of Variance for yield and yield contributing characters in black-seeded soybean genotypes

Source of variation	D F	Mean squares								
		Days to 50 % flowering (No)	Days to maturity (No)	Plant height (cm)	Primary branches (No)	Pods/Plant (No)	Pod length (cm)	Seeds / Pod (No)	100 - seed weight (g)	Yield/plant (g)
Replication	2	0.74	30.36	123.24	6.22	437.66	0.18	0.02	1.82	29.10
Treatment	49	4.82**	91.64**	2012.75*	4.88**	1392.94*	0.44**	0.16**	49.13**	166.12*
Error	98	0.71	9.83	57.86	0.88	85.66	0.03	0.04	1.00	9.69
C.D. at 5%		1.36	5.08	12.32	1.52	14.99	0.31	0.31	1.62	5.04
C.V. (%)		1.66	2.58	8.14	17.57	20.73	5.51	8.11	9.35	26.52

** Significant at $P = 0.01$ level

Table 3. Mean, GCV, PCV, heritability and genetic advance for various traits in black seeded soybean genotypes

Character	Mean	GCV	PCV	Heritability (bs)	Genetic advance as % of mean
Days to 50 % flowering	50.65	2.31	2.85	0.66	3.86
Days to maturity	121.46	4.30	5.02	0.73	7.59
Plant height	93.29	27.30	28.55	0.92	54.02
Primary branches	5.34	21.62	27.86	0.60	34.56
Pods/plant	45.44	48.48	52.58	0.85	92.06
Pod length	3.50	10.61	11.95	0.79	19.38
Seeds/pod	2.38	8.54	11.79	0.53	12.75
100-seed weight	10.69	37.47	38.63	0.94	74.90
Seed yield/plant	7.47	61.82	64.36	0.92	122.31

Character associations (Table 4) revealed significantly negative correlation of days to 50 per cent flowering with plant height (- 0.306) and significantly positive correlation with pods per plant (0.456), 100-seed weight (0.306) and seed

yield (0.448). Plant height was positively correlated with number of branches (0.314) but showed significant negative correlation with pod length (- 0.382) and 100 - seed weight (- 0.510). Pod length exhibited significant positive correlation

with seed per pod (0.444) and 100-seed weight (0.556) but significant negative correlation with plant height (- 0.382) and number of primary branches (- 0.456). Pods per plant showed highly significant and positive correlation with seed yield per plant (0.802). Seeds per pod (0.356) and 100-seed weight (0.597) were also significantly and positively correlated

with seed yield per plant. These results are in accordance with those reported by Singh and Singh (1996), Bhushan *et al.* (2006) and Kamal Pandey *et al.* (2008) in yellow-seeded soybean.

Path analysis revealed positive direct effect of all characters, except days to 50 per cent flowering and number of primary branches, on yield (Table 5). The

Table 4. Phenotypic correlation coefficients for various traits in black-seeded soybean

Character	DFF	DM	PH	BR	NP	PL	SP	SW	SY
DFF	1.000	-0.061	-0.306**	0.190	0.456**	-0.180	-0.062	0.306**	0.448**
DM		1.000	0.258	0.200	-0.035	-0.258	-0.070	-0.108	-0.039
PH			1.000	0.314**	-0.021	-0.382**	0.049	-0.510**	-0.231
BR				1.000	0.317**	-0.456**	-0.126	-0.289*	0.052
NP					1.000	-0.208	0.101	0.103	0.802**
PL						1.000	0.444**	0.556**	0.202
SP							1.000	0.202	0.356**
SW								1.000	0.597**

DFF - Days to 50% flowering; DM - Days to maturity; PH - Plant height; BR- Primary branches; NP - Pods/plant; PL - Pod length; SP-Seeds/pod; SW- 100 seed weight; SY- Seed yield/plat; *Significant at P=0.05 level; ** P=0.01 Significant at level

direct positive effect of pods per plant was highest (0.815) followed by 100-seed weight (0.503). Days to 50 per cent flowering and primary branches both exhibited high positive indirect effect on seed yield mainly through pods per plant. Plant height exhibited negative indirect effect on seed yield through 100-seed weight. Pod length negatively influenced seed yield through number of pods and positively through 100-seed

weight. The low magnitude of effect of residual factor (0.014) on seed yield suggested that the characters studied influenced yield considerably. The overall results of the experiment suggested that pods per plant and 100-seed weight may be considered most reliable and effective selection criteria for improvement of yield in black-seeded soybean.

Table 5. Direct (diagonal) and indirect effects of different characters on seed yield in black-seeded soybean

Character	DFE	DM	PH	BR	NP	PL	SP	SW	SY
DFE	-0.056	0.014	-0.013	-0.012	0.455	-0.010	-0.001	0.204	0.580**
DM	-0.007	0.118	0.010	-0.012	-0.042	-0.037	-0.015	-0.075	-0.059
PH	0.021	0.033	0.036	-0.021	0.000	-0.042	0.005	-0.272	-0.240
BR	-0.014	0.029	0.015	-0.049	0.400	-0.061	-0.009	-0.187	0.124
NP	-0.031	-0.006	0.000	-0.024	0.815	-0.023	0.013	0.062	0.807**
PL	0.006	-0.046	-0.016	0.031	-0.194	0.096	0.040	0.313	0.230
SP	0.001	-0.020	0.002	0.005	0.121	0.044	0.088	0.145	0.386**
SW	-0.023	-0.018	-0.019	0.018	0.101	0.060	0.025	0.503	0.648**

DFE- Days to 50% flowering; DM - Days to maturity; PH - Plant height; BR- Primary branches; NP -Pods/plant; PL - Pod length; SP-Seeds/pod; SW- 100 seed weight; SY- Seed yield/plat; *Significant at P=0.05 level; ** P=0.01 Significant at level; Residual factor: 0.014

Black-seeded soybean, by virtue of being rich in both protein and oil, is a potential crop for meeting the dietary needs of large masses in the vast rainfed regions of the country. The existence of wide variation in the local germplasm for traits of agronomic importance offers substantial scope for genetic

enhancement of the crop by exploiting the naturally existing variation. The information generated in this study on various genetic parameters will be useful for workers engaged in crop improvement programmes on black-seeded soybean.

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Stability Analysis in Soybean [*Glycine max* (L.) Merrill]

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ABSTRACT

Stability analysis was carried out in eight soybean genotypes over four environments to identify phenotypically stable genotypes for grain yield and other plant characters. Pooled analysis of variance showed significance for number of branches per plant, number of pods per plant, 100 seed weight (g), days to maturity, number of seed per pod, seed yield (kg/plot), protein content (%) and oil content (%) indicating that the materials selected possessed significant variation for all the characters under the study. Significant mean squares due to environment are indicative of confirming that the nature of environments which influenced the expression of most of the traits selected for stability studies. Mean squares arising due to GxE interaction were significant for most of the traits except that of plant height (cm) and days to 50 per cent flowering. Considering the high mean yield along with unit regression coefficient and non-significant deviation from regression, soybean genotype BS 1 was found highly stable across environment.

Key word: Genotype – environment interaction, soybean, stability

In India, soybean has emerged as one of the major oilseed crops. It belongs to family Leguminosae and subfamily Papilionaceae. It is commercially grown in the states of Uttarakhand, Punjab, Himanchal Pradesh, Madhya Pradesh and Maharashtra. Yield is a complex quantitative character governed by large number of genes and is greatly influenced by genotype and agro-climatic conditions. Environment constitutes all the physical, chemical and biological conditions that surround

and influence the plant habitat. It is a fact that the phenotypic performance of a genotype is not necessarily the same under diverse environmental conditions and all the genotypes may not approach the same level of phenotypic expression under all environmental conditions. Some genotypes perform well in some environments but not well in others. For all these situations a phenomenon is responsible called, “genotype-environment interaction”. Stability on the

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other hand has been defined as the ability of genotypes to buffer under environmental fluctuations to maintain uniform development of the crop and crop yield. The capacity of a genotype to remain consistent over wide range of environmental condition is called stability. The stability of genotypes with respect to varying environments has always been a matter of great concern to plant breeders. A number of statistics have been proposed to measure the phenotypic stability of different genotypes over the fluctuating environments. In soybean breeding programme it is, therefore, important to screen and identify the phenotypically stable genotypes the phenotypically stable genotypes which could perform uniformly under different environmental conditions. In view of lack of information with respect to adaptability of newly developed soybean genotypes, the present investigation was carried out to determine interaction between genotype and environment for various economic traits and to identify stable genotype(s).

MATERIAL AND METHODS

The experimental materials for the present investigation comprised of eight soybean genotypes including two commercial checks (JS 335 as national check and BS 1 as local check). These genotypes were grown over four environments (four locations i.e., Birsa Agricultural University (BAU) Farm (E1), ZRS, Darisai (E2), ZRS, Dumka (E3) and ZRS, Chianki (E4) during 2007 in a randomized block design with three replications at each location. There were

eight rows of each genotype in a plot with a row spacing of 45 cm and 8 cm plant to plant. Recommended dose of fertilizers [20:60:40:: N:P₂O₅:K₂O kg/ha] was applied in all locations. Observations were recorded on five competitive plants for seven characters namely plant height (cm), number of branches per plant, number of pod per plant, 100 seed weight (g), number of seed per pod, protein content (%) and oil content (%). Days to 50 per cent flowering, days to maturity and seed yield (kg/plot) were recorded on plot basis.

The analysis of variance pooled over locations was carried out to detect the differences among the genotypes, environments and G×E interaction. The traits having non-significant G×E interaction were not analyzed for stability analysis. The stability parameters were estimated using Eberhart and Russell model (1966). They used two parameters for selection of stable genotype, (i) Linear regression or regression coefficient (bi) should be unit and (ii) Mean square deviation from the regression (S^2_{di}) should be equal to zero or non-significant. In addition to these parameters, high mean value (X_i) of a trait is also considered desirable for breeders. Mean provides a measure of comparing the different genotypes, while the coefficient of regression and deviation from regression provide an estimate of G×E interaction.

RESULTS AND DISCUSSION

The analysis of variances for the individual environment revealed significant differences for all the characters in all the four environments indicating existence of genetic difference among the soybean genotypes. Pooled analysis of

variance for different genotypes (Table 1) showed highly significant difference for all the characters studied. Significant mean square due to environment confirming random and variable nature of environments selected, which influenced the expression of most of the traits studied. Similar findings were also reported by Dev Jai *et al.* (2009). Mean square arising due to genotype x environment (G x E) interaction revealed that significant differential response to the changing environments except for the plant height (cm) and days to 50 per cent flowering. Similar results have been reported by Ramteke and Husain (2008). Hence, no further stability analysis was done for these two traits. The significant GxE interaction for 100 seed weight has also been reported earlier by Morais *et al.* (2001), Rao *et al.* (2002) and Mondal *et al.* (2005). The significant G X E interaction for grain yield, days to maturity, number of branches per plant, number of pods per plant, protein and oil content (%) have also been reported earlier by Sudaric *et al.* (2006), Deka and Talukdar (1997), Chandrakar *et al.* (1998), Alam *et al.* (1999), Singh *et al.* (2001), Alghamdi (2004), Ramana and Satyanaryana (2005), Mondal *et al.* (2005) and Rajanna *et al.* (2000).

Component analysis of environment + (genotype x environment) is partitioned in to linear and non-linear components. The mean squares due to environments (linear) were found significant for all the characters. Therefore, it is concluded that the environments were random and different variation could have arisen due to the

linear response of the regression. The significance of linear component of variance due to environment has also been reported by Mahto and Mahto (2007). The mean squares due to G x E (Linear) were significant for all the traits viz. number branches per plant, number of pods per plant, 100 seed weight (g), days to maturity, number of seed per pod, grain yield (kg/plot), protein content (%) and oil content (%) revealing that the behavior of the genotypes could be predicted over the environments more precisely and accurately as the G x E interaction was the outcome of the linear function of the environmental components. However, mean squares due to G x E (linear) were non-significant for plant height (cm), and days to 50 per cent flowering indicating possible absence of genetic differences among the genotypes for their regression on the environmental index making difficult the prediction for the performance of these traits. Similar results were also reported by Deshmukh *et al.* (2009), Ramteke and Husain (2008), and Alghamdi (2004).

The non-linear component (pooled deviation) arising due to heterogeneity measured as mean square due to pooled deviation was significant for number of pods per plant, 100 seed weight (g), days to maturity, number of seed per pod, grain yield (kg/plot), protein content (%) and oil content (%) revealing presence of non-linear response of the genotypes to the changing environments. The significance of pooled deviation for above characters confirmed contribution of non-linear component to total G x E interaction. The genotype differed with respect to stability of these traits making its prediction more

difficult. However, the magnitude of linear component i.e., environment (Linear) and genotype x environment (Linear) was many times higher than the non-linear component (pooled deviation) for number of pods per plant, 100 seed

weight (g), days to maturity, number of seed per pod, grain yield (kg/plot), protein content (%) and oil content (%) revealing that the prediction of stability could be reliable though it may get affected to some extent.

Table 1. Pooled analysis of variance for stability of different characters of soybean

Characters	Geno- type (G)	Environ- ment (E)	G x E	Environ- ment (linear)	G x E (linear)	Pooled deviation	Pooled error
DF	7	3	21	1	7	16	56
Plant height	92.94**	20.02	8.51	60.08*	8.11	7.62	4.84
Branch/ plant (No)	0.08**	0.13**	0.03**	0.41**	0.08**	0.02	0.01
Pod/ plant (No)	36.75**	963.29**	13.62**	2889.89**	11.52*	4.35**	1.65
Days to 50 % flowering	5.30**	18.28**	0.86	54.84**	0.56	0.88*	0.46
100 seed weight	6.03**	2.27**	1.29**	2.76**	1.76**	0.41**	0.12
Days to maturity	15.41**	21.23**	12.09**	42.78**	15.51**	3.82**	1.12
Seed/ pod (No)	2.83**	0.96**	0.54**	1.20**	0.83**	0.18**	0.02
Grain yield (kg/plot)	0.33**	1.45**	0.06**	4.35**	0.09**	0.02*	0.01
Protein content (%)	4.53**	2.06**	1.10*	6.18**	1.63**	0.38*	0.22
Oil content (%)	2.92**	0.07*	0.04*	0.23**	0.06**	0.02**	0.00

*Significant at 5%; **Significant at 1%

Table 2a. Stability parameters (regression coefficient bi and deviation from regression S²di)

Genotypes	Number of branches per plant			Number of pods per plant			100 seed weight			Days to maturity		
	Mean	bi	S ² di	Mean	bi	S ² di	Mean	Bi	S ² di	Mean	bi	S ² di
BAUS-96	2.81	0.84	-0.00	39.75	0.83*	4.54	12.88	-0.20	-0.06	107.81	0.99	12.8**
BS-1	2.96	1.02	0.02	41.50	1.06*	3.00	13.05	0.97	0.32*	108.83	0.89	3.80
BAUS-31	2.93	0.75	0.07**	38.17	1.09	0.25	12.74	1.47	0.41*	108.31	0.18	-1.50
BAUS-40	2.71	1.04	0.00	32.83	0.98	2.52	13.96	2.52*	0.22	107.80	1.09	-0.70
JS-97-52	2.93	1.51	-0.01	38.66	0.99	-0.03	9.79	-0.61	1.06**	112.80	1.93*	2.30
JS-80-21	2.65	0.69	0.06*	36.58	1.09	1.52	13.0	1.64	0.12	106.81	1.32	-1.80
RAUS-5	2.78	0.48	-0.01	33.91	0.84	1.95	12.99	5.39*	-0.11	107.60	1.04	-2.00
JS-335	3.10	1.64*	-0.00	40.00	1.08*	15.24**	13.08	-3.18	0.41*	107.50	0.53	0.00
Mean	2.86	-	-	37.67	-	-	12.70	-	-	108.4	-	-
SE(m)	0.11	-	-	1.45	-	-	0.37	-	-	1.10	-	-
Mean of bi	-	1.00	-	-	1.00	-	-	1.00	-	-	1.00	-
SE (bi)	-	0.84	-	-	0.13	-	-	0.32	-	-	0.80	-

Table 2b. Stability parameters (regression coefficient bi and deviation from regression S²di)

Genotypes	Number of seed per pod			Protein content (%)			Oil content (%)			Grain yield per plot (Kg.)		
	Mean	Bi	S ² di	Mean	bi	S ² di	Mean	Bi	S ² di	Mean	bi	S ² di
BAUS-96	2.58	1.19	0.26**	41.29	0.81	-0.75	18.67	1.42	-0.01	2.67	0.95*	0.01
BS-1	2.50	0.97	0.41**	41.32	0.62	-1.18	20.49	1.99*	-0.00	2.89	0.96*	-0.00
BAUS-31	2.16	0.86	-0.01	41.60	-0.19	-0.90	18.48	0.99	-0.00	2.20	1.00	-0.01
BAUS-40	2.16	1.29	0.03	42.00	0.97*	-0.94	18.24	0.95	0.01	2.60	0.99	-0.01
JS-97-52	2.50	2.59	0.04	40.56	2.21	0.37	18.54	2.11	-0.01	2.27	1.02	0.00
JS-80-21	2.75	1.40*	0.00	40.98	0.03	-1.21	17.60	-0.46	-0.01	2.16	1.08	0.08**
RAUS-5	2.58	-1.29	0.24**	41.18	1.51	-1.21	19.38	1.75*	-0.02	2.86	0.95*	0.03*
JS-335	2.75	0.97	0.29**	41.10	2.01	-1.20	18.71	-0.80	-0.01	2.47	1.00	-0.01
Mean	2.50	-	-	41.25	-	-	18.76	-	-	2.51	-	-
SE(m)	0.24	-	-	0.35	-	-	0.07	-	-	0.08	-	-
Mean of bi	-	1.00	-	-	1.00	-	-	1.00	-	-	1.00	-
SE (bi)	-	1.19	-	-	0.70	-	-	0.74	-	-	0.20	-

In the present study (Table 2a and b) the genotypes showing average stability ($bi=1$) and high to moderate mean performance than the population mean was JS 80-21 for number of branches per plant; BS 1, BAUS 31, JS 97-52 and JS 80-21 for number of pods per plant; BAUS 96, BAUS 40 and JS 97-52 for 100 seed weight; JS 80-21 for less number of days to maturity; JS 97-52 and JS 80-21 for number of seed per pod; BAUS 96, BS 1, BAUS 40 and RAUS 5 for protein content (%); BAUS 96, BS 1, BAUS 40 and JS 335 for grain yield per plant (kg/ha). Genotype showing below average stability (bi significant and >1) with average to high mean than the population mean and specially adapted to favourable environments was RAUS 5 for 100 seed weight (g); JS 97-52 for days to maturity and number of seed per pod; JS 335 for protein content (%); BS 1 for oil content (%). Genotypes showing above average stability (bi significant < 1) under poor environment with average or higher mean than the population mean and specially adapted to poor environment were BAUS 96 for number of pods per plant; BAUS 31 and JS 97-52 for protein content (%); JS 335 for oil content (%).

From the table 3, it is clear that no variety was stable for all the seven characters under study, however, the genotype BAUS 96 exhibited stable performance with respect to number of pods per plant and economic trait like grain yield. The other genotype BS 1 expressed stable performance for number of pods per plant, grain yield and oil content (%), while the genotype BAUS 40 showed stable performance for 100 seed

weight and protein content. The genotype JS 80-21 revealed stable nature for days to maturity and number of seeds per pod, while RAUS 5 exhibited stable performance for grain yield and oil content (%). The genotype JS 335 showed stable performance for number of branches per plant, 100 seed weight and number of seeds per pod. Among the eight genotypes BS -1 proved to be the most stable genotype for majority of the characters including grain yield (kg/plot).

The compensating mechanism of component characters in imparting homeostasis being important, these genotypes would be useful in future breeding programme, the component characters may shift in a compensatory manner in changing environment to give consistent performance of the economic characters. The adaptability and performance of the genotype depends upon the nature of environment influencing the expression of the traits. In the present experiment it was found that Kanke (E1) is average, Darisai (E2) is poor and Dumka (E3), Chianki (E4) is favourable environment for the expression of different quantitative characters.

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Table 3. Stable and non-stable genotypes of soybean for different traits

Characters	Genotypes		
	Stable with higher mean	Stable with lower mean	Non- stable
Branches/plant (No)	BS 1, JS 97-52, JS 335	BAUS 96, BAUS 40, RAUS 5	BAUS 31, JS 80-21
Pods/ plant (No)	BAUS 96, BS 1, BAUS 31, JS 97-52	BAUS 40, JS S-80-21, RAUS 5	JS 335
100 seed weight	BAUS 96, BAUS 40, JS 80-21, RAUS 5		BS 1, BAUS 31, JS 92-52, JS 335
Days to maturity	BS 1, JS 97-52	BAUS 31, BAUS 40,, JS 80-21, RAUS 5, JS 335	BAUS 96
Seed/pod (No)	JS 97-52, JS 80-21	BAUS 31, BAUS 40	BAUS 96, BS 1, RAUS 5, JS 335
Protein content (%)	BAUS 96, BS 1, BAUS 31, BAUS 40	JS 97-52, JS 80-21, RAUS 5, JS 335	
Oil content (%)	BS 1, JS 335	BAUS 96, BAUS 31, BAUS 40, JS 97-52, JS 80-21, RAUS 5	
Grain yield (kg/plot)	BAUS 96, BS 1, BAUS 40	BAUS 31, JS 97-52, JS 335	JS 80-21, RAUS 5

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Effect of Vermicompost in Combination with Fertilizers on Nodulation, Growth and Yield of Soybean (*Glycine max*) in Soybean-Wheat Cropping System

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ABSTRACT

A field experiment was conducted during 2007-08 and 2008-09 to evaluate the response of soybean in soybean-wheat cropping system to vermicompost and NPK fertility levels under Malwa plateau. A conjunctive use of vermicompost at 5 t per ha along with 15:45:15 kg per ha N, P₂O₅ and K₂O in soybean followed by an application of 90 kg N, 45 kg P₂O₅ and 30 kg K₂O per ha in succeeding wheat crop recorded significantly higher plant population, dry matter accumulation and root nodulation, thereby found to be more profitable and productive over recommended doses of fertilizers and control. The nutrient uptake was also higher under this treatment. The treatment gave 9.12 per cent higher production and 8.22 per cent higher net monetary returns as compared to the 100 per cent recommended dose of fertilizers without vermicompost.

Key words: NPK uptake, nodulation, plant population, soybean, wheat, vermicompost

Soybean is an important oil and protein yielding rainy season crop. It covers the largest area of 9.67 million ha among all the oilseeds in India (Shrivastava, 2010). Soybean-wheat is a predominant and more remunerative system as compared to other cropping systems in Malwa plateau. Soybean occupied 5.12 million ha under soybean and 3.79 million ha under wheat in 2008-09 (http://eands.dacnet.nic.in/latest_2006.htm) in Madhya

Pradesh and in most of the area wheat followed soybean. In spite of significant contribution of both the crops in total production, the productivity of both the crops is much below the potentials under real farm situations. Sub-optimal and skewed nutrition in practice in soybean (Joshi, 2004) is considered to be one of the limiting factors in productivity from soybean-wheat cropping system. Nutrient management plays a key role in

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augmenting the productivity of crops in the system. Integrating chemical fertilizers with organic manures has been found to be quite promising not only in maintaining higher productivity but also in improving greater stability to the crop production (Nambiar and Abrol, 1992). The farmyard manure (FYM) is traditionally used as organic manure, but its limited availability and suboptimal quality are important constraints with its use as a source of nutrients. Vermicompost has been advocated as good organic manure for use in integrated nutrient management practices in field crops (Shroff and Devasthali, 1992). It is being an excellent source of supplying multiple nutrients, can effectively be used employing integrated approach and thereby reducing the dependence on fertilizers. Keeping this in view, a study was carried out to visualize the effects of integration of chemical fertilizers with vermicompost on soybean in soybean-wheat cropping system. Present paper deals with the results pertaining to the nutrient management practices, which constituted a part of a large experiment conducted to assess the effects of different land configuration, mulching and nutrient management practices in soybean-wheat cropping system.

MATERIAL AND METHODS

A field experiment was conducted during *kharif* and *rabi* seasons of 2007-08 and 2008-09 at instructional farm of *Krishi Vigyan Kendra*, Kasturbagram, Indore. The experiment consisted of 4 levels of land configurations with mulching and 7

levels of nutrient management practices, which were provided in soybean and succeeding wheat crop and replicated thrice in strip plot design. The nutrient management practices comprised (i) no fertilizers and manure application (control) to soybean and wheat, (ii) vermicompost @ 5 t per ha in soybean followed by no fertilizers and manure to wheat, (iii) 100 per cent recommended dose of fertilizers (RDF) to soybean followed by 100 per cent RDF to wheat, (iv) vermicompost @ 5 t per ha + 100 per cent RDF to soybean followed by 50 per cent RDF to wheat, (v) vermicompost @ 5 t per ha + 75 per cent RDF to soybean followed by 50 per cent RDF to wheat, (vi) vermicompost @ 5 t per ha + 75 per cent RDF to soybean followed by 75 per cent RDF in wheat, and (vii) vermicompost @ 5 t per ha + 50 per cent RDF to soybean followed by 75 per cent RDF to wheat. The soil of experimental field was clay in texture with 0.54 per cent organic carbon and 0.52 dSm⁻¹ EC. It was low in available N (231 kg/ha), medium in available P (13 kg/ha) and high in available K (505 kg/ha). The seasonal rainfall recorded during the cropping period was 819.6 mm (35 rainy days) and 510 mm (31 rainy days), respectively during 2007 and 2008. All the other operations were carried out as per the recommendations. The crop was sown on 02 July 2007 and 27 June 2008 during the experimentation. The recommended dose of nutrients for soybean (20: 60: 20 kg N: P₂O₅: K₂O/ha) through urea, single super phosphate and muriate of potash was applied as basal. The recommended dose of nutrients for wheat (120: 60: 40 kg N:P₂O₅: K₂O/ha), was also applied using the same nutrient carriers. Full dose of phosphorus and potassium

along with one third dose of nitrogen were applied as basal and the remaining dose of nitrogen was applied in two equal splits at the time of first and second irrigation to wheat. Vermicompost was applied before the sowing of soybean as per the treatment. Vermicompost was prepared by using *Eisenia foetida* earthworms and contained 1.51 per cent N, 1.12 per cent P and 0.86 per cent K. The data on plant population, various growth/yield attributes and yields were recorded in different treatments and analyzed statistically (Panse and Sukhatme, 1978). The economics of different treatments was also worked out and analyzed statistically. The cost of nutrients applied in both the crops of the system was included in the cost of cultivation. The data of both the years were pooled after subjecting them to homogeneity test.

RESULTS AND DISCUSSION

Effect on plant population

No significant difference was observed in plant population due to different nutrient management treatments at 20 DAS. However, the plant population at harvest of soybean was significantly affected during both the years (Table 1). Significantly highest plant population (47.5 plants/ m²) was recorded under vermicompost @ 5 t per ha + 75 per cent RDF to soybean followed by 75 per cent or 50 per cent RDF to wheat which were statistically at par with vermicompost @ 5 t per ha + 100 per cent RDF to soybean followed by 50 per cent RDF to wheat, vermicompost @ 5 t per ha + 50 per cent RDF in soybean followed by

75 per cent RDF to wheat and 100 per cent RDF in cropping system. Lowest plant population was recorded in control and all the values were found at par except control. It appears that the use of vermicompost improved soil physico-chemical and biological properties (Das and Dkhar, 2011) leading to better plant stand.

Effect on dry matter accumulation

Dry matter accumulation increased gradually with advancement of crop age and the rate of increase in dry matter was recorded maximum between 45 and 60 DAS in almost all the treatments. This was altered significantly due to different nutrient management practices and was recorded maximum with vermicompost @ 5 t per ha + 75 per cent RDF to cropping system, which was superior to remaining treatments except vermicompost @ 5 t per ha + 75 per cent RDF to soybean followed by 50 per cent RDF to wheat and vermicompost @ 5 t per ha + 100 per cent RDF to soybean followed by 50 per cent RDF to wheat. Agrawal *et al.* (2003) also recorded significant increases in biomass production in wheat due to the application of vermicompost.

Effect on root nodulation

In general, nodule number per plant and their dry weight increased by applying different nutrient management treatments at 45 and 60 DAS (Table 2). Maximum values of both the parameters were recorded in vermicompost @ 5 t per ha + 75 per cent RDF to cropping system at 45 as well as 60 DAS. The effect of treatments was more conspicuous in case of dry weight of nodules. The improvement in

Table 1. Effect of nutrient management practices on plant population, dry matter accumulation, and root nodulation of soybean

Treatments	Plant population (No/m ²)		Dry matter accumulation (g/plant)				Nodules (No/ plant)		Nodule dry weight (g/plant)	
	20	At	30	45	60	At	45	60	45	60
	DAS	harvest	DAS	DAS	DAS	harvest	DAS	DAS	DAS	DAS
Control	51.1	44.4	3.1	9.2	21.0	24.9	14.3	26.8	210	378
Vermicompost @ 5 t/ha to soybean followed by in wheat	51.6	45.5	3.2	9.7	22.0	27.3	14.7	27.4	219	386
100 % RDF to soybean followed by wheat	51.0	46.5	3.2	9.8	22.7	27.3	14.9	27.6	221	389
Vermicompost @ 5 t/ha + 100 % RDF to soybean and 50 % RDF to wheat	50.4	47.0	3.3	10.8	24.6	29.75	16.5	28.9	226	406
Vermicompost @ 5 t/ha + 75 % RDF to soybean and 50 % RDF to wheat	50.6	47.5	3.3	10.7	24.3	28.8	15.6	28.5	225	401
Vermicompost @ 5 t/ha + 75 % RDF to soybean and 75 % RDF to wheat	51.1	47.5	3.3	11.0	24.9	30.2	16.6	29.1	232	408
Vermicompost @ 5 t/ha + 50 % RDF to soybean and 75 % RDF to wheat	51.1	47.0	3.2	9.9	22.9	27.6	15.3	28.0	221	395
SEm (±)	0.35	0.32	0.04	0.21	0.44	0.62	0.34	0.24	2.4	3.5
CD (P=0.05)	NS	1.00	NS	0.64	1.36	1.9	1.04	0.75	7.3	10.9

nodule number and dry weight by different treatments might be the result of improved soil physico-chemical properties of the soil (Das and Dkhar, 2011). Lowest number of root nodules and dry weight of nodules per plant were under control.

Effect on yield and economics

The seed and stover yields and net returns of soybean were significantly influenced by different nutrient management practices but no significant difference was observed on harvest index and B: C ratio (Table 2). The treatment with the application of vermicompost @ 5 t per ha + 75 per cent RDF in soybean and 75 per cent RDF in wheat gave highest seed as well as stover yield of soybean. Since the process of vermicomposting increases microbial diversity and activity dramatically, it is possible that vermicomposts could be a definitive source of plant growth regulators produced by interactions between microorganisms and earthworms, which could contribute significantly to

enhancement of plant growth and yields. Mandal *et al.* (2000) also reported similar findings with organic manure. Similar findings were also reported by Sabale (2005). The economic evaluation of soybean cultivation indicated that the net monetary returns per hectare for different nutrient management ranged between ₹ 19, 602 under control to ₹ 25, 479 under the use of vermicompost @ 5 t per ha + 75 per cent % RDF to soybean followed by 75 per cent RDF to wheat and statistically similar to 100 per cent RDF in the cropping system, vermicompost @ 5 t per ha + 100 per cent RDF to soybean followed by 50 per cent RDF to wheat and vermicompost @ 5 t per ha + 75 per cent RDF to soybean followed by 50 % RDF to wheat. This shows that the use of vermicompost resulted into higher net returns. Benefit: cost ratio did not show any significant differences due to different nutrient management practices. The findings are in close agreement with the findings of Ranwa and Singh (1999).

Table 2. Effect of nutrient management practices (N) on seed and stover yields of soybean and harvest index

	Seed yield (kg/ha)	Stover yield (kg/ha)	Harvest index (%)	Net return (₹/ha)	benefit: cost ratio
Control	2041.4	2234.7	47.97	19602	1.93
Vermicompost @ 5 t/ha to soybean followed by control in wheat	2243.2	2499.3	47.45	21074	1.89
100 % RDF in cropping system	2323.5	2617.0	47.17	23542	2.03
Vermicompost @ 5 t/ha + 100 % RDF to soybean and 50 % RDF in wheat	2486.3	2771.0	47.23	23989	1.94
Vermicompost @ 5 t/ha + 75 % RDF to soybean and 50 % RDF to wheat	2459.6	2700.4	47.72	23871	1.95
Vermicompost @ 5 t/ha + 75 % RDF to soybean and 75 % RDF to wheat	2535.6	2793.2	47.42	25479	2.02
Vermicompost @ 5 t/ha + 50 % RDF to soybean and 75 % RDF to wheat	2348.2	2677.0	47.01	22271	1.90
SEm (±)	42.06	51.30	0.77	764.8	0.03
CD (P=0.05)	129.60	158.07	NS	2357	NS

Effect on uptake of major nutrients

Different nutrient management practices invariably enhanced the uptake of N, P and K by soybean plant but amount varied in proportion to the nutrient input in different treatments. The highest uptake of NPK was recorded under vermicompost @ 5 t per ha to soybean + 75 per cent RDF to soybean-wheat cropping system and statistically significant to RDF application to cropping system but did not differ to the

treatment with application of vermicompost @ 5 t per ha + 100 per cent RDF to soybean followed by 50 per cent RDF to wheat and vermicompost @ 5 t per ha + 75 per cent RDF to soybean followed by 50 per cent RDF to wheat. Dikshit and Khatik (2002) found similar results on NPK uptake by soybean with organic manure and 50 per cent reduced NPK doses (Table 3).

Table 3. Year wise and pooled data on total NPK uptake by soybean under different nutrient management practices

Treatments	Total N uptake (kg/ha)	Total P uptake (kg/ha)	Total K uptake (kg/ha)
Control	175.3	12.6	56.9
Vermicompost @ 5 t/ha to soybean followed by control in wheat	193.3	13.5	66.1
100 % RDF in cropping system	200.4	14.7	67.4
Vermicompost @ 5 t/ha + 100 % RDF to soybean and 50 % RDF in wheat	214.3	15.6	73.0
Vermicompost @ 5 t/ha + 75 % RDF to soybean and 50 % RDF to wheat	211.0	16.1	71.3
Vermicompost @ 5 t/ha + 75 % RDF to soybean and 75 % RDF to wheat	218.3	17.1	75.1
Vermicompost @ 5 t/ha + 50 % RDF to soybean and 75 % RDF to wheat	203.2	14.9	69.5
SEm (±)	2.84	0.51	1.31
CD (P=0.05)	8.74	1.56	4.03

The results reported in the investigation clearly establish that the integration of vermicompost along with RDF or with curtailed fertilizer schedule increases in productivity of soybean significantly. The application of vermicompost @ 5 t per ha integrated with 75 per cent recommended dose of fertilizers to the soybean-wheat cropping systems led to highest yield and net returns and offers fertilizer economy. The other integrated nutrient management treatments with vermicompost @ 5 t per ha and fractional RDF as well offer this advantage over 100 per cent recommended dose of fertilizers with fertilizer economy to evaluated cropping

system. The choice lies with the farmers depending on his resourcefulness.

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Productivity Quality and Profitability of Soybean (*Glycine max* L.) as Influenced by Sulphur and Boron Nutrition

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ABSTRACT

A field experiment was carried out during kharif 2007 and 2008 on Vertisols of Kota region to find out the effect of different levels of sulphur (0, 10, 20, 30 and 40 kg/ha) and boron (0.0, 0.5, 1.0, 1.5 and 2.0 kg/ha) on productivity, quality and profitability of soybean. Soybean responded significantly to the application of sulphur (S) and boron (B). Among the S levels, 30 kg per ha increased the number of branches per plant, pods per plant and seeds per pod by 29, 23.9 and 11.6 per cent, respectively and produced 16.1 per cent higher seed yield (1588 kg/ha) over the control. In case of different levels of B an increase in growth i.e., number of branches per plant (3.8) and major yield components viz., pods per plant (36.8) and seeds per pods (3.1) were recorded along with higher seed (1534 kg/ha) and biological yield (3612 kg/ha) with the application of 1.0 kg B per ha as compared to control. Results showed that application of 30 kg S per ha and 1 kg B per ha found suitable for obtaining higher productivity and quality of soybean with higher profitability under south-eastern plain zone of Rajasthan.

Key words: Boron, soybean, sulphur, yield and yield attributes

Soybean [*Glycine max* (L.) Merrill] with its 40-42 per cent protein and 20-22 per cent oil has already established as one of the major oilseed crop in India. In spite of its high yield potential (4-4.5 t/ha), soybean productivity is much less in India (0.95 t/ha) than the world average of 2-3 t per ha in different soybean growing countries of the world (FAI, 2006). Inadequate fertilizer use and emergence of multiple-nutrient deficiencies due to

poor recycling of organic sources and imbalanced use of fertilizers are main causes for low productivity. Particularly micronutrients and sulphur (S) deficiency is extensively observed primarily due to high crop yield and therefore higher rate of S removal by oilseed crops and lesser use of S - containing fertilizers (Messick, 2003). S application has beneficial effect on soybean in improving growth parameters, yield and quality (Havlin *et al.*, 1999).

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Boron (B) is an essential element for soybean playing many important role in nodulation, flowering, pollen germination, fruiting, seed setting and synthesis of protein and oil (Malewar *et al.*, 2001). Thus, a suitable combination of major, secondary and micronutrients is, by and large, the most important single factor that affects the yield and quality of soybean. However, meager information is available on secondary and micronutrient with respect to fertilization for soybean. Keeping In view of above, a field experiment was conducted to study the effect of S and B nutrients on productivity, quality and profitability of soybean.

MATERIAL AND METHODS

A field experiment was conducted during *kharif* season of 2007 and 2008 on Vertisols of Agricultural Research Station, Kota. The experimental soil was clay loam in texture, neutral in soil reaction with (pH 7.5), medium in organic carbon (0.56 %), available N (320.0 kg/ha), P₂O₅ 23.0 kg/ha) and K₂O (275.0 kg/ha) and was also low in available S (9.5 kg/ha) and B (0.46 mg/kg soil). The treatments consisted of five levels of S (0, 10, 20, 30 and 40 kg/ha) and five levels of B (0.0, 0.5, 1.0, 1.5 and 2.0 kg/ha). Soybean 'JS 93-05' was grown during July to October at a row spacing of 30 cm with the recommended package of practices. The fertilizer N, P, K and Zn 20, 60, 20 and 5 kg/ha respectively were applied just before sowing. S and B were applied through gypsum (CaSO₄ 2H₂O) and Borax (Na₂B₄O₇.10H₂O), respectively before sowing and mixed thoroughly in 0

- 15 cm soil. The observation on growth, yield attributes and yield were recorded at harvest. The oil content in seeds was determined by 'Nuclear Magnetic Resonance' procedure at Nuclear Research Laboratory, IARI, New Delhi. Since data followed the homogeneity test, pooling was done over the season and mean data are discussed as under.

RESULTS AND DISCUSSION

Growth and yield attributes

Growth parameters i.e. plant height, dry matter production, number of branches per plant, number of nodules and nodule dry weight per plant differed significantly due to different treatments (Table 1). The maximum plant height (40.26 and 39.91 cm) was recorded with the application of 30 kg S per ha and 1.5 kg B per ha, respectively, but found statistically non-significant over the treatments. Application of 30 kg S per ha produced significantly higher dry matter production (15.88 g/plant) and branches per plant (4.00) than control, which was statistically at par with other levels of S application. Amongst the B levels, application of 1.0 kg B per ha produced significantly more dry matter (15.34 g/plant) and branches per plant (3.77) over lower levels but remained statistically on par with higher levels of 1.5 and 2.0 kg B per ha. Number of nodules per plant and nodule dry weight per plant in soybean was also influenced with graded levels of S and B application. The maximum number of nodules per plant (51.92 and 51.08) and nodules dry weight (88.72 and 87.31) were obtained with 40 kg S per ha and 2.0 kg B per ha, respectively

being conspicuously higher than control but remained at par with other levels of S and B application. The lowest values of all the growth parameters were noted with the control among the nutrient sources.

The number of pods per plant and seeds per pod was significantly higher in S and B fertilized soybean over control. Application of 30 kg S per ha produced higher number of pods per plant (38.67) and seeds per pod (3.18) and registered increases to the tune of 23.86 and 11.58 per cent, respectively over control however, found on par with 40 kg S per ha. Graded levels of B fertilization also caused significant difference the yield component in soybean over no fertilization.

Application of 2.0 kg B per ha, produced significantly higher number of

pods per plant but almost equal to that under 1.5 and 1.0 kg B per ha. Number of seeds pod was increased significantly due to application of B however, the differences due to levels were not much pronounced. Application of S might have increased the synthesis of proteins and chlorophyll along with the assimilation area thereby producing higher photosynthetic rate. The above findings are also in conformity with the results of Sarkar *et al.* (2002) who reported that the highest yield components were found when the soybean was fertilized with 30.0 kg S and 1.0 kg B per ha as compared to control. Havlin *et al.* (1999) reported that flowering and fruit development were restricted by a shortage of B, whereas the seed index of soybean was non-significantly influenced by the different levels of S and B.

Table 1. Effect of sulphur and boron levels on growth and yield attributes of soybean (Pooled data of 2 years)

Nutrient level (kg/ha)	Plant height at harvest (cm)	Dry matter (g/plant) at 60 DAS	Branches (No/ plant)	Nodule (No/ plant)	Nodule dry weight (mg/plant)	Pods (No/ plant)	Seeds (No/ pod)	Seed index (g/100 seeds)
<i>Sulphur</i>								
S ₀	39.43	13.68	3.10	49.71	84.97	31.22	2.85	9.99
S ₁₀	39.47	14.58	3.53	50.42	86.20	33.48	3.02	10.04
S ₂₀	39.82	15.28	3.76	50.66	86.61	36.67	3.05	10.09
S ₃₀	40.26	15.88	4.00	51.47	87.95	38.67	3.18	10.17
S ₄₀	39.62	16.10	4.07	51.92	88.72	39.17	3.22	10.22
CD (P=0.05)	NS	0.80	0.33	0.52	0.98	3.40	0.14	NS
<i>Boron</i>								
B _{0.0}	39.38	13.93	3.30	50.26	85.91	31.67	2.79	9.99
B _{0.5}	39.68	14.74	3.52	50.68	86.63	34.74	3.08	10.02
B _{1.0}	39.87	15.34	3.77	50.91	87.02	36.76	3.13	10.10
B _{1.5}	39.91	15.71	3.88	51.05	87.26	37.46	3.16	10.18
B _{2.0}	39.74	15.80	4.00	51.08	87.31	38.65	3.16	10.23
CD (P=0.05)	NS	0.80	0.33	0.52	0.98	3.40	0.14	NS

Application of S might have increased the synthesis of protein and chlorophyll along with the assimilation area thereby producing higher photosynthetic assimilation helped in increased growth and translocation of the net photosynthates to sink and thus increased the dry matter, yield attributes and ultimate yield. A significant increase in the yield attributes was observed due to application of B. Boron might have resulted in increased flowering, pollen germination, fertilization, cell division and improved plant water relationship. B fertilization also increased the pollen-producing capacity, anthesis and pollen grain viability (Havlin *et al.*, 1999).

Yields and quality

It is revealed from mean data that seed and biological yield of soybean were significantly influenced by different levels of S and B (Table 2). Among the levels of S, 30 kg S per ha produced significantly higher seed (1,588 kg/ha) and biological yield (3,732 kg/ha) over the control registering increase to the tune of 16.08 per cent and 15.58 per cent, respectively but found statistically at par with 40.0 kg S per ha. The high seed and biological yield of soybean with application might have resulted due to its favourable effect on the yield attributing characters and plant metabolism (Tiwari *et al.*, 1997). The results obtained are consistent with that of Sarkar *et al.* (2002).

Increasing levels of B application showed an increasing trend in seed and biological yield of soybean. Application of B @ 1.0 kg per ha produced 1,534 kg per ha seed and 3,612 kg per ha biological

yield that was found statistically on par with higher doses up to 2.0 kg per ha but significantly higher than control. The lowest seed and biological yield was produced from control (Table 2). This might be due to the favorable role in nodulation and seed formation processes. The results of study are consistent with that of Sarkar *et al.* (2002) and Ahmed *et al.* (1991). They also reported that seed and straw yield increased significantly with each incremental dose of S and B. Similarly, Panwar *et al.* (1998) reported that straw yield was less influenced by high levels of B. The interaction effect of S and B in relation to seed and straw yield was found non-significant. The results revealed that there was no significant effect of different levels of S and B fertilization on harvest index of soybean.

S and B nutrition significantly increased oil content and oil yield over control in soybean (Table 2). There has been an increasing trend with increase in levels of the two nutrients. Application of 30 kg S per ha (18.96 % and 302.4 kg/ha) remained on par with 40 kg S per ha in terms of oil content and oil yield. It is evident from the results that S had remarkable influence of protein, oil content and oil yield in soybean because S is required for the synthesis of fatty acids and S-containing amino acids, (Havlin *et al.*, 1999). B application also influenced the content and yield of oil over control in soybean. The result exhibits that B application significantly increases oil content over the control but the effects of graded levels of B application were found statically on par with each other. Application of 1.0 kg B per ha recorded

Table 2. Effect of S and boron levels on yield, quality and economics of soybean (Pooled data of 2 years)

Nutrient level (kg/ha)	Seed yield (kg/ha)	Bio-logical yield (kg/ha)	Harvest index (%)	Oil content (%)	Oil yield (kg/ha)	Cost of cultivation (₹/ha)	Net returns (₹/ha)	B:C ratio
<i>Sulphur</i>								
S ₀	1,368	3,229	42.23	18.20	249.3	13,063	7,073	1.88
S ₁₀	1,459	3,439	42.26	18.51	270.6	13,117	8,084	2.00
S ₂₀	1,528	3,596	42.33	18.74	286.9	13,171	8,828	2.08
S ₃₀	1,588	3,732	42.39	18.96	302.4	13,225	9,451	2.15
S ₄₀	1,610	3,784	42.39	19.05	307.9	13,279	9,827	2.18
CD (P=0.05)	74.4	165.7	NS	0.18	15.92	-	900	0.09
<i>Boron</i>								
B _{0.0}	1,393	3,289	42.19	18.55	259.2	12,851	7,335	1.91
B _{0.5}	1,474	3,472	42.32	18.64	275.6	13,016	8,215	2.02
B _{1.0}	1,534	3,612	42.33	18.72	288.2	13,181	8,883	2.09
B _{1.5}	1,571	3,694	42.38	18.77	295.9	13,346	9,291	2.12
B _{2.0}	1,581	3,715	42.38	18.79	298.0	13,461	9,530	2.14
CD (P=0.05)	74.4	165.7	NS	0.18	15.92	-	900	0.09

significantly higher oil yield (288.2 kg/ha) over 0.5 kg and control however, remained on par with higher doses up to 2.0 kg B per ha. Increase in oil content due to B might be attributed to the increased conversion of primary fatty acid metabolite to end products of fatty acid by increased activity of acetyl Co-A, resulting in higher oil content (Hemantarajan *et al.*, 2000). Since, the oil yield is mainly the function of seed yield and their respective oil content in the seed. Hence, the application of S and B increased oil yield with the respective increase in the oil content of seed.

Economics

Among different levels of S application, net returns and B: C ratio increased from no S application to 40 kg S per ha (Table 2). S application @ 30 kg per ha fetched significantly higher net return (₹ 9451/ha) and B: C ratio (2.15) which was 33.6 and 12.6 per cent higher over no S application, and was statistically on par with the 40 kg S per ha. Application of 1.0 kg B per ha recorded significantly higher net returns (₹ 8883/ha) and B: C ratio (2.09) and found on par with that obtained

under application of 1.5 kg and 2.0 kg B per ha.

Based on results discussed, it is concluded that application of 30 kg S and 1.0 kg B per ha individually or in combination along with the recommended dose of NPK and Zn was found optimum for higher productivity, better quality and profitability of soybean under South-Eastern plain zone of Rajasthan.

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Morphological, Growth and Yield Attributes Variations in Soybean Variety JS 335 as Influenced by Imazethapyr Herbicide

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ABSTRACT

Soybean varieties differ in their sensitivity to imazethapyr herbicide damage. In these experiments, we examined the impact of herbicide-imazethapyr spray on the growth and yield of two soybean varieties. Morphological changes resulted from herbicide application, including leaf elongation and formation of large shoots at the cotyledonary node in JS 335 only. Herbicide treatment significantly reduced the vegetative and reproductive growth and yield variables relative to the control (unsprayed with herbicide). The application of herbicide substantively reduced the weed load in soybean. Although herbicide application significantly impacted several growth variables, it had no significant impact on yield. This is the first record from India.

Keywords: *Glycine max*, herbicide, imazethapyr, nodes, plant height, seed weight, soybean variety JS 335

Soybean [*Glycine max* (L.) Merrill] is an important commercial crop of India and worldwide. The complete exploitation of soybean yield could be possible by timely management of pest complex. Among the different components of pest complex, weeds ranked first which caused maximum yield loss ranging from 35 to 70 per cent (Billore *et al.*, 2001). The use of herbicides in soybean crop is increasing day by day due to shortage and high wages of labour and incessant rains during crop season. Among the herbicides, imazethapyr, 2-

[4,5-dihydro-4-methyl-4-(1-ethylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid) is often used for the post-emergence control of weeds in legumes, especially soybeans. This compound belongs to the imidazolinone (imazethapyr) herbicide class, which causes phytotoxicity through the inhibition of acetohydroxy acid synthase and the synthesis of branched chain amino acids. Selectivity of these herbicides is based on the rate and/or extent of metabolism (detoxification) of the

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active ingredient by the plant (Brown, 1990; Shaner and Mallipudi, 1991). In the hours following imazethapyr application to soybeans, fresh weight of shoots and roots was increased, but dry weight was decreased, indicating higher water concentrations in imazethapyr-treated plants (Scarponi *et al.*, 1996). Enzyme activities and glucose and starch contents were also affected within hours after imazethapyr application (Scarponi *et al.*, 1996; Scarponi *et al.*, 1995). Imazethapyr has been shown to decrease protein and branched-chain amino acid contents of legumes (Scarponi *et al.*, 1997). Some studies have indicated no growth (Adcock and Banks, 1991) or yield response to soybean treatment with imazethapyr (Krausz *et al.*, 1992). Plants may exhibit different susceptibility to herbicide damage when under moisture or other stresses than when they are not stressed (Gerber *et al.*, 1983; Reynolds *et al.*, 1988). Multiple stressors affecting plant growth and metabolism have the potential to result in increased (or decreased) plant response. Limited information exists on the response of soybeans to herbicide treatment i.e. imazethapyr.

MATERIAL AND METHODS

Field experiments were conducted during rainy season of 2009 and 2010 at research farm of Directorate of Soybean Research, Indore. The treatments included two soybean variety namely, JS 335 and Ahilya 3 (NRC 7) and 6 herbicides viz., fluchloralin @ 1 kg a i per ha and trifluralin @ 1 kg a i per ha (both as PPI), Clomazone @ 1 kg a i per ha and

pendimethalin @ 1 kg a i per ha (both as PE) and imazethapyr @ 100 g a i per ha and quizalofop ethyl @ 50 g a i per ha (both as PoE) and were replicated thrice under randomized block design. Formulated herbicide i. e. ammonium salt of imazethapyr (Pursuit) is labeled for post-emergence weed control in soybeans. Imazethapyr was applied at 100 g a i per ha with ammonium sulphate @ 2 g per litre of water and cyspread (sticker, spreader and activator) @ 2 ml per litre water according to the label instructions. Seeds were sown on 4th July and 23rd June in 2009 and 2010, respectively and harvesting was done in 2nd week of October in both the years. A knapsack sprayer was used to apply 750 litres of herbicide solution per ha uniformly to the treated area, with much of the solution intercepted by the leaf surfaces. The post-emergence herbicides were applied at 18 days after sowing. Plant growth variables were measured 30 days after herbicide treatment. Plant height was measured from the soil surface to the main shoot apex. The number of nodes on the main stem was counted, including the cotyledonary node. Some plants exhibited significant growth from shoots emerging from the cotyledons, so the number of nodes on the cotyledonary shoots was also measured. Leaf area was measured separately for leaves on the primary shoot.

RESULTS AND DISCUSSION

The present experiment was conducted with 2 soybean varieties and six herbicides viz., two each in PPI, PE and PoE group. The plant reaction with reference to morphological and yield attributes were recorded only in soybean cultivar JS 335 and imazethapyr treatment because the changes were observed only in

this treatment. Morphological changes were observed in herbicide imazethapyr treated soybean JS 335. Since there were no morphological variations noted in NRC 7, hence the data of the variety was not presented.

Plant height (cm): Soybeans not treated with herbicide (controls) were 53.87 cm (28.35%) taller than plants treated with imazethapyr (Table 1). Herbicide treatment significantly reduced the height of the main stem of soybean cultivar JS 335 to the tune of 22.09 per cent. However, the variability in plant height was higher under untreated plants than imazethapyr treated plants.

Branches per plant: Untreated soybean produced higher number of branches per plant as compared to imazethapyr treated one which reduced branches to the extent of 16.09 per cent. The higher variability in

number of branches was recorded under imazethapyr treated plants (Table 1).

Nodes per plant: Application of imazethapyr significantly reduced the node number per plant (7.23%) along with high variability as compared to untreated plants (Table 1).

Length of first internode (cm): Untreated soybean plants first internode length was higher (11.83%) than herbicide treated plants which reduced by 10.58% and also showed lower variation in first intermodal length (Table 1).

Mean internodal length (cm): Herbicide application decline the internodal length by 22.19 per cent than untreated plants and untreated plants showed higher variability as compared to treated one (Table 1).

Table 1. Effect of imazethapyr (10% SL) on vegetative characters*

Plant character	Control (untreated)	SD	CV (%)	Imazethapyr @100 g a i / ha as post-emergence	SD	CV (%)	Change over control (%)
Plant height (cm)	53.87	6.158	18.61	41.97	3.695	15.76	-22.09
Branches/plant	3.48	1.833	44.63	2.92	1.986	52.67	-16.09
Number of nodes/plant	12.44	0.667	6.19	11.54	0.972	9.95	-7.23
Length of I internode (cm)	5.01	0.964	21.80	4.48	0.364	9.83	-10.58
Length of mean of internodes (cm)	3.11	0.638	20.51	2.42	0.372	15.37	-22.19

* Data pooled for two years

Leaf characters: No leaf necrosis was evident for herbicide treatment in experiment. Morphological changes were observed in herbicide treated plants (Table 2). Herbicide treated soybean significantly reduced the leaf number per plant (28.27 %) as compared to untreated plants. However, the higher variability was observed in untreated plants with reference to this character. Plants treated with imazethapyr demonstrated narrow elongated leaves uncharacteristic of soybeans var. JS 335 (Fig. 1). These elongated leaves developed after herbicide application, and these leaves were not yet formed when the herbicides were foliar-applied. On these plants, while the leaves formed prior to herbicide application were normal, nearly all leaves formed after herbicide application exhibited elongation. Leaf area measurements further indicated the morphological changes observed in herbicide-treated plants. The maximum length and width of normal leaves was also reduced by 11.17 per cent and 28.65 per cent with the herbicide treated plants which indicated that the leaf width was more sensitive than length and also indicated higher variation in leaf length. The length of changed leaf has been increased and the width of changed leaf decreased significantly due to herbicide as compared to normal leaf i.e. untreated plant which led to a significant decrease in photosynthetic surface area (total leaf area) by 43.88 per cent. The changes in

leaf morphology occurred on 2 to 3 nodes of treated plants (Table 2).

Plant dry biomass (g/plant): Untreated soybean produced higher plant dry biomass to the tune of 30.81 per cent as compared to herbicide treated soybean plant (Table 3).

Weed count and their dry matters: The application of imazethapyr @100g a.i. per ha significantly reduced the monocot and dicot weed count to the tune of 85.71 and 100 per cent, respectively as compared to weedy check. The similar trend was also observed in weed dry matters (Table 3).

Seed yield per plant (g): The morphological variations caused by imazethapyr resulted in 20.03% lower yield as compared to healthy plant (untreated plants). Since the few soybean plant showed morphological variations while, other plants did not. However, on the basis of plant yield there were no yield difference between imazethapyr treated and two hand weeding treatment (Table 3).

Seed index (g/100 seed) and seed yield per plant (g): Imazethapyr treated soybean plant showed a substantial reduction in seed index to the extent of 8.25 per cent as compared to untreated plants. Similarly, seed yield per plant was also showed a decrease trend which was to the tune of 20.03 per cent over untreated soybean (Table 3).

Table 2. Effect of imazethapyr (10 % SL) on soybean leaf characteristics*

Plant character	Control (un-treated)	SD	CV (%)	Imazetha-pyr @100 g ai/ha as post emergence	SD	CV (%)	Change over control (%)
Total leaves (No)	17.33	5.077	29.30	12.43 (7.66 + 4.77)	2.645, 1.856	21.28	-28.27
<i>Length (cm) and width (cm) of leaf</i>							
Maximum length - normal	7.43	0.670	9.02	6.60	1.434	21.73	-11.17
Maximum width (cm)- normal	5.41	0.485	8.96	3.86	1.156	29.95	-28.65
Maximum length - changed	-	-	-	8.26	1.162	14.07	100.0
Maximum width - changed	-	-	-	3.77	5.843	154.98	100.0
<i>Leaf area (cm²)</i>							
Normal	40.41	6.611	16.35	43.60	13.23	30.34	-
Changed	-	-	-	31.24	7.711	-	100.00
Total leaf area (cm ²)	1017.94	-	-	571.29	-	-	-43.88
<i>Number of leaves changed</i>							
Unchanged	17.33	5.077	29.29	7.66	2.645	289.60	-55.80
Changed	-	-	-	4.77	1.856	38.91	100.00
Position of leaf changed in size (Node)	-	-	-	2 to 3	-	-	-
Percentage change in leaf number	-	-	-	38.42	-	-	-

* Pooled data of two years

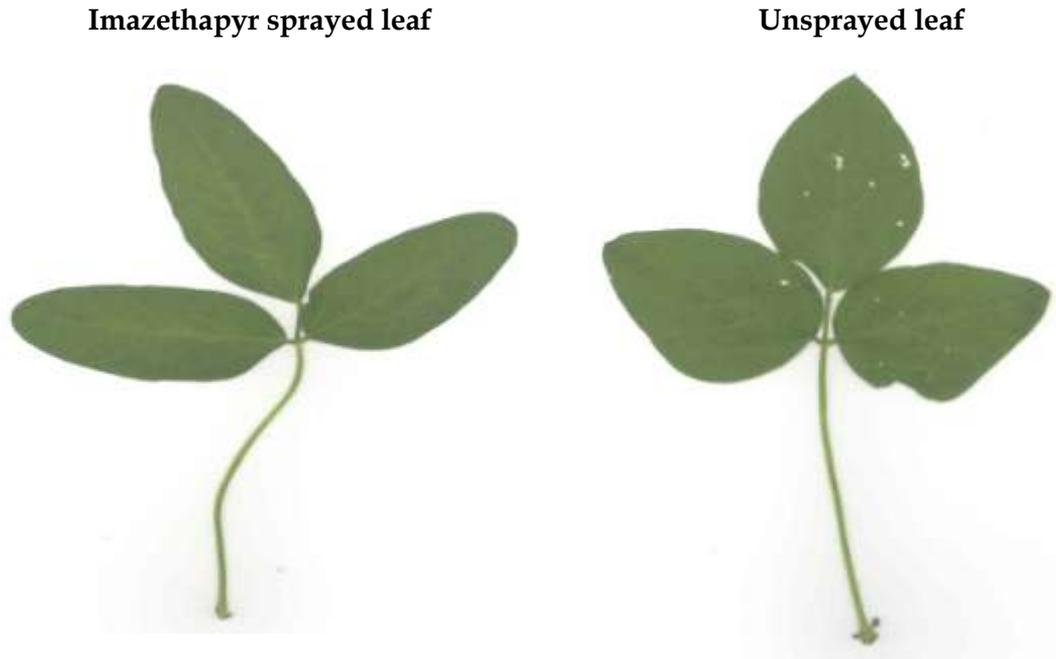


Fig.1. Morphological changes in soybean variety JS 335 leaf due to imazethapyr application (30 days after spraying)

Table 3. Effect of imazethapyr (10% SL) on soybean yield and weeds*

Plant character	Control (un-treated)	Imazethapyr @100 g ai/ha as post emergence	Change over control (%)
Plant dry weight (g/ plant)	4.67	3.57	-23.55
Seed yield/plant (g)	13.78	11.02	-20.03
Seed Index	8.73	8.01	-8.25
Weed count and their dry matter (30DAS)			
Monocot (nos.)	30.16	4.31	- 85.71
Monocot (dry weight-g/m ²)	13.75	2.15	-84.36
Dicot (nos.)	17.65	0.00	-100.00
Dicot (dry matter- g/m ²)	12.47	0.00	-100.00

* Pooled data of two years

Thus, the herbicide treatment significantly impacted several growth variables and yield per plant of surviving plants. Although a large number of plants exhibited morphological effects in which the main stem comprised only a small portion of the total plant biomass, yield was generally not impacted by these morphological changes on a large area basis. Other researchers have also reported no loss in yield with imazethapyr application (Krausz *et al.*, 1992; Newsom and Shaw, 1992), while others have reported yield reductions under certain conditions (Newsom and Shaw, 1992; Griffin and Habetz 1989; Stapleton and Whitwell, 1989). After screening of literature it has been found that this observation has not yet reported from India, hence it forms first record.

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Toxicity Symptoms of Plant Extracts on *Spodoptera litura* Fab. (Lepidoptera : Noctuidae) Larvae

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ABSTRACT

Several plant species have been reported to possess insecticidal properties. In order to ascertain exact cause of mortality and elucidate toxicity symptoms, histological studies were conducted with *Spodoptera litura* larvae died due to application of plant extracts viz., *Acacia arabica* (leaves and seeds), *Annona squamosa* (leaves and seeds), *Datura stramonium* (leaves and seeds), *Eucalyptus globulus* (leaves), *Ipomoea carnea* (leaves), *Lantana camara* (leaves), *Nicotiana tabacum* (leaves) and *Pongamia pinnata* (leaves), with emphasis on cuticular and midgut layers. The results revealed that plant extracts of *A. arabica*, *A. squamosa*, *D. stramonium*, *E. globulus* and *I. carnea* disrupted the cuticular layer of insect showing contact toxicity. The most characteristic effect observed in contact toxicity was black lesions on cuticle, presence of tumors between epidermis and cuticle, ruptured epidermis and formation of vacuoles. Extracts of *L. camara*, *N. tabacum*, *P. pinnata*, *A. arabica* seed, *A. squamosa* seed and *D. stramonium* seed were found damaging the midgut epithelial layer of alimentary canal exhibiting stomach toxicity. In this case, maximum damage was found in midgut microvilli and peritrophic membrane. The epithelial gut cells were found separated and vacuoles were formed. Interestingly, extracts of *A. arabica* leaf, *D. stramonium* leaf and *E. globulus* leaf exhibited both contact as well as stomach toxicity symptoms.

Key word: Plant extracts, *S. litura*, toxicity symptoms

Over 2400 plant species have been reported to possess anti-insect properties like insecticidal, antifeedant, repellent, oviposition deterrent, chemosterilants, insect growth regulatory, etc (Grainge and Ahmed, 1988; Banrejee, 1995; Raheza, 1998; Singh, 2000). Unlike conventional insecticides that are based on single active ingredient, plant derived insecticides comprise an array of chemical compounds which act concertedly on both behavioral and physiological processes. Several of

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such plant species viz., neem, marigold, custard apple, balsam fir, Ipomoea, Pongamia etc. have been widely worked upon to assess diverse types of effects on crop pests (Arivudainambi and Nachiappan 1993; Dhaliwal and Arora, 2001 a, b; Medhini *et al.* 2009). These studies were aimed to assess the effects of plant extracts on mortality, feeding, oviposition, growth, neuro-endocrine system, etc. Information with respect to effect on morphological and anatomical structures of insects is very meager and scanty. Since most of the plant extracts are reported to act as contact or stomach poisons, an attempt was made to elucidate the effect of some selected plant extracts on the cuticle and midgut portion of alimentary canal of *Spodoptera litura* larvae.

MATERIAL AND METHODS

Since the effect of plant extracts was to be studied on integument and on alimentary canal, more specifically on mid-gut, whole cadavers obtained from efficacy experiment were used for this study. For this, the larvae died due to different extracts were frozen immediately at -20 °C to stop any further histological changes before they were subjected to microtomy. The larvae were then cut transverse with sharp surgical scalpel between 4th and 7th abdominal segments so that mid-gut (stomach) portion is ensured in the section. This portion of cadaver was used to cut 40 micron sections through freeze

microtome (SLEE Technik, GMBH, Germany) using special medium called 'Optimum Cutting Temperature (OCT) Compound'. The sections were gently transferred on glass slide. Five sections could be easily accommodated in one slide. At least 20 sections were taken from each sample. All the sections were then stained with haematoxylin and eosin using following standard protocol (Lillie, 1965; Ausubel *et al.*, 1995):

Sections washed in tap water; stained with haematoxylin; rinsed in running tap water; de-stained with acid water; rinsed in running tap water; stained with eosin for 2 minutes; cleared with xylol and finally mounted in Canada balsam or D.P.X.

The sections so prepared were thoroughly examined under microscope (Olympus, Japan; model - CX-41RF) and suitable images were captured with the help of high resolution digital camera attached to the microscope (Olympus, Japan; model - CX-21).

RESULTS AND DISCUSSION

The purpose of this study was to examine the effect of plant extracts especially on integument and the midgut of 3rd instar *S. litura* larvae. In general, the dead larvae showed swelling and development of black lesions on thoracic and abdominal segments, expulsion of gut contents and rupture of integument (Figs. 1-1 to 1-3).

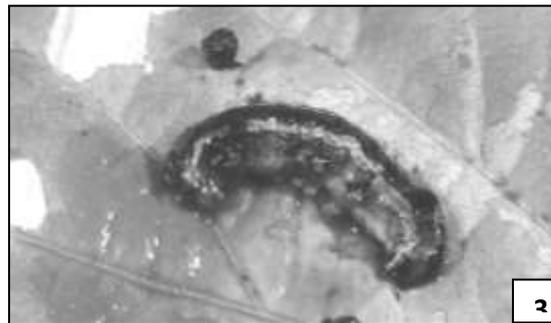


Fig. 1. 1. Swollen thoracic segments and black lesions on integument; 2. Expulsion of gut contents; 3. Dissolved integument

The histological investigations with *S. litura* larvae died due to application of plant extracts revealed that some extracts had a strong adverse effect on the integument while some on food consumption and gut histology.

Characteristically, the insect integument consists of epidermis and cuticle. The outer layer - epidermis stands on basement membrane (Fig. 2-1). Secretion of the epidermis is called cuticle, which covers the whole of the outside of body as well as ectodermal invaginations (Chapman, 1988). In present study, the sections of larvae

treated with *A. arabica*, *A. squamosa*, *D. stramonium*, *E. globulus* and *I. carnea* leaf extracts exhibited cracking of cuticle (Fig. 2-2) which was followed by severe lysis of epidermis (Fig. 2-3). Endocuticle was separated from epidermis. There were no traces of food material in the alimentary canal lumen of the larvae as evident from empty microvilli (Fig. 2-4). It could be, therefore, inferred that the larva died due to severe damage in cuticular structure. This suggested that these extracts exerted strong **contact toxicity** and prevented larvae from feeding.

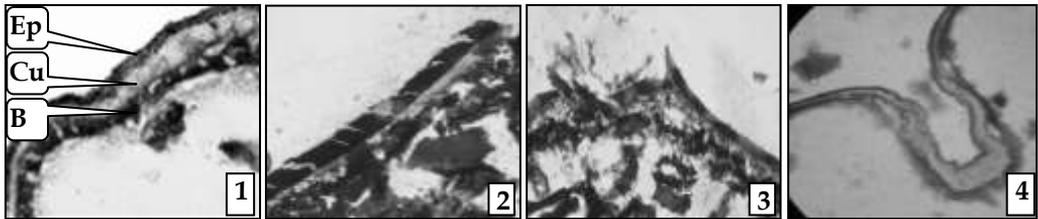


Fig 2. Symptoms of contact toxicity in *Spodoptera litura* larva exhibited by extracts of *A. squamosa* and *I. carnea*

1. Integument of untreated larva [Cu - Cuticle, Ep - Epidermis, Bm - Basement membrane] ; 2. Lesion and crack development in cuticle; 3. Lysis of integument; 4. Midgut microvilli showing no trace of food

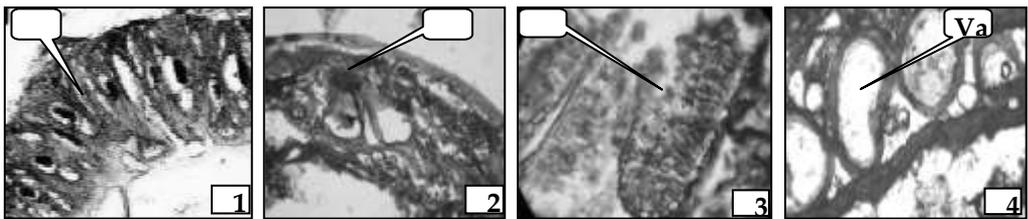


Fig 3. Symptoms of stomach toxicity in *Spodoptera litura* larva exhibited by extracts of *L. camara*, *N. tabacum*, *P. pinnata*, *A. arabica* seed, *A. squamosa* seed and *D. stramonium* seed

1. Peritrophic membrane of midgut of untreated larva; 2. Damaged peritrophic membrane; 3. Damaged microvilli with food material; 4. Vacuoles formation in midgut

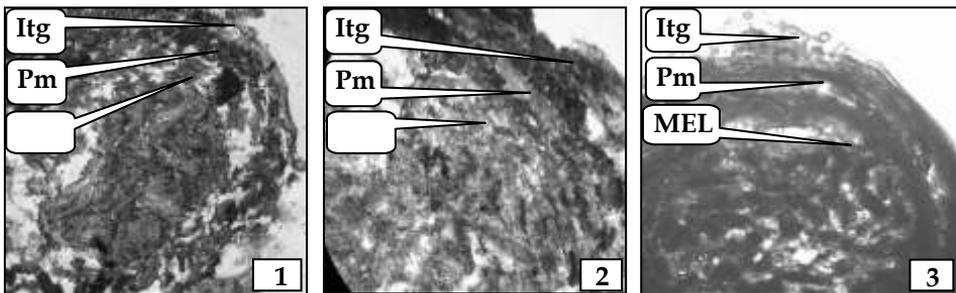


Fig 4. Symptoms of contact as well as stomach toxicity in *Spodoptera litura* larva exhibited by leaf extracts of *A. arabica*, *D. stramonium* and *E. globulus*

1, 2 and 3: Damage of Integument (Itg), Midgut epithelial layer (MEL) and Peritrophic membrane (Pm) due to contact and stomach toxicity

The alimentary canal of normal insects comprises three regions, foregut, midgut and hindgut. The midgut of an insect is considered to be the principal region of digestion and absorption of food. The midgut is also lined with a characteristic infolded peritrophic membrane and is associated with longitudinal or circular muscle layers. The anterior midgut is provided with numerous microvilli (Fig. 3-1) (Chapman, 1988). The larvae treated with extracts of *L. camara*, *N. tabacum*, *P. pinnata*, *A. arabica* seed, *A. squamosa* seed and *D. stramonium* seed damaged entire midgut area along with the basement membrane and muscular layers revealed complete distortion. Maximum damage was in the form of ruptured gut epithelial layer and peritrophic membrane (Fig. 3-2). Circular muscles were also found damaged. Fused cell mass of undifferentiated epithelial cells was also found with the presence of undigested treated food particles in gut lumen and microvilli. The microvilli were also found damaged but contained food particles (Fig. 3-3). The epithelial gut cells got separated from each other and several vacuoles were formed (Fig. 3-4). These symptoms suggested that these extracts acted as **stomach poisons** on the larvae.

Interestingly, some of these extracts viz. *A. arabica* leaf, *D. stramonium* leaf and *E. globulus* leaf extracts exhibited **contact as well as stomach toxicity**, showing combinations of all the above symptoms in the insect body (Figs. 4-1 to 4-3).

The natural insecticides are reported to work in several ways on

insects: antifeedant, insect growth regulator, affect the hormone system, affect the nervous system, and have direct effect on organs and tissues such as the stomach and muscle (Schmutterer, 1990; Mordue and Blackwell, 1993; Nathan *et al.*, 2006). In present study, possibly the active ingredients of plant extracts were directly toxic to the peritrophic membrane or the microvilli of the midgut of the insect body. Because of certain surface active properties, these natural products might enhance the absorption of toxic peptides into cell membranes, including those of the insect midgut. *D. stramonium* leaf extract is reported to exert strong antifeedant reaction on *S. litura* larvae (Rajguru *et al.*, 2011) as reflected in terms of least food consumption and only traces of food particles present in midgut lumen, as compared to other extracts. In present study, larvae exposed to *D. stramonium* leaf extract (topically on larvae as well as on the food material) died due to contact toxicity. This indicated that *D. stramonium* leaf extract acted both as stomach poison as well as antifeedant. Triterpenes in *Calendula officinalis* interfere with the digestion and absorption of ingested food and also with the conversion of absorbed food to biomass (Medhini *et al.* 2009). *Ipomoea carnea* Jacquin, at different concentrations, viz., 1, 2 and 3 percent was tested for antifeedant property against the castor semilooper, *Achaea janata* Linn. All the treatments were significantly superior to control (Arivudainambi and Nachiappan, 1993). *Pongamia pinnata* oil, cake and water extract have been reported to act as antifeedant against several insect pests on tobacco, groundnut, citrus and rice (Dhaliwal and Arora, 2001 a). However, in present study, traces of food

could be seen in mid gut of larvae exposed to *P. pinnata* leaf extract. It implied that whatever quantity of extract consumed with food was sufficient to damage mid gut region and to inflict larval mortality. Different parts of custard apple plant have been evaluated against a variety of insect pests and found promising in many cases (Dhaliwal

et al., 1996; Dev and Koul, 1997; Dhaliwal and Arora, 2001 b).

Apart from being supported by earlier studies, this investigation additionally gave an insight into the precise morphological and anatomical parts of *S. litura* larvae that got affected by the plant extracts and could be differentiated as contact, stomach and contact-stomach poisons.

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Characterization of Local Isolates of *Trichoderma*, *in-vitro* Evaluation against *Sclerotium rolfsii* (Causal Organism of Collar Rot of Soybean) and Compatibility with Seed Dressing Fungicides*

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ABSTRACT

Collar rot of soybean caused by *Sclerotium rolfsii* is a major obstacle in increasing soybean production. It is very difficult to manage the pathogen because of its diverse nature of survival. Biological agent, *Trichoderma* has emerged as an alternative means of management of soil-borne diseases. Two spp. of *Trichoderma* were isolated with different farming systems. There is no specific correlation between the habitats or farming system with species. *Trichoderma viride* was found dominating over *T. harzianum*. Both the species check the growth of *S. rolfsii*. Seed dressing fungicides were evaluated for their compatibility. Vitavax power and thiram were compatible with *Trichoderma* and can be integrated for the management of the collar rot of soybean.

Key words: Collar rot, compatibility, fungicides, management, soybean, *S. rolfsii*, *T. harzianum*, *T. viride*

Collar rot of soybean [*Glycine max*] caused by *Sclerotium rolfsii* is one of the diseases, which reduces the production of crop significantly. Under certain conditions, yield reduction up to 65 per cent has been reported (Agarwal and Kotasthane, 1971). *S. rolfsii* is a soil-borne polyphagous pathogen survives in the soil in the form of sclerotia. Factors, such as temperature, moisture and proximity to a susceptible host influence their survival. Sclerotia on or near the surface survive

longer than those buried in the soil (Beute and Rodriguez-Kabana, 1981). Though chemical control of pathogens, especially of sclerotial pathogens viz. *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani* etc., reduce the disease to some extent, it is not cost-effective, essential and in some cases eco-friendly. An alternative and effective method, to control these pathogens, is the use of biological control agents (Harman *et al.*, 2004).

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Different species of *Trichoderma* have high potential to control soil-borne plant pathogens more effectively than chemicals. Use of these fungi is not as harmful to the environment as chemical pesticides. These are present in substantial quantity in nearly all agricultural soils and in other environments such as an alternative method in plant disease control (Harman *et al.*, 2004). Several strains of *Trichoderma* developed as bio-control agents against fungal diseases of plants (Harman, 2006; Verma *et al.*, 2007). *Trichoderma* strains have also been recognized for their ability to increase plants root growth and their development, crop productivity, resistance to abiotic stresses and uptake and use of nutrients (Ranasingh *et al.*, 2006). The present study was, therefore, undertaken to find out the bioefficacy of local isolates of *Trichoderma* and its compatibility with seed dressing fungicides.

MATERIAL AND METHODS

A. Isolation of pathogen and bioagents

(i) *Isolation of pathogen*

Collar rot infected soybean plants were collected from the field of Directorate of Soybean Research (DSR), Khandwa Road, Indore. Infected portion of stems were cut with sterile blade into small pieces and sterilized with 0.5 per cent sodium hypochlorite for 1 min and then the pieces were washed with sterile distilled water. Two to three washings were done with sterile distilled water to remove excess sodium hypochlorite and then pieces were transferred aseptically into Petri plates containing potato

dextrose agar medium (PDA). The plates were incubated at $27 \pm 1^\circ\text{C}$ for 96 h. After growth of the fungus, the pure culture was further transferred to potato-dextrose agar medium and incubated.

(ii) *Isolation of Trichoderma spp using serial dilution technique*

Soil samples were also collected from different fields of DSR having different cropping systems (Table 1). Ten grams of soil sample was suspended in 100 ml of sterile distilled water and shaken well. From this suspension, one ml was pipetted out and transferred to 9 ml of sterile

Table 1. Origin of *Trichoderma* isolates

Isolate	Field history	Species identified
TRS-1	Soybean -wheat cropping system	<i>T. viride</i>
TRS-2	Mango orchard, with soybean	<i>T. viride</i>
TRS-3	Soybean-fallow	<i>T. viride</i>
TRS-4	Soybean-maize	<i>T. viride</i>
TRS-5	Soybean-wheat	<i>T. viride</i>
TRS-6	Soybean, broad-bed sowing	<i>T. harzianum</i>

distilled water and then subsequent dilutions were made. From 4th dilution 1000 μl (1 ml) suspension was transferred to *Trichoderma* selective medium (TSM) (Elad *et al.*, 1981) and plates were incubated for 7-10 days at $27 \pm 1^\circ\text{C}$. After incubation, the *Trichoderma* colonies were picked up individually on PDA plates and further incubated for 3-4 days for full growth (Johnson *et al.*, 1959).

(iii) *Characterization*

Characterization of species was done on the basis of morphological and cultural characters like colony appearance, growth pattern, radial diameter, pigment production, reverse pigmentation, biomass production, and shape of phialides, conidiophores and spores size and shape (Domsch *et al.*, 1980; Samuels *et al.*, 2004).

B. Efficacy studies of bio-agents against the pathogen

(i) *Dual culture*

Trichoderma species were evaluated against *S. rolfsii* using dual culture technique (Morton and Strouble, 1955). A 5 mm diameter mycelia disc from the growing margin of the 5 days old culture of *Trichoderma* spp. and the pathogen were placed equidistantly on opposite side of the plate at equal distance. In control plates (without *Trichoderma*), a sterile agar disc was placed at opposite side of the pathogen. Inoculated plates were incubated at $27 \pm 1^\circ\text{C}$. After 2, 3 and 4 days of incubation period, radial growth of pathogen was measured and per cent inhibition of average radial growth was calculated in relation to growth of the controls as follows (Datta *et al.*, 2004).

$$L = (C - T)/C \times 100$$

Where, L is inhibition of radial mycelial growth; C is radial growth measurement of the pathogen in control; T is radial growth of the pathogen in the presence of *Trichoderma* isolates.

(ii) *Slide culture method*

For pathogen - *Trichoderma* interaction, method proposed by Siameto *et al.* (2010) was used. A clean slide was placed in 9 cm diameter Petri plate and sterilized. Then a small amount of autoclaved melted potato dextrose agar was spread over the slide to make a thin PDA film on it. The 5 mm discs of 5-days-old growing colonies, cut from the margin of *S. rolfsii* and *Trichoderma* spp. were placed on the opposite sides of the slide, 3 cm apart on the PDA surface. Then five ml of sterile double distilled water was added to the plate to prevent drying up and then incubated for $27 \pm 1^\circ\text{C}$ for a week. At the end of incubation period, meeting area of *Trichoderma* and pathogen hyphae was observed under a light microscope for the presence of coiling structures and for cell wall disintegration.

(iii) *Poisoned food technique*

Poisoned food technique (Bhanumathi and Ravishankar, 2007) was followed to determine the inhibitory effect of *Trichoderma* isolates on *S. rolfsii* and its sclerotia. Conidial suspension (1 ml) of *Trichoderma* isolates prepared from the 5 days old *Trichoderma* cultures. *Trichoderma* spores was harvested in 10 ml of sterile distilled water and filtered through muslin cloth. Then the filtrate was further diluted with sterile distilled water to have 10^4 spore per ml. One ml of the spore suspension was placed in Petri plate, followed by 20 ml molten PDA medium and mixed thoroughly by rotating the plate. One ml of sterile distilled water used, instead of conidial suspension in control. Five mm diameter discs were obtained from the

actively growing region of the five days old culture of *S. rolfsii* on PDA and transferred aseptically to the centre of each *Trichoderma* amended PDA medium. Simultaneously, three *Trichoderma* amended plates were inoculated with mature sclerotia. The treatment was replicated three times. Plates were incubated at $27 \pm 1^{\circ}\text{C}$. Growth of *S. rolfsii* was determined after 2, 3 and 5-days after inoculation by measuring the radial mycelial growth, while sclerotia germination and viability was observed after transferring the sclerotia to PDA plates.

(iv) *Non-volatile compounds*

The effect of cell free culture filtrate of *T. harzianum* and *T. viride* on *S. rolfsii* was studied following the methods of Dennis and Webster (1971). In each 200 ml Erlenmeyer flask, 100 ml potato dextrose broth (PDB) was poured and sterilized after plugging. The broth was inoculated with 5 mm disc of 5 day old cultures of *T. harzianum*, and *T. viride* and incubated at $27 \pm 1^{\circ}\text{C}$ for 21 days with constant shaking in water bath. The culture filtrate was obtained by filtering the broth through Whatman's filter paper No 1. The culture filtrates of each bioagent was syringe filtered with 0.22 μm size filters and then mixed in molten medium to have 5.0, 10.0 and 15.0 per cent concentration. After solidification the plates were inoculated with 5 mm disc of 3 days old mycelium of *S. rolfsii* and incubated at $27 \pm 1^{\circ}\text{C}$ for 5 days and the radial growth of the test pathogen was recorded and per cent inhibition was calculated. PDA plates without

amendment of culture filtrate served as control.

(v) *Volatile compounds*

The antagonists, *T. harzianum* and *T. viride* were centrally inoculated by placing the 5 mm disc from the 3 day old cultures aseptically on PDA plates and incubated at $27 \pm 1^{\circ}\text{C}$ for 1, 2 and 3 days. The top of the each Petri plate was replaced with bottom of PDA plates, inoculated with *S. rolfsii*. The paired plates were sealed with parafilm to create airtight conditions and further incubated at $27 \pm 1^{\circ}\text{C}$. Plates having only test pathogen served as check. Observations were recorded by measuring the radial growth of test pathogen and check (Kucuk and Kivance, 2003).

C. *Compatibility of seed dressing fungicides with bioagents*

Three fungicides viz., Vitavax power (carboxin 37 % + carbendazim 37 %), thiram 75 WP (Tetra methylthiuram disulphide) and carbendazim 50 WP (Methyl 2-benzimidazole carbamate) (Table 2), which are commonly used as seed treatments in soybean, were evaluated against pathogen and antagonists by using poisoned food method described earlier. PDA plates with 3 concentrations 10, 20 and 30 μg a.i. per ml for vitavax power, 25, 50 and 75 μg a.i. per ml for thiram and 1, 5 and 10 μg a.i. per ml for carbendazim were prepared after adding the exact amount of fungicides in molten PDA. Plates were inoculated with antagonists as well as pathogen and incubated at $27 \pm 1^{\circ}\text{C}$ and observation on radial growth was recorded and per cent inhibition was calculated. Unamended plates served as check.

Table 2. Details of seed dressing fungicides used in soybean

Commercial Name	Chemical Name	Dose ($\mu\text{g/ml}$)	Manufacturer
Thiram (75 %WP)	Tetramethylthiuram disulphide	25	Devidayal Agro Chemicals Ltd
		50	
		75	
Bavistin/Carbendazim (50 %WP)	Methyl 2-benzimidazole carbamate	1	BASF, India Ltd
		5	
		10	
Vitavax power	5,6-dihydro-2-methyl-1,4-oxathinn-3-carboxanilide	10	Dhanuka Agritech Ltd
		20	
		30	

RESULTS AND DISCUSSION

1. Identification, occurrence and species dominance

Six isolates of *Trichoderma* spp. isolated from soil samples of different origin. On the basis of growth pattern on media, morphological and cultural characters like colony appearance, size, colour, mycelial growth, spore shape and colour and phialides, they were identified as *T. viride* and *T. harzianum*. *T. viride* was isolated from 5 soil samples while, *T. harzianum* from one sample. There was no specific correlation between the *Trichoderma* species and cropping systems (Table 1). The genus *Trichoderma* was universally present in all soil, although individual species may be either cosmopolitan (*T. harzianum*) or limited (*T. viride*) in their geographic distribution (Venkateshwarlu *et al.*, 2009). Our results showed dominance of *T. viride* over *T. harzianum*. Zhang *et al.*, (2005) also reported variation in frequency of the

Trichoderma species between north and south-west areas of China.

2. Mycoparasitism by *Trichoderma* spp.

(i) Colonization of the pathogen

There were no difference in *T. viride* and *T. harzianum* with regards to the colonization over test pathogen. After 2nd, 3rd and 5th day of parasitization, sclerotia were transferred to the PDA plates for their viability. It was found that 3rd day onwards all sclerotia were parasitized by *Trichoderma* spp. There was no germination after 5 days of parasitization, the mycelium of *S. rolfsii* was also removed aseptically and transferred to PDA plates and incubated. The results showed that the mycelium could not grow, instead mycoparasite i.e. *Trichoderma* grew, which concludes that the host pathogen was lysed by *Trichoderma* spp. Mycoparasitism includes both hyphal interaction and sclerotial parasitization and is the most vital mechanism of

antagonism of fungal antagonist to give protection to the plants from pathogen attack. Mycoparasitism as principal mechanism of biological control, is widely advocated by many scientists (Elad *et al.*, 1993; Howell, 1982). The directed growth of *T. viride* and *T. harzianum* towards *S. rolfsii* hyphae indicated a positive tropism probably chemotropism of the parasite towards its host (Chet, 1987).

(ii) Effect of volatile and non-volatile substances on the growth of *S. rolfsii*

Results presented in (Fig 1 and 2) showed that the volatile and non-volatile substances produced by the *T. viride* as well as *T. harzianum* were effective against the pathogen. Volatile compounds produced by *T. viride* and *T. harzianum* inhibited 73.0 and 70.0 per cent radial growth of the host pathogen at 96 h as compared to control. Non-volatile compounds produced by *T. viride* inhibited 13.3, 52.2 and 100.0 per cent radial growth at 5.0, 10.0 and 15.0 per cent cell free culture filtrate whereas, *T.*

harzianum inhibited 11.0, 51.0 and 100.0 per cent at 5.0, 10.0 and 15.0 per cent culture filtrate, respectively (Fig. 3a and b). Antibiosis is another type of antagonism, mediated by specific or non-specific metabolites of microbial origin, by lytic agents, enzymes, volatile compounds or other toxic substances. Cell free culture filtrates or extracts of these filtrates have been used to demonstrate the possible role of antibiosis in biocontrol. Filtrates of various mutants of *T. harzianum* are suppressive to the white rot pathogen *S. cepivorum* (Kay and Stewart, 1994). Volatile metabolites identified as alkyl pyrones have been reported by Vey *et al.* (2001) from *T. harzianum*. These compounds suppressed the *R. solani* causing damping-off of lettuce. Fungi are known to produce volatile organic metabolites viz. ethanol, isobutanol, isoamyl alcohol and isobutyric acid (Fravel, 1988). Michrina *et al.* (1995) reported antagonistic action of *T. harzianum* against *Fusarium culmorum* due to volatile and non-volatile antibiotics.

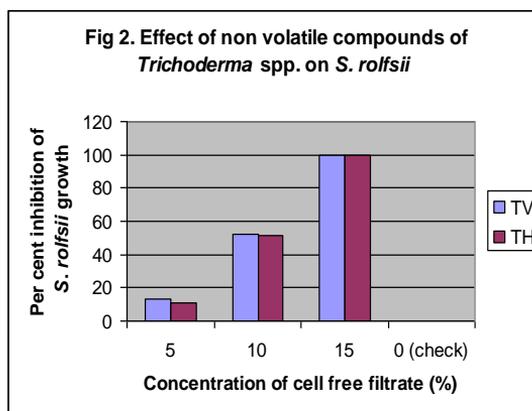
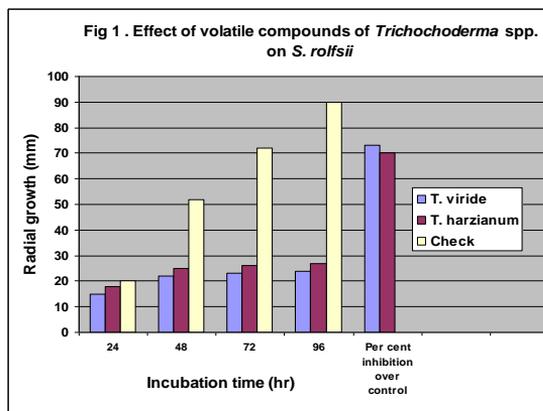




Fig. 3 (a). Effect of volatile compounds produced by *T. viride* on growth of *S. rolfsii*

Fig. 3 (b). Effect of volatile compounds produced by *T. harzianum* on growth of *S. rolfsii*

(iii) *Coiling or parallel running around pathogen*

T. harzianum and *T. viride* were observed readily interacting with *S. rolfsii*. The mycoparasites grew towards pathogen, either ran parallel (Fig. 4) or coiled (Fig. 5) around the pathogen hyphae. The mycoparasite produced

haustoria like structure and penetrated in the host hyphae and finally the protoplasm of host got coagulated, vacuolated and lysed. *T. viride* infrequently coiled around *S. rolfsii* resulting in coagulation of protoplasm and shrunk hyphae leading to lysis.

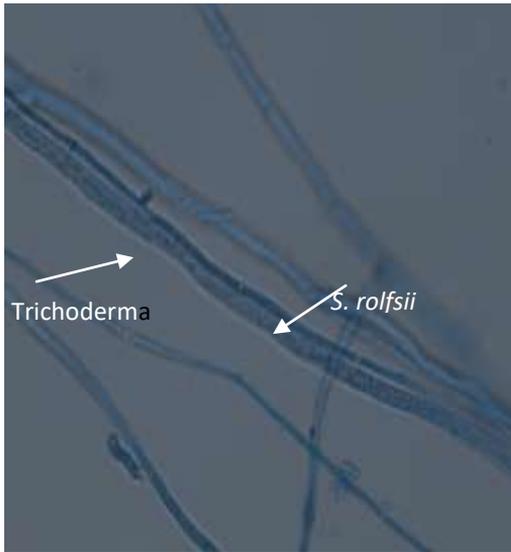


Fig. 4. *T. harzianum* mycelium running parallel to *S. rolfsii*

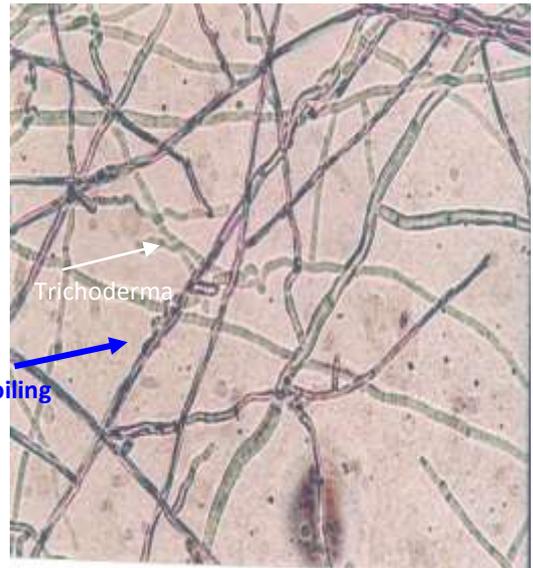


Fig. 5. *T. viride* hyphae making coil over *S. rolfsii* hyphae

Microscopic examination of the hyphal interactions between the antagonists and *S. rolfsii* revealed that the hyphae of *Trichoderma*, either ran parallel (*T. harzianum*) or coiled (*T. viride*) and got pressed to the hyphae of *S. rolfsii*. Therefore, the contact was established resulting into complete or partial digestion of protoplasmic contents and lysis of the pathogen. Finally the protoplasm of host got coagulated, vacuolated and lysed (Fig. 5). Lysis of the host cell wall of the plant pathogenic fungi has been demonstrated to be an important step in the mycoparasitic attack (Chet *et. al*, 1998; Pant and Mukhopadhyay, 2001; Benitez *et. al*, 2004). Mycoparasitism involved a complementary action of antibiosis, nutrient competition and cell wall degrading enzymes such as chitinases, β -1,3-glucanases and proteases. Since,

chitin is the major component of most fungal cell walls; a primary role has been attributed to chitinases in the biocontrol activity of *Trichoderma* (Harman, 2000).

3. Compatibility between *Trichoderma* and seed dressing fungicides

Fungicides *viz.*, thiram, vitavax power and bavistin were evaluated against collar rot pathogen and the antagonist. Different doses of thiram i.e. 25, 50 and 75 μ g a.i. per ml was tested against *S. rolfsii* and *Trichoderma* spp. *Trichoderma* spp. could grow in all the test concentrations whereas, *S. rolfsii* grew well at lower concentrations (i.e. 25 μ g a.i./ml) but the higher concentration (50 and 75 μ g a.i./ml) were inhibitory as it produced 62 and 59 mm diameter growth after 96 h (Fig 6 and

7). Vitavax power (10, 20 and 30 μg a.i./ml) was also tested along with check. The results presented in (Fig 8 and 9) showed that *Trichoderma* spp grew in all the test concentrations whereas, *S. rolfsii* was highly inhibited. In case of carbendazim (bavistin), the reverse was true (Fig 10 and 11). These observations

suggested that vitavax power and thiram can be successfully integrated with *T. harzianum* and *T. viride* for management of seed and seedling rot disease of soybean. Insensitivity of *T. harzianum* to vitavax has been reported previously (Sarmah, 1990; Shrestha, 1992).

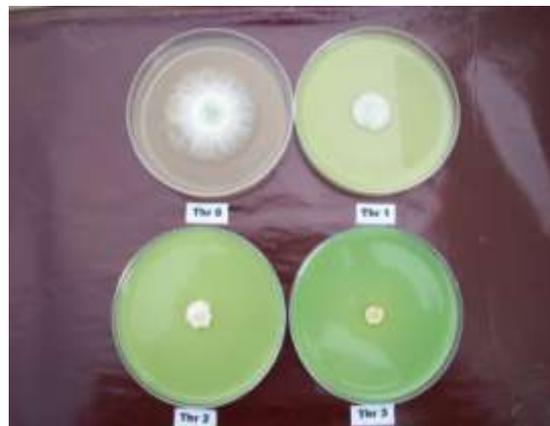
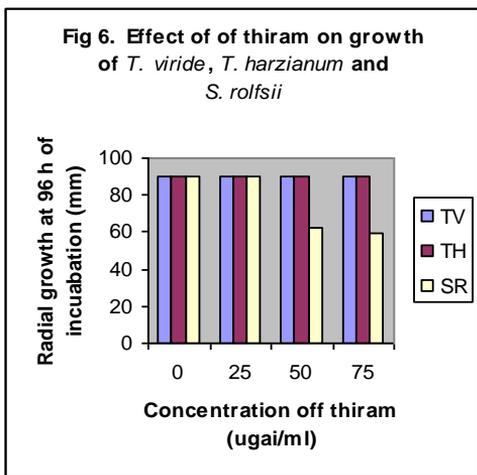


Fig 7. Compatibility between thiram and bioagents

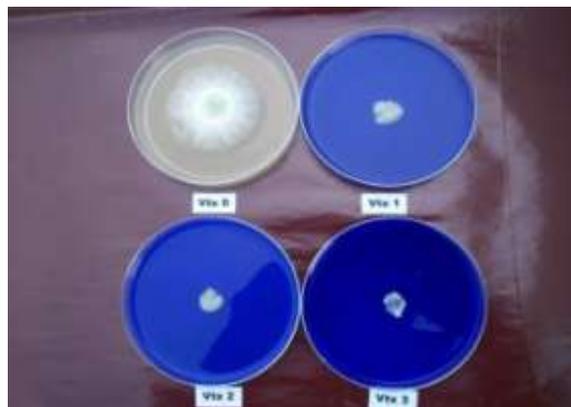
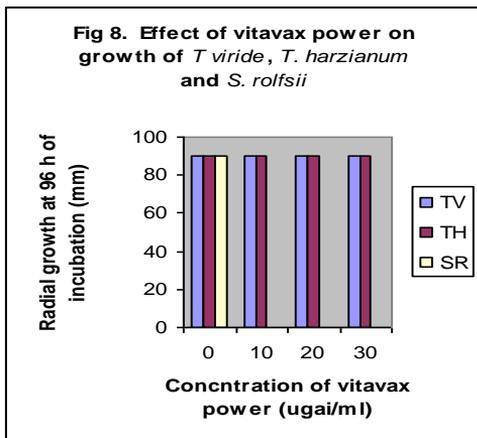


Fig 9. Compatibility between vitavax power and bioagents

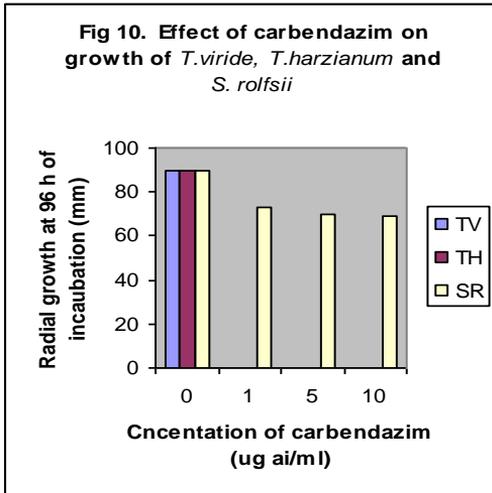


Fig 11. Compatibility between carbendazim and bioagents

The fungicide, vitavax power provides initial protection to seed and seedlings from the attack of many soil-borne pathogens particularly to *R. solani* and *S. rolfsii*, thereby helping in establishment and multiplication of antagonist which provide protection throughout the crop growth period. Use of sub-lethal dose of chemical weakens the pathogens; thereafter antagonist becomes more effective to parasitize them (Mukhopadhyay *et al.*, 1992). Vyas (1994) also reported that the integration of carbendazim with *T. harzianum* or *T. viride* was promising for control of root rot of soybean caused by *M. phaseolina*. Dharamputra and Retnowati (1994) also reported the similar pattern after inoculation of soybean seeds with *T. aureoviride* and tebuconazole for controlling *S. rolfsii*. Successful control of several diseases by integration of biocontrol agents with chemicals has been reported by many workers

(Mukhopadhyay *et al.*, 1992 Hwang and Chakravarty, 1993).

Trichoderma species are naturally resistant to many toxic compounds, including herbicides, fungicides and insecticides, and above all to antibiotics liberated by other microorganisms, which makes *Trichoderma* strains to grow easily under extremely competitive conditions (Hjelyord and Tronsmo, 1998). Most *Trichoderma* species have been described as saprophytic fungi, although some of them may also become mycoparasites (Barnett and Binder, 1973), and so, they may attack directly to other fungi in order to utilize their cell wall and cytoplasmic components as nutritional sources. *Trichoderma* species are highly producer of extra cellular proteins and are best known for their abilities to produce enzymes that degrade cellulose or chitin as well as produce other enzymes that may have to our commercial applications (Kubicek and Penttila, 1998).

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Effect of Ratio (n/v) on Optimizing Bite Length and Weeding Efficiency of the Rotary Tool

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ABSTRACT

A study was conducted on bite length as a design parameter of L shaped tractor PTO operated rotary tool attachable to the tractor PTO weeding machine. The parameter bite length (tillage pitch) (P) of soil was tested against the ratio of revolutions per minute (n) and forward speed (v) for smaller and longer slices/bites during weeding operation with the rotary tool. Often the said tool fails to serve the purpose as larger slice holds the un-slashed weed plant due to moist soil sticking on to the roots which help it to re-establish in the field. Therefore the bite length (P) was studied against the range of n/v from of 2:1 to 14:1. It was evaluated to optimize the bite length design parameter. An equation for rotary tool was developed and used to calculate the bite length (P) for optimum value for the tractor PTO operated rotary weeding tool. These theoretically optimized results showed that optimum value of bite length (1.67 to 2.50 cm) was on keeping the n/v ratio between 8:1 and 12:1. Field test data verification also showed the n/v ratio for optimizing the bite length was in the range of 8:1 to 12:1. Bite length of tool was found inversely proportional to ratio of the n/v .

Validation for optimization of bite length 2.50 cm to 1.67 cm in the field revealed that ratio of n/v from 8:1 to 12:1 led to 87- 90 per cent weed elimination. Results suggest that for efficient operation the tool needs to be operated between the ranges of ratios of 8:1 to 12:1 to achieve maximum weed elimination.

Keywords: Bite length, optimization, power take off, ratio of RPM vs. forward speed, rotary tool

The weeding is the most critical field operation, after sowing of the crop and affects the final yields if not accomplished effectively. The yield loss due to weed infestation in soybean was to the tune of 20-77 per cent (Muniyappa *et al.*, 1986; Tiwari and Kurchania, 1990; and Kurchania *et al.*, 2001). Substantial yield loss can be avoided by efficient management of weeds. Manually operated tools and tractor drawn sweep type weeders work on the principle of horizontal shearing of the soil and are limited to slicing the soil and fail to prevent

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re-establishment of the weed plants. In order to perform efficient weed management, an attempt has been made to develop a rotary weeding machine. The basic principle behind the working of the rotary weeding machine is centrifugal force acting on to cut the soil, uproot and cut the weed plant, and throw the cut slice of soil. This sequence is repeated continuously.

One of the important design parameter is bite length of the tool which depends upon forward speed of the prime mover responsible for pulling the machine and revolution per minute of the rotary tool. The direction of rotation of the tool is a basic rotary design parameter; contrary to the common practice in this country to use only the forward direction of rotation (the rotor follows the direction of the tractor wheels).

Habibi and Singh (2009) evaluated the blades on the basis of specific work requirement in various numbers of blades per flange, forward speed and rotor speed. Specific work had an exponential relation with bite length, whereas linear relationship was observed with velocity ratio for all the blades tested. RC-type blade with less specific work requirement and more volume of soil tilled, had better performance than other blades.

The present study is aimed at optimization of bite length as design parameter of the tool to optimize the weeding efficiency by tractor Power take off (PTO) operated rotary weeding tool. A mathematical equation was developed for the bite length as design parameter,

which permits to decide optimum range of operation and performance can be decided and selected immediately for production of rotary tools for specific soil and field conditions. Optimization of the design parameter of the tractor PTO operated tool is helpful in designing and development of rotary weeding tool for different operating speeds, soil conditions and moisture conditions. The purpose of slicing the soil was not over by just slicing the soil but it was more important to slash the uprooted plant and shake the uprooted weed plant in order to prevent the weed plant roots to re establish.

In rotary tool, there is predetermined length of soil, which is cut every time, thus having a control over bite length and soil is confined from more than one site. The transfer of power directly to soil by using tractor rather than through inefficient drawbar and movement of rotary tools in a direction and speed other than the basic machine, are some of the inherent advantages of rotary or active powered tools for considering them as an alternative to tractive tools. Niyamapa *et al.* (1994) found that power requirement for cutting and throwing of soil, increased with increase in rotor speed, forward speed and tillage depth. These three parameters also affected soil breakage. Larger clod size was formed when tillage depth and forward speed were high and the rotor speed was low. Smaller clod sizes were formed when tillage depth and forward speed were low and the rotor speed was high. The optimisation of bite length of weeding tool would enable to design of the tool to solve the problems associated with the various types of weed elimination situations. This would also realise the true potential of the mechanization of weeding methods.

MATERIAL AND METHODS

The study comprises of (i) development of equation for bite length (tillage pitch) of the L shaped tool, (ii) validation of the developed equation for bite length (tillage pitch) as design parameter theoretically, and (iii) optimisation of bite length (tillage pitch) as design parameter and with field data.

Forward speed of the tool was controlled with the help of gear selection with the ground PTO type tractors. The tool rpm was controlled with the help of PTO speed which was taken from independent PTO system of the tractor. The bite length (Fig 1) was measured as horizontal distance of two slices made by successive trochoids made by the tool / tools operated by the tractor Power take off operated rotary machine.

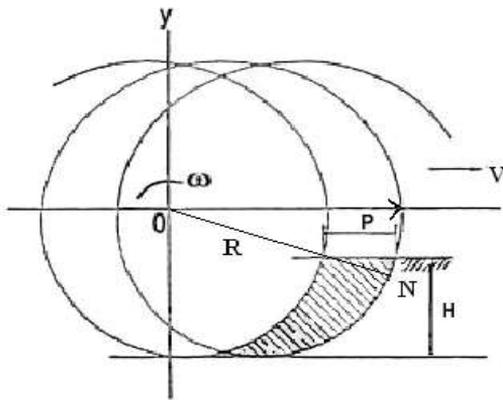


Fig 1. Bite Length (P) between loci of two successive trochoids

Study on the major design parameter of weeding tool i. e. bite length was conducted for different speed of rotary tool and the prime mover (n/v). The length of the cutting tool was taken $R = 30$ cm and

was mounted on a flange which was fixed on a shaft of the tool. This cutting tool has been made of spring steel (5 mm thickness). Number of tools (z) per flange was taken 1, 2 and 3 for the study.

Bite length

This developed equation helps to select bite length (tillage pitch) under suitable limits against n/v so that the tool cuts a slice with minimum of effort and does not leave slice holding the weed plant. Thus, following assumptions were taken into consideration for the development of the equation.

$$P = 60.v/z.n$$

where, z = number of tools, n = number of revolution of the tool, v = forward velocity of machine (cm/sec)

Validation of the equation for bite length (P) of the tool

Validation was conducted by changing the values of n/v by changing values of n and keeping v constant and calculating the bite length (P) cut by the tool. The data was recorded on revolutions per minute and forward speed for 1, 2 and 3 numbers of tools on the flange of the rotary shaft of the tractor PTO driven machine.

The data of different values of n , v and z was put in the equation to calculate the bite length and graph was drawn (Fig. 2). The result from data and the graph clearly depict that with the increase in n/v the bite length decreases and *vice versa*. Thus, the developed equation was found to be valid for different ratios of n/v . Celik *et al* (2008) also observed that soil slice size

increased as rotor radius and tractor forward speed increased and the number of blades on one side of a flange decreased. The number of cuts of a soil slice increased as the number of blades on one side of a flange, the rotor radius, and the rotor rotational speed increased. As the number of cuts of a slice increased, the size of each part decreased and soil fragmentation increased.

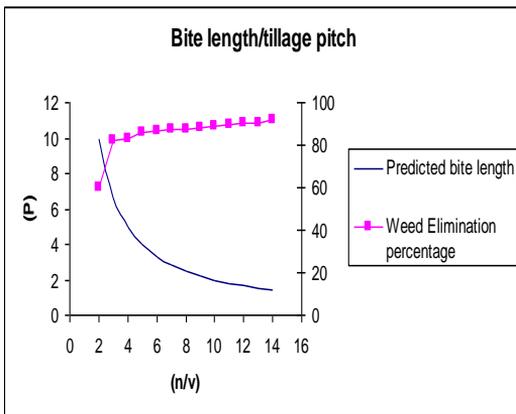


Fig 2. Optimization of Bite length (tillage pitch) vs n/v for $z=3$

Optimization of bite length of the tool

Optimum range of n/v for the bite length was achieved with changing the n/v by changing the forward speed of the machine which is operated with the prime mover (tractor). The equation of bite length was tested with the change of n/v for different values of n and keeping v constant to find out bite length for optimization with the change of revolutions per minute of the tool which respect to its forward speed. The optimization was evaluated with the performance of weed count with the various lengths of bite / slice. The values

of the lengths of three sets were used in the equation developed for finding out the bite length to establish the optimum value or range of n/v . Field testing was conducted to collect data for measurement of bite length against n/v and elimination of weeds. The tests were conducted for three sets for each tool. The data for the bite length against different ratios n/v of the tractor power take off operated rotary tool was analyzed.

The bite length cut by the tool was measured after taking the sample from the field after the operation of the different number of tools with various ratios of n/v . The number of weeds were collected before and after the operation of the tool and recorded with different forward speeds and revolutions per minute from 10 randomly selected places from ten replicated plots (each plot- 50 m x 2.25 m) for each of the above two treatments. The bite length data from each plot was recorded.

The length of the slices cut between two consecutive slices made by the tool was evaluated by the equation in the field test along with weeds. The data of weeds/ m^2 was recorded. The pattern followed by the change of ratio upon the length of cut was evaluated. Test was conducted with tool length $R = 30$ cm. Forward speed selected for the study was $v = 20$ cm / seconds which was kept constant. This revolution per minute of the tool was varied from $n = 20$ onwards. The above said data was selected to vary the ratio of n/v and the bite length cut by the tool was recorded for the study.

RESULTS AND DISCUSSION

The results of the validation and optimization of the bite length tool design

are presented in table 1. The testing of the mathematical equation developed was accomplished to measure the length of bite, $P = v / n \cdot z$ for different ratios of rotor rpm and forward speed. The study clearly indicates that the bite length variation affects the change in the size of the slice. Similarly with increase in the number of tools in the same flange with the same ratio of the bite length was found inversely proportional.

This ratio of n / v 8:1 to 12:1 was the range in which the length of bite as per the developed equation showed that with increase in the ratio of n/v the size of the bite length decreased proportionately. This reduction of bite length with the increasing the ratio n/v when put in the equation proves the validity of the equation for the bite length.

Field test results

Study on weeding operation for first set was done with the tool for measurement of bite lengths theoretically and in the field, there was significant difference in the weed population. The reduction in weed population with $z=1$ when n/v was kept in the ratio in the range of 8:1 to 12:1 for bite length weed elimination was 81 to 84 per cent. Bite length for the ratio of n/v below 8:1 eliminated weeds less than 85% which was of quite low value similarly for the ratio above of n/v 12:1 not much variation/increase of weed elimination was observed. Results for $z=2$ reveals that the reduction in weed population was 86 to 87 percent when n/v was kept in the ratio in

the range of 8:1 to 12:1. The result for $z=3$ showed that reduction in weed population was 87 to 90 per cent when n/v was kept in the ratio from 8:1 to 12:1.

Ratio of n/v of 8:1 to 12:1 was found to be optimum for bite length. At this range maximum weeding efficiency was highest.

Optimization of the bite length as design parameter

The data for optimization of P in the equation provide value of Bite length which can draw the curves for number of tools ($z = 1, 2$ and 3) on the flange when (P) is plotted against n/v . Weeding efficiency increased with the increase in the ratio of n/v . Bite length keeps on reducing as per the equation and practice.

The optimization of bite length (P) for rotary tool was different with the number of tools on the flange with ratio of forward speed and rotation of the tool. But it is important to mention that if the P is reduced below a certain limit the n/v will have to be reduced to very low value which will cause excessive consumption of power and energy besides the tool walking out of the field.

As the bite length increased the tool eliminate lesser weeds and also failed to slash the plant and shake away the soil from the weed plant root. The bite length with the $R=30$ cm and $z=3$ was found to be optimum as the ratio of n/v ranging from 8:1 to 12:1 was sufficient to uproot slash and shake away around than 87 to 90 per cent of the weeds from the soil. Thus $z=3$ and the range of ratios of n/v 8:1 to 12:1 (Fig. 2) was considered to be optimum for the design parameter of bite length (tillage pitch).

Table 1. Effect of ratio (n/v) on optimizing bite length and weeding efficiency

RP M (n)	Forward speed (v) (cm/sec)	Ratio of (n/v)	Number of tools z= 1			Number of tools z= 2			Number of tools z= 3		
			Predicted bite length (cm)	Bite length in the field test (cm)	Weed elimination (%)*	Predicted bite length (cm)	Bite length in the field test (cm)	Weed elimination (%)*	Predicted bite length (cm)	Bite length in the field test (cm)	Weed elimination (%)*
40	20	2	30.00	29.20	31.00	15.00	15.30	41.3	10.00	10.10	60.25
60	20	3	20.00	21.50	35.00	10.00	8.90	65.70	6.67	6.60	82.30
80	20	4	15.00	14.50	41.00	7.50	7.40	77.00	5.00	5.10	83.00
100	20	5	12.00	12.10	50.30	6.00	6.10	80.00	4.00	4.10	85.80
120	20	6	10.00	10.20	60.70	5.00	4.80	83.30	3.33	3.30	86.50
140	20	7	8.57	8.30	74.00	4.28	3.20	85.00	2.86	2.80	87.50
160	20	8	7.50	7.40	77.00	3.75	3.70	86.30	2.50	2.60	87.70
180	20	9	6.67	6.70	81.00	3.33	3.30	86.00	2.22	2.10	88.30
200	20	10	6.00	5.90	82.30	3.00	2.90	86.70	2.00	1.90	88.80
220	20	11	5.45	5.50	83.00	2.72	2.70	87.00	1.82	1.80	89.50
240	20	12	5.00	4.80	83.50	2.50	2.55	87.40	1.67	1.60	90.00
260	20	13	4.62	4.60	84.10	2.30	2.35	87.60	1.54	1.60	90.50
280	20	14	4.29	4.10	85.70	2.14	2.20	87.80	1.43	1.40	91.50

**Mean of three years*

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Studies on Synbiotic Spray Dried Soymilk Powder

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ABSTRACT

The fermented milk products are gift of nature to mankind. The probiotic count, quality and shelf life of such products always remain in question for mass production and utilization. The present experiment explores the possibilities of utilization of soymilk for production of synbiotic food. The spray drying conditions viz. temperature (X_1), slurry concentration (X_2) and pressure (X_3) were optimized for survival of bacteria. The fresh soymilk used in spray drying contains protein 3.4 - 3.6 per cent, fat 1.7 - 2.0 per cent, total solids 7.0 - 7.5 per cent and acidity 3.0 - 3.7 per cent. The synbiotic food contains Bifidobacterium bifidum counts from 3.79×10^9 - 17.78×10^9 cells per g dry weight and their per cent survival was 4.26 - 37.05 per cent. The bulk density and solubility index of synbiotic food varies 0.26 - 0.32 g per ml and 2.3 - 4.8, respectively. High temperature and pressure of spray drying markedly influenced the color and appearance of product and shown significant effect on survival of Bifidobacterium bifidum in the product. After reconstitution of synbiotic food with water, pineapple and strawberry juice, product was highly acceptable. The product blend made of mango juice was liked very much. The maximum survival of Bifidobacterium bifidum in synbiotic food could be obtained by spray drying condition i. e. temperature 164.02°C , slurry concentration 17 per cent TSS and pressure 3.2 kg per cm^2 .

Key wards: Prebiotic, probiotic and synbiotic food, soymilk, spray drying

The human gastro intestinal tract is a kinetic micro-ecosystem that enables normal physiological functions of the host organism unless harmful and potentially pathogenic bacteria dominate it. Maintaining a proper equilibrium of the micro-flora may be ensured by systematic

probiotics, prebiotics or synbiotics. The most widely used definition of probiotics is a live microbial feed supplement that beneficially affects the host by improving its intestinal microbial balance (Fuller, 1989, 1992). Health advantages associated with probiotic intake are alleviation of symptoms of lactose mal-absorption,

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increase in natural resistance of infectious diseases of the intestinal tract, suppression of colon cancer, reduction in serum cholesterol concentration, improved digestion and stimulation of gastrointestinal immunity (Collins and Gibson, 1999) although well characterized strains with proven clinical effects are not numerous. The bacteria belonging to genera *Bifidobacterium* and *Lactobacillus* are most often used as probiotic supplement for food. They exert only beneficial effects with regards to human health. In an analogy with probiotics, prebiotics are the non-digestible oligosaccharides (NDO) used as food ingredients to enhance or modify the composition of endogenous gut microflora. Further, the definition was developed by Gibson and Robrefroid (1995) who named a prebiotic as "a non digestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or more or a limited number of bacteria in the colon, and thus improve host health". The synbiotic, as a combination of probiotics and prebiotics is of more interest today. Synbiotic food promises to give both the benefits of probiotics and prebiotics.

Generally there is a lack of studies on the development of synbiotics from properly selected probiotics and prebiotics. Among the group of prebiotic ingredients i. e., non-digestible oligosaccharides, soy-oligosaccharides is one of the best prebiotic ingredient, which is abundantly present in soybean and usually termed as 'Soy-Oligosaccharides' and act as a

promising prebiotic ingredient, stimulates the growth of *Bifidobacterium* spp. in the large intestine (Amarowicz, 1999). Soymilk is one of the novel products of soybean which contains all the soy- oligosaccharides.

In India the soybean is available abundantly for food uses, including soymilk. The spray drying is one of the most affective processes to extend the shelf-life of dairy product. This process offers the advantage of long-term preservation, convenience in handling, storage, marketing and consumption of food. The information on optimized conditions for production of soymilk powder, synbiotic food, its nutritional value, therapeutic value and their effect on quality of spray-dried products are meager. Therefore, the present investigation was undertaken and findings are reported as under.

MATERIAL AND METHODS

The whole soybean, seeds of variety JS 97 52 were used for soymilk preparation (Tomar and Chauhan, 1998). Seeds were soaked in water for 08 hr, then washed with fresh water and drained. The soaked seeds were grind with small quantity of hot water, then hot water was added to make soy slurry in 1:9 ratio. The slurry was filtered with muslin cloth and soy milk was collected in a clean stainless steel container. For inoculation of soy milk with bifidus culture, the freeze dried culture of *Bifidobacterium bifidum* was propagated in 100 ml sterilized skimmed milk or pan media by aseptically transfer of content of ampoules to the flask incubated for 24 h at 37°C and stored under refrigerator. The starter culture was examined for proper coagulation and sign

of defect, if any. To attain optimum activity of strain two or three serial transfer of starter culture were carried out prior to addition in bulk culture. The scale up system of propagation of culture was followed for further day to day need. The enumeration of total *Bifidobacterium bifidum* was done on modified MRS agar media by standard plate count technique (Indian Standards, IS 1960).

The synbiotic food was prepared, according to procedure of Kumar (1981). Fresh soymilk was standardized, pasteurized and supplemented with predetermined

level of gelatin and soymilk powder to obtain slurry of desired concentration (Table 1). The slurry was stirred well, filtered through sieves, sterilized and cooled at 40°C (Gandhi and Nambudripad, 1979). The 24 h old bulk culture of *Bifidobacterium bifidum* strain was inoculated into slurries, @ 10 per cent and incubated at 37°C for 24 h. Immediately cooled to 10°C and stirred well to get free flowing. The bifidus milk slurries were dried in SMST Lab Model Spray Dryer following standard procedure.

Table 1. Composition of slurry used in spray drying

Component	Slurry concentration (TSS, %)				
	11-12	11-12	11-12	11-12	11-12
Whole Soymilk	11-12	11-12	11-12	11-12	11-12
Soy Milk Powder	1.0	3.0	5.0	7.0	9.0
Gelatin	1.0	1.0	1.0	1.0	1.0
Total Concentration	13	15	17	19	21

The rate of survival of microorganisms during spray drying depends upon factors such as culture, its adaptation to acquire resistance of processing conditions (inlet/outlet air temperature, air pressure, flow rate, concentration of slurry) and amount of inoculum used during spray drying (Espina and Packard, 1979; Pien, 1978 and Sharma, 2000). Therefore, on the basis of information available in the literature and preliminary trials, two and three independent variables were selected to study the effect on various responses of spray dried soymilk powder and synbiotic food. The

experiments were planned in central composite rotatable design (Myers, 1976). To achieve best quality product, the spray dryer was operated at predetermined, spray drying conditions i. e. air inlet/outlet temperatures (164°C - 205°C / 85°C - 110°C), air pressure (2.2 kg/cm² - 3 kg/cm²), slurry concentration (20 - 30%) and flow rate (20 - 40%) as per Table 2 and 3. The product, synbiotic food was collected from stainless steel cyclone side in attached glass jar. It was packed in glass bottle and stored at room temperature for further analysis.

Table 2: Experimental design matrix for production of synbiotic food

Expt. No.	Coded Form			Uncoded Form				
	X ₁	X ₂	X ₃	Inlet temp. (°C) X ₁	Outlet temp. (°C)	Flow rate	Slurry conc. (%) X ₂	Pressure (kg/ cm ²) X ₃
1.	1	+1	+1	205	90±5	30	19	3.5
2.	-1	+1	+1	175	80±5	20	19	3.5
3.	1	-1	+1	205	85±5	30	15	3.5
4.	-1	-1	+1	175	80±5	20	15	3.5
5.	1	1	-1	205	95±5	30	19	2.9
6.	1	1	-1	175	80±5	20	19	2.9
7.	1	1	-1	205	95±5	30	15	2.9
8.	-1	1	-1	175	80±5	20	15	2.9
<i>Second Order Interaction</i>								
9.	+1.682	0	0	215.23	105±5	30	17	3.2
10.	-1.682	0	0	164.77	80±5	20	17	3.2
11.	0	1.682	0	190	100±5	40	21	3.2
12.	0	1.682	0	190	90±5	20	13	3.2
13.	0	0	-1.682	190	95±5	20	17	3.8
14.	0	0	-1.682	190	100±5	20	17	2.6
<i>Centre Point</i>								
15.	0	0	0	190	90±5	20	17	3.2
16.	0	0	0	190	90±5	20	17	3.2
17.	0	0	0	190	90±5	20	17	3.2
18.	0	0	0	190	90±5	20	17	3.2
19.	0	0	0	190	90±5	20	17	3.2
20.	0	0	0	190	90±5	20	17	3.2

To determine the effect of independent variable on some predominant responses viz. *Bifidobacterium bifidum* count, bulk density, solubility index, acidity and moisture content of product, response surface methodology was used. A full second order equation (1) was fitted in each response to describe it mathematically and to study the effect of variables. Where β_0 , β_i and β_{ij} are the constant co-efficient and X_i is the coded independent variable. The adequacy of model was tested using F-ratio and co-efficient of determination (R^2). The model is generally considered adequate when (I) the calculated F ratio was more than that of table value and (II) the R^2 value is more than 70%. The effect of variables at linear, quadratic and interactive terms on the response was described using significance at 1, 5 and 10% level of significance. The best fit models were as also developed using step wise regression analysis (Diwan, 2000; Parwar, 1999; Shukla, 1997; Heinka, 1982).

$$Y = \beta_0 + \sum_{i=1}^4 \beta_i X_i + \sum_{i=1}^3 \sum_{j=i+1}^4 \beta_{ij} X_i X_j + \sum_{i=1}^4 \beta_i X_i^2$$

Evaluation of synbiotic food: The milk fat, SNF, total solid and acidity of fresh soymilk were determined according to procedure by IS (1960). The colour and appearance of spray dried product was determined by the procedure of De (1985), the solubility index of soymilk and synbiotic food by Kumar (1981) and moisture, protein, carbohydrates and total ash content by AOAC (1960) procedure. The per cent survival of microorganism was calculated from the initial value of count in the slurry before drying and in the powder just after drying. The synbiotic food was

reconstituted @ 10 per cent in distilled water and used for determination of fat titrable acidity (IS, 1973 and IS, 1962). The total *Bifidobacterium bifidum* count in slurry and synbiotic food were determined according to procedure described by Prajapati *et al.* (1987). A simple hedonic rating test was used for sensory evaluation of synbiotic food.

RESULT AND DISCUSSION

The composition of fresh soymilk showed that it contains fat, total solids and titrable acidity from 1.7 - 2.0 per cent, 7.0 - 7.5 per cent, 3.0 - 3.7 per cent, respectively. The organoleptic taste of soymilk samples was also acceptable. The minimum amount of soymilk powder (102 g) was obtained at inlet temperature $205 \pm 5^\circ\text{C}$, outlet temperature $85 \pm 5^\circ\text{C}$, and maximum (111.9 g) respectively, at $190 \pm 5^\circ\text{C}$, $75 \pm 5^\circ\text{C}$, 3.2 kg per cm^2 and 3.5 kg per cm^2 (Table 3). The bulk density, solubility index, moisture, acidity, fat and total ash content of soymilk powder varied from 0.22 - 0.28 g per ml, 1.8 - 2.5, 2.8 - 3.8 per cent, 3.0 - 3.8 per cent, 1.0 - 1.2 per cent, 0.3 - 0.5 per cent, respectively (Table 3).

Survival of bifidobacterium bifidum in spray dried synbiotic food: The *Bifidobacterium bifidum* cell count varied from 1.99×10^9 to 17.78×10^9 cell per g dry weight (Table 4). The analysis of variance of multiple regression models for *Bifidobacterium bifidum* cell counts indicated that F-ratio was higher (7.59707) than that of table value (3.02). The standard error of estimate and co-efficient of determination (R^2) were 1.59822 and 87 per cent, respectively (Table 6). The co-efficient of model (Table 5) for linear, interactive and quadratic terms shows

that, the temperature has negative linear significant effect on *Bifidobacterium bifidum* cell count in synbiotic food at 1 per cent level of significance. However, it shows positive response at quadratic terms. The inlet temperature and slurry concentration also interactive

significant effects at 10 per cent level of significance. All other interactions, linear and quadratic terms of the model were non-significant. The best fit model is:

$$Y_1 = 40401503 - 1.826208X_1 + 2.728018 X_1 * X_1 \quad (2)$$

Table 3. Experimental design matrix and responses related to recovery of spray dried soymilk powder

Expt No	Inlet temp. (°C) X ₁	Outlet Pressure temp. (g/cm ²) X ₃	Total SYMP recover UNIT	SYMP Left over in spray drier (g)	Total weight (g)	Bulk density (g/ml)	Solubility index	Mois-ture (%) d.b	Aci-dity (%)	Fat (%)	Total ash (%)	
1.	205±5	95±5	2.9	109	3	112	0.25	2.2	3.3	3.3	1.1	0.4
2.	205±5	85±5	3.2	102	10	112	0.27	2.3	3.8	3.1	1.1	0.3
3.	205±5	80±5	3.5	110	2	112	0.28	2.0	3.3	3.3	1.1	0.4
4.	190±5	100±5	2.9	111	1	112	0.26	2.1	3.7	3.8	1.1	0.5
5.	190±5	90±5	3.2	111.5	0.5	112	0.26	2.4	3.0	3.3	1.1	0.4
6.	190±5	80±5	3.5	111.9	0.1	112	0.27	1.8	2.8	3.0	1.2	0.3
7.	175±5	80±5	2.9	111.7	0.3	112	0.24	2.0	3.2	3.0	1.1	0.4
8.	175±5	90±5	3.2	111.8	0.2	112	0.28	2.2	3.4	3.7	1.1	0.3
9.	175±5	80±5	3.5	111.8	0.2	112	0.22	2.5	3.2	3.3	1.0	0.3

Note: Slurry concentration (%) X₂ =12 and flow rate=30

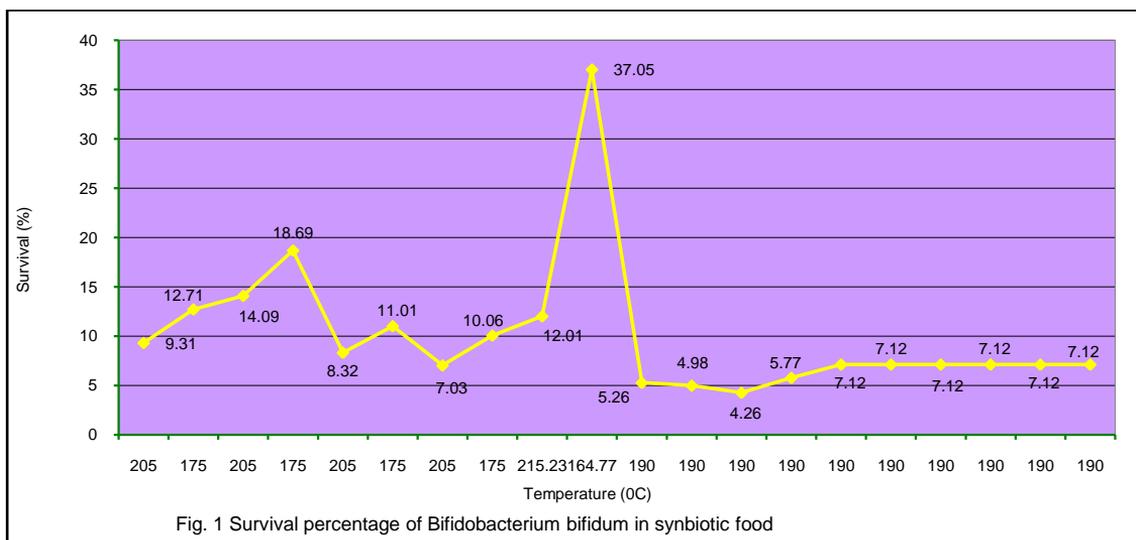


Fig. 1 Survival percentage of Bifidobacterium bifidum in synbiotic food

Table 4. Bifidobacterium bifidum, coliform count, physical and chemical composition of synbiotic food

Expt. No.	Bifido-bacterium bifidum/g dry weight (x 10 ⁹)	Survival of Bifido-bacterium bifidum (%)	Bulk density (g/ml)	Solubility index	Moisture (%)	Total solids (%)	Acidity (%)	Fat (%)	Protein (%)	Carbohydrates (%)	Total ash (%)
1.	4.16	9.31	0.27	2.4	3.8	96.2	0.60	1.1	62.40	26.12	5.8
2.	7.28	12.71	0.28	2.3	4.3	95.7	0.65	1.2	62.5	25.62	5.6
3.	8.33	14.09	0.29	2.5	3.8	96.2	0.63	1.1	62.62	25.71	6.0
4.	10.41	18.69	0.27	2.4	4.1	95.9	0.68	1.1	62.8	25.93	5.3
5.	5.79	8.32	0.30	3.2	3.8	96.2	0.71	1.0	63.2	25.11	6.1
6.	6.91	11.01	0.31	3.7	3.0	97.0	0.66	1.1	64.5	25.14	5.5
7.	5.21	7.03	0.28	3.8	4.0	96.0	0.71	1.2	62.8	26.11	5.1
8.	6.34	10.06	0.30	2.6	3.6	96.4	0.61	1.1	63.01	26.30	5.2
9.	7.38	12.01	0.32	4.8	3.2	96.8	0.82	1.1	64.9	25.01	5.0
10.	17.78	37.05	0.27	3.2	5.1	94.9	0.67	1.0	62.01	25.01	6.1
11.	4.31	5.26	0.29	3.8	2.6	97.4	0.61	1.0	65.07	25.04	5.5
12.	4.02	4.98	0.28	3.6	3.4	96.6	0.72	1.1	64.58	25.01	5.1
13.	1.99	4.26	0.26	2.3	4.8	95.2	0.76	1.0	63.13	25.03	5.2
14.	4.56	5.77	0.27	2.5	3.2	96.8	0.65	1.1	63.66	26.03	5.2
15.	5.15	7.12	0.31	2.9	2.8	97.2	0.66	1.1	64.93	25.07	5.3
16.	5.15	7.12	0.31	2.9	2.8	97.2	0.66	1.1	64.93	25.07	5.3
17.	5.15	7.12	0.31	2.9	2.8	97.2	0.66	1.1	64.93	25.07	5.3
18.	5.15	7.12	0.31	2.9	2.8	97.2	0.66	1.1	64.93	25.07	5.3
19.	5.15	7.12	0.31	2.9	2.8	97.2	0.66	1.1	64.93	25.07	5.3
20.	5.15	7.12	0.31	2.9	2.8	97.2	0.66	1.1	64.93	25.07	5.3

Note: Percent survival of Bifidobacterium bifidum cell in dry product considering the count in the slurry as 100 %: Coliform count/g dry weight = Absent

Table 5. Regression co-efficient of full second order model and significant terms for synbiotic food

Co-efficient	Bifidobacterium Count Y ₁	Bulk Density Y ₂	Solubility Index Y ₃	Moisture Y ₄	Acidity Y ₅
Constant	5.135457	0.308094	2.91712	2.799101	0.661655
<i>Linear</i>					
β ₁	-1.826208***	0.004693	0.262933*	-0.204697*	0.022133
β ₂	-0.414564	0.002696	0.046595	-0.142449	-0.014279
β ₃	0.117677	-0.007089***	-0.295528**	0.314184**	0.004028
<i>Interactive</i>					
β _{1,2}	-0.12875	-0.0025	-0.2125	0.025	-0.00625
β _{1,3}	-0.36875	0.005	-0.0625	-0.25	-0.03125
β _{2,3}	-1.05625*	-0.005	-0.0875	0.125	-0.01375
<i>Quadratic</i>					
β _{1,1}	2.6389***	-0.003143	0.276519*	0.483079***	0.019188
β _{2,2}	-0.335519	-0.006677*	0.170479	0.076593	-0.009089
β _{3,3}	-0.650104	-0.013747***	-0.289027**	0.430059***	0.005049

*** Significant at 1%; ** Significant at 5%; * Significant at 10%; β₁ Inlet air temperature; β₂ slurry concentration (TSS); β₃ air pressure

Table 6. ANOVA of full second order regression model for synbiotic food

Source	Bifidobacterium Count Y ₁	Bulk Density Y ₂	Solubility Index Y ₃	Moisture Y ₄	Acidity Y ₅
Model SS	174.648	0.00469606	5.61388	8.31767	0.0266544
Model MS	19.4053	0.000521785	0.623765	0.924185	0.00296160
Model DF	9	9	9	9	9
Error SS	25.5432	0.00162394	2.24362	1.63983	0.0248656
Error MS	2.55432	0.000162394	0.224362	0.163983	0.00248656
Error DF	10	10	10	10	10
F-Ratio	7.59707	3.21308	2.78017	5.63585	1.19104
F-Table	3.02	3.02	3.02	3.02	3.02
Standard Error of Estimate	1.59822	0.0127434	0.473668	0.404949	0.0498654
R Squared	0.872406	0.743048	0.714462	0.835317	0.517361

The survival value of *Bifidobacterium bifidum* cell (Table 4, Fig 1) during spray drying varied from 4.26 - 37.05 per cent. The survival of bacteria in synbiotic food was obtained maximum during spray drying condition viz., inlet/outlet temperature 164.77 ± 5°C/80.00 ± 5°C,

pressure 3.2 kg per cm² and slurry concentration 17 per cent T.S.S. The percentage value of survival of *Bifidobacterium bifidum* cell in synbiotic food are two fold higher than that of reported value of Kumar (1981) and Prajapati *et al.* (1987). The survival of microorganism during spray drying can be further increased by use of chemicals and thermal protectant and pH adjustment. Porubcan (1976) reported 10⁹ cfu per g viable cells of *Lactobacillus acidophilus* and *Lactobacillus helveticus* in spray dried product, which also confirms the present finding. The survival rate of 37.05 per cent appears promising and could be attributed to culture selection and process standardization.

Bulk Density and Solubility Index:

The bulk density (g/ml) and solubility index value of synbiotic food varied from 0.26 - 0.32 g per ml and 2.3 to 4.8, respectively (Table 4). The F-ratio was higher (3.21308) than table value (3.02). The R² value was 74 per cent. The pressure variable has negative significant effect at linear and quadratic terms. The co-efficient of determination, (R²) value for solubility index was 71 per cent. The best fit model is:

$$Y_2 = 0.300781 - 0.012859 X_3 * X_3 \quad (3)$$

$$Y_3 = 3.249982 - 0.329444 X_3 * X_3 \quad (4)$$

Moisture, Total Solids and Acidity:

The moisture and total solid content (Table 4) of synbiotic food were minimum (2.6%, 94.9%) and

maximum (5.1%, 97.4%), respectively. The R² value was 83 per cent. The temperature and pressure has shown significant effect (5% level) for linear and quadratic term of model. The temperature and pressure during spray drying also contribute positive significant quadratic effect, (1% level). The interaction between temperature and pressure was also significant at (10% level) for linear terms model. The increase in temperature during spray drying reduced the moisture content for desired optimum moisture content. The present findings are in conformity with reported value (Sharma, 2000; Kumar, 1981 and Prajapati *et al.*, 1987). The moisture content of synbiotic food is also within the prescribed limit of ISI and ADMI standards for spray dried products. The calculated optimum value of moisture content of synbiotic food was 5.16 per cent. The spray drying conditions were temperature 171.45°C, slurry concentration 16.96 per cent, TSS and pressure 3.56 kg per cm². The best fit model was:

$$Y_4 = 2.861806 + 0.314184 X_3 + 0.475465 X_1 * X_1 + 0.422445 X_3 * X_3 \quad (5)$$

The minimum and maximum (0.60% and 0.82%) acidity (Table 4) was obtained from synbiotic food in Exp. No. 6 and 4 and 1 and 9, respectively. These experiments represent the combination of spray drying variables as 205, 19, 3.5 and 215.23, 17, 3.2 of temperature (°C), slurry concentration (% TSS) and pressure (kg/cm²),

respectively (Table 5).

Fat, protein, carbohydrate and total ash: The synbiotic food contains fat 1.0 - 1.2 per cent, protein 62.01 - 65.07 per cent, carbohydrates 25.01 - 26.30 per cent and total ash 5.0 - 6.1 per cent (Table 4). The difference in fat, protein, carbohydrate and total ash, in different experimental product were due to variation of different component used for preparation of slurry. Sharma (2000), Kumar, (1981), Prajapati *et al.* (1987), and De (1985) had also reported the similar results about milk spray drying.

Sensory evaluation of synbiotic food: The results of hedonic rating test (Table

7) for different sensory attributes viz., color, taste, flavor and overall acceptability varied from 6.6 - 8.5, 6.4 - 8.2, 6.3 - 8.0 and 6.4 - 8.2, respectively. The findings of overall acceptability score revealed that synbiotic food reconstituted in mango juice was ranked as liked very much. However, in pineapple juice, strawberry juice and water it was liked moderately and liked slightly, respectively. The panelists have also recommended the use of sweetener and different kind of fruit flavor for more acceptable product. Thus, soymilk can be successfully utilized to develop spray dried synbiotic food using proposed optimized conditions of spray drying.

Table 7. Sensory evaluation of synbiotic food

Product	Color	Taste	Flavor	Overall acceptability
Synbiotic food and Water (10g : 90ml)	6.6	6.4	6.3	6.4
Synbiotic food and Mango (20g : 80ml)	8.5	8.2	8.0	8.2
Synbiotic food and Pineapple (20g : 80ml)	6.7	7.1	7.2	7.0
Synbiotic food and Strawberry (20g : 80ml)	7.4	7.3	7.3	7.3

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Ansari M M and Sharma A N. 2000. Compatibility of *Bacillus thuringiensis* with chemical insecticides used for insect control in soybean (*Glycine max*). *Indian Journal of Agricultural Sciences* **70**: 48-9. **(Journal)**

Joshi O P, Billore S D, Ramesh A and Bhardwaj Ch . 2002. Soybean-A remunerative crop for rainfed farming. *In: Agro technology for dry land farming*, pp 543-68. Dhopte AM (Eds.). Scientific Publishers (India), Jodhpur. **(Book chapter)**

Ansari M M and Gupta G K. 1999. Epidemiological studies of foliar diseases of soybean in Malwa plateau of India. Proceedings, World Soybean Research Conference VI, Aug 4-7, 1999, Chicago, Illinois, USA, 611p. **(Symposium/Conf./Workshop)**

Pansae V G and Sukhatme P V. 1978. Statistical Methods for agricultural workers. Indian Council of Agricultural Research, New Delhi. pp.186. **(Book)**

Table: Each table should be typed on separate page and numbered sequentially. Tables should have descriptive heading. Authors are advised to avoid large table with complex columns. Data are restricted to only one or two decimal figures only. Transformed values should be included if these are discussed in the text.

Illustrations: Number all illustrations consecutively in the text. Line drawing should be made in undiluted black ink on smooth white card or tracing paper. Original and two Photostat copies should be drawn approximately twice the size of reproduction. Original should not be labeled and should also not be numbered. Line diagrams of plants, fungi etc. should indicate the scale.

Photographs: Photographs should be on glossy paper and have good contrast. Trim unnecessary areas. Three copies of the photographs should be provided. On the back of the photographs write names of authors, figures numbers and indicate top of the photographs with an arrow using a soft pencil. Show magnification with a bar scale. **Coloured photographs can be printed on payment of full printing cost by the authors.** Legends for figures should be typed separately and numbered consequently.

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They should not exceed more than 1300 words (total 5 typed pages, which deal with (i) research results that are complete but do not warrant comprehensive treatment, (ii) description of new material or improved techniques or equipment, with supporting data and (iii) a part of thesis or study. Such notes require no heading of sections. It should include key words. Figures and tables should be kept to a minimum.

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Authors with in-depth knowledge of the subject are welcome to submit review articles. It is expected that such articles should consist of a critical synthesis of work done in a field of research both in India and/or abroad, and should not merely be a compilation.

Proofs

Authors should correct the proof very critically by ink in the margin. All queries marked in the article should be answered. Proofs are supplied for a check-up of the correctness of the type settings and facts. Excessive alterations will be charged from the author, Proof must be returned immediately to shorten the reproduction time.

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