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# **SOYBEAN RESEARCH**

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Khandwa Road, Indore 452 001  
Madhya Pradesh, India**

# Society for Soybean Research and Development

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## Reduction of Endogenous Plant Ethylene Levels by Rhizobia to Influence Nodulation in Legumes with Emphasis on Soybean

SUSHIL K SHARMA<sup>1</sup>, MAHAVEER P SHARMA<sup>2</sup> AND A RAMESH<sup>3</sup>

National Research Centre for Soybean (ICAR)  
Khandwa Road, Indore 452 001, Madhya Pradesh, India  
(E mail: sks\_micro@rediffmail.com)

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### ABSTRACT

Nitrogen-fixing nodules are formed as a result of series of interactions between rhizobia and host legumes. Ethylene inhibits nodulation in various legumes except soybean wherein regulation of ethylene level did not show any effect on root nodulation. *Bradyrhizobium elkanii* produces rhizobitoxine, a phytotoxin, which causes chlorosis in leaves of soybeans. However, recent studies have revealed that rhizobitoxine enhances the nodulation process by inhibiting the ACC (1-aminocyclopropane-1-carboxylate) synthase in the ethylene biosynthesis of legumes roots and thereby positively influence symbiotic interaction between *B. elkanii* and host legumes. In addition, it has been also reported that some rhizobia possess ACC deaminase, which also facilitates symbiosis by decreasing ethylene levels in the roots of host legumes. The available evidence suggests that rhizobitoxine-producing bacteria modulate plant-microbe interactions via ethylene in the rhizosphere environments. The capability of rhizobitoxine-producing rhizobia could be utilized as tools in agriculture and biotechnology.

**Key words :** ACC deaminase, ethylene, nodulation, rhizobia, rhizobitoxine, soybean

The group 'rhizobia' is a collective term that includes various genera *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, *Sinorhizobium*, and *Methylobacterium* in the  $\alpha$ -Proteobacteria, as well as *Burkholderia* and *Ralstonia* in the  $\beta$ -Proteobacteria (Moulin *et al.*, 2001). Biological nitrogen fixation (BNF) by the rhizobia in symbiotic association with legumes provides significant contribution

of nitrogen to enhance and maintain agriculture productivity. The seat of nitrogen fixation by rhizobia is the nodules formed on root and or on shoot of legumes where they symbiotically fix nitrogen for plant but in return rhizobia utilize carbon fixed by the legumes (Sprent, 2003). This exchange is controlled by an exchange controls system, amino acid cycle that

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<sup>1</sup>, <sup>2</sup> and <sup>3</sup>Senior Scientists

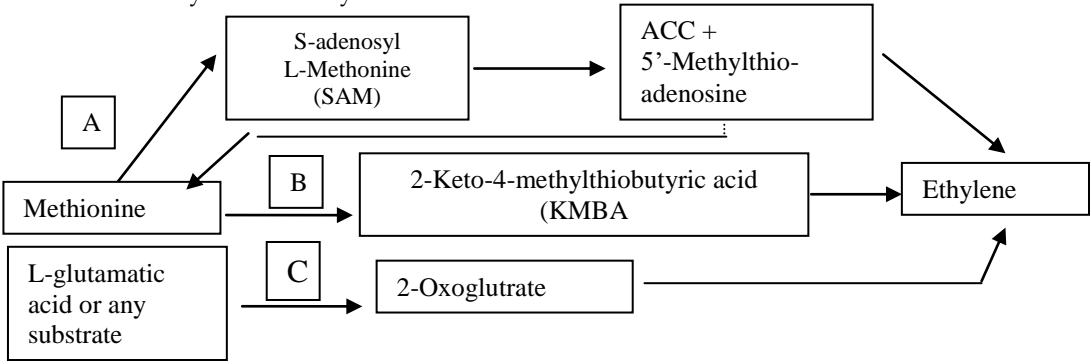
enables two partners to share their resources without either one becoming dominant (Ludwig *et al.*, 2003). Thus, the formation of a huge number of effective nodules is crucial for better nitrogen fixation in plants and thereby enhances plant growth and yield. Besides nitrogen fixation, plant growth and nodulation are too regulated indirectly by reduction or inactivation of phytopathogen or directly via phosphorus solubilization, and iron sequestration by siderophores, ACC deaminase (Glick *et al.*, 1999; Penrose, 2000; Penrose and Glick, 2003), phytohormones production such as auxin gibberellin, cytokinin and ethylene etc. It has been noticed that ethylene level in plant can be reduced by some strains of rhizobia either by rhizobitoxine or ACC deaminase and thereby reducing the negative effect of ethylene on nodulation (Yuhashi *et al.*, 2000). This review provides information on strategies used by rhizobia to lower down endogenous ethylene levels in legumes and consequently influence nodulation in legumes.

**Ethylene biosynthesis in plant and microorganism**

Ethylene is a ubiquitous hormone and it is either synthesized by microbes

(Arshad and Frankenberger, 1988) or by plants (Cristescu *et al.*, 2002). In higher plants, ethylene is produced from L-methionine via the intermediates, S-adenosyl-L-methionine (SAM) and aminocyclopropane-1-carboxylic (ACC) (Yang and Hoffman, 1984). The enzyme involved in this sequence are SAM synthetase, which catalyzes the conversion of methionine to SAM (Giovannelli *et al.*, 1980), ACC synthase, which is responsible for hydrolysis of SAM to ACC and 5'-methylthioadenosine (MTA) (Kende, 1989) and ACC oxidase, which metabolizes ACC to ethylene, carbon dioxide and cyanide (John, 1991). The presence of an efficient detoxification pathway prevents the accumulation of cyanide, even in plant with high rates of ethylene biosyntheses (Penrose and Glick, 1997).

Bacteria and fungi synthesized ethylene either by 2-keto-4-methylthiobutyric acid (KMBA) or by oxoglutarate pathways. So far no report that bacteria synthesized ethylene via ACC (Fukuda *et al.*, 1993) but some soil microbes utilize ACC when applied as external source for ethylene production by unknown pathways in soil (Frankenberger and Phelan, 1985).



**Fig 1. Ethylene biosynthetic pathway in higher plant (A) and microorganisms (B and C)**

## Response of ethylene on plants

Ethylene mediates a range of different plant growth stages including seed germination, tissue differentiation, formation of root and shoot primordia, root elongation, lateral bud developments, flower initiation, anthocyanin synthesis, flower opening and senescence, fruit ripening and degreening, production of organic volatiles, compound responsible for aroma in fruits, storage product hydrolysis, leaf and fruit abscission, and the responses of plant to biotic and abiotic stresses. In some instances the presence of ethylene is stimulatory while in other it is inhibitory. It inhibits root elongation (Ma *et al.*, 1998), shoot and root dry weight, nodulation as well as nitrogen fixation to some extent (Nukui *et al.*, 2000; Peters and Crist-Estes, 1989). Microbial production of ethylene in soil is implicated as possible cause of soil fungistasis property (Lynch, 1975). Recently, ethylene of microbial origin such as *Pseudomonas* sp, *Enterobacter sakazakii* and *Klebsiella oxytoca* has been exploited for controlling *Striga* in African soils (Babalola, 2002) by inducing abortive germination of *Striga* seed in absence of host (Eplee, 1981).

## Effect of ethylene on nodulation

Now, it is well established that ethylene inhibits infection of rhizobia and nodulation of the most legumes as shown in table 1 (Okazaki *et al.*, 2004). It has been thought a decade back that ethylene inhibits nodulation by inhibition of root growth (Hirsch and Fang, 1994). Later on, it was found that it is not the root growth that affects nodulation but ethylene regulates it (Frankenberger and Arshad, 1995). However, nodulation in soybean is not

affected by ethylene levels (Lee and La Rue, 1992; Schmidt *et al.*, 1999). The ethylene may reduce nodule formation process by affecting later stage of infection at the epidermal-cortex interface in various legumes (Guinel and Geil, 2002). The evidence for involvement of ethylene in decreased nodule formation is supported by use of certain inhibitors such as aminoethoxyvinylglycine (AVG) which on application inhibits endogenous plant ethylene levels and thus increase nodule formation (Peters and Crist-Estes, 1989). For instance, nodule formation on *Macroptilium atropurpureum*, *Medicago sativa*, *Lotus japonicus* and *Pisum sativum* roots sharply enhanced by treatment with AVG in presence of light and nitrate (Ligero *et al.*, 1986 and 1991; Nukui *et al.*, 2000; Van Spronsen *et al.*, 2001). Furthermore, an ethylene-insensitive mutant of *Medicago truncatula* "sickle" is hyper-infected by its symbiotic partner (Penmetsa and Cook, 1997). On the contrary, an ethylene-hypersensitive or ethylene-overproducing mutant of *Pisum sativum* R50, form fewer nodules as compared to wild-type plant (Guinel and Sloetjes, 2000).

Several mechanisms involving ethylene may be responsible for regulation of rhizobial infection by plant hosts. One possible mechanism is through feedback inhibition of infection, which causes a transient susceptibility to rhizobial infection in root hair cells and results in a narrow zone of infection and nodule differentiation in root hairs.

It is proposed that low level of ethylene is required to allow proper

**Table 1. Effects of ethylene on nodulation and nitrogen fixation in legumes**

<b>Rhizobia</b>	<b>Cultivar of legume</b>	<b>Treatment</b>	<b>Effect on nodulation</b>	<b>Reference</b>
<i>Bradyrhizobium japonicum</i>	<i>Glycine max</i> L. cv Ransom	Ethylene gas	No effects on nodule number	Lee and La Rue , 1992
<i>Rhizobium</i> sp.	<i>Phaseolus vulgaris</i> L. cv Pencil podded black wax	Ethylene gas	Decrease in nodule number and nitrogen fixation	Grobbelaar <i>et al.</i> , 1971
<i>Rhizobium leguminosarum</i> bv. <i>viciae</i>	<i>Pisum sativum</i> L. cv Feltham First	Ethylene gas	Decrease in nodule number and nitrogen fixation  Inhibition of root extension	Goodlass and Smith, 1979
<i>Rhizobium leguminosarum</i> bv. <i>viciae</i>	<i>Pisum sativum</i> L. cv Sparkle E13f (sym13) mutant (small ineffective nodule)	Ethylene gas	Decrease nodule number	Lee and La Rue , 1992
	<i>Pisum sativum</i> L. cv Rondo nod-3 mutant (hyper nodulation)	Ethylene gas	Decrease nodule number	Lee and La Rue , 1992
<i>Rhizobium trifolii</i>	<i>Trifolium repens</i> L. cv Hula	Ethylene gas	Decrease in nodule number and nitrogen fixation	Goodlass and Smith, 1979



deposition of the cytoskeleton and thus results in a successful entry of the infection thread in the outermost layer of cortical cells (Guinel and Geil, 2002). Kijne *et al.* (1988) reported that the presence of a cytoplasmic bridge is necessary for the infection of cortical cells during nodulation. Under normal circumstances, rhizobial Nod factor induces the formation of ethylene that activate plant enzyme to locally modify the cell wall which result in the formation of cytoplasmic bridge. Higher level of ethylene induced by Nod factor could induce cross-linking of glycoprotein of the infection thread and block its growth and thus result in the abortion of the infection thread (Guinel and Geil, 2002; Wisniewski *et al.*, 2000).

The second mechanism involves the early arrest of rhizobial infection within the nodulation zone. It is well known that only minority of rhizobial infection can persist to colonize differentiating nodule tissue (Penmesta and Cook, 1997). After the first nodules have formed, alfalfa plant reacts to the infection to homologous wild type *Rhizobium* by eliciting a defense response similar to the hypersensitive reaction (HR) in the compatible plant-pathogen interaction (Vasse *et al.*, 1993). This localized HR response could be one of the mechanisms employed by plant to auto-regulate the infection process, and ethylene is well known to be involved in plant defense mechanisms against pathogens (Abeles *et al.*, 1992). The observation that AVG causes an increase in persistent rhizobial infection, has given rise to the suggestion that ethylene may be involved

in controlling the persistence of rhizobial infections (Peters and Crist-Estes, 1989).

It is reported that, in soybean, exogenous ethylene did not inhibit nodulation and treatment with AVG and silver did not enhance nodulation (Hunter, 1993; Schmidt *et al.*, 1999). However, ethylene is required for nodulation in *Sesbania* (D'Haeze, 2001). Furthermore, some environmental factors such as inoculation of rhizobia, nod factor, nitrate and illumination increase ethylene evolution in host roots and thereby regulating nodulation in legumes. These data indicated that the regulation of nodulation among plant species is significantly different and the effect of ethylene on nodulation depends on the host species (Guinel and Geil, 2002; Schmidt *et al.*, 1999).

#### **Mechanisms to reduce endogenous ethylene levels in legumes**

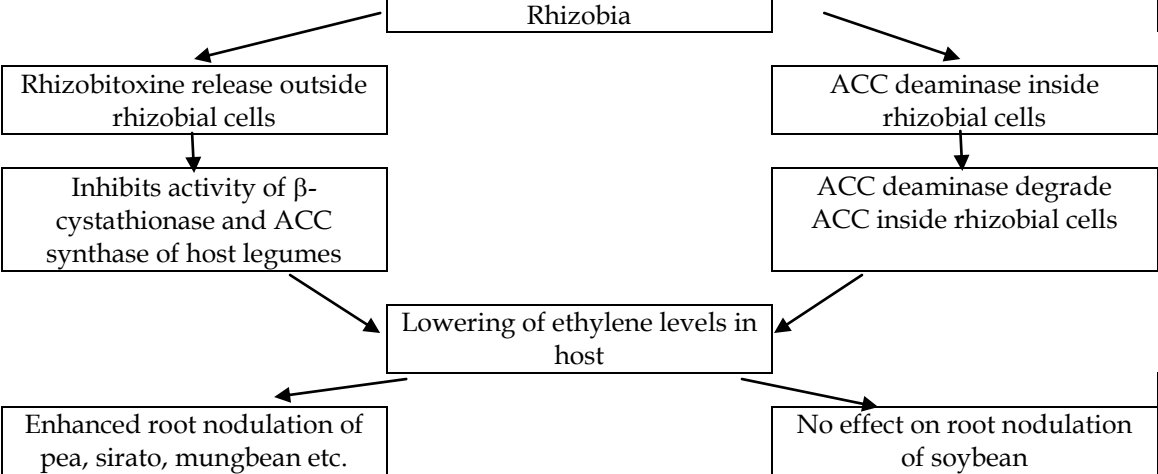
Recently, it has been observed that ethylene levels in plant roots can be reduced by some strains of rhizobia, however, not all strains of rhizobia are able to do so (Fig. 2). Till date only two mechanisms are known by which rhizobia reduce levels of ethylene in plants (Ma *et al.*, 2002); (i) rhizobitoxine production and (ii) ACC deaminase production. The details of such mechanisms are describes below.

##### ***Rhizobitoxine production by rhizobia***

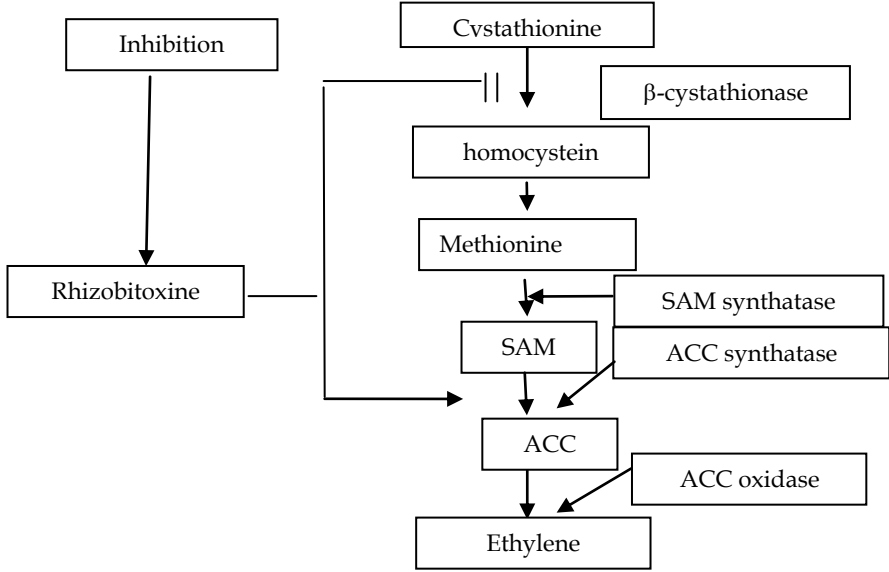
Erdman and co-worker (1956) reported first time that certain strains of rhizobia induce chlorosis in new leaves of soybean. Later, the toxic molecules produced by *Bradyrhizobium elkanii* USDA76 inducing chlorosis was identified as rhizobitoxine [2-amino-4-(-2-amino-3-hydropropoxy) - trans-but-3-enoic acid]

(La Favre and Eaglesham, 1986; Owens *et al*, 1968; Owens, 1973).

It induces foliar chlorosis in early stage in soybean as the compound synthesized in nodule but translocated up to leaves (Owens and Wright, 1965a and b).



**Fig. 2. An overall schematic representation of reduction of ethylene levels by rhizobia and their influence on nodulation of legumes**



**Fig. 3. Rhizobitoxine inhibition of enzymes involved in pathway of ethylene biosynthesis (Yasuta *et al*, 1999)**

Contrary to phytotoxic effects of rhizobitoxine on soybean, Owens *et al.* (1971) have demonstrated that rhizobitoxine inhibits the production of ethylene by measuring incorporation of C<sup>14</sup>-labelled methionine into ethylene. The rhizobitoxine is reported to inhibit  $\beta$ -cystathionase in methionine biosynthesis pathway and ACC synthase in the ethylene pathway (Giovanelli *et al.*, 1972; Yasuta *et al.*, 1999) (Fig. 3). The ethylene inhibitor, AVG, is a structural ethoxy analogue of rhizobitoxine also found to inhibit ACC synthase. The above statements explain role of rhizobitoxine in inhibition of ethylene in root nodules of legumes.

Now, rhizobitoxine is also described as a nodulation enhancer in legume- *Rhizobium* symbiosis (Yuhashi *et al.*, 2000). They reported that rhizobitoxine enhances nodulation and competitiveness of *B. elkanii* on *Macroptilium atropurpureum* (sirato) possibly by reducing level of endogenous ethylene in the host plant. In an experiment conducted by Yuhashi *et al.* (2000), wherein when a wild type *B. elkanii* USDA 94 and rhizobitoxine-deficient mutant of *B. elkanii* USDA94 inoculated simultaneously to *M. atropurpureum*, the wild type was found to be more competitive than mutant. This wild type strain is also able to reduce ethylene levels in roots. This suggests that the production of rhizobitoxine enhances both the nodulation and competitiveness of *B. elkanii* USDA94 because of its inhibitory effect of ethylene synthesis in plant roots (Yuhashi *et al.*, 2000). Rhizobitoxine produced by *B. elkanii* USDA61 appeared to act as a nodulation enhancer for *M. atropurpureum*

and *Vigna radiata* (Duodu *et al.*, 1999) but did not have a positive effect on nodulation of soybean cultivars. Thus, it is suggested that some other sort of regulation is operating in soybean. To elucidate the mechanism by which ethylene inhibits nodulation, a mutated ethylene receptor gene transformed *Lotus japonicus* B-129 with reduced ethylene sensitivity was developed. Inoculation of *Mesorhizobium loti* to the transformed *L. japonicus* remarkably enhanced nodule primordia on host root hairs and also number of infection threads when compared to the wild *L. japonicus*. The above said experiment explains how does endogenous ethylene levels in *L. japonicus* inhibits the formation of nodule primordia and infection processes (Nukui *et al.*, 2004).

#### **ACC deaminase production by PGPR and rhizobia**

ACC deaminase was first isolated in 1978 from *Pseudomonas* sp. ACP and from the yeast, *Hansenula saturnus*, reclassified as *Williopsis saturnus* (Honma, 1983), since then ACC deaminase has been detected in some fungi and many soil-borne bacteria (Table 2) (Campbell and Thompson, 1996; Glick *et al.*, 1995; Ghosh *et al.*, 2003; Jacobson *et al.*, 1994). This enzyme catalyzes the ACC degradation to produce ammonia and  $\alpha$ -ketoglutarate (Honma and Shimomura, 1978). It is proposed that uptake and cleavage of ACC by plant promoting bacteria can lower plant ethylene levels in developing or stressed plants (Glick *et al.*, 1998). In a model proposed by Glick and coworker (1995), bacteria attached to the surface of root of developing plant take-up some of the ACC exuded from plant and degrade

it through the action of ACC deaminase and thus bacteria act as sink for ACC. In order to maintain equilibrium between internal and external ACC levels, more ACC is exuded by the plants. As a consequence decrease the level of ACC inside plant cell and thereby reduce the production of ethylene (Fig. 4). Thus, if plant inoculated with ACC deaminase producing PGPR have longer

roots in gnotobiotic conditions (Glick *et al.*, 1999) and are able to resist the inhibitory effects of stress ethylene that is synthesized as a consequence of stressful conditions such as heavy metals (Burd *et al.*, 2000), phytopathogens (Wang *et al.*, 2000) and flooding (Grichko and Glick, 2001), drought and high salt.

**Table 2. Some bacteria and fungi other than rhizobia possess ACC deaminase activity**

Microorganisms	Reference
<b>Bacteria</b>	
<i>Pseudomonas chloroaphis</i>	Drahos <i>et al.</i> 1998; Klee and Kishore, 1992
<i>P. putida</i>	Glick <i>et al.</i> , 1995; Shah <i>et al.</i> , 1997
<i>P. marginalis</i>	Belimov <i>et al.</i> , 2001
<i>P. fluorescense</i>	Campbell and Thompson, 1996
<i>P. oryisihabitans</i>	Belimov <i>et al.</i> , 2001
<i>Enterobactor cloacae</i>	Glick <i>et al.</i> , 1995; Shah <i>et al.</i> , 1997
<i>Escherichia coli</i>	Itoh <i>et al.</i> , 1996
<i>Kluyvera ascorbata</i>	Burd <i>et al.</i> , 1998
<i>Alkaligenes xylosoxidans</i>	Belimov <i>et al.</i> , 2001
<i>Rhodococues sp.</i>	Belimov <i>et al.</i> , 2001
<i>Bacillus pumilus</i>	Belimov <i>et al.</i> , 2001
<i>B. circulans</i>	Ghosh <i>et al.</i> , 2003
<i>B. firmus</i>	Ghosh <i>et al.</i> , 2003
<i>B. globisporus</i>	Ghosh <i>et al.</i> , 2003
<b>Fungi</b>	
<i>Hansenula saturnus</i>	Honma and Shimomura, 1978; Minami <i>et al.</i> , 1998
<i>Penicillium citrinum</i>	Honma, 1993; Jia <i>et al.</i> , 1999
The ability of rhizobitoxine synthesis has been reported to confine only in slow-growing <i>B. elkanii</i> but not in fast-growing rhizobia and thus it is assumed that some other mechanisms might be operating in these rhizobia to reduce ethylene (Sugawara <i>et al.</i> , 2006). First report of presence of ACC deaminase in rhizobia has given by Ma <i>et al.</i> (2003a) wherein five fast rhizobial strains viz. <i>Rhizobium</i>	<i>leguminosarum</i> bv <i>viciae</i> 128 C53, <i>R. leguminosarum</i> bv <i>viciae</i> 128 C53G, <i>R. leguminosarum</i> bv <i>viciae</i> 99A1, <i>R. leguminosarum</i> bv <i>phaseoli</i> 657, <i>R. hedysari</i> , out of thirteen tested were found positive for ACC deaminase. It is, now, confirmed that nodulation and nitrogen fixation by <i>R. leguminosarum</i> bv <i>viciae</i> can be enhanced by reducing the ethylene level in roots (Ma <i>et al.</i> , 2003b). To support the

involvement of ACC deaminase on nodulation process of *R. leguminosarum* bv *viciae*, two knockout mutants of 128 m (AcDs) which do not produce ACC deaminase and a mutant which over-expressed this protein were

examined in nodulation assays with *Pisum sativum* cv. Sparkle. Both these knockout mutants without ACC deaminase activity reduced nodule number, nitrogen fixation and shoot dry weight of plant.

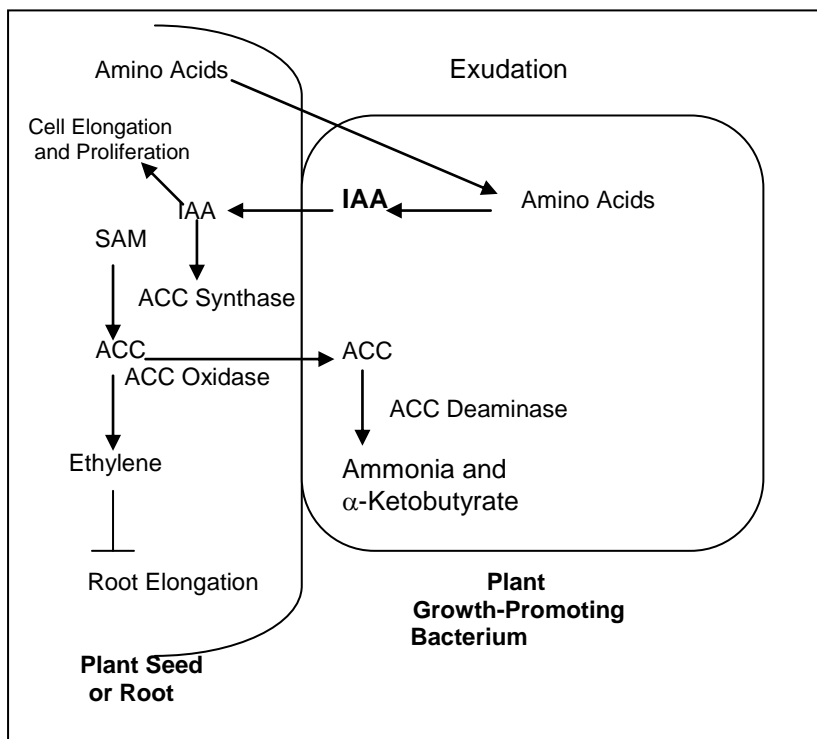


Fig. 4. A model represents role of ACC deaminase in promotion of plant root elongation (Glick *et al.*, 1998)

In addition to the ACC deaminase gene in *R. leguminosarum* bv *viciae* 128 C53K, there are four other potential ACC deaminase genes that were revealed by genome sequencing of four rhizobial strains, *Agrobacterium tumefaciens* C58, *Bradyrhizobium japonicum* USDA 110 and *Mesorhizobium loti* MAFF 303099 and ICMP 3150. The deduced amino acid sequences of these different ACC deaminase genes are highly conserved. It is interesting to note that gene encoding the ACC deaminase in *M.*

*loti* ICMP 3153 and DNA fragment encoding the truncated ACC deaminase in *Sinorhizobium* sp. strain NGR 234 are located on symbiotic island (Sullivan *et al.*, 2002) and the symbiotic plasmid, respectively, in the bacteria. It was found that *R. leguminosarum* bv *viciae* 128 C53K *acdS* gene is located on one of the endogenous large plasmid (> 100 kb) as revealed by southern hybridization. Thus, this suggests the possibility that there was horizontal transfer of the putative ACC

deaminase gene transfer from other microorganisms particularly other soil bacteria. After rhizobia obtained the ACC genes by this means, there regulation system evolved later depending on different response of the host legume to ethylene. For instance nodulation of soybean is insensitive to ethylene (Schmidt *et al.*, 1999) but, in contrast, *Pisum sativum* is ethylene sensitive (Ma *et al.*, 2003b). Moreover, ethylene is essential for nodulation in *Sesbania* (D' Haeze, 2001).

As it is expected that rhizobia that do not contain ACC deaminase are unable to nodulate their cognate legume to the same extent to that if they possess this enzyme. Moreover, it would be possible to genetically manipulate strains that normally lack ACC deaminase with the gene encoding this enzyme with the expectation that the transformed strain will nodulate its cognate legume to a greater extent than the non-transformed strain. For example, *Sinorhizobium meliloti* Rm1021, which does not have ACC deaminase activity, can nodulate its host legume, alfalfa most efficiently and competitively when it transformed with the *acdS* and *lrpL* genes from *R. leguminosarum* bv *viciae* 128C 53K (Ma *et al.*, 2003b).

In conclusion, presence of rhizobitoxine, an inhibitor and ACC deaminase, an enzyme, in rhizobia can decrease production of ethylene in host plants and consequently improve nodulation. However, not all rhizobial strains possess these mechanisms; it may be possible to transform them with gene encoding rhizobitoxine or ACC deaminase to enhance other nodulation efficiency in legumes. Although, nodulation of soybean is

insensitive to ethylene concentration and thus it may be regulated by other mechanism but not by ACC deaminase-producing bacteria. Nevertheless, some of the ACC deaminase-producing bacteria are being tested to promote growth of soybean in the field.

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## Character Association and Path Coefficient Analysis in Advance Breeding Lines of Soybean [*Glycine max* (L.) Merrill.]

KAMAL PANDEY<sup>1</sup>, KAMENDRA SINGH<sup>2</sup>, B V SINGH<sup>3</sup>, PUSHPENDRA<sup>4</sup>, M K GUPTA<sup>5</sup> AND NARENDRA SINGH YADAV<sup>6</sup>

Department of Genetic and Plant Breeding, College of Agriculture,  
G. B. Pant University of Agriculture and Technology,  
Pantnagar-263 145 (U.S. Nagar, Uttarakhand)  
(E-mail: k.genetics@rediffmail.com)

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### ABSTRACT

Character association and path coefficient analysis was carried out using 59 genotypes of soybean [*Glycine max* (L.) Merrill.] derived from 23 diverse crosses and 3 checks for 12 component characters including seed yield during kharif, 2005 at the soybean breeding block of the Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. Dry matter per plant, harvest index, seed yield efficiency, pods per plant, plant height, hundred seed weight and number of primary branches per plant had significant and positive correlation with seed yield both at genotypic and phenotypic level, while number of seeds per pod showed significant and negative correlation with seed yield at genotypic level. Path coefficient analysis showed that, among all the traits studied, dry matter per plant contributed most directly to the seed yield.

**Keywords:** Soybean, character association, path-coefficient analysis, seed yield

Soybean [*Glycine max* (L.) Merrill.], an important leguminous crop, is recognized as golden or miracle bean due to its high nutritive value and various uses, viz., for feed, oil and soy food products. It is rich in oil (18-22 %) and protein (38-42 %). Soybean ranked first in the world in oil production (57 %) in the international trade markets among the major oilseed crops, viz., cottonseed, peanut, sunflower seed, rapeseed, coconut etc. In India area under soybean cultivation is about 8.87 million hectares and production is about 9.46 million tonnes, whereas its contribution to total production of oil seeds and total oil availability from 9 major oil seeds would be about 37 per cent and 25 per cent, respectively (Anonymous, 2008). Soy oil contains 85 per cent unsaturated fatty acids that include high

<sup>1,2,4,5</sup> Professor; <sup>3</sup>Ex-Professor; <sup>6</sup>Technical Assistant

content of essential fatty acids such as linoleic acid and linolenic acid. Productivity of soybean is very low in India; about 1t per ha as compared to world's average yield of 2.240 t per ha. Therefore, organized and concerted efforts are required to enhance its productivity. Yield is a polygenic trait and function of various traits. Thus, direct selection would not be a reliable approach on account of its being highly influenced by environmental factors. The knowledge of the association between yield and its components and among components themselves is of immense practical value in crop improvement through selection. Path coefficient analysis (Wright, 1921) brings out the direct and indirect effects of component traits on yield. The present investigation was carried out with 62 genotypes of soybean to explore the association of certain characters, their direct contribution to yield and indirect effects through other characters on yield.

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## MATERIAL AND METHODS

The present investigation was carried out during *kharif*, 2005 at the soybean breeding block of the Crop Research Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. Experimental material comprised 59 bulks (representing F<sub>5</sub>-F<sub>8</sub> generations) derived from 23 diverse crosses along with 3 checks. The experiment was sown in a completely randomized block design with two replications. Each plot had three rows of four meter length spaced at 45 cm apart

and within row spacing was 7-10 cm. The N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O were applied in a ratio 20: 60: 40 before sowing and standard agronomic practices were followed to raise a normal crop. Observations were recorded from the central row of each plot on five random competitive plants. The characters studied were plant height, number of primary branches per plant, number of pods per plant, number of seeds per pod, basal pod height, hundred seed weight, dry matter per plant, seed yield per plant, harvest index and seed yield efficiency (represented as a ratio of grain yield to non seed dry matter weight). Days to fifty per cent flowering and maturity data were recorded on whole plot basis. The mean values of each character were subjected to analysis. The analysis of variance (ANOVA) was done with the help of statistical method described by Panse and Sukhmate (1969). The genotypic and phenotypic correlations were estimated according to the method given by Searle (1961) and path analysis was done according to the method of Dewey and Lu (1959).

## RESULTS AND DISCUSSION

On the basis of statistical analysis, ANOVA revealed significant differences among the genotypes for all the characters, except seeds per pod. This indicated the existence of significant amount of variability among the genotypes for all the characters studied. In general, genotypic correlations were higher than phenotypic correlations. Directions of phenotypic and genotypic correlations were almost same for all the character combinations. This indicated the role of environment in the expression of character, which alters magnitude of association between the characters, was low.

**Table 1. Inter character association (phenotypic and genotypic levels) between different character pairs in soybean**

Character	Days to 50% flowering	Days to maturity	Pods/ plant	Seeds/ pod	Plant height	Primary Branches / plant	Basal pod height	100-seed weight	Dry matter/ plant	Seed yield/ plant	Harvest index	Seed yield efficiency
Days to 50% flowering		-0.0722 (-0.0380)	-0.1211 (-0.1404)	-0.0107 (0.3823**)	-0.1204 (-0.1297)	0.1522 (0.1637)	-0.1461 (-0.1854)	0.0591 (-0.0167)	-0.1142 (-0.1300)	-0.1237 (-0.1258)	-0.0472 (-0.0399)	-0.0584 (-0.0541)
Days to maturity			0.0057 (0.0151)	-0.0796 (-0.2200)	-0.0040 (-0.0003)	0.0180 (0.0026)	-0.0407 (-0.0210)	0.0552 (0.1013)	-0.0805 (-0.0821)	-0.0120 (-0.0094)	0.0851 (0.0878)	0.0692 (0.0693)
Pods/ plant				-0.2226 (-0.5405**)	0.9751** (0.9813**)	0.4395** (0.5117**)	0.0653 (0.0789)	0.4024** (0.5097**)	0.8359** (0.8404**)	0.9578** (0.9622**)	0.4456** (0.4560**)	0.4766** (0.4884**)
Seeds/ pod					-0.2189 (-0.5446**)	-0.1222 (-0.5315**)	0.1774 (0.6005**)	-0.0596 (-0.4087**)	-0.1201 (-0.3031 *)	-0.2252 (-0.5871**)	-0.2480 (-0.6170**)	-0.2373 (-0.5834**)
Plant height						0.4553** (0.5247**)	0.0306 (0.0406)	0.3751** (0.4736**)	0.8457** (0.8430**)	0.9869** (0.9898**)	0.4717** (0.4882**)	0.5051** (0.5230**)
Primary branches/ plant							-0.2900 * (-0.3891**)	0.2490 (0.2701 *)	0.4212** (0.4707**)	0.4554** (0.5167**)	0.1790 (0.2172)	0.2034 (0.2494)
Basal pod height								0.0688 (-0.0401)	-0.0210 (-0.0240)	0.0088 (0.0116)	0.0371 (0.0460)	0.0541 (0.0573)
100-seed weight									0.3021 * (0.3877**)	0.3717** (0.4701**)	0.2046 (0.2672 *)	0.2363 (0.3110 *)
Dry matter/ plant										0.8463** (0.8439**)	-0.0395 (-0.0333)	-0.0018 (0.0082)
Seed yield/ plant											0.4866** (0.4982**)	0.5189** (0.5332**)
Harvest index												0.9896** (0.9940**)

Genotypic correlation values in parenthesis; \*: significant at 5 % level of significance; \*\*: significant at 1 % level of significance

**Table 2. Path coefficient analysis showing the direct and indirect effect of twelve characters on seed yield at genotypic level**

Character	Indirect effect											
	Correlation with grain yield	Days to 50% flowering	Days to maturity	Pods/plant	Seeds/pod	Plant height	Primary branches/plant	Basal pod height	100-seed weight	Dry matter/plant	Harvest index	Seed yield/plant
Days to 50% flowering	-0.125	<b>-0.03200</b>	-0.00080	0.03100	0.02900	-0.05700	0.00100	0.00600	-0.00040	-0.08800	-0.02600	0.01200
Days to maturity	-0.009	0.00100	<b>0.01900</b>	-0.00300	-0.01600	-0.00010	0.00003	0.00080	0.00200	-0.05600	0.05800	-0.01500
Pods/plant	0.962**	0.00400	0.00030	<b>-0.22000</b>	-0.04100	0.43700	0.00500	-0.00200	0.01100	0.57300	0.30200	-0.10900
Seeds/pod	-0.587**	-0.0200	-0.00440	0.11900	<b>0.07600</b>	-0.24200	-0.00600	-0.02100	-0.00900	-0.20700	-0.40900	0.13000
Plant height	0.989**	0.00400	-0.00001	-0.21700	-0.04100	<b>0.44500</b>	0.00600	-0.00100	0.01100	0.57500	0.32300	-0.11700
Primary branches/plant	0.516**	-0.00500	0.00010	-0.11300	-0.04000	0.23300	<b>0.01100</b>	0.01400	0.00600	0.32100	0.14400	-0.05600
Basal pod height	0.011	0.00600	-0.00040	-0.01700	0.04500	0.01800	-0.00400	<b>-0.03600</b>	-0.00090	-0.01600	0.03000	-0.01300
100-seed weight	0.470**	0.00050	0.00200	-0.11300	-0.03100	0.21100	0.00300	0.00100	<b>0.02300</b>	0.26400	0.17700	-0.07000
Dry matter/plant	0.843**	0.00400	-0.00160	-0.18600	-0.02300	0.37500	0.00500	0.00090	0.00900	<b>0.68200</b>	-0.02200	-0.00200
Harvest index	0.498**	0.00100	0.00170	-0.10100	-0.04700	0.21700	0.00200	-0.00170	0.00600	-0.02200	<b>0.66300</b>	-0.22200
Seed yield efficiency	0.533**	0.00100	0.00130	-0.10800	-0.04400	0.23300	0.00200	-0.00210	0.00700	0.00500	0.65900	<b>-0.22300</b>

Seed yield per plant showed highly significant positive correlations with harvest index, seed yield efficiency, dry matter per plant, hundred seed weight, number of primary branches per plant, plant height and number of pods per plant at both phenotypic and genotypic levels (Table 1). Similar results were reported for number of primary branches per plant and hundred seed weight by Singh and Singh (1996), for harvest index by Mehtre *et al.* (1997), for dry matter by Chamundeswari and Aher (2003) and for plant height, branches per plant, pods per plant, hundred seed weight, biological yield per plant and harvest index by Bhushan *et al.* (2006). Seed yield per plant also showed highly significant negative correlation with number of seeds per pod at the genotypic level only.

Correlation does not provide the true contribution of the characters towards the yield; therefore; the path coefficient analysis was used to partition the correlation coefficients with seed yield, into direct and indirect effects (Table 2). Dry matter per plant had the highest direct effect (0.682) on seed yield per plant followed by harvest index (0.663) and plant height (0.445).

Dry matter per plant, plant height and harvest index showed highly significant positive correlation with seed yield and this was due to the direct effect of these characters. Hundred seed weight showed highly significant positive correlation with seed yield, which was the result of direct

effect of hundred seed weight supported by indirect effect of dry matter weight/plant and plant height. Number of pods per plant had highly significant positive correlation with seed yield, but the direct effect of this character was negative which counter balanced by positive indirect effect of dry matter and plant height (Iqbal *et al.*, 2003). Thus, the number of pods was effective in determining the yield *via* dry matter weight per plant and plant height. Seed yield efficiency had highly significant positive correlation with seed yield but its direct effect was found negative which counter balanced by positive indirect effect of harvest index. Thus, dry matter assumes major role in determining yield in soybean followed by plant height and harvest index, similar results were also reported by Patirana and Guzhov (1979). It was observed that for most of the characters dry matter is effective in determining yield either through direct or indirect effects.

Therefore, it is suggested that dry matter per plant, plant height and harvest index should have prime consideration (Chamundeswari and Aher, 2003; Bhushan *et al.*, 2006). Dry matter per plant is further important as it had highly significant phenotypic and genotypic correlation with number of pods per plant, plant height, number of primary branches per plant and seed yield per plant; which have been proved to be an important seed yield components.

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## **Performance of Soybean [*Glycine Max* (L.) Merrill] Varieties under Different Sowing Dates during *Rabi* in Vertisols of Krishna Zone in Andhra Pradesh**

**B PRAMILA RANI<sup>1</sup>, M V RAMANA<sup>2</sup> AND B KRISHNAVENI<sup>3</sup>**

*All India Coordinated Project on Soybean, Regional Agricultural Research Station, Lam, Guntur-522 034, Andhra Pradesh  
(E-mail: pramilaranib@yahoo.com)*

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### **ABSTRACT**

*Field experiment was conducted during the rabi season of 2006-07 and 2007-08 at the Regional Agricultural Research Station, Lam, Guntur with three soybean genotypes planted at four different dates of sowing to assess the optimum time of sowing soybean during rabi with protective irrigation. Soybean sown around 15<sup>th</sup> September and 25<sup>th</sup> September gave significantly higher seed yield as compared with two October sowings. The yield attributes viz., number of pods per plant and 100 seed weight contributed to higher seed yield. Pre-release soybean genotype LSb 23 was comparable with JS 335 and was significantly superior over PS 1029.*

**Key words:** Sowing date, *rabi*, soybean

Soybean is mostly grown as *kharif* crop with the onset of monsoon and the optimum time of sowing for soybean varies between June and July in different parts of Andhra Pradesh. However, in the Krishna zone of Andhra Pradesh, the June - July sown soybean generally gets caught in rains at harvest and the crop duration is prolonged. Moreover field weathering sets in leading to loss of seed quality (Joshi and Bhatia, 2003). This region receives 200-300 mm rain through N-E monsoon during October- November

months, which makes *rabi* crops feasible in black soils under rainfed conditions. Soybean comes up well as a pre-*rabi* or as a *rabi* crop in this zone with one or two protective irrigations. Hence, there is a need to study the optimum time of sowing soybean during *rabi* and to evaluate suitable soybean variety for *rabi* to get maximum yield with good quality seed. This will make it possible to increase soybean area in non-traditional season to augment production (Bhatnagar and Tiwari, 1993).

<sup>1</sup>Senior Scientist (Agronomy,) presently working at Agricultural Research Station, Garikapadu-521175, Krishna district, A.P.; <sup>2</sup> Senior Scientist (Plant Breeding); <sup>3</sup> Scientist (Plant Breeding)

## MATERIAL AND METHODS

A field experiment was conducted at Regional Agricultural Research Station, Lam, Guntur, Andhra Pradesh (16°18' N; 80°29' E) during the *rabi* season of 2006-07 and 2007-08. The experimental soil was clay in texture, slightly alkaline (pH 7.6) and low in available nitrogen (220 kg N/ha), medium in available phosphorus (40.2 kgP<sub>2</sub>O<sub>5</sub>/ha) and high in available potash (538 kg K<sub>2</sub>O/ha). The treatments consisted of four dates of sowing (16.09.2006/11.09.2007; 29.09.2006/22.09.2007; 22.10.2006/10.10.2007; 13.11.2006/25.10.2007) as main plot treatments and three varieties viz., JS 335, PS 1029, LSb 23 as sub-plot treatments. The experiment was laid out in split-plot design with three replications. Soybean was sown at the spacing of 30 cm x 7.5 cm. Fertilizers were supplemented @ 30:60:40 kg of N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O per ha. The crop was sown on the onset of monsoon under rainfed conditions at all the sowing dates. However, irrigation was given at later growth stages as and when required. The normal annual rainfall of the region is 925

mm; of which 65 per cent is received during *kharif* (June to September) and 26 per cent during *rabi* (October to December) seasons.

The observations on plant height, pods per plant and 100 seed weight were recorded at harvest. The yield data was recorded and expressed as kg per hectare. The pooled data for the two years of experimentation was analyzed using standard statistical procedure.

## RESULTS AND DISCUSSION

### Effect of sowing dates

Soybean seed yield was significantly influenced by dates of sowing and varieties (Table 1). The highest seed yield was recorded in crop sown on 4<sup>th</sup> week of September (29.09.2006/22.09.2007), which was at par with 2<sup>nd</sup> week of September (16.9.06/11.09.07) sown crop. Soybean crop sown later dates showed a decline in yield by 20 per cent (22.10.06/10.10.07) and 40 per cent (13.11.06/25.10.2007) as compared to highest yield achieved in crop sown on 4<sup>th</sup> week of September (Table 1).

**Table 1. Soybean seed yield as influenced by sowing dates and varieties during Rabi (Pooled two year data)**

Variety	Date of sowing				Mean
	16.09.2006/ 11.09.2007	29.09.2006/ 22.09.2007	22.10.2006/ 10.10.2007	13.11.2006/ 25.10.2007	
JS 335	1702	1728	1419	1183	1508
PK 1029	1474	1614	1272	952	1328
LSb 23	1806	1982	1557	1057	1601
Mean	1661	1775	1416	1064	
	Date of Sowing	Variety	Date of Sowing x Variety		
SEm (±)	54	49	69		
CD (p = 0.05)	187	146	NS		

The expression of higher yield can be substantiated by higher values of yield attributing characters like plant height and number of pods per plant (Table 2). The reduction in plant height and total biomass of plant with delayed time of sowing was reported earlier by Pramila Rani and Kodanda Ramaiah (1999) and Singh *et al.* (2000). However, the 100 seed weight of later sowing dates was significantly higher than the early dates which may be due to decreased number of pods in the late sown crop. Similar results were also reported by Yadav and Sharma (2000).

#### Effect of varieties

Of the three varieties evaluated, the soybean genotype LSb 23 (numerically 6 % higher) recorded

comparable seed yield with variety JS 335 (1508 kg/ha). Both the varieties yielded significantly higher than variety PS 1029 (Table 1). Number of pods per plant and 100 seed weight, respectively appears to be contributory in better yield expression of JS 335 and LSb 23 over PS 1029 (Table 2).

#### Interaction effect

The interaction of sowing dates with varieties was not found significant for seed yield and yield attributes except the number of pods per plant. The soybean genotype, JS 335, sown around 15<sup>th</sup> September recorded significantly more number of pods per plant as compared with the other two genotypes and sowing dates (Table 2).

**Table 2. Soybean yield attributes as influenced by sowing dates and varieties during *rabi* (Pooled)**

Treatment	Plant height (cm)	Pods (No/ plant)	100 seed weight (g)
<b><i>Date of sowing</i></b>			
16.09.2006/ 11.09.2007	28.2	27.9	13.4
29.09.2006/ 22.09.2007	27.3	23.1	13.3
22.10.2006/ 10.10.2007	25.5	21.7	13.7
13.11.2006/ 25.10.2007	24.1	19.5	14.1
SEm (+)	0.76	1.5	0.17
<b>CD(P=0.05)</b>	<b>2.6</b>	<b>5.2</b>	<b>0.6</b>
<b><i>Variety</i></b>			
JS 335	26.9	26.4	12.7
PK 1029	24.5	20.1	13.6
LSb 23	27.5	22.7	14.6
SEm (+)	0.56	0.77	0.14
<b>CD(P=0.05)</b>	<b>1.7</b>	<b>2.3</b>	<b>0.4</b>
<b>Interaction</b>			
SEm (+)	0.79	1.08	0.2
<b>CD (P=0.05)</b>	<b>NS</b>	<b>3.3</b>	<b>NS</b>

The results of the investigation establishes that soybean as *rabi* crop sown during later half of September in the black soil regions of Krishna zone of Andhra Pradesh to get higher yield with good seed quality. The genotype LSb 23 was comparable with the best adopted variety of the region i.e. JS 335 and provides opportunity for varietal diversification. The adoption of crop in non-traditional season in the said region can augment area and production of crop in this upcoming state.

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## **Genotype x Environment Interaction Analyses for Seed Yield and Various Characters in Rainfed Soybean (*Glycine max*. (L.) Merrill)**

**S HOLKAR<sup>1</sup>, INDU SWAROOP<sup>2</sup>, O P GIROTHIA<sup>3</sup> AND S K SHARMA<sup>4</sup>**

*All India Coordinated Research Project on Dryland Agriculture,*

*College of Agriculture (JNKVV), Indore 452 001*

*(E mail : holkar.s@rediffmail.com)*

*Received : 02.12.2006*

### **ABSTRACT**

*Nine promising soybean genotypes belonging to different maturity groups were tested for seed yield, water use efficiency and other attributes under rainfed conditions during 2003 to 2005. Impact of environment (linear) was significant on most of the characters except for leaf area per plant and harvest index. Genotype x environment (linear) interaction was also significant for days to flower initiation, physiological maturity and relative water content (RWC) indicating predominance of linear component over nonlinear component. Genotype JS 93 05 was the only stable genotype, which had consistency in performance for the characters, namely days to flowering, days to maturity and reproductive phase. Mean relative water content at podding stage ranged from 67.9 per cent (NRC 12) to 83.1 per cent (JS 93 05). Genotypes JS 93 05, JS 90 41 and NRC 12 showed stable performance over years for water use efficiency. Soybean genotypes namely NRC 37 (2117 kg/ha) followed by JS 335 (1839 kg/ha), JS 93 05 (1689 kg/ha) and NRC 12 (1662 kg/ha) were found stable for yield under all the environmental conditions.*

**Key words:** Seed yield, soybean, physiological maturity, relative water content, water use efficiency

Soybean [*Glycine max* (L.) Merrill] is predominantly cultivated as a rainfed crop in central India on Vertisols (Sharma and Dixit, 1988). The performance of the varieties under rainfed farming system is mainly a function of water use efficiency, relative water content, days to reproductive phase and other contributing characters during crop growth period that help in producing phyto-mass and seed yield. The soybean yield is a function of time of onset of monsoon, its quantum and distribution during crop growth period. Singh (1988) also emphasized on sustainable

<sup>1,3</sup>Scientist; <sup>2,4</sup>Senior Scientist

productivity through efficient water and nutrient utilization. Stability analyses for various yield-contributing characters along with water use efficiency and relative water content which contribute towards drought management would be desirable. Therefore, the present study was undertaken to evaluate the performance of promising soybean genotypes grown under rainfed conditions of *Malwa* plateau of Madhya Pradesh.

## MATERIAL AND METHODS

Nine soybean genotypes, viz. JS 335, JS 95 60, JS 90 91, JS 93 05, JS 90 41, MAUS 47, Samrat, NRC 12 and NRC 37 belonging to different maturity groups were evaluated for consecutive three years i.e. from 2003 to 2005 at Dryland Agriculture Farm, College of Agriculture, Indore. Nine treatments were arranged in a randomized block design and replicated four times. Soybean was planted on 25<sup>th</sup> June 2003, 28<sup>th</sup> June 2004 and 2<sup>nd</sup> July 2005 with the onset of monsoon. Each plot (4.5 m x 3.20 m) comprised eight rows of soybean

spaced at 40 cm. Water use efficiency was determined for each year using simple water budget method taking into account the seasonal rainfall, profile moisture content up to 90 cm depth at the time of sowing and harvesting and addition/depletion of water. The details of the rainfall received during the crop growth period and profile moisture contents over years are presented in table 1. During the year 2003 and 2004, there was only one dry spell of seven days and fourteen days respectively, which occurred in the second fortnight of July. In the year 2005, two dry spells during 33<sup>rd</sup> and 35<sup>th</sup> SMW were experienced. Relative water content (RWC) was calculated as per the formula  $RWC (\%) = \frac{\text{fresh weight of leaves} - \text{dry weight of leaves}}{\text{turgid weight of leaves} - \text{dry weight of leaves}} \times 100$  (Barrs and Weatherly, 1962). The observations on yield and yield attributes were recorded at the time of harvest. The stability analyses were carried out as per Eberhart and Russell (1966).

**Table 1. Soil profile moisture content and Rainfall received during crop growth period (2003-05)**

Parameters	Year		
	2003	2004	2005
Total rainfall received during the year (mm)	982.0	846.8	740.8
Rainfall received during crop growth period (mm)	963.5	699.0	664.0
Soil moisture (90 cm depth) at the time of sowing (mm)	304.7	378.4	328.8
Soil moisture (90 cm depth) at the time of harvest (mm)	331.4	213.2	221.5
Addition/depletion of moisture (mm)	26.7	165.2	107.3
Water use by soybean	936.8	864.2	771.2

## RESULTS AND DISCUSSION

The pooled data showed highly significant differences among the genotypes for most of the characters under study except harvest index. Soybean genotype NRC 37 possessed the significantly longest juvenile period followed by JS 335 and NRC 12, JS 90 91 and MAUS 47 while the shortest juvenile period was recorded in JS 95 60, which was closely followed by JS 93 05, JS 90 41 and Samrat. A similar trend was also observed in case of physiological maturity. The relative water content in soybean genotypes was higher at podding as compared to flowering. The significantly highest relative water content was recorded in NRC 12 and JS 93 05 at flowering and podding, respectively. Genotype MAUS 47 and NRC 12 showed the lowest relative water content at flowering and podding. The highest photosynthetic area was observed

in NRC 37, which was at par with NRC 12 and MAUS 47. The significantly lowest photosynthetic assimilation area was noted in JS 93 05. Significantly highest pods per plant was noted in genotype JS 95 60 and was at par with the rest of the genotypes except JS 90 91, JS 93 05 and JS 90 41. The highest seed yield was recorded in NRC 37 which differed non-significantly with JS 335 (Table 2). The lowest yield was recorded in JS 95 60 and was statistically at par with rest of the genotypes. Genotypes Samrat, JS 93 05, NRC 12 and JS 90 91 achieved good yield potential (1749 to 1576 kg/ha) but did not differ significantly among themselves. In general late maturing (>35 days to flower initiation and >90 days to physiological maturity) genotypes NRC 37, JS 335 and NRC 12 gave higher yield as compare to early maturing (30 days to flower initiation and 82 days to physiological maturity) ones namely, JS 95 60, JS 93 05, JS 90 41 and Samrat.

**Table 2. Mean performance of genotypes for seed yield and other yield contributing characters of soybean over years. (2003-05)**

Genotypes	Days to flower initiation	Days to physiological maturity	RWC (%) on flowering	RWC (%) on podding	Leaf area/plant (cm <sup>2</sup> )	No. of pods/plant	Harvest Index (%)	WUE (kg/ha /mm)	Seed yield (kg/ha)
JS 335	37.9	91.8	53.9	80.7	940.8	52.5	37.31	2.00	1839
JS 95 60	30.1	80.7	50.6	77.0	733.7	54.9	34.33	1.52	1395
JS 90 91	34.7	85.4	50.7	77.5	976.7	44.2	35.36	1.70	1576
JS 93 05	31.0	82.7	51.7	83.1	590.9	43.1	33.89	1.83	1689
JS 90 41	31.7	82.7	51.1	76.7	698.7	42.6	32.26	1.52	1411
MAUS 47	34.3	82.4	48.1	75.3	1088.0	45.3	33.42	1.71	1587
Samrat	31.0	82.3	51.3	72.8	912.9	48.4	34.09	1.89	1749
NRC 12	36.1	95.2	59.1	67.9	1080.0	47.5	32.62	1.80	1662
NRC 37	42.3	98.3	56.0	77.3	1155.1	49.5	36.74	2.31	2117
Mean	34.3	86.8	52.4	76.5	908.6	45.3	34.44	1.80	1669
S Em (±)	0.7	0.45	0.83	1.57	46.38	4.3	4.68	0.12	118
CD (P = 05)	<b>2.1</b>	<b>1.31</b>	<b>2.43</b>	<b>4.60</b>	<b>135.4</b>	<b>12.5</b>	<b>NS</b>	<b>0.37</b>	<b>344</b>
CV %	4.1	1.01	3.36	4.13	10.2	9.8	20.8	11.6	11.7

The productivity of late maturing genotypes might be due to higher photosynthetic surface area, higher pod bearing abilities and longer reproductive phase. The genotypic differences in soybean under variable environmental

conditions have also been reported by Valerio *et al.* (2002), Murakami and Cruz (2004) and Yan and Hunt (1998). The water use efficiency of different soybean genotypes showed more or less similar trend as was observed in seed yield.

**Table 3. Grouping of soybean genotypes on the basis of regression coefficient and deviation from regression showing suitability for different environmental conditions**

Characters	Genotypes stable over environment ( $g_i > \text{mean}$ , $b_i = 1$ , $S^2d_i = 0$ )	Genotypes stable for poor environment ( $g_i > \text{mean}$ , $b_i < 1$ , $S^2d_i = 0$ )	Genotypes stable for favourable environment ( $g_i > \text{mean}$ , $b_i > 1$ , $S^2d_i = 0$ )
Days to flower initiation	JS 95 60	JS 90 91, JS 93 05, JS 90 41, Samrat, NRC 12, NRC 37	JS 335, JS 95 60
Days to physiological Maturity	JS 93 05, MAUS 47	JS 95 60, JS 90 91, JS 90 41, Samrat	JS 335, NRC 12, NRC 37
Days to reproductive phase	-	JS 95 60, JS 90 91, JS 93 05, JS 90 41, MAUS 47, Samrat	JS 335, NRC 12, NRC 37
RWC on flowering	-	JS 93 05, JS 90 41, MAUS 47, NRC 12	JS 335, JS 95 60 JS 90 91, Samrat, NRC 37
RWC on podding	JS 90 41, MAUS 47 NRC 12, NRC 37	JS 90 91, Samrat	JS 335, JS 95 60, JS 93 05,
Leaf area/plant		JS 335, JS 90 91, JS 93 05, JS 90 41, Samrat, NRC 12	JS 95 60 MAUS 47
Number of pods/plant	JS 335, MAUS 47	JS 95 60, NRC 12, NRC 37	JS 90 91, JS 93 05, JS 90 41, Samrat
Harvest Index (%)	-	JS 90 91, JS 93 05, Samrat, NRC 12	JS 335, JS 95 60, JS 90 41, MAUS 47, NRC 37
Water use efficiency (kg/ha/mm)	JS 93 05, JS 90 41, NRC 12	JS 335, JS 95 60, NRC 37	JS 90 91, MAUS 47 Samrat
Seed yield (kg/ha)	JS 93 05, JS 90 41, NRC 12, NRC 37	JS 335, JS 95 60	JS 90 91, MAUS 47 Samrat



None of the genotype showed stable performance for relative water content at flowering stage, indicated that soybean is more responsive to the environmental variation in terms of amount and distribution of rainfall, plant canopy and other influencing factors (Table 3). Stable performance for relative water content at podding showed that late maturing NRC 12 (67.9 %), NRC 37 (77.3 %) and early maturing MAUS 47 (75.3 %) and JS 90-41 (76.7 %) have wider adaptability for this character. The stability of relative water content in these genotypes could be due to better soil moisture drawing capacity at pod formation stage. Water use efficiency (WUE) of different genotypes ranged from 1.52 kg per ha per mm (early maturing JS 95 60) to 2.31 kg per ha per mm (late maturing NRC 37) with above average stability. Genotypes JS 93 05, JS 90 41 and NRC 12 showed stable performance for WUE over years. Sharma *et al.* (2003) also reported similar findings for this character in soybean. Higher value of pooled deviations than the G x E interaction (Linear) for water use efficiency and seed yield suggested that environmental factors were unpredictable in nature. On the contrary, Khurana and Yadav (1982) observed that the variation in performance of soybean varieties due to environment was predictable in nature. Similar findings were also reported by Manivannan *et al.* (1996) in case of green gram. Pooled deviation was significant for the character viz. RWC at podding, WUE and seed yield emphasized that unpredictable

nature of environmental factors played important role in governing these traits.

Considering the stability parameters, a genotype should have higher mean values, unit regression and least deviation from regression. On the basis of genotype x environment interactions, genotypes like MAUS 47, JS 90 91, Samrat and JS 90 41 did well under favourable environmental conditions (Table 3) while the remaining genotypes showed wider adaptability under unfavourable environmental conditions. Among the genotypes, JS 90 91 and JS 95 60 were found to possess highest and lowest regression coefficient (bi) respectively.

On the basis of three years results, soybean genotypes namely JS 93 05, JS 90 41, NRC 12, and NRC 37 showed stable performance over all the environments with reference to yield, while genotype JS 335 and JS 95 60 did well under harsh environmental conditions. Soybean genotypes viz. JS 90 91, MAUS 47 and Samrat required favourable environment for better performance.

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## **Evaluation of Potassium Uptake and Utilization Efficiency in Soybean Genotypes**

**S D BILLORE<sup>1</sup>, A RAMESH<sup>2</sup>, A K VYAS<sup>3</sup>, O P JOSHI<sup>4</sup> AND N PANDYA<sup>5</sup>**

*National Research Centre for Soybean, Khandwa Road, Indore 452 001,*

*Madhya Pradesh, India*

*(E-mail : billsd@rediffmail.com)*

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### **ABSTRACT**

*A pot culture experiment was conducted on Vertisols (Sarol series) with four levels of K (0, 7.41, 14.82 and 22.23 ppm) to assess the K response of seven soybean genotypes (JS 335, JS 93 05, JS 71 05, NRC 7, NRC 12, NRC 37 and Hardee) and their K use efficiency. The graded levels of K exerted influence on physical root parameter and the yield. An early maturing genotype, JS 93 05 with an edge over other genotypes in root length and root volume associated with maximum number of nodules indicated the capability of the variety to effectively draw the nutrient and moisture from the soil and higher symbiotic nitrogen trait. The lower values of rooting characteristics and lowest root to shoot ratio associated with late maturity duration genotype NRC 37 suggests that this variety may need optimum nutrition and adequate availability of moisture throughout the growing period to perform. All the growth parameters and soybean yield were linearly related to K application rates. The response of soybean genotypes to potassium suggests that it shall be appropriate to revise the present recommended level of K (7.41) applications to soybean. Since the agronomic and physical efficiency increased only up to 14.82 ppm, this level appears to be adequate.*

**Key words:** Potassium, soybean, use efficiency

There has been a growing concern about the low use efficiency of nutrients (Saurbeck and Hillel, 1990) which ranges from 2 to 50 per cent (Rai, 2008). The improvement of nutrient efficiency in crops is an important issue both, for reducing cost of agricultural production and for protecting the environment. In

Indian context, it is estimated that just by raising the nutrient use efficiency by 10 per cent, the country can save almost 20 million ha of land at the current level of productivity (Rai, 2008). The lower K use efficiency of K fertilizers applied to soybean (Carpenter, 1975) along with the sub-optimal recommendation of 20 kg K

<sup>1,2</sup> Senior Scientist; <sup>3,4</sup> Principal Scientist; <sup>5</sup> Technical Officer

per hectare (Tiwari *et al.*, 2001) against K uptake of 101-120 kg per hectare (Nambiar and Ghosh, 1984), the need for revision of recommendations was felt (Joshi, 2008). It is equally important to look for genotypes efficient in utilizing native/applied K from soil to use them appropriately for general cultivation as well as for their inclusion in crop improvement programmes. To readjust/refine the recommendation of K fertilizer application and to identify K efficient genotypes, the present investigation was taken up.

## MATERIAL AND METHODS

A pot culture experiment was conducted during *kharif* 2005 at National Research Centre for Soybean, Indore on *Sarol* soil series with the following pertinent soil characteristics: pH, 7.86; EC, 0.14 dS per m; organic carbon, 0.30 per cent; available P and K 4.80 and 120 kg per hectare, respectively to study the genotypic variation for potassium utilization. The treatments included seven soybean genotypes (JS 335, JS 93 05, JS 71 05, Hardee, NRC 7, NRC 12 and NRC 37) and four levels of potassium (0, 7.41, 14.82 and 22.33 ppm). All the 21 treatments were arranged in completely randomized design with three replications. Another set of experiment was also laid out for taking the observation at flowering. A uniform dose of nitrogen (8.93 ppm) and phosphorus (11.92 ppm) along with different K treatments was applied as basal. Recommended package of practices were followed for the successful raising of soybean crop.

Each pot was filled with ten kg soil. Five seeds were planted and three seedlings were raised up to maturity. Soybean was planted on 4<sup>th</sup> July 2005 and harvested at maturity of the respective genotypes. Observations on root and nodule parameters and shoot dry matter were taken at the time of flowering (R<sub>2</sub> stage). Yield and yield attributes were recorded at the time of harvest. Potassium content in seed, straw and soil were estimated by standard procedures. The partial factor productivity (seed yield with fertilized K/ amount of fertilizer K applied), K harvest index (K uptake by seed/ total K uptake by seed and straw), agronomic K efficiency (seed yield with fertilizer K - yield without K/ amount of applied fertilizer K) and physiological K efficiency (seed yield with fertilizer K - yield without K/total K uptake in fertilizer K - total uptake in without K uptake) were worked out.

## RESULTS AND DISCUSSION

A significant variation in the evaluated root and symbiotic parameters as influenced by genotype and K application (Table 1) was noted. Genotype JS 93 05 produced significantly higher root length at flowering over rest of the soybean genotypes. The minimum root length was in NRC 37 and remaining genotypes were identical in this character. The highest dry matter accumulation in the root was noticed with cultivar Hardee followed by JS 93 05. The highest root volume was recorded with JS 93 05, which was closely followed by NRC 12. The lowest root volume was recorded with NRC 37. The maximum nodule number was recorded with JS 93 05 followed by JS 335. However, reverse was true in case

Table 1. Root characteristics of soybean genotypes as influenced by graded levels of potassium

Treatment	Root length (cm)	Root dry weight (mg/plant)	Root volume (cm <sup>3</sup> )	Nodule number/plant	Nodule dry weight/plant (mg)	Root shoot ratio	Dry matter at flowering (g/plant)	Plant height (cm)	Bran-ches/plant	Pods/plant	Seed Yield (g/plant)	Straw yield (g/plant)	HI (%)
<i>Genotype</i>													
JS 93 05	67.67	764	352.13	61.5	171.36	1.81	3.26	43.4	3.7	21.7	2.89	4.14	41.1
JS 335	53.58	497	225.43	53.9	197.55	1.34	3.20	42.3	4.3	21.1	2.83	4.03	41.1
NRC 37	40.67	618	196.40	30.8	128.25	1.22	4.49	49.9	3.8	34.7	3.27	4.62	41.5
Hardee	55.00	776	300.58	34.9	104.57	1.52	4.11	49.5	3.0	15.5	2.21	3.49	38.3
JS 71 05	56.33	583	276.60	44.4	139.84	1.63	3.55	36.9	3.3	20.1	2.92	3.78	44.5
NRC 7	50.17	498	247.56	37.5	108.59	1.54	3.48	36.8	2.7	20.5	3.30	4.01	45.1
NRC 12	56.92	635	343.37	20.2	62.34	1.86	2.59	41.0	4.3	17.2	2.48	3.60	40.7
SEm (+/-)	1.889	2.10	17.98	0.501	1.220	0.066	0.039	1.12	0.29	2.57	0.0002	0.0002	0.68
<b>CD</b>	<b>5.387</b>	<b>6.00</b>	<b>51.26</b>	<b>1.429</b>	<b>3.479</b>	<b>0.187</b>	<b>0.111</b>	<b>3.19</b>	<b>0.85</b>	<b>7.33</b>	<b>0.0005</b>	<b>0.0004</b>	<b>1.93</b>
<b>(P=0.05)</b>													
<i>K level (ppm)</i>													
0.0	50.38	475	237.85	29.9	88.41	1.58	2.68	38.8	2.8	13.2	2.12	3.07	40.7
7.41	52.72	580	269.08	35.6	113.11	1.52	3.31	41.9	3.4	19.0	2.60	3.57	41.9
14.82	55.86	680	304.67	45.3	144.06	1.55	3.64	43.9	3.8	23.7	3.20	4.44	42.3
22.23	60.52	764	312.01	51.0	175.85	1.58	4.33	46.5	3.8	30.2	3.44	4.73	42.1
SEm (+/-)	1.428	1.60	13.59	0.379	0.922	0.049	0.029	0.85	0.23	1.94	0.0001	0.0001	0.51
<b>CD</b>	<b>4.073</b>	<b>4.60</b>	<b>38.76</b>	<b>1.080</b>	<b>2.630</b>	<b>NS</b>	<b>0.083</b>	<b>2.41</b>	<b>0.64</b>	<b>5.54</b>	<b>0.0004</b>	<b>0.0005</b>	<b>1.46</b>
<b>(P=0.05)</b>													

of nodule dry biomass. The maximum root to shoot ratio was recorded in NRC 12 followed by JS 93 05, JS 71 05, NRC 7, Hardee, JS 335 and NRC 37. Sum total of rooting characters and nodulation traits goes in favour of JS 93 05 indicating its efficiency in N fixation. Root length, dry weight of roots, root volume, nodules per plant and their dry biomass linearly increased as the levels of K increases. Different levels of K did not alter the root to shoot ratio. The genotypic differences in soybean due to variable levels of potassium were also reported by (Wang *et al.*, 1996). Bansal *et al.* (2001) also stipulated that the potassium had beneficial effects on nodulation and their dry biomass. The maximum and minimum plant dry matter accumulation was recorded in NRC 37 and NRC 12.

Significantly tallest plants were observed in genotype NRC 37 followed by Hardee while the lowest plant height was with NRC 7 and JS 71 05. The maximum branches per plant was with JS 335 and NRC 12 which remained at par with NRC 37 and JS 93 05.. Significantly highest pods per plant were recorded in NRC 37 while remaining genotypes showed non-significant differences among themselves. These results are in agreement with the findings of Billore and Joshi (1997). Plant height, branches and pods per plant invariably increased as the levels of potassium increases. The evaluated genotypes varied in yield performance (Table 1). Seed yield was the maximum in NRC 7 and the differences were significant over Hardee and NRC 12. NRC 37 was next to give higher yield followed by JS 71 05 and JS 93 05. Highest

straw yield was recorded with NRC 37, which differed significantly over Hardee and JS 71-05. This is logical on account of large amount of foliage produced by NRC 37 as also obvious from the lowest root to shoot ratio. The varietal differences may be accounted for their genetic makeup and maturity duration (Billore and Joshi, 1997). Potassium application significantly enhanced the seed yield to the tune of 22.49, 54.08 and 61.92% due to 7.41, 14.82 and 22.23 ppm over control, respectively. Though, the difference between 14.82 and 22.23 ppm was non-significant. Effect of increasing levels of potassium on straw yield was similar to that of seed yield. Similar increases in soybean yield and yield attributes were reported by (Kundu *et al.*, 1990; Borges and Mallarino, 2000; Chaturvedi and Chandel, 2005).

Statistically significant maximum total K uptake was associated with NRC 7 over Hardee and NRC 12 (Table 2). Total K uptake significantly increased as the levels of K increased. Though, the difference between 14.82 and 22.23 ppm was found non-significant. According to Sauerbeck and Hellal (1990), some plant and soil parameters affect nutrient uptake. Significantly highest K-harvest index was recorded in NRC 7 and minimum K-harvest index was with NRC 37 followed by Hardee and JS 71 05. The K-harvest index remained unaffected due to different levels of potassium.

The trend of partial factor (K) productivity was as follows: NRC 7 > NRC 37 > JS 71-05 > JS 93 05 > JS 335 > NRC 12 > Hardee. The trend of agronomic efficiency was in order: JS 71 05 > JS 93 05 > JS 335 > NRC 7 > NRC 37 > Hardee > NRC 12. Physiological efficiency

indicated the following trend: JS 71 05 > Hardee > NRC 7 > JS 335 > NRC 37 > JS 93 05 > NRC 12 (Table 2). The partial factor productivity decreased as the levels of K increases. Agronomic and physiological efficiency increased only up to 14.82 ppm K/ha and then it decreased. Kolar and Grewal (1994) also reported a depression in agronomic K use efficiency with increase in K application.

On the basis of foregoing results it could be concluded that the soybean genotypes namely JS 93 05, NRC 7, JS 335 and JS 71 05 can be utilized for further breeding programme for enhancing efficient utilization of K. The study also suggested that the potassium recommendation for soybean should be revised for achieving the optimum productivity of soybean in Vertisols.

**Table 2. K uptake, partial factor productivity, agronomic and physiological K use efficiencies of soybean genotypes as influenced by graded levels of potassium**

<b>Treatment</b>	<b>Total K uptake (g/pot)</b>	<b>K harvest index</b>	<b>Partial factor productivity</b>	<b>Agronomic K use efficiency (kg seed/kg K)</b>	<b>Physiological K use efficiency</b>
<i>Genotype</i>					
JS 93 05	0.23	55.82	49.14	16.01	16.06
JS 335	0.21	54.54	48.93	15.80	20.93
NRC 37	0.25	51.09	54.90	10.17	19.82
Hardee	0.20	51.53	36.20	11.35	22.43
JS 71 05	0.23	52.94	53.98	20.85	23.71
NRC 7	0.25	66.26	56.04	11.79	21.65
NRC 12	0.20	58.27	41.38	8.26	15.81
SEm (+/-)	0.02	1.614	-	-	-
<b>CD (P=0.05)</b>	<b>0.05</b>	<b>4.60</b>	<b>-</b>	<b>-</b>	<b>-</b>
<i>K level (ppm)</i>					
0.0	0.15	55.70	-	-	-
7.41	0.20	54.67	70.57	12.93	18.94
14.82	0.26	56.70	44.37	15.57	21.61
22.23	0.29	56.17	31.08	11.88	18.97
SEm (+/-)	0.01	1.219	-	-	-
<b>CD (P=0.05)</b>	<b>0.04</b>	<b>3.479</b>	<b>-</b>	<b>-</b>	<b>-</b>

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## **Effect of Micronutrients and *Bradyrhizobium japonicum* Inoculation on Nodulation, Growth, Nutrient Uptake by Plant and Yield of Soybean in Mollisol**

**MAHENDRA SINGH<sup>1</sup> and NARENDRA KUMAR<sup>2</sup>**

Department of Soil Science, College of Agriculture,  
Govind Ballabh Pant University of Agriculture and Technology,  
Pantnagar - 263 145, Uttarakhand, India  
(E-mail: nk5278@rediffmail.com)

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### **ABSTRACT**

A field experiment was conducted at Corp Research Centre, Pantnagar for two years during rainy season (kharif) of 2006 and 2007 to study the effect of Mo, B and Zn with *Bradyrhizobium japonicum* inoculation on nodulation, plant dry weight, nutrient uptake by plant and yield of soybean (*Glycine max* L. Merrill) var. PS 1347 in a Mollisol. Application of Zn (@ 5 kg/ha) along with *B. japonicum* inoculation and *B. japonicum* plus Mo (@ 4 g/kg seed) plus B (@ 0.5 kg/ha) have significantly increased nodule number per plant by 28.16 and 48.50 per cent at 60 DAS than uninoculated control in the first year. Treatment consisting *B. japonicum* plus Mo (@ 4 g/kg seed) plus B (@ 0.5 kg/ha) plus Zn (@ 5 kg/ha) gave maximum nodule number (51 and 80 nodules/plant, respectively) in both the years. This treatment also produced highest nodule dry weight of 400.0 and 673.6 mg per plant which was significantly more by 108.0 and 154.1 per cent, respectively over control in both the years with maximum plant dry weight of 23.61 g per plant at 60 DAS in 2006. Highest N and P uptake of 103.1, 98.1 kg per hectare, and 32.5, 30.4 kg per hectare, respectively by plant at harvest, maximum Zn and Mo content (1.04 and 0.63 ppm, respectively) in 2006 and maximum Zn, B and Mo content (1.04, 0.66 and 0.07 ppm, respectively) in 2007 in soil and highest grain yields of 3117.2 and 2462.0 kg per hectare were recorded with *B. japonicum* plus Mo (@ 4 g/kg seed) plus B (@ 0.5 kg/ha) plus Zn (@ 5 kg/ha) during both the years.

**Key words:** Micronutrients, *B. japonicum*, nodulation, N, P uptake, soybean, yield

Soybean has high nitrogen requirement due to its high content of proteins (40 %). However, being a leguminous crop, it can meet out its most of the N demand through fixation atmospheric nitrogen in root nodules with *Bradyrhizobium japonicum*

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<sup>1</sup>Research Scholar; <sup>2</sup>Professor

symbiosis provided sufficient number of effective soybean bacteria are present in the soil. The use of chemical fertilizers in agriculture has become indispensable to feed ever growing population of the country. But, the excessive use of chemical fertilizers has adverse effect on soil quality, and hence most of the soils are being deficient in micronutrients resulting in declining crop yields. Therefore, present investigation was conducted to study the effect of micronutrients with *B. japonicum* inoculation on nodulation, plant dry weight, N uptake and P uptake by plant and yield of field grown soybean.

## MATERIAL AND METHODS

The field experiment was conducted for two consecutive years (2006 and 2007) at Crop Research Centre of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar during rainy season (*khari*) to study the effect of micronutrients and *B. japonicum* inoculation on nodulation, plant dry weight, N and P uptake by plant, and yield of soybean *var.* PS 1347. The experimental soil was well drained Aquic Hapludoll silty clay loam having pH 7.4, organic carbon 0.86 per cent, available phosphorus 19.19 kg per hectare, available potassium 130.71 kg per hectare and nitrogen 240.17 kg per hectare. The soil was low in zinc and molybdenum (0.42 and 0.028 ppm, respectively) but medium in boron (0.35 ppm) content. The experiment included ten treatments, viz. Uninoculated control, *B. japonicum*, local strain, *B. japonicum* plus boron (@ 0.5 kg/ha), *B. japonicum* plus zinc (@ 5

kg/ha), *B. japonicum* plus Mo (@ 4 g/kg seed), B (@ 0.5 kg/ha), Zn (@ 5 kg/ha), Mo (@ 4 g/kg seed) and *B. japonicum* plus B (@ 0.5 kg/ha) plus Zn (@ 5 kg/ha) plus Mo (@ 4 g/kg seed) replicated thrice in randomized block design (RBD).

Each plot measured 2.75 m x 4 m and soybean was sown at 45 cm row to row spacing. Plant population was maintained to 40 plants per meter square. Basal dose of N, P and K @ 20, 40 and 60 kg per hectare, respectively was applied through urea, single super phosphate and muriate of potash while Zn (@ 5 kg /ha ) as zinc sulphate, B (@ 0.5 kg/ha) as borax and Mo (@ 4 g/ kg seed) as sodium molybdate was applied through seed treatment at the time of sowing. *B. japonicum* inoculant ( $3.52 \times 10^8$  c.f.u. /g) was obtained from the Division of Microbiology, IARI, New Delhi. The *B. japonicum* inoculation was done through seed (@ 500 g/ 75 kg seed) as per requirement of the treatment. For recording observations at 60 days after sowing (DAS), five plants were randomly selected from each plot, uprooted and nodules were carefully separated from the washed roots and counted. The nodules of each plot were dried in open glass petri dishes at  $65 \pm 2^\circ\text{C}$  for 48 h in hot air oven. Similarly, the plant dry weight was recorded by keeping five plants in hot air oven at  $65 \pm 2^\circ\text{C}$  for 48 h. Available Zn in soil was determined by using diethylene triamine penta acetic acid (DTPA) extraction (Lindsay and Novell, 1978), Mo by Grigg's reagent (Grigg, 1953) and available boron (B) by hot water soluble B method (Berger and Truog, 1939). The total nitrogen content in soybean plant was determined by micro Kjeldahl method (Bhargava and Raghupati, 1998) and total phosphorus content was analyzed by vanadomolybdate phosphoric

acid yellow color method in nitric acid system (Bhargava and Raghupati, 1998). N and P uptake of plant was computed to express the results.

After threshing and proper cleaning, the grain yield of individual plot was recorded with single pan balance and expressed as kg per hectare after conversion.

## RESULTS AND DISCUSSION

*B. japonicum* alone inoculation numerically increased nodulation in terms of nodule number and their dry weight per plant over uninoculated control (Table 1) in both the years because of symbiotic relationship between compatible host and *B. japonicum*. Similar findings have been reported by Vijayapriya *et al.* (2003) who found increasing nodulation in soybean by the application of *Bradyrhizobium* over the control.

Application of Zn (@ 5 kg/ha) along with *B. japonicum* inoculation and *B. japonicum* plus Mo (@ 4 g/kg seed) plus B (@ 0.5 kg/ha) plus Zn (@ 5 kg/ha) have significantly increased nodule number per plant by 28.16 and 48.5 per cent, respectively (Table 1) than control in 2006. It might be due to increased efficiency of *B. japonicum* by added micronutrients that enhanced the nodule formation. These findings corroborate with Zaghloul and Aly (2002), who reported that inoculation of *B. japonicum* along with Mo (@ 4 g/kg seed) increased the nodulation. Maximum nodule number (51 and 80 nodules/plant, respectively) at 60 DAS were recorded by the combined application of *B. japonicum*

plus B (@ 0.5 kg/ha) plus Zn (@ 5 kg/ha) plus Mo (@ 4 g/kg seed) in both the years. These findings are in conformity with Kumar *et al.* (2005) who reported that combined application of 100 per cent NPK plus Zn plus B plus Mo plus PSB plus *B. japonicum* resulted in the highest number of nodules per plant over the control.

Application of Zn (@ 5 kg/ha) with and without *B. japonicum* inoculation was found to significantly increase plant dry weight over control and *B. japonicum* alone inoculation in 2006. It is well known fact that *Rhizobium* inoculation enhances nodulation and nitrogen fixation in legume plants, and use of micronutrients particularly Mo leads to increased nitrogenase activity (Deng, 1990) whereas Zn is involved in the synthesis of IAA and metabolism of auxins and increased photosynthesis, which might have resulted more dry matter accumulation. Supplemented Zn increased the plant biomass as the crop was grown on Zn deficient soil. Srimathi *et al.* (2002) observed that pelleting of seed with zinc sulfate resulted in the highest dry matter production in soybean. Combined inoculation of micronutrients along with *B. japonicum* inoculation resulted maximum and significantly higher plant dry weight than uninoculated control and *B. japonicum* alone in the first year with numerical increase of 11.6 per cent over uninoculated control in the second year. This may be due to the increased activity of nitrogenase and more availability of nutrients to the plant. These findings corroborate with Sakr *et al.* (1990) who reported that combined application of Zn, B, Mo and *Rhizobium* resulted in the highest shoot dry weight over control.

**Table 1. Effect of micronutrients with *B. japonicum* inoculation on soybean nodulation, plant dry weight and nutrient uptake by plant**

Treatment	At 60 DAS						At harvest			
	Nodule (No/plant)		Nodule dry weight (mg/plant)		Plant dry weight (g/plant)		N uptake (kg/ha)		P uptake (kg/ha)	
	I year	II year	I year	II year	I year	II year	I year	II year	I year	II year
Uninoculated control	34.3	34.6	192.23	265.00	15.37	60.33	60.46	59.93	19.00	23.22
<i>B. japonicum</i>	37.6	41.3	235.43	366.33	16.23	65.00	63.32	76.08	20.56	27.48
Local strain	34.0	35.3	376.91	383.66	22.73	48.33	90.32	60.71	27.68	21.95
<i>B. japonicum</i> + Boron @ 0.5 kg/ha	40.0	28.6	326.71	336.66	20.09	58.33	76.60	70.94	24.90	22.14
<i>B. japonicum</i> + Zinc @ 5 kg/ha	44.0	57.0	390.65	456.33	22.06	55.66	74.42	82.28	26.66	29.13
<i>B. japonicum</i> + Mo@ 4 g/kg seed	40.0	79.6	315.48	406.66	20.57	63.66	77.55	66.10	27.46	23.22
Boron @ 0.5 kg/ha	35.3	67.3	314.75	433.33	20.12	70.66	76.27	70.43	24.55	24.73
Zinc @ 5 kg/ha	34.0	73.6	300.35	426.66	21.10	55.33	69.59	74.39	20.63	21.90
Mo@ 4 g/kg seed	40.0	69.6	294.48	464.00	21.99	84.00	83.62	72.33	27.13	20.13
<i>B. japonicum</i> + Boron @ 0.5 kg/ha + Zinc @ 5 kg/ha + Mo@ 4 g/kg seed	51.0	80.0	400.00	673.66	23.61	67.33	103.15	98.13	32.52	30.45
SEM ( $\pm$ )	2.75	13.74	10.37	57.08	0.99	8.87	7.81	6.49	1.94	2.45
<b>CD (P = 0.05)</b>	<b>8.0</b>	<b>NS</b>	<b>30.22</b>	<b>169.52</b>	<b>2.90</b>	<b>NS</b>	<b>22.84</b>	<b>18.99</b>	<b>5.75</b>	<b>NS</b>
CV (%)	11.94	13.64	5.42	21.90	8.31	23.97	17.18	15.03	13.37	17.03

**Table 2. Effect of micronutrients with *B. japonicum* inoculation on soybean yield and micronutrient status in soil after crop harvest**

Treatment	Micronutrients in soil (ppm)						Grain yield (kg/ha)	
	2006			2007			2006	2007
	B	Mo	Zn	B	Mo	Zn		
Uninoculated control	0.40	0.035	0.70	0.45	0.03	0.57	2561.51	1862.00
<i>B. japonicum</i>	0.41	0.036	0.77	0.43	0.05	0.77	2623.45	2190.66
Local strain	0.43	0.031	0.81	0.44	0.03	0.73	2685.18	2072.00
<i>B. japonicum</i> + Boron @ 0.5 kg/ha	0.57	0.038	0.91	0.58	0.04	0.79	2839.50	2372.00
<i>B. japonicum</i> + Zinc @ 5 kg/ha	0.43	0.033	1.00	0.53	0.04	1.00	3089.41	2462.00
<i>B. japonicum</i> + Mo@ 4 g/kg seed	0.41	0.055	0.95	0.45	0.06	0.86	2870.36	2012.00
Boron @ 0.5 kg/ha	0.53	0.036	0.86	0.59	0.04	0.85	2839.50	2105.33
Zinc @ 5 kg/ha	0.40	0.037	0.99	0.42	0.03	0.95	2832.09	2012.00
Mo@ 4 g/kg seed	0.39	0.063	0.91	0.41	0.06	0.93	2737.03	2222.00
<i>B. japonicum</i> + Boron @ 0.5 kg/ha + Zinc @ 5 kg/ha + Mo@ 4 g/kg seed	0.49	0.063	1.04	0.66	0.07	1.04	3117.28	2462.00
SEM ( $\pm$ )	0.06	0.01	0.05	0.05	0.006	0.05	181.63	10.21
<b>CD (P = 0.05)</b>	<b>NS</b>	<b>0.015</b>	<b>0.14</b>	<b>0.14</b>	<b>0.014</b>	<b>0.15</b>	<b>NS</b>	<b>29.87</b>
CV (%)	40.2	21.35	9.33	16.95	21.36	10.62	10.99	0.80

Inoculation of *B. japonicum* along with Zn (@ 5 kg/ha), Mo (@ 4 g/kg seed) and B (@ 0.5 kg/ha) resulted in the maximum and significantly more N uptake (Table 1) by plant at harvest than all the treatments except Mo (@ 4 g/kg seed) with or without *B. japonicum* inoculation in first year and Zn (@ 5 kg/ha) in second year. This treatment also gave maximum and significantly more P uptake than all the treatments except local strain, Mo (@ 4 g/kg seed) with or without *B. japonicum* inoculation in the first year and maximum P uptake (30.45 kg/ha) in second year. This may possibly be because of increased enzymatic activity both in plant and *B. japonicum*. Similar results were reported by Amit *et al.* (2007) who found that *Rhizobium* along with Mo, B and Zn significantly increased N and P uptake by plant in mung bean over control. Hossain *et al.* (2005) also reported that combined application of P plus Mo plus *Bradyrhizobium* produced higher N uptake (132.2 kg/ha) in cv. G-2 (120.6 kg/ha). It is well known fact that *Rhizobium* inoculation enhance the nodulation and nitrogen fixation in plants while the use of micronutrients was found to be beneficial in enhancing the N and P uptake as Mo replaces the nitrogen fertilizers to legume and enhance the rhizobial infection, B helps in the transport of sugars and a close relationship is found between Zn supply and N content (Jyung and Krishna, 1975).

Maximum Zn and Mo content (1.04 and 0.63 ppm, respectively) in 2006

and maximum Zn, B and Mo content (1.04, 0.66 and 0.07 ppm, respectively) in 2007 in soil was recorded with inoculation of *B. japonicum* along with Zn (@ 5 kg/ha), Mo (@ 4 g/kg seed) and B (@ 0.5kg/ha). The increase in Zn, B and Mo content in soil was due to addition of these micronutrients in soil.

Application of Mo, B and Zn along with *B. japonicum* inoculation significantly increased grain yield by 21.69 per cent in 2006 and gave 32.22 percent numerical increase in 2007 over the control. It may be due to addition of micronutrients along with *B. japonicum* inoculation, Mo is the part of nitrogenase enzyme and responsible for the increased biological nitrogen fixation and zinc showed beneficial effect on chlorophyll content and so, it indirectly influenced the photosynthesis and reproduction. Application of Mo along with *B. japonicum* numerically increased grain yield over the control in both years. It may be due to the soybean in symbiosis with *B. japonicum* and is able to satisfy its nitrogen (N<sub>2</sub>) demand with biological nitrogen fixation (BNF). However, BNF can be affected by molybdenum deficiency because this micronutrient is part of the nitrogenase enzyme responsible for the process. These findings corroborate with Sonare *et al.* (5

Zn, B and Mo or FYM than the control. Kumar *et al.* (2005) also reported that combined application of 100 per cent NPK with Zn, B, Mo and *Rhizobium* resulted in the highest seed yield (2165 kg /ha) over the control.

The response of crop to the added micronutrients particularly Zn and Mo in terms of nodulation, plant biomass, nutrient uptake by plant, and grain yield was due to the deficiency of these micronutrients in soil. Thus, the results of this experiment emphasized the need of the proper use of micronutrients with microbial inoculant to maintain soil health and to optimise the seed yield of soybean.

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## Effect of Integrated Nutrient Management on Growth and Yield of Soybean [*Glycine max* (L.) Merrill] in Jhabua Hills Zone of Madhya Pradesh

R K TRIPATHI<sup>1</sup>, I S TOMAR<sup>2</sup>, SUNITA MISHRA<sup>3</sup> AND D K VANI<sup>4</sup>

Zonal Agricultural Research Station (RVSKVV, Gwalior), Jhabua, Madhya Pradesh  
(E-mail: kvkjhabua@rediffmail.com)

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### ABSTRACT

A field experiment was conducted for two years (2004-2005) during kharif season on sandy clay soil classified as typic Ustochrept of Jhabua, India to study the effect of different nutrient combinations organic, inorganic and bio-fertilizers on yield attributes, seed and stover yield of soybean. The values of different attributes associated with 50 percent RDF coupled with application of vermin compost @ 2 t/ha was maximum and followed by 100 percent RDF coupled with seed treatment with rhizobium and PSB. In compared with no fertilizer, the enhancement in seed and stover yield by best treatment amounted by 60 percent and 39 percent respectively. Thus, the combined use of manures, bio fertilizer and inorganic fertilizer played a significant role in increasing seed and stover yield of soybean.

**Key words:** Soybean, fertilizer, organic, inorganic, yield, rhizobium culture, PSB culture.

Soybean [*Glycine max* (L.) Merrill] is one of the major *kharif* oilseed crops in India, mainly in the semi-arid tropics of Central India. The limited area of 0.03 m ha in 1970 has increased 317 fold during year 2008 (9.5 m ha) (Anonymous, 2008). However, its productivity gap between achievable seed yield (> 2.5 t/ha) and current yield level of about 1.0 t/ha remains very wide (Gupta and Rajput, 2001). Low productivity of the crop is primarily because of inappropriate soil, water and crop management practices.

There exists a considerable potential to bridge the yield gap between actual and achievable yield through the adoption of appropriate resource management strategies. Soybean draws its nutrient need from soil, if not fertilized properly, adversely affects soil fertility. One such strategy is to maintain soil health and fertility for sustainable production of soybean through judicious use of fertilizers (Bobde *et al.*, 1998) coupled with organic resources (Joshi and

<sup>1</sup>Technical Officer (Agromet); <sup>2</sup>Programme Coordinator, KVK; <sup>3</sup>Programme Coordinator, KVK; <sup>4</sup>Subject Matter Specialist (Agril. Engg.), KVK



Billore, 2004). It has been realized from long- term fertilizer experiments that to achieve sustainability in production, the use of organic manures alone is not sufficient (Prasad, 1996, Nambiar and Ghosh, 1984, Singh and Dwivedi, 1996). It has also been brought out that use of organic manures in integration with fertilizers meets the need of micro nutrients in soybean (Joshi *et al.*, 2000) This calls for intergraded use of organics, inorganic and bio-fertilizers for highly intensive production system to maintain soil health and to augment the efficiency of nutrients.

Hence, the present investigation was undertaken to study the effects of judicious and combined use of inorganic fertilizers (NPK), organic manures and bio-fertilizers on growth and yield of soybean.

## MATERIAL AND METHODS

A field experiment was conducted during *kharif* seasons of 2004 and 2005 at a fixed site of Zonal Agricultural Research Station, Jhabua, Madhya Pradesh. The soil of experimental site was sandy clay classified as typic Ustochrept with pH 6.8, organic carbon 4.8 g per kg and EC 0.29 dSm<sup>-1</sup>. The available N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O contents were 218, 11.3 and 426 kg per ha, respectively. The experimental was laid out in a randomized block design (RBD) with ten treatments namely, no fertilizer, vermi compost @ 2.0 t per ha, FYM @ 5.0 t per ha, 100 per cent recommended dose of fertilizer (RDF), 75 per cent RDF plus rhizobium culture plus PSB culture, 50 per cent RDF plus rhizobium culture plus PSB culture,

50 per cent RDF plus vermi compost @ 2.0 t per ha, vermi compost @ 2.0 t per ha plus rhizobium culture plus PSB culture, rhizobium culture plus PSB culture and 100 per cent RDF plus rhizobium culture plus PSB culture with three replications. The recommended dose of N : P<sub>2</sub>O<sub>5</sub> : K<sub>2</sub>O (100% RDF) used for soybean were 30 : 60 : 30 kg per ha applied as basal. The carriers used for these nutrients were urea, single super phosphate and muriate of potash, respectively. The rhizobium and PSB culture were applied for seed treatment 5 g per kg after the treating with recommended fungicides, wherever applicable. FYM (N: P: K :: 0.6: 0.5: 1.4%) and vermi compost (N: P: K :: 2.1: 1.2: 1.7%) were applied at the time of field preparation of specified plots.

Soybean (JS 93 05) at 30 cm x 10 cm spacing, was sown in first week of July during *kharif* and harvested at 96 days in first year and at 90 days in second year. The total rainfall received during the first and second year (June to October) was 1239.4 and 678.4 mm, respectively.

The observations on yield attributes were recorded at harvest from randomly selected five plants of each plot. Statistical analysis was carried out using standard analysis of variance (Gomez and Gomez, 1984). The significance of the treatment effect was determined using the F- test and to determine the significance of the difference between the mean of the two treatments, least significance (LSD) were estimated at the 5% probability level.

## RESULTS AND DISCUSSION

In general, yield attributing characters namely, plant height, branches per plant and pods per plant as well as seed and

stover yields of soybean were higher during 2005. The lower yield in 2004 in spite of above normal precipitation (1239 mm) may be accounted for incessant rains between 26<sup>th</sup> July and 24<sup>th</sup> August (982 mm in 27 rainy days) followed by a dry spell of 27 days. The cloudy weather during the flowering period and subsequent dry weather led to a set back to the crop during 2004.

Different nutrient management treatments were effective in improving the yield attributing characters of soybean over no fertilizer and the same trend was observed during both the years (Table 1). This improvement has proportionally been reflected in enhancement in seed yield, stover yield and harvest index of soybean. Use of bio-fertilizers (rhizobium and PSB) alone, although revealed numerical increase over no fertilizer application, the difference was not statistically significant in plant height, branches per plant, pods per plant, seed yield, stover yield and harvest index. This appears to be logical as the biofertilizers are only facilitator for nutrient availability. In rest of the treatments external application of nutrient through organic or inorganic sources existed and that resulted in significant yield responses. The maximum values were associated with 50 per cent RDF coupled with application of vermi compost @ 2 t per ha followed by

100 per cent RDF coupled with seed treatment with rhizobium and PSB. This implies that a fertilizer economy by 50 percent can be availed with enhancement in seed and stover yield by nearly 13 and 9 percent, respectively. In comparison with no fertilizer, the enhancement in seed and stover yield by best treatment amounted by 60 per cent and 39 per cent respectively. It also needs attention that application of RDF with rhizobium and PSB improves the growth attributes and seed (by 11%) and stover (by 7%) yield over application of RDF only. In case of other treatments, the seed and stover yield enhancement over control ranged between 4 - 42 per cent and 2 - 28 per cent, respectively. Similar results on combined use of organic, inorganic and bio-fertilizers which play a significant role in increasing the growth, yield attributing parameters, seed and stover yield as well as maintaining soil health on long- term basis has been reported by Mishra *et al.* (1990) and Ghosh *et al.* (2005).

The cumulative results for two years brings out that integrating inorganics with organic sources is a better option for optimum performance of soybean and there exists a possibility of rationalizing the application of inorganic fertilizers by employing integrated approach.

**Table 1. Effects of different nutrient management practices on yield attributes and yield of soybean**

Treatment	Plant Height (cm)			Branches/ plant			Pods/plant			Seed Yield (kg/ha)			Stover (kg/ha)			Harvest Index		
	2004	2005	Pooled	2004	2005	Pooled	2004	2005	Pooled	2004	2005	Pooled	2004	2005	Pooled	2004	2005	Pooled
No fertilizer	50.5	52.3	51.4	1.70	1.87	1.78	16.67	17.66	17.17	1090	1202	1146	1460	1482	1471	42.7	44.8	43.8
Vermi compost @ 2.0 t/ha	52.0	53.5	52.7	2.08	2.18	2.13	19.13	20.05	19.59	1335	1399	1367	1604	1652	1628	45.4	45.8	45.6
FYM @ 5 t/ha	52.2	54.6	53.4	2.18	2.23	2.21	19.79	21.05	20.42	1391	1465	1428	1635	1727	1681	46.0	45.9	45.9
100% recommended dose of fertilizer (RDF)	53.5	54.8	54.2	2.33	2.29	2.31	21.69	21.68	21.69	1416	1535	1476	1697	1829	1763	45.5	45.6	45.6
75% RDF + rhizobium culture @ 5 g/kg seed+ PSB culture @ 5 g/kg seed	54.6	56.1	55.4	2.43	2.44	2.44	23.23	23.28	23.25	1458	1641	1549	1762	1873	1817	45.3	46.7	46.0
50% RDF + rhizobium culture @ 5 g/kg seed + PSB culture @ 5 g/kg seed	51.7	53.4	52.6	2.01	2.16	2.09	22.23	20.11	21.17	1274	1399	1337	1640	1669	1654	43.7	45.6	44.7
50% RDF + vermi compost @ 2.0 t/ha	56.3	56.4	56.4	3.01	3.17	3.09	27.33	26.55	26.94	1765	1913	1839	1988	2106	2047	47.0	47.6	47.3
Vermi compost @ 2.0 t/ha + rhizobium culture @ 5 g/kg seed + PSB culture @ 5 g/kg seed	51.6	53.7	52.7	2.13	2.28	2.21	18.83	20.75	19.79	1313	1464	1389	1658	1753	1706	44.1	45.5	44.8
Rhizobium culture @ 5 g/kg seed + PSB culture @ 5 g/kg seed	52.0	53.6	52.8	1.73	1.93	1.83	17.16	18.38	17.77	1138	1252	1195	1486	1527	1506	43.4	45.0	44.2
100% RDF + rhizobium culture @ 5 g/kg seed + PSB culture @ 5 g/kg seed	54.2	56.2	55.2	2.68	2.90	2.79	26.16	25.05	25.60	1513	1752	1633	1783	1991	1887	45.9	46.8	46.4
CD (P = 0.05)	2.18	2.05	1.27	0.19	0.23	0.11	1.77	1.95	1.29	143	141	84	118	136	79	1.96	0.75	1.0

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## Evaluation of Agricultural By-products for Mass Multiplication of *Trichoderma* sp.

**M M ANSARI\***

National Research Centre for Soybean, Khandwa Road,  
Indore 452 001, , Madhya Pradesh, India  
(E mail : ansarimasud@rediffmail.com)

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### ABSTRACT

Eight locally available agro-wastes, viz. straw of wheat, lentil, chickpea, safflower, mustard, soybean and grass, and maize cobs were tried individually and in combination for mass multiplication of *Trichoderma* sp. It can be grown on any of these substrates. Enriching the substrates with either one per cent urea or sugar solution enhanced faster and good growth of *Trichoderma* sp. and produced more spores per gram of substrate. Mixture of substrate and Farmyard Manure (FYM) (1:1, w/w) also produced good number of spores. Pasteurization of substrate was found better to multiply the organism in a short time. A method was also developed to mass multiply the *Trichoderma* sp. easily within 1-2 months and can be used in controlling soil borne diseases of crops.

**Key words:** Agro-wastes, chickpea, grass straw, lentil, maize cob, mass multiplication of bioagents, mustard, safflower, soybean, wheat straw, *Trichoderma*

*Trichoderma* species, as biocontrol agent, have been reported to act against soil borne plant pathogens causing serious diseases of crops (Elad *et al.*, 1980; Vyas, 1994; Carver *et al.*, 1996). Now the commercial formulations of *Trichoderma* are available for field application in India. However, it is also known that these biocontrol agents should be native for their efficacy against their target pathogens. Application of antagonistic fungi to the rhizosphere of crop plants for the control of soil borne diseases requires

their mass production within a short time using commonly available cheap substrates. Several attempts in this direction have been reported (Backman and Rodriguez Kabana 1975; Kelly, 1976; Sawant *et al.*, 1995; Rukmani and Mariappan, 1993; Muthamilan, 2007). In the present investigation, eight locally available agro-wastes were tried so that antagonistic fungi along with organics can be added as soil application in the integrated disease management (IDM) system.

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\*Principal Scientist, Plant Pathology

## MATERIAL AND METHODS

### *Selection of effective strains of Trichoderma*

It is well established that the local strains are more effective and virulent as compared to exotic strains. Isolations were carried out from the soil of experimental field of National Research Centre for Soybean on *Trichoderma* selected medium (Elad *et al.*, 1981) and a number of isolations were made. The effective and fast growing strain was identified after screening the isolates for dry mycelium production and radial growth on synthetic medium i.e. Czapekdox agar medium.

### *Agro wastes*

Eight agro-wastes, viz. straw of wheat, lentil, chickpea, safflower, mustard, soybean and grass, and maize cobs were screened individually and in combinations thereof for their efficacy to produce mass inoculum of fungal antagonist and their shelf life. All the substrates including spent cob (maize) was cut into small pieces (2-3 cm). All the substrates, were soaked in tap water overnight and excess water was drained out (final moisture content was approximately 80 %). Each substrate (100 g) was filled in conical flasks and autoclaved twice at 121°C for 30 minutes on two successive days. The flasks were allowed to cool down to room temperature prior to inoculation. The combination of all substrates (mixed in equal quantity w/w) was also evaluated. Three replications were made for each treatment.

In another experiment, the agro-waste substrate (wheat straw) was enriched either with one per cent sucrose or urea or yeast before sterilization and the growth and multiplication of *Trichoderma* was observed as per the method mentioned above along with suitable control.

### *Trichoderma inoculum*

Ten discs of five mm size of seven-day-old cultures grown on potato-dextrose agar medium were transferred in 10 ml sterile distilled water and mixed thoroughly using Cyclomixer ( $10^8$ /ml spore concentration), was added aseptically into the flasks. The flasks were incubated at room temperature (25-30 °C). After 15 days of growth, the colonized agro-wastes were dried at room temperature and ground to powder using a laboratory blender.

### *Calculation of colony forming units in stored formulations*

The estimation of colony forming units (cfu) of *Trichoderma* sp in different substrates was done after 15 days of full growth, by suspending one gram of dried product prepared on different agro-wastes by serially diluting the powder and finally plated on fresh *Trichoderma* selective medium (Elad *et al.*, 1981). The plates were incubated at  $25 \pm 2$  °C. There were three replicates for each case.

### *Shelf life study of Trichoderma culture*

Shelf life was studied in the culture prepared in wheat straw. After 30 days of full growth, it was dried at room temperature and homogenized with the help of mixer and kept in flask at room temperature (25-30°C). One gram of

culture substrate was drawn from the flask and mixed in 10 ml of sterile distilled water and then serial dilutions were made. From 4<sup>th</sup> dilution 100 µL was drawn and streaked on previously poured *Trichoderma* selective medium and incubated at 27 °C for 48-72 h. After incubation the *Trichoderma* colonies were counted and per gram population were calculated.

#### ***Effect of different methods of pasteurization on Trichoderma multiplication***

For this experiment wheat straw was used and the following four procedures were tried. In each case 300 g dry substrates was used and after the procedure it was equally distributed in three flasks and sterilized and inoculated as per the procedure mentioned above. Un- pasteurized control was maintained for comparison.

- A - Soaking of substrate for 30 minutes in fresh tap water and sterilization under pressure for 30 minutes on two successive days.
- B - Boiling of substrate in water for 30 minutes and sterilization under pressure for 30 minutes on two successive days.
- C - Soaking of substrate in one per cent alkali (NaOH) for 30 minutes and washing under tap water to remove excess alkalinity and sterilization under pressure for 30 minutes on two successive days.
- D - Soaking of substrate in one per cent HCl acid for 30 minutes and washing under water to remove excess acid and sterilization under

pressure for 30 minutes on two successive days.

- E - Control (Un-pasteurized substrate sterilized for 30 min on two successive days).

#### ***Mass multiplication of Trichoderma sp.***

For standardization of procedure for mass multiplication wheat straw and soybean refuse were used. Old and fresh substrates were used. The experiment was conducted at glass house condition during the month of February to April, when temperature was 30-33 °C. Four kg substrate was soaked in water and the excess water was drained out and then one per cent urea was mixed thoroughly. Inoculum was prepared from the 7-10 day old culture having the 10<sup>8</sup> to 10<sup>10</sup> spores per ml. Next day substrates were inoculated with two liters of culture filtrates and the substrates was covered with tarpaulin per gunny bags to maintain moisture. Watering was done in alternate days to maintain moisture. Cover was also moistened to maintain moisture. In another set FYM and wheat straw (1:1 w/w) was also tried for mass multiplication of *Trichoderma* sp.

## **RESULTS AND DISCUSSION**

Out of 11 *Trichoderma* isolates evaluated for their growth parameters in the synthetic medium, i.e. Czapekdox medium, most of the isolates produced around 300 mg per 100 g culture filtrate. Isolate TR- 2 produced minimum dry weight i.e. 222 mg per 100 ml, whereas isolate Ind -2 produced maximum i.e. 363 per 100 ml culture medium (Table 1). Hence, the isolate Ind -2 were employed for further studies in the subsequent experiments.

**Table 1. Evaluation of different *Trichoderma* isolates in synthetic medium (Czapekdox agar medium) at  $26 \pm 2^\circ\text{C}$**

Isolate No.	Dry mycelium wt (mg/100ml)culture
TR 1	0.320
TR 2	0.222
TR 3	0.302
TR 4	0.302
Ind -1	0.224
Ind-2	0.363
TR 7	0.308
TR 6	0.337
TR 8	0.337
TR10	0.329
TR 12	0.295

\* Average of three replicates

In most of the substrates i.e. straw of wheat, chick pea, soybean and grass,

and maize cobs, the growth initiation started within two days. In case of lentil straw it took very less time i.e. only one day and in combination of substrates it took three days, whereas in case of straw of safflower and mustard, it has taken 8 days. Similar trend was observed in completion of full growth of *Trichoderma* in substrates, the least time taken in straw of grass and lentil i.e. 3 and 4 days respectively. In combination of substrates and other substrates it took around 6-7 days for full growth, whereas in case of straw of mustard and safflower it took 11 and 12 days, respectively for full colonization (Table 2). The results indicated that the slow growth in straw of mustard and safflower might be due to hardness of the substrates and lack of suitable nutrients needed for sporulation of the organism.

**Table 2. Growth of *Trichoderma* sp. in individual and mixed substrate at  $26 \pm 2^\circ\text{C}$**

Substrate	Days for initiation of growth	Days for full growth	Spores/g substrate
Wheat straw	2	7	$1.16 \times 10^6$
Maize cob	2	6	$1.81 \times 10^6$
Lentil	1	4	$1.31 \times 10^6$
Chick pea	2	8	$0.87 \times 10^6$
Soybean	2	6	$1.70 \times 10^6$
Safflower	8	12	$0.291 \times 10^6$
Mustard	8	11	$0.139 \times 10^6$
Grass straw	2	3	$1.15 \times 10^6$
Mixed substrates	3	5	$1.16 \times 10^6$

\* Average of three replicates



Among all the substrates used here, wheat straw enriched with either one per cent urea or sugar or yeast extract solution to facilitate faster and good growth of the *Trichoderma* sp. revealed that the initiation of growth took two days in all the treatment irrespective of enrichment. Whereas, enriching the substrate with either urea or sugar led to faster growth of *Trichoderma* sp. as compared to enriching with yeast extract and non-enriched (control). Enriching the substrate with either one per cent urea or sugar solution reduced the full growth time around half, it took 4 and 5 days in

urea and sugar solution treatment respectively , whereas in case of yeast extract treatment it took 7 days for full growth which is as similar as non-enriched substrate (Table 3). Bandyopdhyay *et al.* (2003) also found that treatment of substrate with one per cent yeast-peptone-sugar solution produced better growth and sporulation of *Trichoderma* sp as compared to untreated substrate. So from the results, it can be concluded that enriching the substrate will help mass multiplication of *Trichoderma* sp.

**Table 3. Growth of *Trichoderma* sp. in enriched and non-enriched substrate (wheat straw)**

Substrate	Days for initiation of growth	Days for full growth
Wheat straw+ 1% urea	2	4
Wheat straw+ 1% sugar	2	5
Wheat straw+ 1% yeast extra	2	7
Wheat straw (control)	2	7

Among all the substrates used here, wheat straw pasteurized with different methods and *Trichoderma* sp. and multiplication was studied. The results revealed that all the procedures were effective as compared to un-pasteurized control. In the control the initiation of growth started in two days whereas in others in one day only and the complete growth occurred within 3 - 4 days, whereas in untreated it took seven days. The boiled and sterilized substrate was found better as compared to other treatments, in this case the growth started

in one day and completed within three days. *Trichoderma* sp. multiplied faster as compared to other treatments. Because in boiling the most of the phenolic compounds were removed and the substrates also become soften due to heat treatment (Table 4).

Enumeration of *Trichoderma* spores in the prepared dry powder after 15 days of growth revealed that the colony count was least in mustard and safflower waste, *i.e.*  $0.139 \times 10^6$  and  $0.291 \times 10^6$  cfu,

**Table 4. Effect of pasteurization on multiplication of *Trichoderma* sp at  $26 \pm 2$  °C**

Pasteurization method	Days for initiation of growth	Days for full growth
Soaking of substrate for 30 min in fresh tap water and sterilization under pressure for 30 min on two successive days	1	4
Boiling of substrate in water for 30 min and sterilization under pressure for 30 min on two successive days	1	3
Soaking of substrate in 1% alkali (NaOH) for 30 min and washing under tap water to remove excess alkalinity and sterilization under pressure for 30 min on two successive days	1	4
Soaking of substrate in 1% HCl acid for 30 min and washing under water to remove excess acid and sterilization under pressure for 30 min on two successive days	1	4
Control (Un pasteurized substrate, sterilization for 30 min. on two successive days)	2	7

\*Average of three replicates; Wheat straw was taken as substrate

respectively. The low CFU population was due to slow growth and unfavourable nutrient present in the substrate. The highest population was recorded in maize cob spent ( $1.81 \times 10^6$ ), soybean refuge ( $1.70 \times 10^6$ ) and lentil wastes ( $1.31 \times 10^6$ ) whereas, in wheat straw and in combination of substrates, the spores were same ( $1.16 \times 10^6$  spores /g substrate) (Table 2).

In shelf life study, which was conducted in wheat straw, found that the required CFU per gram, i.e.  $10^6$  CFU per gram culture substrate was maintained up to three months and thereafter it started decreasing. Six months after the population reduced to  $10^2$  CFU per gram at room temperature.

It was observed that it took one month to produce good mass culture of *Trichoderma* if temperature is maintained around  $25-27$  °C, but it took more than two months if temperature increased as the multiplication was slow. Spore count per gram substrate was found to be  $1.25 \times 10^6$ -  $1.38 \times 10^6$  CFU per gram. In case of mixture of substrate and FYM the culture grew better and faster and have more CFU ( $1.57 \times 10^8$  CFU/g substrate) as compared to the wheat substrate alone. Kousalya and Jeyarajan (1988) screened twenty-one substrates for mass multiplication of *T. harzianum* and *T. viride* and observed that tapioca (cassava) rind was superior to other substrates. Jacob and Sivaprakasam (1993) evaluated several organic wastes and found dried effluent from gobar gas

plant and farm yard manure as promising substrates for mass production of *T. harzianum* and *T. viride*. It was concluded that *Trichoderma* sp can be multiplied in any of the locally available substrates, however to obtain good number of spores it may be further enriched with either one per urea or sugar solution and FYM may also be supplemented. In India around 270 million tones of agriculture based biomass (Data Book, 2005) are produced every year, which can be utilized for mass culturing the bioagents like *Trichoderma* sp. It will help in proper utilization of biomass and also add the carbon content in the soil, which is depleting every year and provide low cost sustainable agriculture.

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## Variable Selection and Knowledge Discovery for Disease Clusters

ALKA ARORA<sup>1</sup>, SHUCHITA UPADHYAYA<sup>2</sup> and RAJNI JAIN<sup>3</sup>

Indian Agricultural Statistics Research Institute, Pusa, New Delhi-110 012

(E-mail: alkak@iasri.res.in, alka27@yahoo.com)

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### ABSTRACT

*In this paper, 'Maximum Possible Combination Reduct' derived from rough set theory is employed for variable selection and description of clusters. The proposed multi-stage approach of applying reduct in clustering involves data pre-processing using reduct, cluster formation via clustering algorithm and finding cluster description by utilizing reduct. Proposed approach is demonstrated on soybean disease dataset from machine learning repository. Variable selection with proposed approach resulted in the removal of 17 irrelevant variables out of total 35 variables prior to application of standard clustering algorithm. Cluster description with proposed approach resulted in describing obtained disease clusters with only seven significant variables which contribute towards the occurrence of soybean diseases.*

**Key words:** Clustering, cluster description, data mining, indiscernibility, maximum possible combination reduct, reduct, soybean

In knowledge discovery, clustering is a tool for finding hidden patterns in the dataset. Clustering algorithm partitions the dataset into homogenous clusters such that entities within cluster are similar. Many clustering algorithms are available in literature; one can refer to Han and Kamber (2006) and Mirkin (2005) for details on clustering algorithms. However, majority of clustering algorithms just generate general description of the clusters like which entities are member of each cluster and lacks in generating description in the form of pattern. According to Ganter and Wille (1997), cluster description is able to approximately describe the cluster in the form that "this cluster consists just of all the entities having the pattern P, where the pattern is formulated using the variable and values of the given many valued context". From an intelligent data analysis perspective deriving knowledge in the form of pattern from obtained clusters is as important as grouping the entities into

<sup>1</sup> Scientist Sr. Scale, IASRI, New Delhi; <sup>2</sup> Reader, Kurukshetra University, Kurukshetra; <sup>3</sup> Sr. Scientist, National Center for Agricultural Economic and Policy Research, New Delhi

clusters. Pattern aids in representation and understanding of the cluster in meaningful format.

In this paper, an attempt is being made for variable selection and cluster description using Rough Set Theory (RST). As discussed by Komorowski *et al.* (1999), RST has been successfully applied in the area of variables selection for supervised learning. The rough set ideology of using only the supplied data and no other information is beneficial in variable selection, as it does not require any supplementary knowledge. RST divides the dataset into set of indiscernible/equivalence classes. Hence RST has a natural appeal to be applied in clustering as every indiscernible relation can be considered as natural cluster. Reduct from RST is the set of variables that can differentiate all indiscernible classes. Therefore in our approach, we have tried reduct for variable selection in clustering and then its use for generating cluster description. The proposed multi stage approach of cluster description involves (1) data pre-processing using reduct, (2) cluster formation via clustering algorithm, (3) cluster description employing reduct. For initial investigation and conformity of the

approach small agricultural dataset of soybean disease diagnosis from UCI repository is considered for case study. Using reduct on soybean dataset, objective of this analysis is to find relevant variables which contribute towards the occurrence of a particular disease.

## MATERIAL AND METHODS

### Rough set theory: an overview

RST is a mathematical approach, proposed by Pawlak (1991), to cope with data analysis in the presence of imprecision, vagueness and uncertainty. One can refer to Komorowski *et al.* (1999) and Pawlak (1991) for details on RST. In RST, dataset is represented in the form of information table,  $X = (U, A)$  where  $U$  a non-empty, finite set of entities is called the universe and  $A$  is a non-empty, finite set of variables on  $U$ . With every variable  $a \in A$ , we associate a set  $V_a$  such that  $a: U \rightarrow V_a$ . The set  $V_a$  is called the domain or value set of variable  $a$ . Small table from soybean disease dataset is used for illustration (Table 1). The dataset has ten entities with eight nominal variables.

**Table 1. Small soybean dataset**

Id	Date	Precip	damage	severity	canker_lesion	fruiting_bodies	decay
X1	July	lt-norm	Scattered	pot-severe	tan	absent	absent
X2	October	norm	Scattered	pot-severe	tan	absent	absent
X3	September	lt-norm	whole-field	pot-severe	tan	absent	absent
X4	August	norm	whole-field	pot-severe	tan	present	absent
X5	August	lt-norm	upper-area	pot-severe	tan	absent	absent
X6	September	gt-norm	whole-field	pot-severe	dk-brown-blk	absent	absent
X7	July	gt-norm	Scattered	pot-severe	dk-brown-blk	absent	firm-and-dry
X8	August	gt-norm	low-areas	pot-severe	dk-brown-blk	absent	firm-and-dry
X9	September	gt-norm	upper-area	Minor	dk-brown-blk	absent	firm-and-dry
X10	October	gt-norm	whole-field	Minor	dk-brown-blk	absent	firm-and-dry

### **Indiscernibility relation**

Indiscernibility is core concept of RST. Entities in the information system about which we have the same knowledge form an indiscernibility/equivalence relation. Indiscernibility relation divides the dataset into set of equivalence classes. Formally any set  $B \subseteq A$  there is associated an equivalence relation called B-Indiscernibility relation defined as follows:

$$IND_A(B) = \{ \{x, x' \in U^2 \mid \forall a \in B, a(x) = a(x')\} \}$$

If  $(x, x') \in IND_A(B)$ , then entities  $x$  and  $x'$  are indiscernible from each other by variables from  $B$ . For example from table 1, when  $B = \{\text{damage}\}$  then entities (X1, X2, X7) are indiscernible and therefore form one equivalence class; X3, X4, X6 and X10 are indiscernible and X5 is indiscernible with X9. Formally:

$$IND(\{\text{damage}\}) = (\{X1, X2, X7\}, \{X3, X4, X6, X10\}, \{X5, X9\}, \{X8\})$$

### **Reduct**

Concept approximation is achieved in RST through data reduction i.e., by retaining the minimum subset of variables that can differentiate all equivalence classes in the universe set. Such minimum subset is called reduct. Remaining variables can be deleted from the data set as their removal will not affect clustering. There are many methods as well as many software's available for computation of reducts. These are not discussed here because of space constraint. Mostly reduct is computed relative to decision attribute in the dataset. As clustering is done on unsupervised data where decision/class information is not

present, therefore our approach of reduct computation is purely on the basis of indiscernibility. We have considered 'Genetic Algorithm' (GA) for reduct computation using 'Rough Set Exploring System' (RSES) software as GA produces many reduct set of varying cardinality (length) to suit the need of different applications.

### **Maximum Possible Combined Reduct (MPCR)**

MPCR is defined as the union of variables present in the reduct sets obtained after applying GA (Jain, 2004). Any variable that belongs to at least one of the reduct in the population of reducts from GA also belongs to MPCR. For Example, reduct computation on the table 1 resulted in six reduct sets of cardinality three;  $R1 = \{\text{date, damage, canker\_lesion}\}$ ,  $R2 = \{\text{precip, damage, severity}\}$ ,  $R3 = \{\text{date, precip, severity}\}$ ,  $R4 = \{\text{date, precip, damage}\}$ ,  $R5 = \{\text{date, precip, decay}\}$  and  $R6 = \{\text{precip, damage, decay}\}$ . MPCR set, which is union of variables from these sets, is  $\{\text{date, precip, severity, damage, decay, canker lesion}\}$ . Variable fruiting bodies is filtered out in this process.

### **Variable Selection**

Data pre-processing helps in knowing the important variables before doing clustering and the clustering task becomes more efficient and focused as only important variables are used. There are mainly two methods of data pre-processing - Variable Extraction or Selection. Variable Extraction (VE) is the use of one or more transformations of the input variables to produce new salient variables. VE method like principal component analysis has a major drawback as, it is difficult to understand the data

(and the found clusters) using the extracted variables. Variable Selection (VS) is the process of identifying the most effective subset of the original variables. There are mainly two types of methods for VS - filter and wrapper methods in supervised learning (Dash and Liu, 1997). Filter methods are independent of the algorithm that will use their output and employ some metric dependent on intrinsic properties of data to select the subset. On the other hand in the wrapper method, variable selection algorithm works as a wrapper around the induction algorithm for subset selection. Wrapper method in comparison to filter may result in smaller subset. However wrapper approach is computationally costly as every possible combination of subset of variables needs to be evaluated (Talavera, 2005). In supervised learning both the methods work around criteria of class label prediction for variable selection. According to Dash and Liu (1977) and Dash *et al.* (1997), as clustering is done on unsupervised data without the class information; therefore the traditional variable selection algorithms for classification do not work. Selecting variables in clustering scenario is much harder problem due to absence of class label that guides the search for relevant information.

**Review of literature in variable selection for clustering**

Little work has been done on variable selection for unsupervised data and most of the approaches are customized for a particular clustering algorithm. Devaney and Ram (1997), applied sequential forward and backward

search approach for subset selection and evaluated the same by measuring the category utility of the clusters formed by applying COBWEB (a hierarchical clustering algorithm). Talavera (1999) used a variable dependence measure to select the variables and evaluate the same using COBWEB. Dy and Brodley (2002), proposed a variable subset selection method based on estimating the maximum likelihood criteria, wrapped around EM (Expectation Maximization) algorithm. Dash *et al.* (1997) proposed method of ranking the variables and subset selection based on entropy.

**Cluster Description**

The problem of cluster description comes under the area of interpretation and representation of clusters. Cluster can be described using a pattern, which is formed by conjunction of attributes from the cluster. The problem of producing description for a single cluster without any relevance to other clusters has attracted considerable attention from the researchers (Mirkin, 2005)). As discussed by Mirkin (1999), accuracy of obtained pattern is measured in terms of Precision Error (PE). PE of pattern *P* , PE (*P*) is defined as:

$$PE (P) = \frac{| false\ positive\ C(P) |}{| U - C |} \dots\dots\dots(1)$$

Where numerator, *false positive C (P)* is defined as the number of objects that lies outside cluster *C* , for which pattern *P* is true and denominator denotes the number of objects outside *C* .

**Review of literature in cluster description**

Area of producing cluster description for individual clusters is relatively new;

therefore there are few references of cluster description approaches in literature. In Mirkin (1999) approach, variables with greatest contribution towards a cluster are used to form a conjunctive concept that approximately describes the cluster. The contribution weight of a variable is proportional to the deviation of the variables within-cluster mean from its grand mean, which suggest that more deviant a variable is, the more contributing it is in the cluster description. In his approach, Abidi *et al.* (1998, 2001) have proposed the rough set theory based method for rule creation for unsupervised data using dynamic reduct. Dynamic reduct is defined as the frequently occurring reduct set from the samples of original decision table. However these approaches have its limitations. Mirkin's approach is applicable only to datasets having continuous attributes. Abidi in his approach has used the cluster information obtained after cluster finding and generated rules from entire data with respect to decision variable, instead of producing description for individual clusters. Michalski and Step (1981), has experimented with conceptual clustering and in turn obtained clusters has cluster description in the form of pattern. Other description approaches like decision tree is not directly applicable to clustering as criteria in clustering is to get homogenous clusters with respect to all the attributes (Mirkin, 2005). However in decision tree, homogeneity is with respect to decision attribute. However, our approach is to generate user understandable cluster description for

individual clusters by conjunction of significant variables that define the cluster.

### **Proposed approach**

In the proposed approach, we have applied reduct from RST in clustering. We have first explored the feasibility of rough set for variable selection in clustering as this will lead to comprehensive cluster description with only relevant variables. Clustering algorithm requires similar valued variables for grouping instances and different valued variables for cluster formation. Therefore in the step of VS, we take care of the variables that account for discernibility in the data and are responsible for cluster formation. Reduct analysis is carried out using GA without taking class information into consideration, which resulted in reduct set of varying cardinality (length). For preserving full indiscernibility, MPCR set is computed from the reduct sets produced by GA. Therefore MPCR approach for variable selection is simple, that learns from the data and results in creation of a single set.

In the proposed approach of cluster description based on MPCR, reduct is computed for every cluster. As cluster is formed on the basis of homogeneity, therefore all the variables (MPCR) that account for discernibility within cluster can be removed. This will provide set of variables which have similar value for majority of objects in the cluster, hence significant for that cluster. Pattern formulated with the conjunction of all significant variables can be quite complex, hence in the next step, we propose to rank significant variables. Significant variables are then ranked on Precision Error (PE) which is defined as:



$$PE(a = v) = \frac{|false\ positive\ C(a = v)|}{|U - C|} ..(2)$$

Where numerator defines the number of entities that lies outside cluster  $C$ , for which  $a=v$  ( $a \in A, v \in V_a$ ) is true and denominator defines the number of entities outside cluster  $C$ . An attribute value pair  $a=v$  is said to be more contributing if it has less PE, means majority of objects satisfying this attribute value pair belongs to a single cluster.

Therefore, problem of cluster description can be defined as forming a description  $P$  by combining the significant variables with less PE such that PE for  $P$  is zero or minimum. Hence, pattern  $P$  distinctively describes the cluster without or minimum errors.

**Approach for variable selection and cluster description is mentioned below.**

1. GA based reduct sets computation without taking into consideration class information
2. Compute MPCR from reduct sets
3. Apply clustering algorithm on MPCR variables
4. Compute GA based reduct for individual clusters
5. Compute MPCR set for each clusters
6. Relevant variable set of Cluster  $C$  = Set of MPCR variables - MPCR variables of Cluster  $C$
7. Calculate PE for relevant variables in cluster  $C$
8. Combine variables with less PE to make the description, such that PE for that description equals zero

## RESULTS AND DISCUSSION

### Example of application: Soybean disease diagnosis

In soybean disease set, universal set (U) contains 47 entities and set of variables (A) consist of 35 variables characterizing diaporthe-stem-canker, charcoal-rot, rhizoctonia-root-rot and phytophthora-rot diseases [UCI]. All the variables are nominal in nature. Table 2 shows the variable information. Variables are broadly categorized into environmental descriptors, condition of leaves, condition of stem, condition of fruit pods and condition of root. Variable instance number and class information are not considered for clustering.

### Variable Selection Process

In the VS process reduct is computed based on GA using RSES software. This resulted in 76 reduct sets of varying cardinality of length 5 to 9 variables. VS is experimented with reduct set of minimum and maximum cardinality. Significant results are not observed when clustering algorithm is carried out on reduct set of minimum and maximum cardinality. For better results, MPCR reduct set is considered, that is union of reduct variables in all the reduct sets (Table 3). MPCR resulted in reduced dataset with the elimination of 17 variables.

We have selected Expectation Maximization (EM) algorithm for portioning the data into homogenous clusters, as it can handle both numeric and

**Table 2. Variable information of soybean dataset**

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v1	date: April = 0, May = 1, June = 2, July = 3, August = 4, September = 5, October = 6
v2	plant-stand: normal = 0, lt-normal = 1
v3	precip: lt-norm = 0, norm = 1, gt-norm = 2
v4	temp: lt-norm = 0, norm = 1, gt-norm = 2
v5	hail: yes = 0, no = 1
v6	crop-hist: diff-lst-year = 0, same-lst-yr = 1, same-lst-two-yrs = 2, same-lst-sev-yrs = 3
v7	area-damaged: scattered = 0, low-areas = 1, upper-areas = 2, whole-field = 3
v8	severity: pot-severe = 1, severe = 2
v9	seed-tmt: none = 0, fungicide = 1
v10	germination: '90-100%' = 0, '80-89%' = 1, 'lt-80%' = 2
v11	plant-growth: abnorm = 1
v12	leaves: norm = 0, abnorm = 1
v13	leafspots-halo: absent = 0
v14	leafspots-marg: dna = 2
v15	leafspot-size: dna = 2
v16	leaf-shread: absent = 0
v17	leaf-malf: absent = 0
v18	leaf-mild: absent = 0
v19	stem: abnorm = 1
v20	lodging: yes = 0, no = 1
v21	stem-cankers: absent = 0, below-soil = 1, above-soil = 2, above-sec-nde = 3
v22	canker-lesion: dna = 0, brown = 1, dk-brown-blk = 2, tan = 3
v23	fruiting-bodies: absent = 0, present = 1
v24	external decay: absent = 0, firm-and-dry = 1
v25	mycelium: absent = 0, present = 1
v26	int-discolor: none = 0, black = 2
v27	sclerotia: absent = 0, present = 1
v28	fruit-pods: norm = 0, dna = 3
v29	fruit spots: dna = 4
v30	seed: norm = 0
v31	mold-growth: absent = 0
v32	seed-discolor: absent = 0
v33	seed-size: norm = 0
v34	shriveling: absent = 0
v35	roots: norm = 0, rotted = 1
v36	diaporthe-stem-canker = D1, charcoal-rot = D2, rhizoctonia-root-rot = D3, phytophthora-rot = D4

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nominal variables. EM models the distribution of the entities probabilistically, so that an entity belongs to a cluster with certain probability. The first step, calculation of the cluster probabilities, which are the expected class value, is “expectation”; the second step is calculation of the distribution parameter is “maximization” of the likelihood of the distribution given the data (Mirkin, 2005). EM algorithm has built in evaluation measure for computing the number of clusters present in the dataset. EM selects the number of clusters automatically by maximizing the logarithm of the likelihood of future data, estimated using cross-validation. Beginning with one cluster, it continues to add clusters until the estimated log-likelihood decreases.

To test the significance of variable selection process, clustering process is carried out with EM clustering algorithm on full dataset as well as on reduced dataset using open source software from university of WAIKATO (WEKA). As

suggested by Talvera (2005), clustering is done on reduced variable set and log-likelihood value is compared with the one obtained with full variable set. If the resulting score is as good as the one obtained with full variables, this is an indicator that the non selected variables are not relevant. EM algorithm resulted in the value of log likelihood -10.7932 with full dataset and MPCR subset, this shows that removed variables were not playing any role in clustering. Reduced dataset has 47 entities and 18 variables characterizing these entities. EM clustering algorithm when applied on MPCR attributes grouped the entities into four disease clusters without any incorrectly clustered instances (Table 6).

### Cluster Description

To study the disease characteristics, we carried out reduct analysis using RSES software on obtained four disease clusters. Table 3 shows the MPCR variables in full dataset and in different clusters.

**Table 3. MPCR reducts from full dataset and in different clusters.**

MPCR variables in full data set	v1, v2, v3, v4, v5, v6, v7, v8, v9, v10, v12, v20, v21, v22, v23, v24, v25, v28, v35.
MPCR variables in Cluster1	v1, v5, v6, v7, v8, v9, v10, v20, v22.
MPCR variables in Cluster2	v1, v4, v5, v6, v7, v9, v10, v20.
MPCR variables in Cluster3	v1, v5, v6, v8, v9, v10, v12, v20, v25, v35.
MPCR variables in Cluster4	v1, v3, v4, v5, v6, v8, v9, v10, v21, v24.

Reduct analysis on different clusters shows that it has different MPCR variables, as variables are having different values in different clusters. Variables are not common across clusters and as such some variables are playing role in one cluster and not in other cluster.

Let us consider the cluster description for Cluster1. Removal of MPCR variables (v1, v5, v6, v7, v8, v9, v10, v20, v22) (Table 4) has left the set of significant variables (v2 = 0, v3 = 2, v4 = 1, v12 = 1, v21 = 3, v23 = 1, v24 = 1, v25 = 0, v26 = 0, v27 = 0, v28 = 0, v35 = 0). In the next stage, these

significant variables are ranked on PE. Let us consider computation of PE for variable  $v_2 = 0$  from Cluster1 (Equ. 2). Dataset contains 47 entities, hence  $card\ U$  is 47. Cluster1 has 10 entities, hence  $card\ C_i$  is 10. Descriptor ( $v_2 = 0$ ) has support of 22 objects in full dataset, out of which 10 objects belongs to Cluster 1 (Table 6).

Therefore  $PE\ (v_2 = 0) = (22 - 10) / (47 - 10) = 12 / 37 = (0.32)$ ,

Number of false positive is 12, as 10 entities from Cluster 2 and 2 entities from Cluster3 are satisfying this condition (Table 6). Similarly PE for other variables is computed for individual clusters. Table 4 lists significant variables in corresponding clusters along with value of PE in bracket. PE for descriptors  $v_{21} = 3$  and  $v_{23} = 1$  is zero, hence either of these descriptor is sufficient to describe the Cluster 1 without any error.

**Table 4. Significant descriptors in individual clusters**

Cluster 1	$v_{21} = 3(0)$ , $v_{23} = 1(0)$ , $v_{28} = 0(0.27)$ , $v_2 = 0(0.32)$ , $v_4 = 1(0.37)$ , $v_{24} = 1(0.43)$ , $v_{35} = 0(0.51)$ , $v_3 = 2(0.62)$ , $v_{26} = 0(0.72)$ , $v_{27} = 0(0.72)$ , $v_{12} = 1(0.75)$ , $v_{25} = 0(0.86)$
Cluster 2	$v_3 = 0(0)$ , $v_{21} = 0(0)$ , $v_{22} = 3(0)$ , $v_{26} = 2(0)$ , $v_{27} = 1(0)$ , $v_{28} = 0(0.27)$ , $v_{24} = 0(0.29)$ , $v_2 = 0(0.32)$ , $v_8 = 1(0.48)$ , $v_{35} = 0(0.51)$ , $v_{23} = 0(0.72)$ , $v_{12} = 1(0.75)$ , $v_{25} = 0(0.86)$
Cluster 3	$v_{22} = 1(0.16)$ , $v_4 = 0(0.18)$ , $v_{21} = 1(0.21)$ , $v_{24} = 1(0.43)$ , $v_{28} = 3(0.45)$ , $v_2 = 1(0.45)$ , $v_7 = 1(0.51)$ , $v_3 = 2(0.62)$ , $v_{23} = 0(0.72)$ , $v_{26} = 0(0.72)$ , $v_{27} = 0(0.72)$
Cluster 4	$v_{22} = 2(0)$ , $v_{35} = 1(0.03)$ , $v_2 = 1(0.26)$ , $v_{20} = 1(0.3)$ , $v_{28} = 3(0.33)$ , $v_7 = 1(0.43)$ , $v_{23} = 0(0.66)$ , $v_{26} = 0(0.66)$ , $v_{27} = 0(0.66)$ , $v_{12} = 1(0.7)$ , $v_{20} = 0(0.7)$ , $v_{25} = 0(0.83)$

**Table 5. Cluster Description Results**

Cluster	Pattern	PE
Cluster 1 (diaporthe-stem-canker)	stem-cankers = above-sec-nde <b>or</b> fruiting-bodies = present	0
Cluster 2 (charcoal-rot)	Precip = lt-norm <b>or</b> stem-cankers = absent <b>or</b> canker-lesion = tan <b>or</b> int-discolor = black <b>or</b> sclerotia = present	0
Cluster 3 (rhizoctonia-root-rot)	canker-lesion = brown ^ temp = lt-norm	0
Cluster 4 (phytophthora-rot)	canker-lesion = dk-brown-blk	0

Let us consider another example of Cluster 3 (Table 6) which has ten entities corresponding to disease rhizoctonia-root-rot. There is no single descriptor with zero PE (Table 4), hence as per proposed approach variables with less PE, v22 = 1 with PE 0.16 and v4 = 0 with PE 0.18 are combine together. Pattern (v22 = 1 ^ v4 = 0) is then evaluated, which has support of 10 entities in full dataset and all the 10 entities belongs to Cluster3, therefore PE is zero (Equ. 1).

Descriptions of disease clusters obtained with proposed approach are summarized in table 5 (combining together value of the attribute from Table 1):

#### *Comparison with cluster description approaches*

Mirkin (1999) in his approach on soybean disease dataset, has predicted variables v23 (Cluster 1), v26 (Cluster 2), v4 ^ v24 (Cluster 3) and v35 ^ v12 (Cluster 4) as significant variables for describing soybean disease clusters. However, he has considered these nominal variables as numeric in his approach which is applicable to numeric data. Our approach has predicted v3, v4, v21, v22, v23, v26 and v27 as important variables. In further experiment, correlation is studied between the uncommon variables from these approaches and it was found that these variables are highly correlated.

Michalski and Stepp (1981), in their conceptual clustering approach has predicted following description for disease clusters.

*Cluster 1* {(date = jul...oct) ^ (precip = gt-normal) ^ (leaf\_malf = abs) ^ (stem = abn) ^ (stem-cankers = above-sec-nde) ^

(external decay = firm-and-dry) ^ (fruit-pods = norm)}

*Cluster 2* {(leaf\_mal = abs) ^ (stem = abn) ^ (int-discolor = black)}

*Cluster 3* {(leave = norm) ^ (stem = abn) ^ (stem-canker = below-soil) ^ (canker-lesion = brown)}

*Cluster 4* {(plant-stand = gt-normal) ^ (precipitation = gt-normal) ^ (temp = lt-normal) ^ (plant height = abn) ^ (leaves = abn) ^ (leaf malformation = abs) ^ (stem = abn)}

However, our approach has resulted in more concise cluster description. Variable selection process has taken care off irrelevant variables like (stem = abn), (leaf\_malf = abs) before clustering, as these variables are having same value for all of its instances. Moreover, ranking of significant variables has resulted in concise cluster description.

Reduct based approach for variable selection and cluster description has been presented in this paper. Variable selection with MPCR reduct gave significant results, and it resulted in the removal of 17 irrelevant variables out of total 35 variables. In future, experiments need to be carried out for variable selection using reduct set of different cardinality which is obtained after applying GA for optimum reduct set. Description of clusters using MPCR set helped in generating simple, understandable description with zero PE. This helped in identifying the contributing variable for that cluster and in turn identified contributing variables in full dataset. Further research is required to apply the same approach on more datasets to confirm the existence of relation.

**Table 6. EM Clustering result on MPCR variables**

S No	v	v	v	v	v	v	v	v	v	v	v	v	v	v	v	V	V	V	V	v35	Cluster
	1	3	4	5	6	7	8	9	10	12	20	21	22	23	24	25	26	27	28		
0	4	2	1	1	1	0	1	0	2	1	0	3	1	1	1	0	0	0	0	0	cluster1
1	5	2	1	0	3	1	1	1	2	1	1	3	0	1	1	0	0	0	0	0	cluster1
2	3	2	1	0	2	0	2	1	1	1	0	3	0	1	1	0	0	0	0	0	cluster1
3	6	2	1	0	1	1	1	0	0	1	1	3	1	1	1	0	0	0	0	0	cluster1
4	4	2	1	0	3	0	2	0	2	1	0	3	1	1	1	0	0	0	0	0	cluster1
5	5	2	1	0	2	0	1	1	0	1	1	3	1	1	1	0	0	0	0	0	cluster1
6	3	2	1	0	2	1	1	0	1	1	1	3	0	1	1	0	0	0	0	0	cluster1
7	3	2	1	0	1	0	2	1	2	1	0	3	0	1	1	0	0	0	0	0	cluster1
8	6	2	1	0	3	0	1	1	1	1	0	3	1	1	1	0	0	0	0	0	cluster1
9	6	2	1	0	1	0	1	0	2	1	0	3	1	1	1	0	0	0	0	0	cluster1
10	6	0	2	1	0	2	1	0	0	1	1	0	3	0	0	0	2	1	0	0	cluster2
11	4	0	1	0	2	3	1	1	1	1	0	0	3	0	0	0	2	1	0	0	cluster2
12	5	0	2	0	3	2	1	0	2	1	0	0	3	0	0	0	2	1	0	0	cluster2
13	6	0	1	1	3	3	1	1	0	1	0	0	3	0	0	0	2	1	0	0	cluster2
14	3	0	2	1	0	2	1	0	1	1	0	0	3	0	0	0	2	1	0	0	cluster2
15	4	0	1	1	1	3	1	1	1	1	1	0	3	0	0	0	2	1	0	0	cluster2
16	3	0	1	0	1	2	1	0	0	1	0	0	3	0	0	0	2	1	0	0	cluster2
17	5	0	2	1	2	2	1	0	2	1	1	0	3	0	0	0	2	1	0	0	cluster2
18	6	0	2	0	1	3	1	1	0	1	0	0	3	0	0	0	2	1	0	0	cluster2
19	5	0	2	1	3	3	1	1	2	1	0	0	3	0	0	0	2	1	0	0	cluster2
20	0	2	0	0	1	1	1	1	1	0	0	1	1	0	1	1	0	0	3	0	cluster3
21	2	2	0	0	3	1	2	0	1	0	0	1	1	0	1	0	0	0	3	0	cluster3
22	2	2	0	0	2	1	1	0	2	0	0	1	1	0	1	1	0	0	3	0	cluster3
23	0	2	0	0	0	1	1	1	2	0	0	1	1	0	1	0	0	0	3	0	cluster3
24	0	2	0	0	2	1	1	1	1	0	0	1	1	0	1	0	0	0	3	0	cluster3
25	4	2	0	1	0	1	2	0	2	1	1	1	1	0	1	1	0	0	3	0	cluster3
26	2	2	0	0	3	1	2	0	2	0	0	1	1	0	1	1	0	0	3	0	cluster3
27	0	2	0	0	0	1	1	0	1	0	0	1	1	0	1	0	0	0	3	1	cluster3
28	3	2	0	1	3	1	2	0	1	0	1	1	1	0	1	1	0	0	3	0	cluster3
29	0	2	0	0	1	1	2	1	2	0	0	1	1	0	1	0	0	0	3	0	cluster3
30	2	2	1	1	3	1	2	1	2	1	0	2	2	0	1	0	0	0	3	1	cluster4
31	0	1	1	0	1	1	1	0	0	1	0	1	2	0	0	0	0	0	3	1	cluster4
32	3	2	0	0	1	1	2	1	0	1	0	2	2	0	0	0	0	0	3	1	cluster4
33	2	2	1	1	1	1	2	0	2	1	0	1	2	0	1	0	0	0	3	1	cluster4
34	1	2	0	0	3	1	1	1	2	1	0	2	2	0	0	0	0	0	3	1	cluster4
35	1	2	1	0	0	1	2	1	1	1	0	2	2	0	0	0	0	0	3	1	cluster4
36	0	2	1	0	3	1	1	0	0	1	0	1	2	0	0	0	0	0	3	1	cluster4
37	2	2	0	0	1	1	2	0	0	1	0	1	2	0	0	0	0	0	3	1	cluster4
38	3	2	0	0	2	1	2	1	1	1	0	2	2	0	0	0	0	0	3	1	cluster4
39	3	1	0	0	2	1	2	1	2	1	0	2	2	0	0	0	0	0	3	1	cluster4
40	0	2	1	1	1	1	1	0	0	1	0	1	2	0	1	0	0	0	3	1	cluster4
41	1	2	1	1	3	1	2	0	1	1	1	1	2	0	1	0	0	0	3	1	cluster4
42	1	2	0	0	0	1	2	1	0	1	0	2	2	0	0	0	0	0	3	1	cluster4
43	1	2	1	1	2	3	1	1	1	1	0	2	2	0	1	0	0	0	3	1	cluster4
44	2	1	0	0	3	1	2	0	2	1	0	1	2	0	0	0	0	0	3	1	cluster4
45	0	1	1	1	2	1	2	1	0	1	1	2	2	0	1	0	0	0	3	1	cluster4
46	0	2	1	0	3	1	1	0	2	1	0	1	2	0	0	0	0	0	3	1	cluster4

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## Field Plot Headland Seed Drop Control Mechanism for Tractor Operated Seed Drill

DEVVRAT SINGH<sup>1</sup>, RAJKUMAR RAMTEKE<sup>2</sup> AND I R KHAN<sup>3</sup>  
National Research Centre for Soybean, Khandwa Road, Indore, 452 017,  
Madhya Pradesh, India  
(E-mail: singhdv123@hotmail.com)

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### ABSTRACT

*A simple device "seed drop control mechanism" with the seed drill attachable on top link of three-point linkage system on tractor was conceived, designed, fabricated and farm validated for effective dropping of seed at the headland region of the field. The use of device on the seed drill economizes the seed requirement by 29 per cent. The device costs merely Rs 4000/- in addition to the cost of seed drill and its use can enhance the average seed yield of soybean in headland region by 102 kg per hectare (10.83%). The enhancement in yield and saved seed per hectare itself can recover the three forth cost of device in one year. Local manufacturer on account of its simplicity can conveniently manufacture the device.*

**Key words:** Field plot headland, seed drop control mechanism, three-point linkage, top link

Soybean is a premier oilseed crop of Central India. Among the inputs, seed is major and costs nearly one-sixth of total inputs needed for raising the crop (Tiwari and Joshi, 2002). Although, the mechanized soybean culture is reported to be about 50 per cent (Holt *et al.*, 1999), the planting operation for the crop is largely mechanized in India and completed by the farmers using tractor mounted seed drills. Even the small and marginal farmers resort to planting of soybean with tractor mounted seed drill

using custom-hire arrangement. The normal seed drills in usage releases excessive seeds in the headland region of the field and results in use of more seed than recommended and resultant above-optimum plant population affect the crop performance. The drop of excessive seed is usually uneven that results in clusters of plants in regular rows. To save the wastage of valuable input like seed and save the crop yield losses, an effort has been made to conceive, develop and validate a seed drop mechanism

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<sup>1</sup> Senior Scientist, <sup>2</sup>Scientist Senior Scale, <sup>3</sup>Technical Assistant



attachable to avoid the excess seed drop in the headland region of the field. This device makes it possible to ensure even distribution of seeds in the row avoiding uneven clusters of plants in headland region. The developed seed drop control mechanism with the seed drill can be fabricated locally for better fitment to the seed drill attachable to the individual tractor. The developed mechanism is attachable to top link of three-point linkage system. The tractor operated seed drop control mechanism attached with the seed drill facilitates desired plant population thus saving precious seeds in facilitating appropriate post-planting mechanized operations.

## MATERIAL AND METHODS

A simple seed drop mechanism with the seed drill developed is made of box section mild steel (20 mm diameter) and flat TATA steel with 10 mm thickness (Fig. 1). Feed cut off in the traditional seed drills depend upon the ground wheel of the machine which takes time to stop the seed drop and fails to stop immediately. The

device attached with seed drill is capable of nearly eliminating the seed drop from seed drills as soon as the seed drill is lifted from with the help of top links. The gravity feed type seed metering mechanism was used with the seed drill. The seed drop control mechanism works on opening and closing of the hole in the seed box with the help mild steel plate which is actuated by the lever attached to the top link of the tractor through a pivot on the seed drill (Fig. 2 and 3). This seed drop control mechanism can easily be fixed and detached to and from the top lower link of the tractor and the seed drill. This simple device on the seed drill can be fabricated in mere cost of Rs 4000/- in addition to the seed drill and fitted to the seed drill matching to individual tractor model for better fitment as per three-point linkage category.

Farm validation of seed drill with developed seed drop control mechanism was done consecutively for three years between *kharif* 2001 and 2003 at research farm of National Research Center for Soybean and compared with normal seed drill. The soybean (var. JS 335) was planted

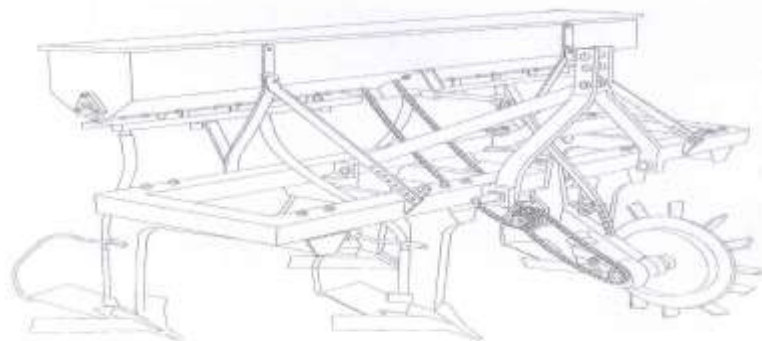
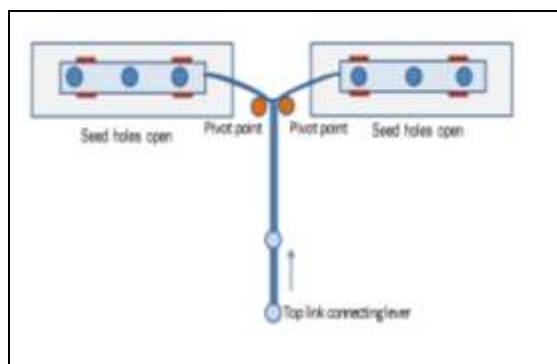
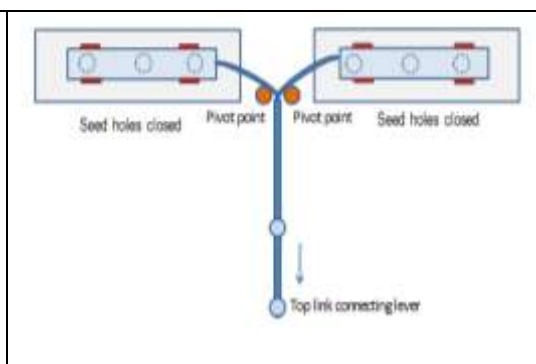


Fig. 1. Five row tractor drawn seed drill fitted with seed drop control mechanism



**Fig. 2. Seed drop control mechanism (Seed holes open)**



**Fig. 3. Seed drop control mechanism (Seed holes closed)**

at row to row distance of 45 cm and seed drop per square meter, resultant plant population and seed yield from 10 randomly selected places from ten replicated plots (50 m x 2.25 m) from the head land region was recorded. The data collected from these plots were subjected to 't' test for each year and cumulative data for three years was analyzed using randomized block design.

## RESULTS AND DISCUSSION

Data (Table 1) on seed drop revealed that use of device with the tractor invariably utilized significantly less seed during each year and for cumulatively three years. Pooled data for three years

showed an average reduction of 29 per cent of seed (year to year variation of 25-31 %) when the seed drop device was used on seed drill than without it. The resultant plant population when the device was used was on an average 18.5 plants per square meter as compared to 26.9 plants per square meter on using seed drill without it (Table 2). It is to mention here that the optimum population recommended is 4 lakh plants per hectare which works out to about 18 plants per square meter sown at row distance of 45 cm (Anonymous, 1995). The effect of appropriate plant population was expressed in terms of seed yield of soybean as well. The sowing of soybean using seed drop device yielded

**Table 1. Seed drop (no/m<sup>2</sup>) through seed drill with and without headland seed drop control mechanism**

Treatment	Year			Grand Mean
	2001	2002	2003	
With headland seed drop control mechanism	22.5	21.3	20.5	21.4
Without headland seed drop control mechanism	30.0	30.8	29.6	30.1
't' value (p = 0.01)	5.62**	10.26**	11.00**	-
CD (p = 0.01)	-	-	-	1.15

10.83 per cent higher than without the use of device (Table 3). An average yield increase of 102 kg per hectare is likely to fetch an amount of Rs 2448/- (considering the prevailing cost to be Rs 24/kg). If we add the amount saved by way of economizing the seed using the

device, the saving shall work out to about Rs 3000 per hectare. Thus, use of the device for only one year can recover almost three forth the cost of manufacture of the seed drop mechanism.

**Table 2. Plant population (no/m<sup>2</sup>) through seed drill with and without headland seed drop control mechanism**

Treatment	Year			Grand Mean
	2001	2002	2003	
With headland seed drop control mechanism	18.5	18.5	18.5	18.5
Without headland seed drop control mechanism	27.2	27.2	26.5	27.0
't' value (p = 0.01)	11.95**	11.15**	9.59**	-
C D (P = 0.01)	-	-	-	0.89

**Table 3. Seed yield (kg/ha) through seed drill with and without headland seed drop control mechanism**

Treatment	Year			Grand Mean
	2001	2002	2003	
With headland seed drop control mechanism	1065.2	979.9	1093.2	1046.1
Without headland seed drop control mechanism	940.8	851.0	1039.9	943.9
't' value (P = 0.01)	11.40**	7.97**	6.97**	
CD (p = 0.01)				16.77

The study suggests that use of seed drop mechanism developed at National Research Center for Soybean is economical, effectively controls the seed drop in head land region and fetches more yield. The saving on one of the major input like seed (29%) and enhanced yield (11%) in the head land region of the field is instrumental in bringing down the cost of cultivation of soybean.

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## **Pant Soybean 1225 - An Improved Variety of Soybean with Broad Genetic Base**

**PUSHPENDRA<sup>1</sup>, KAMENDRA SINGH<sup>2</sup>, B V SINGH<sup>3</sup> and M K GUPTA<sup>4</sup>**

*Department of Genetics and Plant Breeding, College of Agriculture,  
Govind Ballabh Pant University of Agriculture and Technology,  
Pantnagar 263 145, Uttarakhand  
(E-mail - pushpendra\_sb@yahoo.com)*

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**Key words:** Genetic base, soybean, variety

Soybean has emerged as an important crop with established potential to reduce the oil and protein gap in the diet of predominantly vegetarian society like India. Today it has become one of the important oil seed crops and has occupied a coveted position in Indian Agriculture. It has been instrumental in changing the socio-economic status of the soybean growers, particularly in central part of India. At present, it is being grown in more than 9.6 million hectares with a production of about 10.8 million tonnes (Anonymous, 2008). However, India's productivity (1.1 t/ha) still remains just half of that of USA (2.5 t/ha).

The introduction of soybean in India as modern cultivated crop started in 1963-64 with feasibility trials conducted at Pantnagar and Jabalpur using exotic varieties like Bragg, Clark- 63, Davis and Lee, etc. These varieties were used as parents to generate new variability for selection. Till 1980, most of the varieties were either introduction or

selection from exotic material; these have been called as varieties of selection cycle-1. The varieties developed by using the exotic varieties as parent, have been grouped in selection cycle-2. The varieties in selection cycle -1 have produced 4 times more yield than indigenous variety Kalitur by virtue of higher number of pods per plant, seed weight, shorter maturity duration and increased biomass. The varieties in selection cycle- 2 showed 19 per cent higher yield than varieties of selection cycle- 1. This was due to improvement in harvest index and seed filling duration (Karmakar and Bhatnagar, 1996).

Indian soybean breeders have used only a small part of available genetic resource and hence soybean varieties are considered to have narrow genetic base. There is a need for restructuring the breeding strategy through attempting the crosses between widely adapted genotype along with land races/pre-bred lines/

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<sup>1,2</sup>Professor; <sup>3</sup>Ex-Head and Professor; <sup>4</sup>Technical Assistant

alien species specially *Glycine soja* Seib Zucc. Directed introduction, germplasm enhancement and further use of pre-breeding lines in crossing programme for the development of cultivars will eventually lead to realization of high productivity and broadening the genetic base of soybean cultivar at farm level. Yield potential can be enhanced by increasing the contribution of yield components to achieve our targeted national productivity of about 1.6 to 1.8 t per ha.

In order to broaden the genetic base of newly developed varieties of soybean, Pantnagar centre took initiative by utilizing *Glycine soja* Syn. *Glycine formosana* a typical wild looking soybean to improve the genetic background of cultivated soybean by infusing the characters of economic importance. Singh *et al.* (1974) have already identified PI 171443 and *Glycine formosana* as resistance sources for YMV at this centre. *Glycine soja* has distinct morphological characters. The plants are prostrate, tawny, leaflets are narrowly lanceolate, flowers are purple and seeds are small black (0.3 g as compared to 12.0 g/100 seed weight of normal cultivated soybean). It has tawny pubescence, black pod colour with 2-5 seeds per pod. *Glycine soja* possessing single dominant gene conforming the resistance against YMV, and it has long vegetative phase, i.e. 80-85 days as compared to 45-50 days in case of cultivated soybean which could be considered desirable feature as longer juvenility is essential to attain high biomass. The condition is just reverse with respect to reproductive phase, i.e. 45-50 days only when compared with cultivated soybean varieties i.e. 70-85 days. Wenbin and Jinling (1988) has suggested the

introduction of high protein gene from *G. soja* into cultivated types an additional remarkable feature of this wild soybean possessing resistance to hairy caterpillar which has been considered a most serious insect of soybean during rainy season in different parts of country (Ram *et al.*, 1984). However, the resistance has been transferred through conventional breeding approach along with important agronomic traits from a non-recurrent donor parent to an adopted cultivar which lack in these desirable traits. Use of limited backcross has been found quite successful for transferring yellow mosaic virus (YMV) resistance from *G. soja* where F<sub>1</sub> is backcrossed once with the adopted cultivar with resistant. BC-1 is routed through pedigree method of breeding. Following this approach, PK 515 {pre-breeding line from (*G. soja* × Bragg) × Bragg}, a line that is resistant to YMV, moderately resistant to hairy caterpillar has been developed.

Pant soybean 1225 (PS 1225) is an improved genotype derived from a pre-breeding line PK 515 that was developed through interspecific hybridization involving *G. soja*, a wild relative of cultivated soybean having equal number of chromosomes ( $2n = 40$ ) with elite cytoplasm. Following the conventional breeding approach, positive gene with characters of economic importance have been transferred in the back ground of widely adapted variety Bragg (introduced from USA during early sixties ) having almost good agronomic traits except the susceptibility to YMV. YMV is considered most important constraint for not popularizing the soybean in northern part of India.

PS 1225 was derived from a cross PK 515 x PK 327 through hybridization followed by pedigree method of breeding. Where, PK 515 was the female parent possessing YMV resistance and elite cytoplasm from its wild counterpart whereas, PK 327 was known for early maturity as well as better seed longevity. PS 1225 has occupied first rank in coordinated breeding trial conducted in north plain zone. On overall zonal mean

basis it has shown 43.64, 7.89, 18.76 and 56.61 per cent yield superiority over with check varieties viz. Bragg, PK 416, PS 1042 and Pusa 16, respectively (Table 1) (Anonymous, 1998, 1999 and 2000). In State Varietal Trial (SVT) conducted by various RATDS in Uttarakhand during 2003-04 to 2005-06 it was superior in yield by 6.30, 19.50 and 31.07 per cent over check varieties PS 1241, PS 1092 and PK 327, respectively (Table 2).

**Table 1. Performance of PS 1225 under Multilocation Coordinated Trials (North Plain Zone)**

Variety	Yield (kg/ha)			Mean	% increase or decrease over check and qualifying variety	Maturity duration (days)
	Initial varietal trial (1998-99)	Advanced varietal trial I (1999-00)	Advanced varietal trial II (2000-01)			
PS 1225	2185	2211	2041	2146	-	122
SL 459	2332	2126	1760	2073	+3.52	115
SL 695	2231	2013	1363	1869	+14.82	116
Bragg (c)	1059	2436	987	1494	+43.64	118
PK 416 (c)	2148	2028	1791	1989	+7.89	118
PK 1042(c)	1616	2024	1781	1807	+18.76	119
Pusa-16(c)	1454	1334	1376	1388	+54.61	120

**Table 2. Performance of PS 1225 in State Varietal Trial (SVT) conducted at RATDS, Uttarakhand (Plain)**

Variety	Yield (kg/ha)				Per cent superiority over the check	Maturity in days
	2003-04	2004-05	2005-06	Mean		
PS 1225	1914	1875	2185	1991		121
PK 1251	1875	1771	1910	1852	+7.50	122
PK 327(c)	1580	1159	1819	1519	+31.07	112
PK 1092(c)	1701	1398	1896	1665	+28.47	115
PK 1241 (c)	1972	1646	2003	1873	+6.30	124

PS 1225 is quite distinct from all other existing varieties released so far in terms of essential morphological characters viz gray pubescence, light green leaves and creamy yellow seed with light brown hilum. Maturity duration is comparable with the existing check varieties of the zone. It takes about 121 days to mature. PS 1225 has multiple disease resistance. It is resistant to YMV,

bacterial pustules, charcol rot, anthracnose, pod blight and soybean mosaic virus disease (Table 3). As regards resistance against insect-pests, it is at par with other varieties recommended for the North plain zone. It contains about 42.0 per cent protein and 18.0 per cent oil and retained more than 87 per cent germination even after storage under ambient conditions for 8 months

**Table 3. Comparative performance of PS 1225 with respect resistance to disease and other parameters**

Parameters	Check variety					Other qualifying varieties	
	PS 1225	Bragg(c)	PK 416	PS 1042	Pusa 16	SL 459	SL 495
<i>Disease resistance (1-9 scale)</i>							
Yellow mosaic virus	1	5	1	1	5	1	1
Bacterial pustules	1	1	1	1	1	1	1
Rhizoctonia blight	3	3	3	3	5	3	3
Pod blight	1	3	3	1	3	1	3
<i>Quality parameters</i>							
Protein content	42.00	39.75	39.00	40.00	40.00	39.50	38.00
Oil content	18.00	20.30	21.46	20.60	20.10	19.80	21.00
<i>Germination (%) after 8 months storage under ambient conditions</i>	87.00	75.00	85.00	90.00	85.00	85.00	85.00

Considering its wider genetic base, harboring alien cytoplasm from *Glycine soja*, representing distinct at morphological markers, multiple disease resistance with different YMV resistant gene, better plant type, free from lodging and pod shattering, better germinability, high protein content and high yield potential, it has qualified as a promising genotype. Moreover PS 1225 fulfills the essential requirement of DUS testing as distinctness, uniformity and stability which is considered essential for registration of any variety. Uttarakhand

State Variety Release Committee recommended PS 1225 for general cultivation for soybean growers in the plains of Tarai and Bhabar area of Uttarakhand.

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## Genetic Divergence Studies in Soybean [*Glycine max* (L.) Merrill]

**B KRISHNA VENI<sup>1</sup>, B PRAMILA RANI<sup>2</sup> and M V RAMANA<sup>3</sup>**

*Regional Agricultural Research Station, Lam, Guntur- 522 034, Andhra Pradesh*

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**Key words:** Clusters, genetic diversity, germplasm, soybean

Knowledge on genetic divergence of the germplasm lines is of immense important for the breeders in hybridization programme whereas, the diversity among the parents is of utmost importance, as the crosses between the parents with maximum genetic divergence would more likely yield desirable recombinants in the segregating generations. D<sup>2</sup> statistic developed by Mahalanobis (1936) is a powerful tool to measure genetic divergence among genotypes. An attempt was made in the present investigation to study the genetic divergence in 65 germplasm lines of soybean.

Sixty five soybean germplasm lines obtained from National Research Centre for Soybean, Indore and Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, including five checks were studied in a randomized block design with two replications during *kharif* 2007 at Regional Agricultural Research Station, Lam. Each line was raised in two rows of five meter length with a spacing of 30 cm x 7.5 cm between and within the rows, respectively. Observations were recorded on five randomly selected plants from

each plot for ten characters *viz.*, days to 50 per cent flowering, plant height, number of branches per plant, number of nodes per plant, number of pods per plant, pod length, number of seeds per pod, days to maturity, test weight and seed yield per plant. The analysis of genetic divergence was worked out using Mahalanobis D<sup>2</sup> statistics. The soybean germplasm lines were grouped in clusters by Tochers' method as described by Rao (1952).

The analysis of variance for different characters showed significant differences among the genotypes studied. Based on the relative magnitude of D<sup>2</sup> values, all the germplasm lines were grouped into eight clusters (Table 1). Majority of the germplasm lines were grouped in cluster I (42) followed by cluster II (9) and cluster III (7). Rest of the clusters *viz.*, V, VI and VII possessed only one genotype each. The pattern of distribution of genotypes into different clusters was at random. Genotypes belonging to same geographic origin were included in different clusters suggesting that geographic diversity does

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<sup>1</sup>Scientist <sup>2</sup>Senior Scientist <sup>3</sup>Professor

not necessarily represent genetic diversity. This is in conformity with the earlier findings of Ganeshmurthy and

Seshadri (2002) and Ramana and Satyanarayana (2006).

**Table 1. Clustering pattern of soybean germplasm lines**

Cluster No.	No. of genotypes	Genotypes
I	42	TGX 849-294D, JS 143, TGX 1073, UPSM 534, IC 45765, TGX 814-355, EC 18673, JS 98-76, RKS 24, TG X 802-2310, EC 241665, PLSO 57, TGX 854-4F, EC 18671, EC 251470A, AGS 1668, EC 30967, EC 251470, G 11, JS 98-67, EC 241708, MACS 22, JS 98-63, DS 2207, Himso 1602, TG X 813-150, UPMS 34, NRC 67, TG X 824-34A, JS 95-60, SL 886, P 501B, SL 525, Bragg, EC 337990, JS 99-92, D 50269-1-6, JS 98-66, EC 1164, MRSB 345, JS 97-51, DS 2207
II	9	LSb 23, EPS 4728, PK 1029, JS 335, G 47B, MACS 1038, EC 18672,
III	7	MACS 124, MACS 450 EC 241665, NP 2, PI 60269, EC 22999, AGS 1668, WT 182,
IV	2	Cockerstuart TG X 814-44B, TG X 814-28E
V	1	RPSP 722
VI	1	JS 93 05
VII	1	LSb 1
VIII	2	EC 257470, EC 357990

Cluster means showed appreciable differences for all the ten characters studied (Table 2). Highest mean value for number of branches per plant (3.26), number of nodes per plant (11.31), number of pods per plant (38.88), number of seeds per pod (2.76), test weight (13.29 g/100 seeds) and seed yield per plant (5.0 g) were recorded in cluster II. The genotypes that fall under this cluster can be used for hybridization for improvement of seed yield. Cluster III recorded high mean for days to 50 per cent flowering (50.42) and days to maturity (93.17), while cluster VII had lower mean values for both the traits.

Hence the genotypes in cluster VII can be utilized as parents for incorporating earliness. The maximum mean value for pod length was recorded in cluster VI (3.71 cm). Among the characters studied, days to 50 per cent flowering contributed maximum to genetic divergence (39.38 %) followed by seed yield per plant (18.37 %) and number of nodes per plant (18.32 %). The observed results find support from studies conducted by Chandankar *et al.* (2002) and Chowdhury *et al.* (1996), who reported the maximum contribution of days to 50 per cent flowering and seed yield per plant, respectively for genetic divergence in soybean.

**Table 2. Mean values and contribution of different characters of eight clusters for 65 soybean germplasm lines**

Cluster	Days to 50% flower-ing (No)	Plant height (cm)	Bran-ches/ plant (No)	Nodes/ plant (No)	Pods/ plant (No)	Pod length (cm)	Seeds / pod (No)	Days to maturity (No)	Test weight (g /100 seeds)	Seed yield/ plant (g)
I	39.92	26.97	2.63	7.48	26.64	3.15	2.44	86.14	11.80	2.56
II	43.17	32.98	3.26	11.31	38.88	3.62	2.76	92.67	13.29	5.0
III	50.42	24.78	2.78	6.26	23.98	2.99	2.41	93.17	11.30	1.58
IV	31.50	21.30	2.15	5.70	17.75	3.20	2.25	85.0	8.50	1.10
V	29.50	28.90	1.80	4.00	11.35	2.30	2.00	80.0	7.60	0.93
VI	32.0	20.35	2.15	4.30	31.65	3.70	2.70	87.50	12.95	4.95
VII	28.50	20.20	1.10	3.25	11.60	2.40	2.10	77.0	12.65	1.35
VIII	33.0	24.30	2.00	12.0	20.60	2.60	1.90	84.0	13.15	2.85
Contri-bution (%)	39.38	0.53	0.87	18.32	3.80	4.66	0.24	5.91	7.93	18.37

**Table 3. Average intra and inter-cluster distances among 8 clusters of soybean germplasm**

Cluster	I	II	III	IV	V	VI	VII	VIII
I	<b>214.06</b>	339.82	444.53	327.79	515.86	411.20	471.04	421.48
II		<b>237.68</b>	517.05	676.82	979.79	526.08	875.93	547.49
III			<b>241.59</b>	850.73	1112.86	961.51	1167.73	1071.29
IV				<b>0.00</b>	98.37	417.94	190.90	265.84
V					<b>0.00</b>	619.34	198.80	423.10
VI						<b>0.00</b>	295.48	552.06
VII							<b>0.00</b>	332.65
VIII								<b>0.00</b>

*Figures in bold indicate the intra cluster distances, while the others are inter cluster distances*

The magnitude of intra-cluster distance measures the extent of genetic diversity between the genotypes of same cluster while the inter-cluster distance measures the genetic distance between two clusters. The intra cluster distance was maximum in cluster III (241.59) (Table 3). The maximum inter cluster distance of 1167.73 was observed between cluster III and cluster VII followed by cluster III and cluster V (1112.86) and cluster III and cluster VIII (1071.29). The clustering pattern of genotypes in the present study has not been influenced by the source and origin. The

results indicated that the genotypes grouped in cluster III (EC241665, NP2, PI60269, EC22999, AGS1668, WT182, Cockerstuart) and cluster VII (LSb 1) followed by cluster III and cluster V (RPSP722), cluster III and cluster VIII (EC 257470 and EC 357990) recorded high inter cluster distances. The above results indicated that considerable genetic diversity exists in the genotypes used in the present study. Hence, there is scope for varietal improvement through heterosis breeding between cluster III and cluster VII followed by cluster III and

cluster V by selecting the parents based on the combining ability so as to fix them in the hybridization programme.

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## Productivity of Soybean (*Glycine max* L. Merrill) as Influenced by Integrated Nutrient Management Practices under Vertisols of Chhattisgarh Plains

TUKARAM SONKAR<sup>1</sup>, RAJENDRA LAKPALE<sup>2</sup> and S S TUTEJA<sup>3</sup>

Department of Agronomy,  
Indira Gandhi Krishi Vishwavidyalaya, Raipur 492 006, Chhattisgarh  
(E-mail: rlakpale@hotmail.com)

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The national productivity of soybean in India is hovering around one tonne per hectare during past few years. On the contrary, the realizable yield under real farm conditions as has been achieved in Front Line Demonstrations is nearly 2 tonnes per hectare and the varietal potential is 2.5 to 3.5 tonnes per hectare (Tiwari *et al.*, 2001). Constraint analysis brought out that non-judicious and skewed use of chemical fertilizers leading to unbalanced nutrition has been one of the major reasons (Joshi and Bhatia, 2003) for restricting soybean productivity to 1 tonne per hectare in India. To come out of this barrier, it is considered to adopt Integrated Nutrient Management (INM) concept, which aims at maintenance and/or adjustments of soil fertility and plant nutrient supply to an optimum level for sustaining crop productivity through optimization of all possible sources of plant nutrients in an integrated manner. In the present investigation, in addition to combining of organic

manure/biofertilizers with inorganic fertilizers including zinc and magnesium (Wasmatkar *et al.*, 2002), which are reported to be deficient in soils, have been utilized in an integrated approach to assess the impact of balanced nutrition on soybean productivity.

A field experiment was conducted at the Instructional Farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur, (Chhattisgarh) during *kharif* 2006. The soil of experimental site belonged to Vertisols and was clayey in texture. It was low (228.6 kg/ha), medium (13.50 kg/ha) and high (372.3 kg/ha) in available N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O, respectively. The experiment was laid out with 10 treatment combinations replicated thrice in randomized block design. The treatment combinations were: (i) control (no fertilizers), (ii) 100 per cent recommended dose of fertilizers (RDF - 25: 80: 60 kg N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O /ha), (iii) FYM (10 t/ha), (iv) 50 per cent RDF plus FYM (10 t/ha), (v) 50 per cent RDF plus FYM (5 t/ha) plus rhizobium plus PSB, (vi) 100 per cent RDF

<sup>1</sup> M.Sc. Student, <sup>2</sup> and <sup>3</sup> Senior Scientist

plus zinc (5 kg/ha) + magnesium (10 kg/ha), (vii) FYM (10 t/ha) plus zinc (5 kg/ha) plus magnesium (10 kg/ha), (viii) 50 per cent RDF plus FYM (10 t/ha) plus zinc (5 kg/ha) plus magnesium (10 kg/ha), (ix) 50 per cent RDF plus FYM (5 t/ha) plus rhizobium plus PSB plus zinc (5 kg/ha) plus magnesium (10 kg/ha), (x) 100 per cent RDF plus FYM (10 t/ha) plus zinc (5 kg/ha) plus magnesium (10 kg/ha) plus zinc (5 kg/ha) plus magnesium (10 kg/ha) plus rhizobium plus PSB. The carriers for zinc and magnesium were magnesium sulphate and zinc sulphate, respectively. The rhizobium @ 5g per kg seed and PSB @ 5g per kg seed were applied through seed treatment prior to sowing. Soybean variety 'JS-335' was sown in rows with spacing of 30 cm and plant to plant spacing of 10 cm on July 11, 2006 using a seed rate 75 kg per hectare and was harvested on October 27, 2006. The observations on yield attributing characters, seed and stover yields were recorded at harvest.

The results revealed that the soybean seed yield increased significantly (by 19-65%) over control (1297 kg/ha) on imparting various nutrient management treatments. The FYM application alone @ 10 tons per hectare yielded 30 per cent lower than the yield observed at recommended dose of fertilizers (18.60 kg/ha), but the difference was not statistically significant. Application of additional 50 per cent RDF along with FYM @ 10 tons per hectare further stepped up the seed yield by 14 per cent. A further increase (4-5%) in seed yield was noted on combination of 50 per cent RDF plus FYM (5 or 10/ha) plus zinc (5 kg/ha) plus magnesium (10 kg/ha) as compared to 50 per cent RDF plus FYM (10 t/ha). The maximum response in terms of

seed yield of soybean was observed in treatment with application of 100 per cent RDF plus FYM (10 t/ha) plus zinc (5 kg/ha) plus magnesium (10 kg/ha) plus rhizobium plus PSB (65% increase over control), which was at par with 100 per cent RDF, 50 per cent RDF plus FYM (10 t/ha) plus zinc (5 kg/ha) plus magnesium (10 kg/ha), 50 per cent RDF plus FYM (5 t/ha) plus zinc (5kg/ha) plus magnesium (10 kg/ha) plus rhizobium plus PSB and 50 per cent RDF plus FYM (10 t/ha). More or less similar behaviour with respect to different treatments was noted in case of straw yield. The yield levels achieved are in line with the contributing factors. The highest yielding treatment as stated above has the highest values for all the yield attributing characters. Similarly control has the lowest yield and lower values of all the yield attributing characters. The effect of inclusion of zinc and magnesium with 100 per cent RDF is discernable in terms of around 6 per cent increase in yield, but inclusion of these two nutrients along with FYM in treatments did not show any increment indicating that FYM fulfills their requirement (Joshi *et al.*, 2000). The data indicated that the soybean yield increases with the progressive increase in nutrient input and integration of organic and inorganic sources including zinc and magnesium and biofertilizers. The effect of different components like biofertilizers (Kumrawat *et al.*, 1997), phosphorus and biofertilizers (Sharma and Namdeo, 1999; Thanki *et al.*, 2005; Billore *et al.*, 2005), micronutrient (Joshi *et al.*, 2000) in integrated nutrient management for soybean has been documented and renders support to results highlighted in the present manuscript.

**Table 1. Effect of integrated nutrient management on yield attributes of soybean**

Treatment	Pods/ plant	Seeds/ pod	Seeds/ Plant	100 seed weight (g)	Seed yield (q/ha)	Stover yield (q/ha)	Net returns (Rs/ha)	B:C ratio
Control (no fertilizers)	31.80	2.31	80.20	10.40	12.97	16.39	9614	1.29
100% RDF*	40.66	2.65	104.60	11.76	18.60	21.17	14779	1.55
10 t FYM / ha	33.13	2.39	81.20	11.13	15.43	19.94	10867	1.15
50% RDF + 10 t FYM / ha	36.00	2.60	91.26	11.39	17.62	21.93	12648	1.20
50% RDF + 5 t FYM /ha + Rhizobium + PSB	33.60	2.53	85.96	11.53	17.03	20.21	12785	1.34
100% RDF + 5 kg Zn/ha + 10 kg Mg /ha	42.80	2.66	106.20	11.83	19.64	22.91	14875	1.37
10 t FYM / ha + 5 kg Zn/ha + 10 kg Mg/ha	33.20	2.57	84.33	11.36	16.80	19.94	11288	1.05
50% RDF + 10 t FYM / ha + 5 kg Zn/ha + 10 kg Mg/ha	38.60	2.64	94.80	11.60	18.50	21.19	12420	1.05
50% RDF + 5 t FYM /ha + Rhizobium + PSB + 5 kg Zn/ha + 10 kg Mg /ha	37.60	2.62	93.33	11.46	18.37	18.77	13136	1.22
100% RDF + 10 t FYM / ha + 5 kgZn/ha + 10 kg Mg/ha + Rhizobium + PSB	45.73	2.80	113.13	12.36	21.41	26.50	15227	1.18
SEm (+)	2.82	0.08	6.35	0.30	1.34	1.47	-	-
CD (P = 0.05)	8.40	0.26	18.86	0.90	3.98	4.37	-	-

\* 25:80:60 kg NP<sub>2</sub>O<sub>5</sub>K<sub>2</sub>O/ha

The economic evaluation of the one year results revealed that higher net returns (between Rs 14779 and Rs 15227/ha) were associated with treatment/treatment combinations associated with application of 100 per cent RDF. With treatment combinations comprising of 50 per cent RDF, the net returns varied between Rs 12648 and Rs 13136 per hectare and can be adopted in view of long term benefit of sustainability and associated B:C ratio. The data also suggest that the application of zinc and magnesium can be avoided in case FYM is integrated with fertilizer application. For tangible information, the experiment is to run for longer duration.

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## Resistance of Some Soybean Lines to Stem Fly

**S P TAWARE<sup>1</sup>, G B HALVANKAR<sup>2</sup> and PHILIPS VARGHESE<sup>3</sup>**

*Agharkar Research Institute, Genetic Group, Division of Plant Sciences,*

*GG Agharkar Road, Pune 411 004, Maharashtra*

*(E-mail: tawaresoy@yahoo.co.in)*

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India has surpassed China in terms of area under soybean cultivation (8.85 million ha) and has become number four on global scenario. However, in terms of annual production (9.47 mt) it is still on fifth rank ([www.sopaindia.org](http://www.sopaindia.org)). Low productivity (1070 kg/ha) in India is mainly due to biotic and abiotic stresses under which this crop is grown. Among biotic stresses, several insect-pests attacking soybean crop are responsible for considerable reduction in yield. In India, the soybean crop is grown by the marginal farmers who cannot afford input cost to mitigate these biotic stresses. To grow resistant varieties is a better option which can help to minimize the input cost as well as to reduce environmental hazards due to indiscriminate use of pesticides. Present study is aimed at screening some promising soybean lines for their resistance against stem fly (*Melanagromyza sojae* Zehntner).

Ten promising soybean lines were grown in *kharif* seasons of 2005 and 2006 at Agricultural Experimental Farm at Hol, Athphata, Tal Baramati, Pune in

randomized block design with three replications along with Bragg and PK 1029 as susceptible checks for stem fly, MACS as resistant check for stem fly and MACS 450, JS 335 and MAUS 2 as released checks. Each line was represented by three rows of three meter length per replication with 45 cm and 5 cm distance between and within rows, respectively.

Data on stem fly damage was recorded on 10 random plants per replication by measuring the length of stem tunneled by stem fly larvae at physiological maturity stage and expressing it as percentage of total plant height. The percentage data were transformed into square roots and subjected to analysis of variance. The genotypes were categorized as per AICRPS (2001) method. To assess the loss in yield due to insect damage, these soybean lines were grown under protected and unprotected conditions in *kharif* 2006 season and the lines were categorized by using maximin-minimax method (Odulaja and Nokoe, 1993). The categorization for both the methods was done as follows.

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<sup>1</sup>Soybean Entomologist; <sup>2</sup>Soybean Agronomist; <sup>3</sup>Soybean Breeder

AICRPS Method		Maximin-minimax Method			
Category	Discription	Category		Discription	
Highly resistant (HR)	= < Mean LSD (0.01)	Resistant yielder (RHy)	high	= Relative loss < 25% and Relative yield > 75%	
Resistant (R)	= Between mean - LSD (0.01) and mean - LSD (0.05)	Resistant yielder (RLy)	low	= Relative loss < 25% and Relative yield < 75%	
Moderately resistant (MR)	= Between mean - LSD (0.05) and mean	Susceptible yielder (SHy)	high	= Relative loss > 25% and Relative yield > 75%	
Low resistant (LR)	= Between mean and mean + LSD (0.05)	Susceptible yielder (SLy)	low	= Relative loss > 25% and Relative yield < 75%	
Suceptible (S)	= Between mean + LSD (0.05) and mean + LSD + LSD (0.01)				
Highly susceptible (HS)	= >M + LSD (0.01)				

Stem tunneling ranged from 6.55 (TNAUS 55) to 27.01 per cent (PK 1029) in *kharif* 2005 season and 2.28 (MAUS 222) to 40.64 per cent (PK 1029) in *kharif* 2006 season (Table 1). Results showed that MACS 1038 and check variety MACS 124 to be resistant to stem fly during both the years. TNAUS 55 in *kharif* 2005 and AMS 47, MACS 1028, MACS 1058 and MAUS 222 in *kharif* 2006 recorded highly resistant reaction to stem fly damage. However, these lines recorded moderate to low resistance in other season. Based on minimum two years data, Taware *et al.* (2001, 2004, 2005 and 2007) have

reported nine genotypes, viz. JS(SH) 93 01, JS (SH) 93-37, TS 98-21, TS 98-91, MAUS 2, MACS 124, MACS 716, NRC 52 and UGM 20075 to be resistant soybean genotypes to stem fly. Likewise Dubey *et al.* (1998) screened 44 soybean genotypes and found three genotypes resistant to stem fly. Sekhar *et al.* (2000) studied 39 genotypes and found none of them to be resistant to stem fly. Sridhar *et al.* (2003) have screened 70 soybean lines and have reported MACS 57 to be the most resistant variety to stem fly. Sharma and Bhatnagar (1996) reported some of the Bragg mutants to be resistant to stem fly.

**Table 1. Stem fly damage, seed yield and categorization for resistance in 16 soybean lines**

Soybean line	Yield kg/ha (unprotected)					Stem fly damage					
	<i>Kharif</i>	<i>Kharif</i>	Mean	Rank	Cate- gory <sup>@</sup>	<i>Kharif</i> 2005			<i>Kharif</i> 2006		
	2005	2006				Stem tunne- ling (%)	Square root values	Cate- Gory <sup>#</sup>	Stem tunne- ling (%)	Square root values	Cate- gory <sup>#</sup>
NRC 67	3236	3356	3296	3	RHy	14.31	3.77	LR	13.23	3.64	MR
MRSB 345	3119	3075	3097	4	RHy	10.33	3.20	MR	14.25	3.76	LR
AMS 47	2386	2446	2416	11	SLy	15.02	3.86	MR	1.68	1.29	HR
DS 2207	3359	1650	2505	10	RLy	15.25	3.90	MR	17.79	4.21	LR
MACS 1028	3552	4017	3785	2	RHy	15.02	3.86	MR	5.70	2.37	HR
MACS 1038	3875	3698	3787	1	RHy	11.81	3.41	R	9.62	3.10	R
MACS 1058	3077	2845	2961	6	RLy	24.04	4.82	LR	9.43	3.04	R
MAUS 222	2552	3371	2962	5	RHy	14.67	3.79	MR	2.28	1.43	HR
NRC 70	2942	2168	2555	9	RLy	13.66	3.66	R	13.10	3.62	MR
TNAUS 55	2575	2110	2343	12	RLy	6.55	2.55	HR	15.27	3.87	LR
BRAGG (C)	2362	654	1508	16	SLy	35.53	5.96	LR	31.92	5.65	HS
MACS 450 (C)	3572	1993	2783	7	RLy	17.67	4.20	MR	13.42	3.66	MR
JS 335 (C)	3115	2006	2561	8	SLy	22.15	4.71	MR	21.49	4.64	HS
PK 1029 (C)	2907	698	1803	14	SLy	27.01	5.17	LR	40.64	6.37	HS
MAUS 2 (C)	1054	2603	1829	13	RLy	18.85	4.34	MR	16.97	4.08	LR
MACS 124 (C)	2764	468	1616	15	SLy	12.79	3.56	R	8.66		R
Mean	2669	2322	2496	-	-	23.32	4.73	-	15.50	3.71	-
CD (P=0.05)	675	490	-	-	-	-	1.03	-	-	0.61	-
CD (P=0.01)	903	659	-	-	-	-	1.38	-	-	0.82	-

<sup>@</sup> RHy=Resistant High yielding; RLy=Resistant Low yielding; SLy=Susceptible Low yielding<sup>#</sup> HR=Highly Resistant; R=Resistant; MR=Moderately Resistant; LR=Low Resistant; S=Susceptible; HS=Highly Susceptible

Categorization of the soybean lines on the basis of yield data recorded in *kharif* 2006 season under protected and unprotected conditions by using maximin-minimax method (Odulaja and Nokoe, 1993) revealed NRC 67, MRSB 345, MACS 1028, MACS 1038 and MAUS 222 to be resistant high yielding (RHy). Mean yield of these lines in two seasons under protected conditions also indicated promising results for seed yield. DS

2207, MACS 1058, NRC 70 and TNAUS 55 were categorized as resistant low yielding (RLy) and can be used in breeding programme to incorporate resistance in superior agronomic background.

Thus, lines resistant to stem fly reported in the present study, especially MACS 1038, can be used in breeding for stem fly resistance.

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**Choudhary Ravindra Kumar Dr;** Senior Scientist (Entomology), All India Coordinated Research project on Safflower, College of Agriculture, Indore 452 001, Madhya Pradesh

**Gupta GK Dr;** Principal Scientist (Plant Pathology), National Research Centre for Soybean, Indore 452 001, Madhya Pradesh

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**Sharma AN Dr;** Principal Scientist (Entomology), National Research Centre for Soybean, Indore 452 001, Madhya Pradesh

**Sharma MP Dr;** Senior Scientist (Microbiology), National Research Centre for Soybean, Khandwa Road, Indore 452 001, Madhya Pradesh

**Sharma OP Dr;** Ex- Dean, College of Agriculture, (JNKVV), Tikamgarh, WA 10, Scheme No. 94, Ring Road (E), Dewas Naka, Indore 452010, Madhya Pradesh

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