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Soybean (*Glycine max*) genotype–mediated variation in the symbiotic performance of *Rhizobium*

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ABSTRACT

Legume – rhizobium interaction is the result of molecular dialogue involving a succession of events on the perception of signal molecules produced and secreted by both partners. Soybean (*Glycine max* L.) is known to be nodulated by two different genera, *Bradyrhizobium* (slow growing rhizobia) and *Sinorhizobium* (fast growing) species. The present investigation is an attempt to understand the host genotypic effect on the fast and slow growing root nodulating bacteria of soybean which impacts the biological nitrogen fixation and would lead to the selection of best cultivar-strain compatible interaction. Five soybean genotypes of North Plain Zone, viz. DS 12-13, DS 9712, DS 2705, SL 979, SL 982 were evaluated for their symbiotic potential with two slow (KAS-1, MTCC10753) and two fast growing root nodulating bacteria (DS-1, LSR-8). Genotype DS12-13 formed significant number of nodules with KAS-1 with LS mean of 17.3. Genotype DS 2705 was poorly nodulated by the four strains. Among the strains, slow growing KAS-1 and fast growing DS-1 strains were effective across the genotypes. There was significant increase in the specific acetylene reducing activity of these strains 71.1 and 72.6 nmoles of C_2H_4 produced/mg/ndw respectively. These strains conformed to the biochemical identification by failing to grow on citrate, glucose peptone agar and Hoffer's alkaline media.

Key words: Acetylene reducing activity, *Bradyrhizobium*, Genotype, *Sinorhizobium*, Soybean

An essential element of agricultural sustainability is the effective management of N in the environment and legumes derives it through Biological Nitrogen Fixation (BNF). Symbiotic nitrogen fixation involving *Rhizobium*/ *Bradyrhizobium* and legumes depends on the genetic properties of both the bacteria and the host plant because there is a marked specificity between species of legumes and rhizobia (Sangam *et al.* 2015, Simms and Taylor 2002). Taxonomic studies have shown that soybean (*Glycine max* L.) is nodulated by fast- and slow growing rhizobia and collectively referred to as "soybean rhizobia" (Jordan 1982). Soybean nodulating bacteria are distributed over different species belonging to three different genera: *Bradyrhizobium*, *Mesorhizobium* and *Sinorhizobium* (Chen *et al.* 1988). In India soybean is cultivated in over 10m ha under different agroclimatic zones. Over the years there has been a build of native rhizobia able to nodulate soybean in Indian soils. A large genetic diversity was observed in these populations (Satyaprakash and Annapurna 2006, Annapurna *et al.* 2007, Appunu *et al.* 2008). However, ineffective native isolates are unable to form effective symbiotic associations leading to poor performances of the crop and decrease in yields. In soils that have not been previously cropped to soybean, the absence of compatible populations of root-nodule bacteria can pose a challenge to increased yields and farmer adoption. Under these conditions, inoculation with infective strains is needed to achieve effective nodulation. However, host genotype variation in response to inoculation has been observed. Hence, careful selection of the two partners is needed for maximising the performance and accrue benefits. From an applied perspective, a better understanding of these interactions and the implications is required (Benjamin *et al.* 2015). The present study was undertaken to select the most effective combination of soybean genotype and rhizobial strain in terms of symbiotic parameters.

MATERIALS AND METHODS

Hoffer's alkaline medium, Simmons citrate agar, glucose peptone agar, media were used for the biochemical tests to differentiate between *Rhizobium* and *Agrobacterium* as per the standard procedure of Vincent (1970).

Five soybean verieties, i.e. SL 979, SL 982, DS 12-13, DS 9712, DS 2705 all identified for north plain zone (NPZ) were evaluated for the symbiotic parameters, viz. nodule number, nodule dry weight, acetylene reducing activity, root and shoot dry weights upon inoculation with two fast and two slow growing rhizobia. Seeds were surface sterilized using 0.8% $HgCl_2$, washed 6-8 times with sterilized water and placed on 0.8% soft agar for germination. Germinated seeds

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were transferred to the pots filled with sterilized mixture of sand and vermiculite and replenished with 1/4th strength N-free Jensen's nutrient solution. There were 4 replications for each treatment and four seedlings were placed in each pot and inoculated with representative cultures (OD=1.0) @ 2ml/seedling. Plants were grown in National Phytotron Growth Chamber, at IARI, New Delhi with 14 hr photoperiod and temperature of 29/22^{0}C, day and night. Plants were thinned to two per pot eight days after emergence (DAE). Nutrient solution was given as and when required and plants were harvested after 35 days after sowing (DAS). Cross inoculation studies were conducted with heterologous hosts, viz blackgram, greengram, chickpea, cluster bean, lentil, *Lathyrus*, cowpea and pea and the experimental details were similar as previously.

The ethylene produced by reduction of acetylene was assayed using a Gas chromatograph with FID detector having Porapak N column and nitrogen as carrier gas. The operating conditions were: Column temperature: 75^{o}C, Injector temperature:110^{o}C, and Detector temperature:110^{o}C. Standard ethylene (110 vpm) was used to calculate the amount of ethylene produced. Plants were harvested 35 DAS, roots were patted to remove the sand adhering to it, washed and patted dry, separated from shoots and placed in 80ml tubes along with the nodules. Root nodules were incubated with 10% of acetylene for 45 min. Tubes devoid of roots, but having 10% acetylene were used as blank control. The nitrogenase activity was expressed as n moles of C_2H_4 produced h/mg/dry wt. of nodules.

Nodules were detached and counted from each replication. Shoot, root and nodule samples were packed separately in butter paper, dried in hot air oven at 60°C and weights recorded. The observations of treatments for the nodule number, nodule dry mass, root and shoot dry weights and specific ARA were recorded and analysed using statistical analysis software (SAS) system and The Graphical linear module (GLM) procedure and advanced Tukey test for multiple comparisons to study the interaction effects of both genotype and rhizobial strain and Least Means Squares (LSM) were calculated.

RESULTS AND DISCUSSION

Biochemical characterization

None of the four strains grew on glucose peptone medium within 48 hr of incubation. No growth was observed on Hofer's alkaline medium as well and the four strains were unable to use citrate as carbon source and failed to grow in the citrate medium. Colonies were <1 mm on Congo red YEMA plates (KAS-1 and MTCC 10753) even after 7 days of incubation. These were categorised as slow growers. Strains DS-1 and LSR-8 grew on CRYEMA plates as 2-3 mm colonies in 2-3 days of incubation and designated as fast growers.

Symbiotic parameters

Number of nodules: The differences among treatments for nodule number were found to be not statistically significant for the strains but they were significant for the host genotype (Fig 1). Soybean genotypes DS 12-13, DS 9712 and SL 982 performed best with an LS mean of 10.9, 9.2 and 8.21 respectively and soybean genotype DS 2705 performed the least for nodule number with an LS mean of 6.44. Interestingly, strain LSR-8 performed better with the low nodulating genotype DS 2705 with LS mean of 10.13. Among the four strains, individually, slow growing KAS-1 formed more number of nodules across the genotypes followed by fast growing strain DS-1. Differences in plant growth, nodulation and intrinsic water use efficiency of *Rhizobium*-inoculated and un-inoculated promiscuous and commercial soybean genotypes have been reported (Maseko *et al.* 2015).

Nodule dry weights: Nodule dry weights showed a similar trend for the strains, data being not significant for the strains (Fig 2). However, both genotypes DS 12-13 and DS 9712 gave more nodule dry weight with LS mean of 15.73 and 15.29 respectively than DS 2705, SL 982 and SL 979 which had LS mean of 8.6, 9.5 and 8.83 respectively. Highest and lowest nodule dry weight was shown by strain KAS-1 on DS 12-13 (26.5 mg/plant) and DS 2705 (5.8 mg/plant) respectively. Nodule weights are good indicators of the performance of a rhizobial strain. This parameter is

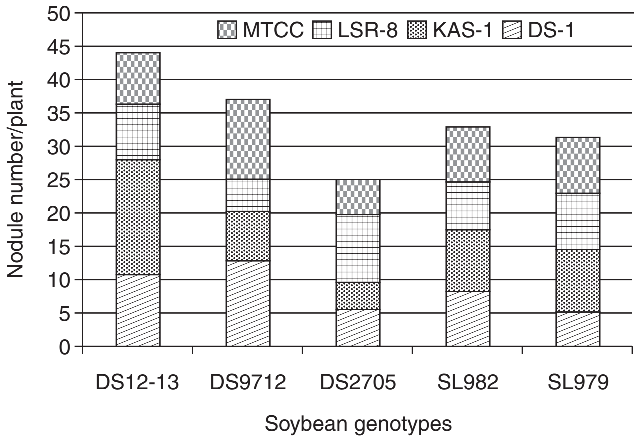

Fig 1 Effect of strain inoculation on soybean genotypes for nodule number. All the values are in Least Square Means.

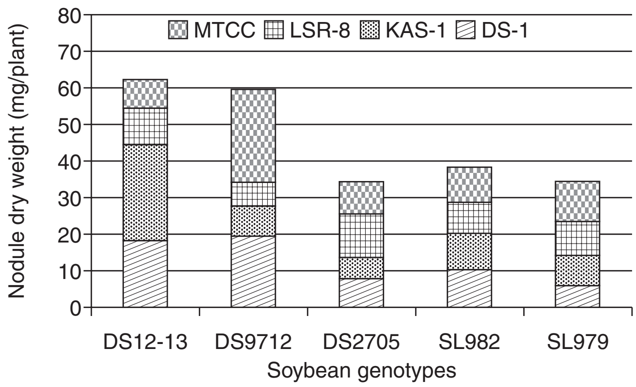

Fig 2 Effect of inoculation of slow and fast growing rhizobia on soybean genotypes. All the values are in Least Square Means.

thought to be more robust than nodule number. Generally, high nodule number is not considered a good trait since it would act as sink, and most of the energy shall be drained towards the nodule rather than the upper parts of the plant affecting its yield.

Nitrogenase activity: The enzyme nitrogenase, occurring in the legume root nodules catalyses the reduction of dinitrogen to ammonia. This enzyme also reduces acetylene to ethylene. Thus acetylene reduction assay (ARA) method indirectly measures the nitrogenase activity as well as nitrogen fixing potential of the root nodules. The nitrogenase activity in terms of nmoles C_2H_4/hr/mg dry weight of nodule was measured using the intact root system in the experiment. The data on ARA is given in Table 1**.** Significant differences were found among the treatments. Host genotype variation as well as strain variation was observed. Host genotype DS 9712 and DS 12-13 outperformed the other three. Individually slow growing strain KAS-1 was found to produce more moles of C_2H_4 (30.37 nmoles/hr/mg nodule dry weight) as compared to fast growing DS-1 (26.38 n moles/hr/mg nodule dry weight), LSR-8 (12.74 n moles/hr/mg nodule dry weight) and MTCC (20.33 n moles/hr/mg nodule dry weight). The two strains KAS-1 and DS-1 showed maximum ARA with both DS 12-13 and DS 9712. Interaction of the isolates with the two genotypes SL 982 and SL 979 was found to be poor.

Root dry weights: Among the four isolates, strains DS-1 and KAS-1 stimulated better root growth. Strain DS-1 provided mean root dry weights across the five host genotypes as 145.28 (mg/plant), followed by KAS-1 (128.42 mg/plant). Strain impact on root growth stimulation was found significant, the means ranging between 96.13 to 145.28 mg/plant. Among the genotypes, DS 9712 responded well for inoculation, giving maximum root biomass of 181.0 mg/plant closely followed by DS 12-13 (176.0 mg/plant) (Fig 3). The other three host genotypes did not show significant variation in the root weights. Inoculation *per se* improves root elongation and biomass, because of the IAA production by the strains. However, some strains may also have negative impact on root growth (Patten and Glick 2002).

Table 1 Effect of strain inoculation on ARA* activity of soybean genotypes after 35 DAS

Variety	*Rhizobium* strains				Mean (variety)
	DS-1	KAS-1	LSR-8	MTCC 10753	
DS 12-13	32.23^{abc**}	36.3^{abc}	13.9^{bc}	16.6^{bc}	24.79^{b}
DS 9712	72.6^{a}	71.1^{a}	10.72^{bc}	53.8^{ab}	52.08^{a}
DS 2705	13.63^{bc}	8.2^{c}	19.57^{bc}	14.76^{bc}	14.05^{b}
SL 982	9.12^{c}	16.91^{bc}	10.91^{bc}	4.50^{c}	10.36^{b}
SL 979	4.34^{c}	19.21^{bc}	8.56^{c}	11.94^{bc}	11.01^{b}
Mean (strain)	26.38^{ab}	30.37^{a}	12.74^{b}	20.33^{ab}	

*ARA activity measured as nmoles of ethylene produced /h/mg dry weight of nodule. ** Least square (LS) Means with same superscripts are not significantly different.

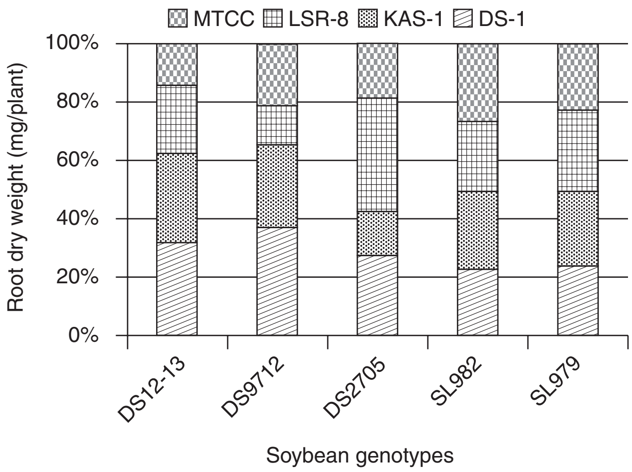

Fig 3 Effect of inoculation of slow and fast growing rhizobia on soybean genotypes. All the values are in Least Square Means.

Shoot dry weights: Inoculation improved the shoot biomass. Here performance of the strains was found to be significantly different among themselves for this parameter (Table 2). Host genotype also responded differentially upon inoculation. Both DS 12-13 and DS 9712 gave maximum LS mean shoot biomass of 458.3 and 465.2 respectively, whereas DS 2705, SL 979 and SL 982 were found insignificant among themselves. Strain KAS-1 gave maximum shoot biomass with DS 12-13 (601 mg/plant), followed by DS-1 with DS 9712 (599.5 mg/plant). Among the strains, LSR-8 gave the lowest shoot dry weight of 297.5 mg/plant. Many studies have shown that inoculation improves shoot biomass for various legumes. This could be because of better nutrient uptake by the plant.

Host range of strains

Screening of the four strains for their ability to form nodules on heterologous hosts was carried out in glass house pot conditions. All the four strains formed nodules on greengram, blackgram, redgram and cowpea but only strain DS-1 showed nodule formation on chickpea. Lentil, pea, clusterbean, were not nodulated by any of the four strains.

Table 2 Effect of strain inoculation on shoot dry weight (mg/plant) in soybean genotypes after 35 DAS

Variety	*Rhizobium* Strains				Mean (variety)
	DS-1	KAS-1	LSR-8	MTCC 10753	
DS 12-13	562 ^{ab*}	601^{a}	316^{cde}	353^{bcde}	458.3^{a}
DS 9712	599.5^{a}	458.6^{abcd}	303.6^{cde}	499.23^{abc}	465.2^{a}
DS 2705	299.2^{cde}	213.2^{e}	273.37^{cde}	257.3^{de}	260.55^{b}
SL 982	356.6^{bcde}	292.2^{cde}	330.2^{bcde}	307.72^{cde}	321.6^{b}
SL 979	279.6^{cde}	297.11^{cde}	264.4^{de}	313^{cde}	288.5^{b}
Mean (strain)	414.9^{a}	372.5^{a}	297.5^{b}	346^{ab}	

*Least square (LS) Means with same superscripts are not significantly different.

Studies have shown that fast growing soybean rhizobia (*S. fredii*) have wider host range (Pueppke and Broughton 1999). Careful observation of the nodule by sectioning showed that nodule formed on greengram and blackgram were pink in centre indicating effectiveness, but nodules on chickpea and red gram were white in centre indicating ineffectiveness. Soybean rhizobia are generally selective in nodulation trait unlike groundnut rhizobia which are promiscuous. Even though nodule formation may happen, nitrogen fixation may be impaired.

For an interaction to be effective, a compatible relationship between the legume and rhizobium is a must which to a large extent is determined by the composition of root exudates and molecular signals exchanged between the two partners (Biate *et al.* 2014a). A compatible partnership will yield better results in terms of plant growth, yield and N content of the plant. There is a large genetic diversity of soybean rhizobia existing in soils (Satyaprakash and Annapurna 2006, Biate *et al.* 2014b, Sameh *et al.* 2014). It is important to select the most effective strain to develop as a bioinoculant.

Many workers have reported differential responses exhibited by a soybean genotype upon inoculation with different rhizobial cultures (Maseko *et al.* 2015, Ronner *et al.* 2016). Judicious selection of a compatible two some of host genotype and rhizobial strain for a particular agro-climatic zone and soil condition is required for improving the performance in the field. Despite the presence of fast growers, it is the slow growing rhizobia which have an edge in performance. Our results are in conformation with earlier studies where it was shown that fast growing soybean nodulating rhizobia are less effective on agronomically improved soybean cultivars.

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