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Original article

Response of soybean cultivars toward inoculation with three arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* in the alluvial soil

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ABSTRACT

The aim of this study was to assess the comparative efficacy of three arbuscular mycorrhizal fungi (AMF) combined with cultivar specific *Bradyrhizobium japonicum* (CSBJ) in soybean under greenhouse conditions. Soybean seeds of four cultivars namely JS 335, JS 71-05, NRC 2 and NRC 7 were inoculated with three AM fungi (*Glomus intraradices*, *Acaulospora tuberculata* and *Gigaspora gigantea*) and CSBJ isolates, individually or in combination, and were grown in pots using autoclaved alluvial soil of a non-legume cultivated field of Ajmer (Rajasthan). Assessment of the data on nodulation, plant growth and seed yield revealed that amongst the single inoculations of three AMF, *G. intraradices* produced the largest increases in the parameters studied followed by *A. tuberculata* and *G. gigantea* indicating that plant acted selectively on AMF symbiosis. The dual inoculation with AMF + CSBJ further improved these parameters demonstrating synergism between the two microsymbionts. Among all the dual treatments, *G. intraradices* + *B. japonicum* brought about the largest increases in the studied characteristics particularly in seed weight per plant that increased up to 115.19%, which suggested that a strong selective synergistic relationship existed between AMF and *B. japonicum*. The cv. JS 335 exhibited maximum positive response towards inoculation. The variations in efficacy of different treatments with different soybean cultivars indicate the specificity of the inoculation response. These results provide a basis for selection of an appropriate combination of specific AMF and *Bradyrhizobium* which could further be utilized for verifying the symbiotic effectiveness and competitive ability of microsymbionts under field conditions of Ajmer region.

1. Introduction

Soybean (*Glycine max* (L.) Merrill.) is the most widely grown legume worldwide and has a high potential as a source of protein and oil, and it also enhances soil fertility for other crops by modifying the soil nitrogen budget. India is the fifth largest producer of soybeans after the United States, Brazil, China and Argentina [25]. Soybean has made an unprecedented expansion in India for the past ten years. The increase in soybean cultivation in India is likely to improve the rural economy

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and socio-economic status of the Indian farmers. Association of soybean with arbuscular mycorrhizal fungi (AMF) increases the uptake of nutrients particularly phosphorus [10,50], zinc [5] and nitrogen as well as increasing crop production [33]. The association of bradyrhizobial strains with the roots of soybean plants also improves soil health and nitrogen fixation, thus further increasing crop production [21]. Recent investigations have brought to light instances where biological activities are markedly enhanced in two or three-membered associations of organisms. Synergistic effects of AMF and *Bradyrhizobium japonicum* have a high potential to improve the nutrient supply of soybean including phosphorus and soil quality [44]. However, published studies [38,42] indicate that a much larger genetic variability of bradyrhizobia and AMF strains exists in different cultivar regions than was assumed previously. Selection of the appropriate AMF is amongst one of the critical issues for the application of AM technology in agriculture [9].

Pioneer studies reported differences among soybean genotypes, with regard to efficacy of symbiotic N_2 fixation [7] as well as in the response of N_2 fixation to fertiliser N addition [53]. The variation in life strategies among the AMF may be linked to host-symbiont compatibility phenomena which represents an important aspect of mycorrhizal soil ecology, particularly regarding the interaction with *B. japonicum* and soybean. In a study, variation was found in the nodulation and N_2 fixation response of Pigeonpea to seven different isolates of AMF. While some AMF stimulated nodule growth and function, others did not, particularly at flowering when the demand for carbon increases [18]. In addition, the initiation and functioning of the tripartite symbiosis is believed to be very complex and despite the progress achieved in the last two decades there are still many unknowns. There is genetic variability in the AM colonization capacity of various genotypes of host species (e.g., barley, [4]; grapevine, [23]; bell pepper and tomato, [28]). Wide variability also exists in populations of mycorrhizal fungi in their hyphal growth and thus competitive ability [8]. Also there is growing evidence of host specific differences in plant responses to AMF and fungal response to plants [3], though not many studies have been carried out to determine the effect of host on the AMF species diversity [39]. In addition, the genotype of the fungus is important because some degree of host specificity exists in AMF [4]. In the tripartite symbiosis (AMF + nodule bacteria + groundnut as a host plant species), there was a significant genotype effect [19], indicating a genetic variability in host capacity to sustain effective symbiosis with AMF and nodule bacteria. However, in the last two decades very few studies have investigated the variability among Indian soybean cultivars in relation to this tripartite symbiosis [14,31].

Considering these aspects, in the present investigation three AMF and four bradyrhizobial isolates were tested individually and or in combination under the greenhouse conditions using autoclaved alluvial soil, in four different soybean cultivars namely JS 335, NRC 2, NRC 7 and NRC 12. The main objective of the present research work was to determine the level of variability in soybean cultivars response toward single/dual inoculation with different AMF and bradyrhizobial isolates so as to screen out the most effective symbiotic system.

2. Materials and methods

2.1. *Procurement of seeds*

Soybean seeds of four cultivars viz., JS 335, JS 71-05, NRC 2 and NRC 7 were collected from Soybean Breeding Research Centre, Jawaher Lal Nehru Krishi Vishwa Vidhyalaya, Jabalpur (Madhya Pradesh), India. These cultivars are commonly grown in central India.

2.2. *AM fungal inoculum preparation*

Individual AMF spores showing hyphal connection were isolated by the wet sieving and decanting method [12] from the air-dried rhizosphere soil samples collected from Jabalpur region of Madhya Pradesh. Characterization of individual AMF spores was carried out after being subjected to morphogenetic and micrometric analysis based on their colour, diameter, shape, wall layers, surface content, hyphal colour, hyphal width and hyphal attachment with the wall. On this basis, three genera of AMF were categorized as *Glomus, Acaulospora, Gigaspora* and the identification was done at species level (*Glomus intraradices, Acaulospora tuberculata and Gigaspora gigantea*) with the help of relevant literature ([27,36,48], http://invam.caf.wvu.edu; http://www.agro.ar.szczecin.pl/~jblaszkowski). Isolated AMF spores from rhizosphere soil were purified and maintained in pot culture on *Zea mays* cv. Shakti under greenhouse conditions. For inoculum preparation of the three AMF, surface sterilized seeds of *Zea mays* cv. Shakti and sterilized substrate (soil and sand 1:1 V/V) were used. The pure inoculum was produced by single spore cultivation (http://invam.caf.wvu.edu). The substrate containing spores and root pieces served as a stock culture of AMF inoculum.

2.3. *Bradyrhizobium inoculant preparation and seed treatment*

From a collection of 57 *Bradyrhizobium japonicum* isolates of soybean being maintained at the Department of Botany, Maharshi Dayanand Saraswati University, Ajmer (Rajasthan) under INCO-DEV Research project, we chose 4 different isolates representing 4 soybean cultivars. These isolates were selected on the basis of non-significant difference with regard to their symbiotic efficiency, which was determined earlier based on results of various pot and field trials (data not shown here). Bradyrhizobial isolates are being maintained in 20E medium [47]. A mixture of phosphorus free sterilized charcoal (pH, 6.8) and sand (3:1) was used as carrier for inoculant production. Sterilized carrier was inoculated with exponentially growing bradyrhizobial cultures. Carrier inoculant having around 10^{10} bacterial cells g^{-1} was applied to surface sterilized soybean seeds before sowing by using 10% sugar (jaggery) solution [41] as a sticker material for proper seed pelleting. Seeds without bacterial treatment served as controls.

2.4. *Earthenware pot preparation and inoculation*

Air dried and sieved autoclaved alluvial soil collected from a non-legume cultivated field of Ajmer was used to fill in earthenware pots (8 kg pot^{-1}). A total of 10 g of mycorrhizal inoculum (containing 20 spores g^{-1}) of each AMF viz., *G. intraradices*, *A. tuberculata* or *G. gigantea* was placed in each pot at a depth of 2 cm below the seed sowing level and was covered with soil. Thus each pot received 200 AMF spores of each AM fungus species. There were 8 treatment combinations for each soybean cultivar (control i.e. no AMF or CSBJ, single treatment of CSBJ, three single treatments of AMF and three dual treatments of AMF + CSBJ). There were six replicates of each treatment. Plants in pots were grown for 90 days in a greenhouse (temperature of 27–35 °C, relative humidity of 70–80%) under natural illumination and were watered with distilled water as needed. A set of plants was harvested after 45 days to determine the frequency of root nodules and later after 90 days the remaining plants with fruits were uprooted and data pertaining to shoot height, shoot dry matter, seed weight and shoot nitrogen (N) and phosphorus (P) content, and intensity of AMF colonization were recorded.

2.5. *Estimation of AMF colonization*

Freshly collected roots were washed in water, cleaned with 10% KOH, acidified with 1N HCl and stained in 0.05% trypan blue [30]. Quantification of AMF root colonization was carried out using the slide method [13].

2.6. *Chemical analysis of soil and estimation of shoot nitrogen and phosphorus*

The Ajmer soil used for filling the pots was analysed before experimentation for pH (1:4; w/v; soil suspension), EC (soil suspension, 1:4, W/V), organic carbon [46], available nitrogen [22] and phosphorus [29]. Shoot N and P were determined by the Kjeldahl method and ammonium molybdate vandate method respectively, as described by Jackson [22].

2.7. *Statistics*

The data were statistically analysed by analysis of variance (ANOVA) and LSD test was carried out according to Misra and Misra [26] to detect differences between treatment means when the ANOVA indicated significant treatment effects. In all procedures, probabilities less than 0.05 were considered to indicate statistical significance. Accordingly, significant increase over control has been marked in Table 1 and figures. The data on shoot N and P were also analysed statistically to obtain the correlation matrices for the various parameters such as shoot dry matter, seed weight and AMF colonization using STATISTI XL 1.7 program (http//:www.statistixl.com).

3. Results

3.1. *Soil characteristics*

Selected properties of the Ajmer soil were: Light brown; alluvial; sandy loam; pH, 8.1; EC, 0.56 dSm^{-1}; OC, 0.17%; available N, 62.91 mg kg^{-1} and Olsen-P, 16.00 mg kg^{-1}.

Table 1 – Effect of three AM fungi and cultivar specific *Bradyrhizobium japonicum* (CSBJ) on vegetative growth and seed weight of four soybean cultivars under greenhouse conditions

Treatments	cv. JS 335			cv. JS 71-05			cv. NRC 2			cv. NRC 7		
	SH	SDM	SW	SH	SDM	SW	SH	SDM	SW	SH	SDM	SW
Control	$38.8^{d} \pm 2.46$	$2.66^{d} \pm 0.45$	$1.54^{f} \pm 0.07$	$34.33^{e} \pm 1.03$	$2.66^{e} \pm 0.12$	$1.68^{f} \pm 0.04$	$40.66^{c} \pm 1.11$	$2.66^{f} \pm 0.09$	$1.69^{f} \pm 0.03$	$42.12^{c} \pm 1.91$	$3.53^{c} \pm 0.32$	$1.79^{f} \pm 0.02$
GI	$81.00^{b} \pm 3.00$	$5.16^{b} \pm 0.09$	$2.76^{c} \pm 0.04$	$64.00^{b} \pm 2.40$	$4.97^{b} \pm 0.16$	$2.79^{c} \pm 0.07$	$78.00^{c} \pm 1.90$	$4.91^{f} \pm 0.14$	$2.69^{f} \pm 0.04$	$56.25^{c} \pm 3.46$	$4.19^{c} \pm 0.15$	$2.75^{f} \pm 0.03$
AT	$74.5^{c} \pm 5.14$	$4.70^{b} \pm 0.67$	$2.63^{d} \pm 0.03$	$57.5^{c} \pm 1.51$	$4.20^{c} \pm 0.05$	$2.61^{d} \pm 0.03$	$50.00^{c} \pm 2.08$	$3.55^{f} \pm 0.15$	$2.57^{f} \pm 0.03$	$45.00^{b} \pm 2.66$	$4.15^{b} \pm 0.16$	$2.48^{d} \pm 0.03$
GG	$70.7^{c} \pm 1.88$	$4.23^{c} \pm 0.17$	$2.46^{e} \pm 0.04$	$43.8^{d} \pm 1.00$	$3.94^{d} \pm 0.10$	$2.37^{e} \pm 0.03$	$46.40^{c} \pm 2.14$	$3.37^{f} \pm 0.18$	$2.34^{f} \pm 0.05$	$43.33^{b} \pm 0.88$	$4.00^{b} \pm 0.24$	$2.38^{e} \pm 0.03$
CSBJ	$74.00^{c} \pm 2.21$	$4.48^{c} \pm 0.23$	$2.45^{e} \pm 0.05$	$59.4^{b} \pm 1.22$	$4.08^{d} \pm 0.02$	$2.31^{e} \pm 0.03$	$51.62^{b} \pm 2.95$	$3.26^{e} \pm 0.06$	$2.07^{e} \pm 0.04$	$47.16^{b} \pm 3.86$	$4.38^{b} \pm 0.26$	$2.40^{e} \pm 0.05$
GI + CSBJ	$89.4^{a} \pm 0.31$	$6.06^{a} \pm 0.14$	$3.93^{a} \pm 0.04$	$79.7^{a} \pm 2.46$	$5.53^{a} \pm 0.17$	$3.07^{a} \pm 0.05$	$88.00^{a} \pm 1.14$	$5.41^{a} \pm 0.16$	$3.00^{a} \pm 0.10$	$61.80^{a} \pm 2.09$	$5.60^{a} \pm 0.22$	$3.15^{a} \pm 0.04$
AT + CSBJ	$76.38^{b} \pm 2.40$	$5.18^{b} \pm 0.10$	$2.95^{b} \pm 0.04$	$60.2^{b} \pm 1.32$	$4.43^{c} \pm 0.17$	$2.98^{b} \pm 0.15$	$56.20^{b} \pm 2.00$	$4.36^{c} \pm 0.17$	$2.70^{b} \pm 0.03$	$48.37^{b} \pm 2.42$	$4.25^{b} \pm 0.14$	$2.70^{b} \pm 0.02$
GG + CSBJ	$72.08^{c} \pm 0.99$	$4.94^{b} \pm 0.14$	$2.77^{c} \pm 0.05$	$48.5^{d} \pm 1.61$	$4.13^{d} \pm 0.02$	$2.75^{c} \pm 0.08$	$51.80^{b} \pm 1.69$	$4.14^{c} \pm 0.08$	$2.52^{c} \pm 0.03$	$46.5^{b} \pm 1.60$	$4.26^{b} \pm 0.04$	$2.65^{c} \pm 0.01$

Values are means of six replicates ± SE; values in a column followed by the same letter are not significantly different at LSD $P < 0.05$. SH, shoot height (cm plant^{-1}); SDM, shoot dry matter (g plant^{-1}); SW, seed weight (g plant^{-1}); GI, *Glomus intraradices*; AT, *Acaulospora tuberculata*; GG, *Gigaspora gigantea*.

3.2. Nodular frequency

Variability in nodular frequency amongst the four soybean cultivars grown under greenhouse conditions in alluvial soil containing population of cultivar specific *Bradyrhizobium japonicum* (CSBJ) with three AMF was verified which indicated differences in symbiotic potential. In cv. JS 335 and cv. NRC 2, only the treatment with *G. intraradices* + CSBJ resulted in significant increase ($P < 0.05$) in nodular frequency compared to single inoculation with CSBJ, whereas, other dual treatments i.e. *A. tuberculata* + CSBJ and *G. gigantea* + CSBJ could not bring about any significant difference. In contrast, in cv. JS 71-05 significantly higher values of nodular frequency were observed in all the dual treatments compared to single inoculation with CSBJ while in cv. NRC 7, only *G. intraradices* + CSBJ and *A. tuberculata* + CSBJ treatments significantly improved the nodulation (Fig. 1). The maximum percent increase in nodular frequency caused by *G. intraradices* + CSBJ treatment was recorded 48.79% in cv. JS 335, 26.49% in cv. JS 71-05, 25.25% in cv. NRC 2 and 46.58% in cv. NRC 7 compared to single treatment of CSBJ suggesting promotory effect of AMF on nodulation caused by CSBJ.

3.3. Shoot height and shoot dry matter

Statistical differences among treatments were also observed for shoot growth parameters of four soybean cultivars. For example, shoot height and dry matter of cv. JS 335 and cv. JS 71-05 significantly increased after single and dual inoculations with AMF + CSBJ. In contrast, the values of these parameters were not significantly different from control in cv. NRC 2 inoculated with three AMF singly, whereas inoculation with CSBJ alone and, with three AMF + CSBJ led to statistically significant increase in shoot height and dry matter compared to control. In case of cv. NRC 7, single inoculation with *G. intraradices* did not significantly improve shoot height and dry matter, while *A. tuberculata* and *G. gigantea* were potential enough to do so. Dual inoculation led to further improvements in shoot height and dry matter when compared with single inoculation, in cv. NRC 7. The greatest increase in shoot height (132.15%) and dry matter (127.81%) compared to control was noted after the treatment with *G. intraradices* + CSBJ in cv. JS 71-05 and cv. JS 335 respectively. The response of cv. NRC 7 toward this treatment, however, was the poorest yielding 46.72% and 58.64% increase in shoot height and dry matter respectively compared to control. The effect of *G. intraradices* + CSBJ in enhancing shoot height and dry matter of four soybean cultivars was most significant ($P < 0.05$) amongst all treatments (Table 1).

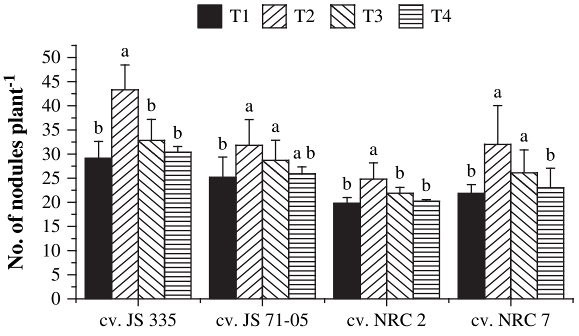

Fig. 1 – Nodular frequency of four soybean cultivars inoculated with cultivar specific *Bradyrhizobium japonicum* (CSBJ) alone and combined with three AM fungi. T1 – CSBJ alone; T2 – *Glomus intraradices* + CSBJ; T3 – *Acaulospora tuberculata* + CSBJ; T4 – *Gigaspora gigantea* + CSBJ. Columns with the same letter are not significantly different at LSD < *P* 0.05. Error bars are SEM.

3.4. Seed yield

Seed weight per plant, in general, improved after single and dual inoculation with AMF and CSBJ. In cv. JS 335 and cv. JS 71-05, seed weight was significantly greater than control in all the treatments. However, no statistically significant difference was encountered between *G. intraradices* and *G. gigantea* + CSBJ treatments with regard to seed weight, in cv. JS 335 and cv. JS 71-05. Among the single treatments with three AMF and CSBJ, the largest amount of seed weight was recorded for *G. intraradices* treated JS 335 and JS 71-05 cultivars, showing 79.22% and 66.07% increase from control respectively. On the contrary, this treatment did not significantly improve the seed weight of cv. NRC 2 and cv. NRC 7 compared to control. Also, the values of seed weight of cv. NRC 2 were not significantly different from control in all the used single treatments. The symbiotic efficiency of three dual treatments considering seed weight was in the following order in all soybean cultivars: *G. intraradices* + CSBJ > *A. tuberculata* + CSBJ > *G. gigantea* + CSBJ (Table 1).

3.5. Nutrient parameters

Statistical analysis revealed that soybean cultivars receiving single and dual treatments of AMF and CSBJ usually had significantly higher levels of shoot N compared to the control plants. Further, higher accumulation of shoot N was encountered in plants receiving dual treatment compared to the single one. However, the effect of *G. intraradices* + CSBJ and *A. tuberculata* + CSBJ was not significantly different from their respective single treatments of AMF with regard to shoot N of cv. JS 335. Contrary to this, shoot N was significantly increased by around 9% in cv. JS 335 inoculated with *G. gigantea* + CSBJ compared to single treatments of *G. gigantea*. In cv. JS 71-05, irrespective of AMF and CSBJ used, all the dual treatments were potential enough to significantly enhance the shoot N, when compared with their respective single AMF treatments. In case of cv. NRC 2 effect of dual inoculation was not encouraging as insignificant increase/reduction in shoot N compared to single treatment was noticed. It was noteworthy that dual inoculation with *G. intraradices* + CSBJ significantly increased the shoot N of cv. NRC 7 compared to single inoculation whereas, *G. gigantea* + CSBJ and *A. tuberculata* + CSBJ did not, suggesting the certain degree of specificity of inoculum response (Fig. 2).

While comparing the effect of AMF and CSBJ on shoot P it was observed that all the single inoculations of AMF/CSBJ were able to significantly improve the shoot P from control. In addition, dual inoculation was superior over single inoculation in almost all cases except for *G. intraradices* + CSBJ in cv. 71-05, where the value of shoot P was not significantly

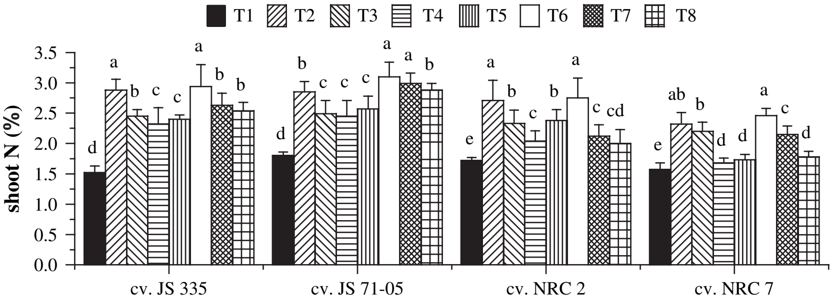

Fig. 2 – Shoot nitrogen (%) of four soybean cultivars inoculated with cultivar specific *Bradyrhizobium japonicum* (CSBJ) alone and combined with three AM fungi. T1 – control; T2 – *Glomus intraradices*; T3 – *Acaulospora tuberculata*; T4 – *Gigaspora gigantea*; T5 – CSBJ; T6 – *Glomus intraradices* + CSBJ; T7 – *Acaulospora tuberculata* + CSBJ; T8 – *Gigaspora gigantea* + CSBJ. Columns with the same letter are not significantly different at LSD < P 0.05. Error bars are SEM.

different from that of *G. intraradices* alone. Moreover, the enhancement of shoot P of cv. JS 335 and cv. JS 71-05 due to single inoculation with *A. tuberculata* was not significantly different from that of *G. gigantea*. Similar trend of non-significant enhancement in shoot P was also observed while comparing these values for *G. intraradices* + CSBJ treatment with the *G. intraradices* treatment alone (Fig. 3).

3.6. *AMF colonization*

The average of mycorrhizal root colonization ranged from 71 to 99% in cv. JS 335, 68–100% in JS 71-05, 72–100% in cv. NRC 2 and 73–100% in cv. NRC 7. Considerable variability among cultivars was noticed in inoculation response. For example, dual inoculation with *A. tuberculata* + CSBJ significantly enhanced the AMF colonization compared to single inoculation whereas, *G. intraradices* + CSBJ and *G. gigantea* + CSBJ could not significantly improve it, in cv. JS 335, indicating promotory effect of CSBJ on specific AMF only. Similarly, in cv. JS 71-05 AMF colonization was not significantly different from single treatment, for *A. tuberculata* + CSBJ and *G. gigantea* + CSBJ. In addition, mycorrhizal colonization of cv. NRC 2 inoculated with AMF + CSBJ did not significantly differ from single inoculation with AMF. A similar variable pattern of inoculation responses in AMF colonization was noted in cv. NRC 7 (Fig. 4).

3.7. *Correlation matrices*

Pearson's correlation analysis revealed that seed weight correlated significantly with shoot dry matter and P. However a considerable variability in correlation matrices could be observed as per their correlation coefficients. For example, correlation between seed weight and shoot dry matter was positive and significant at $P < 0.01$ in cv. JS 335 ($r = 0.943$), cv. NRC 2 ($r = 0.905$) and NRC 7 ($r = 0.911$) while in cv. JS 71-05 it was significant at $P < 0.05$ ($r = 0.766$). On the other hand, positive correlation between seed weight and P was highly significant ($P < 0.001$) in cv. JS 335 ($r = 0.952$), cv. NRC 2 ($r = 0.962$) and NRC 7 ($r = 0.962$) while in cv. JS 71-05 it was significant at $P < 0.01$ ($r = 0.894$). Shoot dry matter and P were positively correlated with variable level of significance (cv. JS 335 – $P < 0.001$, $r = 0.978$; cv. NRC 2 – $P < 0.001$, $r = 0.981$; cv. JS 71-05 – $P < 0.01$, $r = 0.891$; cv. NRC 7 – $P < 0.01$, $r = 0.887$). Correlation between AMF colonization and shoot P was also found statistically significant in cv. JS 335 ($P < 0.01$, $r = 0.888$). In addition, AMF colonization correlated statistically significantly with shoot N also (cv. JS 335 – $P < 0.01$, $r = 0.940$; cv. NRC 2 – $P < 0.05$, $r = 0.858$; cv. NRC 7 – $P < 0.01$, $r = 0.815$).

4. Discussion

Due to the intimacy of the relationship between the AMF and rhizobia, the nature of AMF-*Rhizobium* interactions can determine the efficacy of the tripartite symbiosis in terms of enhancing plant yield. Several reports have suggested that the growth and yield of legumes are influenced by interactions between AMF species and rhizobia [1,31,34,49]. According to a report, the AMF associated with legumes are an essential link for effective phosphorus nutrition, leading to enhanced

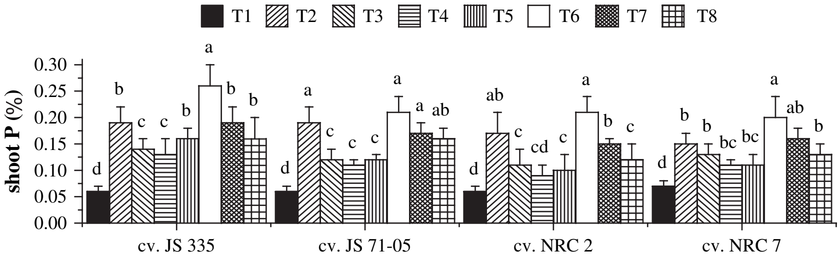

Fig. 3 – Shoot phosphorus (%) of four soybean cultivars inoculated with cultivar specific *Bradyrhizobium japonicum* (CSBJ) alone and combined with three AM fungi. For abbreviations T1–T8 see Fig. 2. Columns with the same letter are not significantly different at LSD < P 0.05. Error bars are SEM.

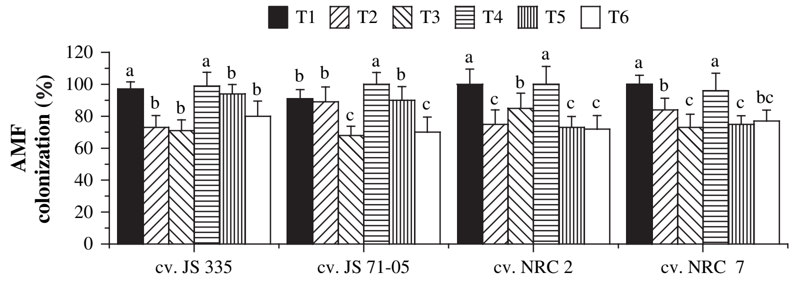

Fig. 4 – AMF colonization (%) of four soybean cultivars inoculated with cultivar specific *Bradyrhizobium japonicum* (CSBJ) alone and combined with three AM fungi. T1 – *Glomus intraradices*; T2 – *Acaulospora tuberculata*; T3 – *Gigaspora gigantea*; T4 – *Glomus intraradices* + CSBJ; T5 – *Acaulospora tuberculata* + CSBJ; T6 – *Gigaspora gigantea* + CSBJ. Columns with the same letter are not significantly different at LSD $< P$ 0.05. Error bars are SEM.

nitrogen fixation that in turn promotes root and mycorrhizal growth [11]. However, in a more recent study, inoculation of *Glomus clarum* in rice under greenhouse conditions decreased the growth or had no significant effects when compared with controls assuming that different crop plants might have different mechanisms to interact with AMF [32]. The growth and yield increase of legumes inoculated with AMF and rhizobia is generally due to enhanced N and/or P uptake as suggested by many researchers [2,35,49].

The existence of host preference by AMF has been investigated by several researchers which provide support for the argument that different AMF produce markedly different levels of root colonization, growth rates and nutritional responses in some plant species compared to others [15,45]. In the present study, *G. intraradices* gave the maximum increases in the parameters studied followed by *A. tuberculata* and *G. gigantea* suggesting that plant acted selectively on AMF symbiosis and a more beneficial relationship could be established with the highly compatible and effective AMF microsymbiont. Similar to this, the responses of lentil (*Lens culinaris* cv. 'Ziba') to co-inoculation with AMF and some indigenous rhizobial strains in a calcareous soil was greatly affected by the fungal species where the *G. intraradices* was superior to *G. mosseae* in increasing plant growth and nutrient uptake [52]. In a more recent investigation, variation in the relative effectiveness was reported for subterranean clover grown on a high P-fixing soil in which *A. laevis* had the most positive effect on P uptake by plants compared to *G. invermaium* and *S. calospora* indicating that compatible pairing between host and fungus is crucial in improving the nutrient uptake [37].

The dual inoculation with AMF + CSBJ further improved all the characteristics studied in the present experiment which is attributed to the synergistic interaction between two microsymbionts. This is in accordance with the previous literature data which shows that dual inoculation of rhizobia plus AMF significantly increases dry weight, N and P contents of the shoot, increases root nodulation, root AMF colonization and improves the height and diameter growth [31,49,52].

Depending upon the AMF-*Rhizobium* interaction, the response of a legume host to a given set of AMF-*Rhizobium* sp. partners may or may not be favorable for plant growth depending on the interaction of the symbionts [50]. While studying the effects of interactions between *G. pallidum*, *G. aggregatum* and *S. microcarpa* and four *Rhizobium phaseoli* strains on the growth and yield of three kidney bean cultivars, it was found that the symbiotic efficiency was "dependent on the particular combination" of the AMF species, *Rhizobium* strain and even the host cultivar [1]. Our results demonstrated that the dual inoculation of *G. intraradices* + *B. japonicum* brought about the maximum increase in root nodulation, shoot growth, N and P concentrations in shoots, and seed yield compared to the dual combination of *A. tuberculata* + *B. japonicum* and *G. gigantean* + *B. japonicum* indicating that the inoculation response was specific for genotypes of different symbiotic partners. Such specific response depending on the particular combination of the AMF species, *Rhizobium* strain and the host genotype has been reported earlier [21,35] which strengthen our findings. The plant genotype can play a significant role in shaping plant-associated microbial communities and determining the biological outcome of such associations [40]. Variation for host responses has been studied by evaluating cultivars or breeding lines, inoculated with different AMF, for variation in growth and nutrient uptake in different plants [6,16]. The inoculation response of different cultivars was considerably variable in our study and cultivar JS 335 was found to be the most compatible host in terms of its positive response towards inoculation compared to other three soybean cultivars. This study, hence, demonstrated the variable level of synergistic interactions between AMF species and *B. japonicum*. Similar to the present findings, variability in the four soybean cultivars (TGX 1805-17F, TGX 1681-3F, TGX 1805-33F and TGX 1740-3F) towards mycorrhization with *Glomus* spp. alone and in combination with *B. japonicum* have been reported [42]. Variable enhancement of mycorrhizal root colonization in soybean cultivars after inoculation with *Glomus* spp. and *Gigaspora* spp., as observed in the present investigation, was also observed earlier [43]. However, according to another published report, there was little variation in mycorrhizal root colonization in the soybean variety Pusa 22 after inoculation with two *Glomus* spp. and one *Gigaspora* spp. [44] which is contrary to our results. Nevertheless, taking into consideration overall data of the present study, it could be unequivocally stated that inoculation response of soybean cultivars may vary with symbiont microorganisms and the effectiveness of the inoculation depends on the degree of contribution of the symbiotic partners. Consequently, inoculation may be highly effective if synergetic interaction is established among effective symbionts of rhizobia and AMF.

Correlation analyses revealed the strong positive relationships between shoot dry matter, seed weight and AMF colonization with shoot N and P. This supported a conclusion of previous researchers who found significant linear correlations between shoot dry weight and total N in shoots [17,51]. The phosphorus absorption ability was reported to be strongly connected with dry matter production [24], which is parallel to our results. An effective mycorrhizal infection may affect nodule weight, amount of leghaemoglobin and nitrogen fixing activity [1,20]. In addition, variability in the correlation matrices for different parameters taken into consideration in the present investigation also indicates variation in the magnitude of interactive effects of AMF and *B. japonicum* with different soybean cultivars.

5. Conclusions

This study demonstrated that by proper selection for AMF and rhizobia which are compatible with each other and with the host plant, the growth, yield and nutrition of the legume crop like soybean can be significantly enhanced. Looking to the considerable variability in cultivar response with symbiotic partners, the identification of cultivars highly compatible for tripartite symbiosis (AMF-*Rhizobium*-legume) is a must when recommending them to farmers, as well as determining cultivars for use as parental genotypes in breeding programs. Better response of cv. JS 335 towards dual inoculation with *G. intraradices* and *B. japonicum* as reported in the present study allow us to suggest that this cultivar should be tested in the arable fields of Ajmer region along with these inoculants so that an appropriate combination of specific AMF and *Bradyrhizobium* could be selected and utilized for improved production of soybean in sustainable manner.

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REFERENCES

[1] M.H. Ahmad, Compatibility and co-selection of vesicular arbuscular mycorrhizal fungi and rhizobia for tropical legumes, Crit. Rev. Biotech. 15 (1995) 229–239.

[2] R. Azcon, R. Rubio, J.M. Barea, Selective interactions between different species of mycorrhizal fungi and *Rhizobium meliloti* strains, and their effects on growth, N_2-fixation (^{15}N) and nutrition of *Medicago sativa* L, New Phytol. 117 (1991) 399–404.

[3] J.D. Bever, Host specificity of AM fungal population growth rates can generate feedback on plant growth, in: S.E. Smith, F.A. Smith (Eds.), Diversity and Integration in Mycorrhizas, Proc. Third International Conference on Mycorrhizas (ICOM 3), Kluwer Academic Publishers, London, 2002, pp. 281–290.

[4] S.M. Boyetchko, J.P. Tewari, Susceptibility of barley cultivars to vesicular-arbuscular mycorrhizal fungi, Can. J. Plant Sci. 75 (1995) 269–275.

[5] B.D. Chen, X.L. Li, H.Q. Tao, P. Christie, M.H. Wong, The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc, Chemosphere 50 (2003) 839–846.

[6] C.R. Clement, M. Habte, Genotypic variation in vesicular-arbuscular mycorrhizal dependence of the pejibaye palm, J. Plant Nutr. 18 (1995) 1907–1916.

[7] L.M. De Chueire, M. Hungria, N_2-fixation ability of Brazilian soybean cultivars with *Sinorhizobium fredii* and *Sinorhizobium xinjiangensis*, Plant Soil 196 (1997) 1–5.

[8] P.Y. De la Bastide, B.R. Kropp, Y. Piche, Population structure and mycelial phenotypic variability of the ectomycorrhizal basidiomycete *Laccaria bicolor* (Maire) Orton, Mycorrhiza 5 (1995) 371–379.

[9] V. Estaun, A. Camprubi, E.J. Joner, Selecting arbuscular mycorrhizal fungi for field application, in: S. Gianinazzi, H. Schueep, J.M. Barea, K. Haselwandter (Eds.), Mycorrhiza Technology in Agriculture: From Genes to Bioproducts, Birkhauser Verlag, Basel, Switzerland, 2002, pp. 249–259.

[10] M.E. Gavito, P. Schweiger, I. Jakobsen, Phosphorus uptake by arbuscular mycorrhizal hyphae: effect of soil temperature and atmospheric CO_2 enrichment, Global Change Biol. 9 (2003) 106–116.

[11] M. Geneva, G. Zehirov, E. Djonova, N. Kaloyanova, G. Georgiev, I. Stancheva, The effect of inoculation of pea plants with mycorrhizal fungi and *Rhizobium* on nitrogen and phosphorus assimilation, Plant Soil Environ. 52 (2006) 435–440.

[12] J.W. Gerdemann, T.H. Nicolson, Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting, Trans. Br. Mycol. Soc. 46 (1963) 235–244.

[13] M. Giovannetti, B. Mosse, An evaluation of technique for measuring vesicular arbuscular infection in roots, New Phytol. 84 (1980) 489–500.

[14] D. Harwani, Biodiversity and efficiency of bradyrhizobial strains and arbuscular mycorrhizal fungi of soybean cultivars grown in haroti region of Rajasthan, Ph. D. Thesis, Maharshi Dayanand Saraswati University, Ajmer, India, 2006.

[15] T. Helgason, J.W. Merryweather, J. Denison, P. Wilson, J.P.W. Young, A.H. Fitter, Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland, J. Ecol. 90 (2002) 371–384.

[16] B.A.D. Hetrick, G.W.T. Wilson, T.S. Cox, Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors, Can. J. Bot. 70 (1992) 2032–2040.

[17] M. Hungria, T.R.J. Bohrer, Variability of nodulation and dinitrogen fixation capacity among soybean cultivars, Biol. Fertil. Soils 31 (2000) 45–52.

[18] D.C. Ianson, R.G. Linderman, Variation in the response of nodulating pigeonpea (*Cajanus cajan*) to different isolates of mycorrhizal fungi, Symbiosis 15 (1993) 105–119.

[19] K.K. Ibrahim, V. Arunachalam, P.S.K. Rao, K.V.B.R. Tilak, Seasonal response of groundnut genotypes to arbuscular mycorrhiza – *Bradyrhizobium* inoculation, Microbiol. Res. 150 (1995) 218–224.

[20] Y.Z. Ishac, J.S. Angle, M.E. El-Borollosy, M.E. Demerdash, M.I. Mostafa, C.N. Fares, Growth of *Vicia faba* as affected by

inoculation with vesicular-arbuscular mycorrhizae and *Rhizobium leguminosarum* bv. viceae in two soils, Biol. Fertil. Soils 17 (1994) 27–31.

[21] C.M.V. Jaarsveld, M.A. Smith, G.H. Kruger, Interaction amongst soybean (*Glycine max* (L.) Merrill) genotype, soil type and inoculant strain with regard to N_2 fixation, J. Agron. Crop Sci. 188 (2002) 206–211.

[22] M.L. Jackson, Soil Chemical Analysis, Prentice Hall of India Ltd, New Delhi, 1973.

[23] N. Karagiannidis, N. Nikolaou, A. Mattheou, Influence of three VA-mycorrhiza species on the growth and nutrient uptake of three grapevine rootstocks and one table grape cultivar, Vitis 34 (1995) 85–89.

[24] J. Lynch, A. Lauchli, E. Epstein, Vegetative growth of the common bean in response to phosphorus nutrition, Crop Sci. 31 (1991) 380–387.

[25] S.K. Mahna, Production, regional distribution of cultivars and agricultural aspects of soybean in India, in: D. Werner, W.E. Newton (Eds.), Nitrogen Fixation in Agriculture, Forestry, Ecology and the Environment, Springer, the Netherlands, 2005, pp. 43–66.

[26] B.N. Misra, M.K. Misra, Introductory Practical Biostatistics, Darbari Prakashan, Kolkata, India, 1983.

[27] J.B. Morton, G.L. Benny, Revised classification of arbuscular mycorrhizal fungi (Zygomycetes); a new order, Glomales, two new suborders, Glomineae and Gigasporineae and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae, Mycotaxon 37 (1990) 471–491.

[28] S. Nemec, L. Datnoff, Pepper and tomato cultivar responses to inoculation with *Glomus intraradices*, Adv. Hortic. Sci. 7 (1993) 161–164.

[29] S.R. Olsen, C.V. Cole, F.S. Wantabe, L.A. Dean, Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate, Cric. US. Agric. (1954) 939.

[30] J.M. Phillips, D.S. Hayman, Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection, Trans. Br. Mycol. Soc. 55 (1970) 158–161.

[31] K. Prasad, M.K. Meghvansi, D. Harwani, S.K. Mahna, D. Werner, Synergistic effects of arbuscular mycorrhizal fungi in soybean (*Glycine max* L. Merrill), Anusandhan 1 (2005) 13–19.

[32] M.P. Raimam, U. Albino, M.F. Cruz, G.M. Lovato, F. Spago, T. P. Ferracin, D.S. Lima, T. Goulart, C.M. Bernardi, M. Miyauchi, M.A. Nogueira, G. Andrade, Interaction among free-living N-fixing bacteria isolated from *Drosera villosa* var. villosa and AM fungi (*Glomus clarum*) in rice (*Oryza sativa*), Appl. Soil Ecol. 35 (2007) 25–34.

[33] K.P. Raverkar, K.V.B.R. Tilak, Improved mycorrhizal colonization, nodulation status, biomass production and nutrient uptake in soybean due to inoculation with *Bradyrhizobium japonicum*, Legume Res. 25 (2002) 32–36.

[34] D. Redecker, P. von Berswordt-Wallrabe, D.P. Beck, D. Werner, Influence of inoculation with arbuscular mycorrhizal fungi on stable isotopes of nitrogen in *Phaseolus vulgaris*, Biol. Fertil. Soils 24 (1997) 344–346.

[35] J.M. Ruiz-Lozano, R. Azcon, Specificity and functional compatibility of VA mycorrhizal endophytes in association with *Bradyrhizobium* strains in *Cicer arietinum*, Symbiosis 15 (1993) 217–226.

[36] N.C. Schenck, Y. Perez, A Manual for Identification of Vesicular Arbuscular Mycorrhizal Fungi, INVAM, third ed. University of Florida, Gainesville, Florida, 1990.

[37] P.F. Schweiger, A.D. Robson, N.J. Barrow, L.K. Abbott, Arbuscular mycorrhizal fungi from three genera induce two-phase plant growth responses on a high P-fixing soil, Plant Soil 292 (2007) 181–192.

[38] A.M. Shalaby, M.M. Hanna, Interactions between VA mycorrhizal fungus *Glomus mosseae, Bradyrhizobium japonicum* and *Pseudomonas syringae* in soybean plants, Egyptian J. Microbiol. 35 (2000) 199–209.

[39] R. Singh, A. Adholeya, Effect of crop rotation on diversity and functionality of arbuscular mycorrhizal fungi (AMF), Mycorrhiza News 15 (2003) 25–26.

[40] K.P. Smith, R.M. Goodman, Host variation for interactions with beneficial plant-associated microbes, Annu. Rev. Phytopathol. 37 (1999) 473–491.

[41] N.S. SubbaRao, *Rhizobium* inoculant, in: Biofertilizers in Agriculture and Forestry, second ed. Oxford and IBH Publishing Co. Pvt Ltd, New Delhi, India, 1988, pp. 16–76.

[42] L.B. Taiwo, A.A. Adegbite, Effect of arbuscular mycorrhiza and *Bradyrhizobium* inoculation on growth, N_2 fixation and yield of promiscuously nodulating soybean (*Glycine max*), J. Agri. Res. 2 (2001) 110–118.

[43] K.V.B.R. Tilak, S.K. Rathi, A.K. Saxena, Field evaluation of different arbuscular mycorrhizae of soybean, in: A. Adholeya, S. Singh (Eds.), Mycorrhizae: Biofertilizers for the Future, Tata Energy Research Institute, New Delhi, India, 1995, pp. 339–344.

[44] K.V.B.R. Tilak, A.K. Saxena, K.V. Sadasivam, Synergistic effects of phosphate-solubilizing bacterium (*Pseudomonas striata*) and arbuscular mycorrhizae on soybean, in: A. Adholeya, S. Singh (Eds.), Mycorrhizae: Biofertilizers for the Future, Tata Energy Research Institute, New Delhi, India, 1995, pp. 224–226.

[45] P. Vandenkoornhuyse, K.P. Ridgway, I.J. Watson, A.H. Fitter, J.P.W. Young, Co-existing grass species have distinctive arbuscular mycorrhizal communities, Mol. Ecol. 12 (2003) 3085–3095.

[46] A. Walkley, I.A. Black, An examination of degradative method for determination of soil organic matter and a proposed modification of chromic acid titration method, Soil Sci. 37 (1934) 29–38.

[47] D. Werner, J. Wilcockson, E. Zimmermann, Adsorption and selection of rhizobia with ion exchange papers, Arch. Microbiol. 105 (1975) 27–32.

[48] T. Wu, W. Hao, X. Lin, Y. Shi, Screening of arbuscular mycorrhizal fungi for the revegetation of eroded red soils in subtropical China, Plant Soil 239 (2002) 225–235.

[49] L.J.C. Xavier, J.J. Germida, Response of lentil to co-inoculation with arbuscular mycorrhizal fungi and rhizobia varying in efficacy, Soil. Biol. Biochem. 34 (2002) 181–188.

[50] L.J.C. Xavier, J.J. Germida, Selective interactions between arbuscular mycorrhizal fungi and *Rhizobium leguminosarum* bv. viceae enhance pea yield and nutrition, Biol. Fertil. Soils 37 (2003) 261–267.

[51] A. Zaidi, M.S. Khan, Co-inoculation Effects of phosphate solubilizing microorganisms and *Glomus fasciculatum* on Green Gram-*Bradyrhizobium* Symbiosis, Turk. J. Agric. For. 30 (2006) 223–230.

[52] M. Zarei, N. Saleh-Rastin, H.A. Alikhani, N. Aliasgharzadeh, Responses of lentil to co-inoculation with phosphate-solubilizing rhizobial strains and arbuscular mycorrhizal fungi, J. Plant Nutr. 29 (2006) 1509–1522.

[53] F. Zhang, M. Dijak, D.L. Smith, J. Lin, K. Walsh, H. Voldeng, F. Macdowell, D.B. Layzell, Nitrogen fixation and nitrate metabolism for growth of six diverse soybean (*Glycine max* (L.) Merr.) genotypes under low temperature stress, Environ. Exp. Bot. 38 (1997) 49–60.