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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