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ORIGINAL RESEARCH

Effect of Dual Inoculation of Arbuscular Mycorrhiza Fungus and Cultivar Specific *Bradyrhizobium japonnicum* On the Growth, Yield, Chlorophyll, Nitrogen and Phosphorus Contents of Soybean (Glycine Max (L.) Merrill.) Grown on Alluvial Soil

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Abstract: The aim of the present study was to investigate the effect of dual inoculation of arbuscular mycorrhiza fungi (*Glomus fasciculatum*), and cultivar specific *Bradyrhizobium japonnicum* on the shoot height, dry matter, arbuscular mycorrhiza fungal colonization, chlorophyll, nutrient contents and yield of soybean variety NRC 37 (Ahilya 4). The results revealed an overall increase in shoot height, dry matter, arbuscular mycorrhiza fungal infection, chlorophyll, nitrogen and phosphorus contents and yield in the arbuscular mycorrhiza fungal mediated plants than uninoculated ones. Maximum increases were recorded in dually inoculated plants with arbuscular mycorrhiza fungi (AM Fungi) and cultivar specific *Bradyrhizobium japonnicum* (CSBJ). The combined application of AM Fungi and CSBJ were also remarkably increased the all parameters tested on soybean. The dual inoculation with microsymbionts revealed synergistic effect on soybean. The results suggest that dual inoculation of AM Fungi and CSBJ have the potential to enhance the growth, nutrient contents and yield of soybean.

Keywords: Glomus fasciculatum; Bradyrhizobium japonnicum; Chlorophyll; Nitrogen; Phosphorus; Soybean

1. Introduction

Alluvial soil has the highest productivity with respect to other soils. The alluvial soil found in India, particularly in the Indo–Gangetic plain. The Gangetic plains of India are one of the most intensively cultivated plains of the world. Alluvial soil is one of the best soils, requiring the least water due to its high porosity. The consistency of alluvial soil ranges from drift sand and rich, loamy soil to silt clays. India is one of the richest countries in the world in terms of alluvial soil (nearly 43%), which covers more than 46% of its total land area (Alka et al., 2017). The morphological, physical, chemical, and mineralogical properties of alluvial soils depend greatly on the characteristics of the alluvial parent material in which the soils formed. According to several studies, organic materials improve soil chemical, physical and biological properties and thereby contribute to the maintenance of overall soil fertility and productivity (Topoliantz, Ponge, & Ballof, 2005). Soil microbial communities are extremely diverse, and the relation between their diversity and function influences soil stability, productivity and resilience; on the other hand, organic matter, water activity, soil fertility, physical and chemical properties influence microbial biomass in soils (Tomich et al., 2011). The alluvial soil is generally covered by grasses and forests, as well as a number of crops grown, such as rice, wheat, sugarcane, tobacco, maize, cotton, soybean, jute, oilseeds, fruits, vegetables, etc.

The rhizosphere of plants is residence to a huge diversity of microbial species, many of which interact naturalistically with plant roots. There has been significant interest recently in exploiting their beneficial microorganisms for the purpose of more sustainable for crop production and soil health. Rhizospheric microorganisms play an important role in sustaining soil health and long-term productivity by performing major soil functions such as decomposition of organic matter, nutrient cycling and formation of soil aggregate. The recognized benefits of plants inoculation with beneficial microorganisms include reduced pathogen infection, improved fertilizers use efficiency and resistance to a biotic stress such as salinity (Yaung, et al., 2009; Kim et al., 2011;) (Chen et al., 2011; Prasad et al., 2019). Most of the legumes possess two types of microbial symbionts namely mycorrhizal fungi and nitrogen fixing bacteria (BNF) thereby establishing triple association, capable of supplying nitrogen (N) and Phosphorous (P) contents to the plants (Silveira and Cardoso, 2004; Prasad et al., 2005; Meghvansi et al., 2008; (Prasad et al., 2005) ; (Meghvanshi et al., 2008; Prasad et al., 2019; K. Prasad, Meghavansi, Harwani, Mahna, & Werner, 2005). Both the AM Fungi and *Bradyrhizobium japonnicum* (BJ) act as biofertilizers and have the unique ability to convert nutritionally important elements unavailable form in soil to available form through biological process (Hedge et al., 1999; Vessey, 2003; ; Meghavanshi et al., 2010; Prasad, 2011; Prasad, 2015; Prasad, 2017; Prasad, 2020) (Meghvanshi et al., 2008).

Mycorrhizal fungi interact with a wide range of microbes in the root rhizosphere, and in the bulk soil. These interactions may be inhibitory or stimulatory; some are clearly competitive and others may be mutualistic. Through, AM Fungi are not capable to fixing atmospheric nitrogen; they are known to increase N_2 fixation and positively interact with N_2 fixers (Bagyaraj and Menge, 1978; Bethlenfalvay, 1992; Barea et al., 1992; Prasad et al., 2005; Prasad et al., 2006; Meghavanshi et al., 2010; Nicholas et al., 2021) (Bagyaraj & Menge, 1978; Barea et al., 1992; Nicholas et al., 2021; K. Prasad et al., 2005). Combined inoculation of phosphate solubilising microorganisms and AMF have shown better N and P uptake and improved crop yields in nutrient deficient soils (Singh, Kapoor, & Wange, 1991).

Soybean (Glycine max (L.) Merrill.) is the most widely grown worldwide as cash crop 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. Soybean, commonly known as Soya, a protein rich pulse/oil crop has growing demand in Asia, especially in India because it can provide high quality protein in Indian diet. The plant has got immense medicinal value and is also used as fodder for cattle. There is need to enhance its productivity to meet the demand of growing population. The increase in soybean cultivation in India is likely to improve the rural economy and socio-economic status of the Indian farmers. Association of soybean with AM Fungi increases the uptake of nutrients particularly phosphorus (; Prasad, 2017) (Gavito, Schweiger, & Jakobsen, 2003; Xavier & Germida, 2003), zinc (Chen et al., 2011) Chen et al., 2003) and nitrogen as well as increasing crop production (Raverkar and Tilak, 2002). The association of bradyrhizobial strains with the roots of soybean plants also improves soil health and nitrogen fixation thus further increasing crop production (Jaarsveld et al., 2002; Prasad et al., 2006; Meghavanshi et al., 2010; Prasad, 2011). Recent investigations have brought to light instances where biological activities are markedly enhanced in two or three membered associations of organisms. Synergistic effects of AM Fungi and BJ have a high potential to improve the nutrients supply of soybean including phosphorus and soil quality (Tilak, Saxena, Sadasivam, Adholeya, & Singh, 1995). However, published studies (Shalaby and Hann, 2000; Taiwa and Adegbite, 2001; Meghavanshi et al., 2010; Prasad, 2011; Prasad et al., 2019) (Prasad et al., 2019; Shalaby & Hanna, 2000) indicate that a much larger genetic variability of bradyrhizobia and AMF strains exists in different cultivar regions than was assumed previously. Researches in the past few decades on various aspects of root symbionts have shown that dual interaction of AM fungi and CSBJ has improved the growth, nodulation and yield (Gill and Singh, 2008; Talaat and Abdallah, 2008; Meghavanshi, et al., 2008) and also nutrient status (Chakrabarty et al., 2007), Meghavanshi et al., 2008; Meghavanshi et al., 2010) in legumes. In the light of these views, an attempt has been made in the present study, to investigate the effect of dual inoculation of AM Fungi (*Glomus fasciculatum*) and CSBJ in growth, mycorrhizal colonization in root, chlorophyll, nitrogen and phosphorus contents and yield of soybean under greenhouse conditions.

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2. Materials and Methods

2.1. Procurement of Seeds

Soybean seeds of cultivar NRC 37 (Ahilya 4) were collected from Soybean Research Centre (NRC), Indore (Madhya Pradesh), India. These cultivars are commonly grown in central India.

2.1.1. ***Preparation of Inoculum***

2.1.2. AM Fungi (Glomus fasciculatum) Inoculum Preparation

Individual AMF spores showing hyphal connection were isolated by the wet sieving and decanting method (Gerdemann & Nicolson, 1963) 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 color, diameter, shape, wall layers, surface content, hyphal color, hyphal width and hyphal attachment with the wall. Identification was done at species level (*Glomus fasiculatum*) with the help of relevant literature (Morton and Benny, 1990; ; Prasad and Rajak, 1999; Wu et al., 2002) (Schenck & Perez, 1990). Isolated AMF spores were purified and maintained in pot culture on Zea mays cv. Shakti under greenhouse conditions. For inoculum preparation of the 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. The substrate containing spores and root pieces served as a stock culture of AMF inoculum. The treatment was replicated ten times (one seed maintained in each pot).

2.1.3. Bradyrhizobium Inoculant Preparation and Seed Treatment

The CSBJ isolates were obtained from root nodules of NRC 37 soybean variety sampling from Jabalpur, Madhya Pradesh. Healthy, pinkish, well formed, unbroken nodules were collected from the roots of NRC 37. The nodules were surface sterilized for 3 minutes in 0.1% mercuric chloride and washed repeatedly with sterile water. Individual nodules were crushed with sterile glass rods in a small aliquot of sterile water and milky fluid was streaked on to sterile 20 E medium (Vincent, 1970; Werner, Wilcockson, & Zimmermann, 1975). The plates were incubated at 28°C for 6-8 days. Colonies were selected and streaked on 20E plate for purity and pure culture of isolated bacteria was subjected to morphological and biochemical (growth on Hofers alkaline medium, growth on Glucose peptone agar) test for characterization as described by Subbarao (1999) A mixture of phosphorus free sterilized charcoal (pH, 6.8) and sand (3:1) were 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 healthy soybean seeds before sowing by using 10% sugar (jaggery) solution (Subbarao, 1999)as a sticker material for proper seed pelleting. Seeds without bacterial treatment served as controls. The treatment was replicated ten times.

2.1.4. Earthenware Pot Preparation and Inoculation

Air dried and sieved autoclaved alluvial soil collected from a non-legume cultivated field was used to fill in earthenware pots (10 kg pot-1). A total of 5g of mycorrhizal inoculum (containing 40 spore's g^{-1}) of AMF 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. There were 4 treatment combinations for soybean (control i.e., no AMF or CSBJ, single AMF, single treatment of CSBJ, dual treatments of AMF + CSBJ). There were ten replicates of each treatment. The experiment was laid out in randomized complete block (RCB) design. Plants were watered every day as per requirement. Plants in pots were grown for 100 days in a greenhouse (temperature of 27–35^{0}C, relative humidity of 70-80%) under natural illumination and were watered as needed. A set of plants was harvested after 45 days to determine the frequency of root nodules, chlorophyll content and later after100 days the remaining plants with fruits were uprooted and data pertaining to shoot height, shoot dry matter, seed weight and, shoot N and P content and intensity of AMF colonization were recorded.

Estimation of AMF Colonization in Root

Freshly collected roots were washed in water, cleaned with 10% KOH, acidified with 1N HCl and stained in 0.05% Trypan blue (Phillips & Hayman, 1970). Quantification of AMF root colonization was carried out using the slide method (Giovannetti and Mosse, 1980).

2.1.5. ***Determination of Chlorophyll contents in Leaves***

Three replicates at random from each treatment were selected for determination of chlorophyll content in leaves. Chlorophyll content of leaves was determined after 60 days of inoculation of plants. Chlorophyll contents were separated and estimated following the method as described by (Sadasivan & Manickam, 1996). Chlorophyll was extracted in 80% acetone and total chlorophyll contents were calculated with the formula:

[*Total chlorophyll content mg* g^{-1} *= 20.2 (A 645) + 8.02(A 663) V/1000*W]*

Where, A = Absorbance at specific wave length; V = Final volume of chlorophyll content in 80% acetone; W = Fresh weight of the tissue extract.

2.1.6. ***Chemical Analysis of Soil and Estimation of Shoot Nitrogen and Phosphorus***

Soil used for filling the pots was analyzed before experimentation for pH (1:4; w/v; soil suspension), EC (soil suspension, 1:4, W/V), organic carbon (Walkley and Black, 1934), available nitrogen (Jackson, 1973) and phosphorous (Olsen et al., 1954). Shoot N and P were determined by the Kjeldahl method and ammonium molybdate vandate method respectively, as described by (Jackson, 1973).

2.1.7. ***Statistical Analysis***

Observations on growth yield and chemical properties in shoot were analyzed using SPSS (SPSS Inc. version 17.0). Results were subjected to one way analysis of variance and the significant difference was determined according to Duncan's Multiple Range Test at significant level of P<0.05.

3. Results and Discussion

3.1. Soil Characteristics

Selected properties of 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}.

3.2. Nodular Frequency

Variability in nodular frequency in NRC 37 soybean cultivar grown under greenhouse conditions in alluvial soil containing population of CSBJ with AMF was verified which indicated differences in symbiotic potential. Significantly higher values of nodular frequency were observed in the dual treatments with the value of 43.66 as compared to single inoculation with CSBJ with the value of 33.33 (Figure 1).

3.3. Shoot Height and Shoot Dry Matter

Statistical differences among treatments were also observed for parameters of soybean. Shoot height and dry matter of cv. NRC 37 significantly increased after single and dual inoculations with AMF and CSBJ. Maximum shoot height and shoot dry matter were noticed in dual inoculation (AMF + CSBJ) followed AMF, CSBJ and control (Table 1). Dual inoculation led to improvements in shoot height (81.29%) and dry matter (120.54%) as compared to control (Table 1). The effect of G. *fasciculatum* and CSBJ in enhancing shoot height and dry matter of soybean was most significant ($P < 0.05$) amongst all treatments (Table 1). SubbaRao et al. (1985) reported that synergistic effect of vesicular arbuscular mycorrhiza and Azospirillum brasilense significantly increased growth of barley in pots conditions.

3.4. AMF Colonization in Root

Significantly (P <0.05) higher values of AMF root colonization was observed in the dual inoculated (AMF + CSBJ) with the value of 77.66% as compared to single inoculation (AMF) with the value of 64.66% (Figure 2).

3.5. Chlorophyll Content

The effects of individual inoculation with both *Glomus fasciculatum* and CSBJ and their combined inoculation on the chlorophyll content of cv. NRC 37 are presented in the figure 3. The results presented in the figure 3 revealed that chlorophyll contents in the treated plants were higher than those in control. G. *fasciculatum* markedly improved the chlorophyll contents in the leaves of cv. NRC 37. However, maximum chlorophyll contents were recorded in the plants dually inoculated with G. *fasciculatum* and CSBJ. AM fungi have been shown to improve the chlorophyll contents on the leaves of many plants (Zuccarni, 2007). It is evident from the results of the present study that the efficacy of AM fungi was influenced by co-inoculation with CSBJ. Increase in the chlorophyll contents due to dual inoculation of AM fungi and Bradyrhizobium might be attributed to increased rate of photosynthesis and transpiration or due to increased growth (Krishna and Bagyaraj, 1984) (K. Prasad et al., 2005). Krishna and Bagyaraj, (1984) reported that increase in chlorophyll content in the inoculated plants might be due to presence of large number of chloroplast bundle sheath in the leaves. Dual inoculation of AM fungi and CSBJ significantly increased chlorophyll contents in *Vigna unguiculata* (L.) (Rajasekaran & Nagarajan, 2005). These findings are supported by the findings of our present study.

3.6. Shoot Nutrient Parameters

Statistical analysis (P<0.05) revealed that soybean receiving single and dual treatments of AMF and CSBJ usually had significantly higher levels of shoot N compared to the control (Figure 4). The results clearly indicated that inoculation of the plants with both G. *fasciculatum* and CSBJ increased the nitrogen and phosphorus contents of soybean cv. NRC 37. There was overall increase in nitrogen and phosphorus contents in the treated plants as compared to control. However, maximum nitrogen and phosphorus contents were recorded in the plants dually inoculated with G. *fasciculatum* and CSBJ. Inoculation of legumes with CSBJ increase the nodulation of legumes causing more nitrogen fixation and making it available for the plants and therefore, it is used as an alternative for urea to minimize the cost of produce (Lynch et al., 1991). Moreover, it also increases rhizospheric microflora viz. acid producers and phosphate solubilizers causing more available phosphorus (Lipman and Conybeare, 1936). CSBJ inoculation has been shown to improve the nitrogen and phosphorus contents of legumes (Abd-Alla and Onar, 2000; (Meghvanshi et al., 2008; M. K. Meghvanshi, Prasad, & Mahna, 2010) which supported the results of our present findings (Figure 5). One of the most significant effects of mycorrhizal inoculation on the host plant is the increase in phosphorus uptake (Bai et al., 2008) due to enhanced capacity to absorb more phosphorus from the soil which is otherwise unavailable to the plants. AM fungi supported nitrogen fixation by providing legumes with phosphorus and other immobile nutrients which are essential for nitrogen fixation (Clark and Zeto, 2000). (Tavasolee et al.,2011) suggested that effective AM fungi can enhance the performance of rhizobial infection and vice versa which was also evident during our present investigation. The results of the present study indicated that combined inoculation of *G. fasciculatum* and CSBJ had a synergistic effect resulting in the improvement of nitrogen and phosphorus. Many reports are available on the increased nitrogen and phosphorus contents of legumes due to dual inoculation of AM fungi and Bradyrhizobium which are supported by the findings of our present study (K. Prasad et al., 2005).

AMF- Glomus *fasciculatum*; CSBJ- cultivar specific *Bradyrhizobium* japonicum; ±SE-Std error; Values in a column followed by the same letter are not significantly different at P< 0.05 according to DMRT

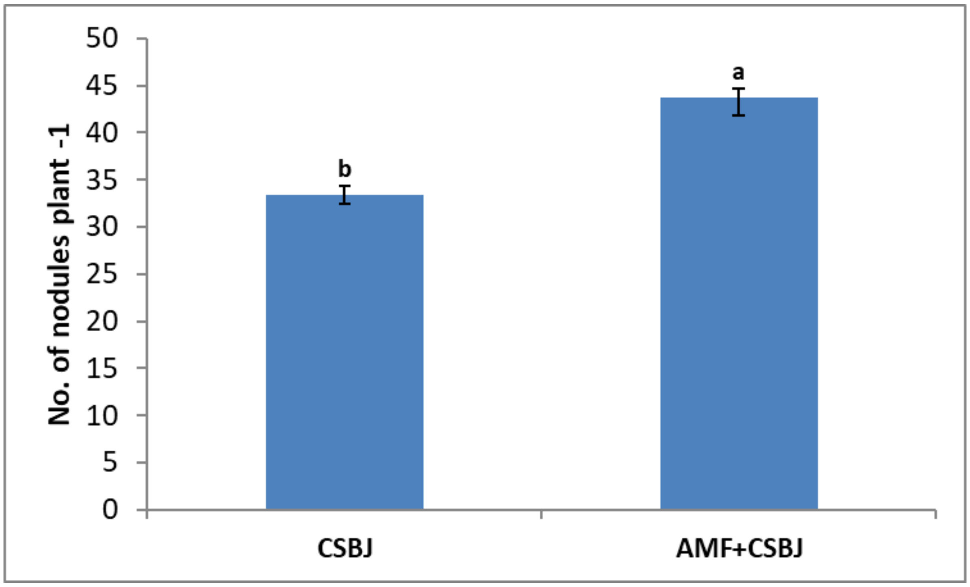

Figure 1. Nodular frequency of soybean inoculated with cultivar specific *Bradyrhizobium japonicum* (CSBJ) alone and combined with AMF; a, b ...values indicate significant different at p< 0.05 according to DMRT

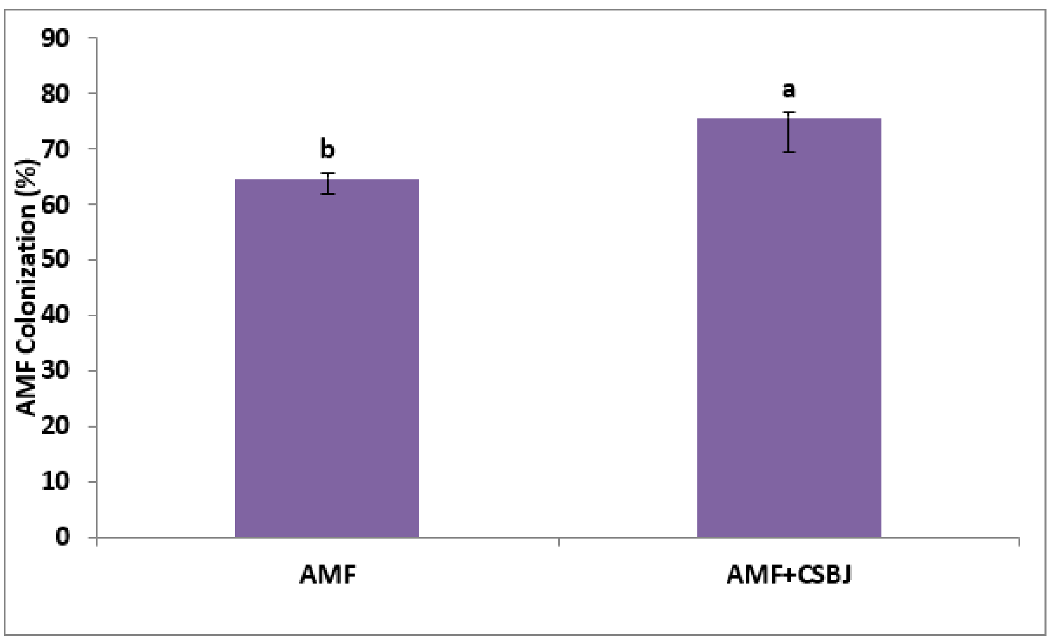

Figure 2. AMF colonization (%) of soybean inoculated with cultivar specific*Bradyrhizobium japonicum* (CSBJ) alone and combined with AMF; a, b ...values indicate significant different at P< 0.05 according to DMRT

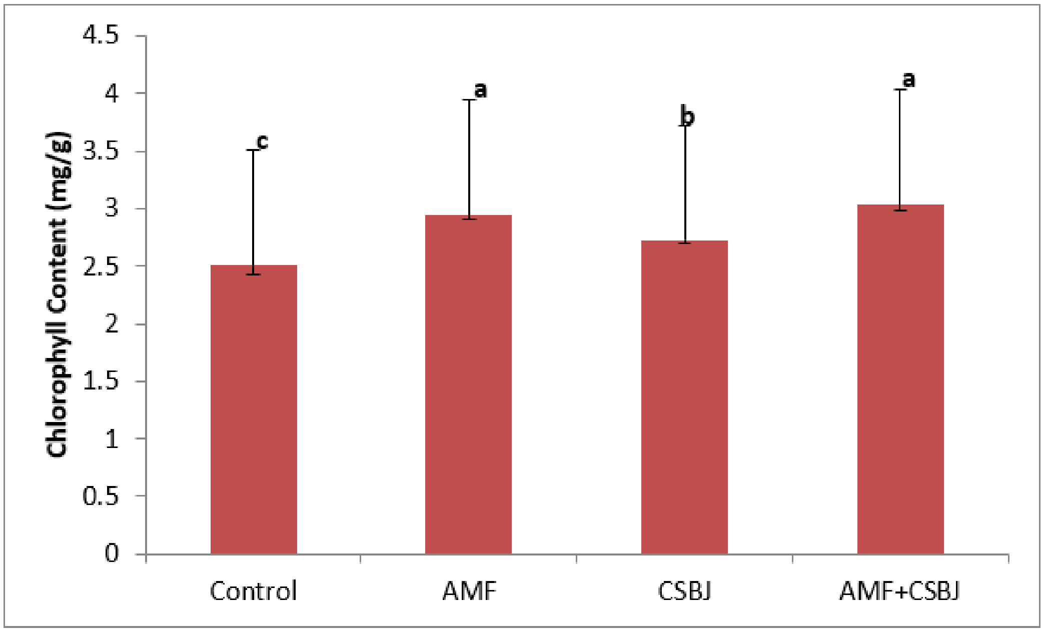

Figure 3. Chlorophyll Content (mg/g) of soybean inoculated with cultivar specific *Bradyrhizobium japonicum* (CSBJ) alone and combined with AMF; a, b …values indicate significant different at P< 0.05 according to DMRT

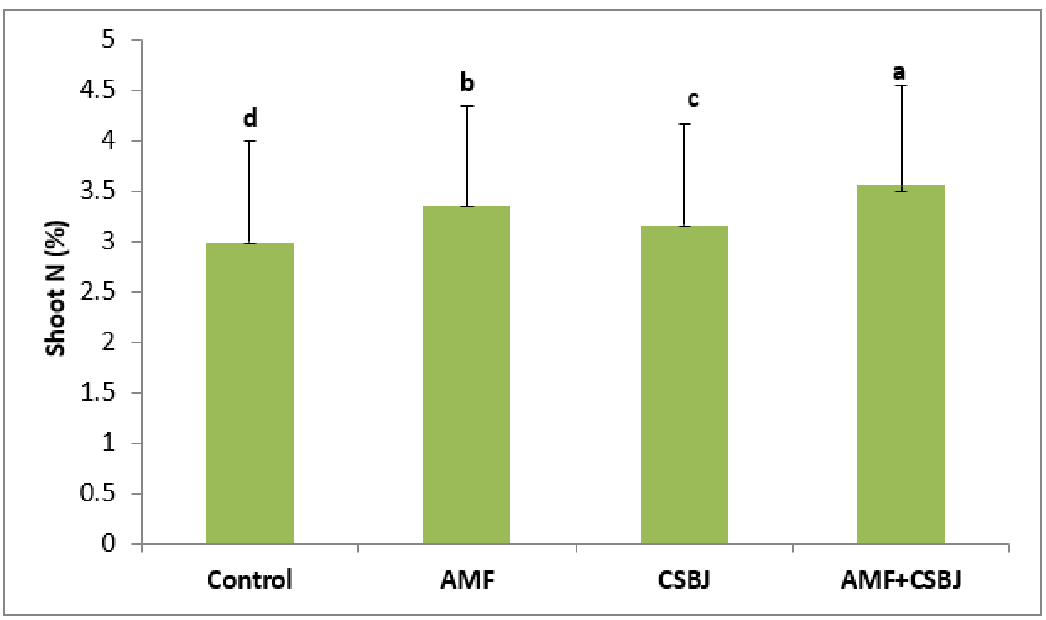

Figure 4. Shoot nitrogen (%) of soybean cultivar inoculated with cultivar specific *Bradyrhizobium japonicum* (CSBJ) alone and combined with AMF; a, b …values indicate significant different at P< 0.05 according to DMRT

Table 1. Effect of AM fungi and CSBJ on growth and yield of soybean cultivars (NRC 37) under greenhouse conditions.

Treat-ments	Shoot Height (cm plant^{-1})	Shoot Dry Matter (g plant^{-1})	Seed Weight (g plant^{-1})
Control	$38.33^{d}\pm1.57$	$2.58^{d}\pm0.03$	$1.56^{d}\pm0.06$
AMF	$62.86^{b}\pm1.69$	$4.85^{b}\pm0.36$	$2.58^{b}\pm0.04$
CSBJ	$54.08^{c}\pm0.95$	$3.93^{c}\pm0.20$	$2.40^{c}\pm0.04$
AMF+CSBJ	$69.49^{a}\pm0.64$	$5.69^{a}\pm0.09$	$3.21^{a}\pm0.05$

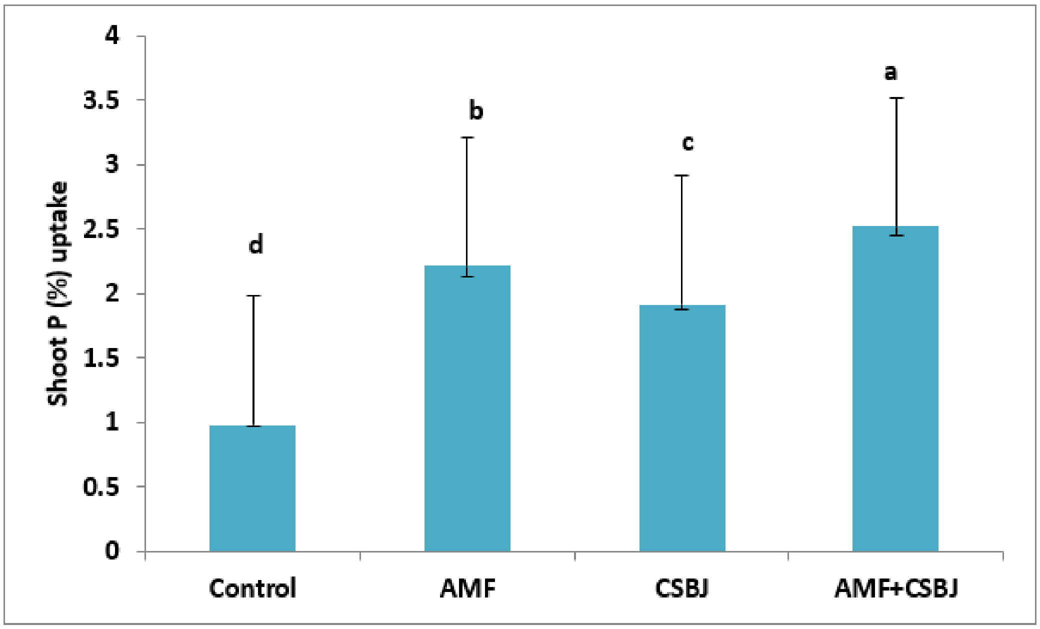

Figure 5. Shoot phosphorus (%) of soybean inoculated with cultivar specific *Bradyrhizobium japonicum* (CSBJ) alone and combined with AMF; a, b ...values indicate significant different at P< 0.05 according to DMRT

4. CONCLUSION

Dual inoculation of G. *fasciculatum* and CSBJ increased shoot growth, dry biomass, AMF root colonization, chlorophyll, nitrogen and phosphorus contents and quality seed yield of soybean variety NRC 37 indicating that their combination may have a potential role to enhance the productivity of the crop.

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6. AUTHOR CONTRIBUTIONS

Conceived the plan: KP; Performed the experiments: KP; Data analysis: KP; Wrote the paper: KP

7. CONFLICT OF INTEREST

The author declares no conflict of interest.

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References

(n.d.-a). *Indian Journal of Agricultural Sciences*, *69*, 73-83.
(n.d.-b).
Abd-Alla, M. H., & Omar, S. A. (2000). Survival of rhizobia/Bradyrhizobium and a rock phosphate solubilising fungus Aspergillus niger on various carriers from some agro industrial wastes and their effects on nodulation and growth of Faba Bean and Soybean. *Journal of Plant Nutrition*, *24*, 261-272.
Abdel, F. G., & Mohamedin, A. H. (2000). Interactions between arbuscular mycorrhizal fungi and Streptomyces and their effects on Sorghum plants. *Biology and Fertility of Soils*, *3*, 401-409.
Bagyaraj, D. J., & Menge, J. A. (1978). Interactions between a VA mycorrhiza and Azotobacter and their effects on rhizosphere microflora and plant growth. *New Phytology*, *80*, 567-573.
Barea, J. M., Azcon, A., Guilar, C., Azcon, R., Read, D. J., & Varma, A. K. (1992). Vesicular-Arbuscular mycorrhizal fungi in nitrogen fixing systems. *Methods in Microbiology; Norris* (p. 391-416). Academic Press.
Bhat, M. I., Rashid, Faisulur-Rasool, A., Ahdi, S. S., Haq, S. A., & Bhat, R. A. (2010). Effect of Rhizobium and vesicular arbuscular mycorrhizae fungi on green gram (Vigna radiata L. Wilczek) under temperate conditions. *Research Journal of Agricultural Sciences*, *1*(2), 113-118.
Chakrabarty, et al. (2007). Interactive effect of VAM and Rhizobium on nutrient uptake and growth of Vigna mungo. *Journal of Mycopathology Research*, *45*(2), 289-291.
Chen, et al. (2011). Effect of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on soybean growth related to root architecture and available of N and P. *Mycorrhiza*, *21*(3), 173-181.
Gavito, M. E., Schweiger, P., & Jakobsen, I. (2003). Phosphorus uptake by arbuscular mycorrhizal hyphae: effect of soil temperature and atmospheric CO2 enrichment. *Global Change Biology*, *9*, 106-116.
genotype, soil type and inoculant strain with regard to N2 fixation. (n.d.). *Journal of Agronomy& Crop Science*, *188*, 206-221.
Gerdemann, J. W., & Nicolson, T. H. (1963). Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society*, *46*, 235-244.
Jackson, M. L. (1973). *Soil Chemical Analysis*. New Delhi: Prentice Hall of India Ltd.
Meghvanshi, et al. (2008). Response of soybean cultivars towards inoculation with three arbuscular mycorrhizal fungi (AMF) and Bradyrhizobium japonicum in alluvial soil. *European Journal of Soil biology*, *44*, 316-323.
Meghvanshi, M. K., Prasad, K., & Mahna, S. K. (2010). Symbiotic potential, competitiveness and compatibility of indigenous Bradyrhizobium japanicum isolates to three soybean genotypes of two distinct agro-climatic regions of Rajasthan. *India. Saudi Journal of Biological Sciences*, *17*, 303-310.
Nicholas, O., Igichon, Olubukola, O., Babaloba, Xavier, C., & Baldwyn, T. (2021). Effect of rhizobia and arbuscular mycorrhizal fungi on yield, size distribution and fatty acid of soybean seed grown under drought stress. *Microbiological Research*, 126640-126640.
Phillips, J. M., & Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, *55*, 158-161.
Prasad, et al. (2005). (Vol. 1). Anusandhan.
Prasad, et al. (2019). Management of soilborne pathogens to improve productivity of pulses using organic technologies for sustainable agriculture. *International Journal of Research and Analytical Reviews*, *6*(2), 82-101.
Prasad, K., Meghavansi, M. K., Harwani, D., Mahna, S. K., & Werner, D. (2005). Synergistic effect of arbuscular mycorrhizal fungi and Bradyrhizobium japonicum on growth, yield and nutrient status of Soybean (Glycine max. *L.) Merrill). Anusandhan*, *1*, 23-29.
Production in sustainable manner. (n.d.). *Journal of Basic & Applied Mycology*(11), 5-13.
Rajasekaran, S., & Nagarajan, S. M. (2005). Effect of dual inoculation (AM Fungi and Rhizobium) on chlorophyll content of Vigna unguiculata (L), Walp. Var. Pusa 151. *Mycorrhiza News*, *17*(1), 10-11.
Sadasivan, S., & Manickam, A. (1996).
Schenck, N. C., & Perez, G. L. (1990). *A Manual for identification of vesicular arbuscular mycorrhizal fungi, INVAM, third edition*. Gainesville, Florida.
Shalaby, A. M., & Hanna, M. M. (2000). Interactions between VA mycorrhizal fungi Glomus mosseae, Bradyrhizobium japonicum and Pseudomonas syringae in soybean plants. *Egyptian Journal of Microbiology*, *35*, 199-209.
Singh, C. S., Kapoor, A., & Wange, S. S. (1991). The enhancement of root colonization of legumes by vesicular arbuscular mycorrhizal (VAM) fungi through the inoculation of the legume seed with commercial yeast (Saccharomyces cerevisiae). *Plant & Soil*, *131*, 129-133.
Subbarao. (1999). *Rhizobacterium and Legume root nodulation, Soil Microbiology* (and others, Ed.). New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd.
Subbiah, B. V., & Asija, G. L. (1956). A rapid procedure for determination of available nitrogen in soils. *Current Science*, *25*, 259-260.
Taiwo, L. B., & Adegbite, A. A. (2001). Effect of arbuscular mycorrhiza and Bradyrhizobium inoculation on growth, N2 fixation and yield of promiscuously nodulating soybean (Glycine max). *Journal of Agricultural Research*, *2*, 110-118.

Tilak, K. V. B. R., Saxena, A. K., Sadasivam, K. V., Adholeya, & Singh, S. (1995). Synergistic effects of phosphate solubilizing bacterium (Pseudomonas striata) and arbuscular mycorrhizae on soybean. *Mycorrhizae: Biofertilizers for the Future* (p. 224-226).
Topoliantz, S., Ponge, J. F., & Ballof, S. (2005). Manioc peel and Charcoal: a potential organic amendment for sustainable soil fertility in the tropics. *Biology & Fertility of Soil*, *41*, 15-21.
Vincent, J. M. (1970). *A manual for the practical study of the root nodule bacteria*. Oxford: Scientific Publications.
Werner, D., Wilcockson, J., & Zimmermann, E. (1975). Adsorption and selection of rhizobia with ion exchange papers. *Archives of Microbiology*, *105*, 27-32.
Xavier, L. J. C., & Germida, J. J. (2003). Selective interactions between arbuscular mycorrhizal fungi and Rhizobium leguminosarum cv. viceae enhance pea yield and nutrition. *Biology & Fertility of Soils*, *37*, 261-267.
Zuccarni, P. (2007). Mycorrhizal infection ameliorates chlorophyll content and nutrient uptake of Lettuce exposed to saline irrigation. *Plant Soil & Environment*, *53*(7), 283-289.