BIOASSOCIATIVE EFFECT OF RHIZOSPHERIC MICROORGANISMS ON GROWTH, NUTRIENT UPTAKE AND YIELD OF MUNG BEAN (*VIGNA RADIATA* L. WILCZEK)

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ABSTRACT

Nitrogen applications have generated great interests in agriculture, with much of its success associated with increasing the uptake of nitrogen by crops while reducing pollution by this chemical fertilizer. The aim of the present study was to evaluate the interactive effect of rhizospheric microorganisms on nutrient uptake, yield and growth of mung bean grown in pots under glasshouse conditions. The results revealed that the growth, in terms of morphology and physiology, of all the inoculated plants was better than that of the control plants. In terms of growth, plant height, fresh and dry weights and length of the roots plants inoculated with both *Funneliformis mosseae* + *T. viride* did best. Total chlorophyll content, alkaline and acidic phosphatase activities were greatest when inoculated with only *F. mosseae* and fresh and dry weights of shoots when inoculated only with *T. viride*. Significant increase in N and P uptake was recorded when inoculated with both *F. mosseae* + *T. viride*. Overall the significant increase in growth and development was due to positive interactions among rhizospheric microorganisms leading to healthy and vigorously growing plants. However, there is now a need for field trails of this technique.

Keywords: Arbuscular mycorrhizal fungi, nutrient uptake, phosphatase activity, nitrogen fixing bacteria, *Trichoderma viride*, mung bean

Introduction

Legumes are important land plants as they are able to grow in water-deficient and low-nutrient environments. Many legumes form symbiosis with both nitrogen fixing rhizobia and arbuscular mycorrhizal (AM) fungi. Dual inoculation with both microorganisms results in a tripartite mutualistic symbiosis and increases plant growth to a greater extent than inoculation with only one of them (Chalk et al. 2006). *Vigna radiata* (L.) Wilczek commonly known as mung bean is an important grain legume crop in South East Asia and Africa, and a source food that has a high nutritive value (Kumar et al. 2002; Salunke et al. 2005). It is not only a rich and economical source of protein, phosphorus, carbohydrate, minerals and provitamin A, but also commonly used as fodder and green manure. Mung bean contains bioactive components with antioxidant, antimicrobial and insecticidal properties (Bounce 2002; Kaprelynts et al. 2003; Madhujith et al. 2004; Ahmad et al. 2008).

The scarcity of food containing high levels of protein, micronutrients and various vitamins sources is an increasing problem affecting millions of people in developing countries (Burchi et al. 2011). The need for innovative technologies to meet the growing challenges of scarcity of food and malnutrition in the poor and hungry parts of the world must be addressed (Clugston 2002). To improve the quality and yield of economically important legumes, particularly mung bean, farmers apply large quantities of chemical fertilizer, which has detrimental effects on the soil, such as the accumulation of toxic salts. Application of environmentally friendly and potentially cost effective microbial bio-fertilizer could be a better solution (Mia and Shamsuddin 2010).

Structural and physiological studies have revealed that legumes form tripartite symbiotic associations with nodule forming rhizobia and AM fungi (Barea et al. 2005). The micro-symbionts in both associations are benefited by photo assimilates from the plant. The macro-symbionts fix nitrogen in the case of the bacterial symbiosis in root nodules (Brewin 1991; Crespi and Galvez 2000) and immobile nutrients, especially phosphate, in the case of AM symbiosis (Stract et al. 2003; Miransari et al. 2009). Nitrogen availability and P uptake from less soluble sources is an important issue, thus it is important to understand the tripartite association of legumes with *Rhizobium* and AM (Kwapata and Hall 1985; Satter et al. 2006). There is increasing evidence that diverse microbial populations in the rhizosphere play a significant role in agricultural sustainability (Barea et al. 2002). However, due to the obligatory biotrophy of AM fungi, the two symbioses are rarely studied together. Moreover, the initiation and functioning of the tripartite symbiosis is complex and despite efforts made over the last two decades or so, there are still many facts about this symbiosis that are unknown (Gianinazzi-Pearson et al. 1995). Considering the beneficial effect of AMF on other crops, more attention should be paid to combining appropriate mycorrhizal fungi with other soil microbes in order to increase yields of mung bean. With this in mind, a pot experiment was carried out under glass house conditions to investigate the effect of AM fungi alone or in combination with other bio-inoculants on mung bean.

Material and methods

Mass multiplication of bio-inoculants

The two dominant AM species used in this study were *Funneliformis mosseae* (T.H. Nicolson and Gerd.) Walker and Schüßler and *Acaulospora laevis* Gerdemann and Trappe. These species were isolated from the rhizosphere of mung beans collected from the Botanical Garden, Kurukshetra University, Kurukshetra and kept in sterilized polythene bags at 10 °C for further processing. The AM species were propagated in association with barley under standard pot culture conditions. The culture of *Rhizobium* sp. (procured from Department of Microbiology, CCS Haryana Agricultural University, India) was mass multiplied using nutrient broth medium and that of *T. viride* on a modified wheat bran-saw dust medium (Mukhopadhyay et al. 1986).

Preparation of pot mixture

The experiment was laid out in a randomized complete block design, with five replicates of each treatment. Top soil (0–30 cm) was collected from the Botanical garden of Botany Department, Kurukshetra University, Kurukshetra, which consisted of 20.8% silt, 3.78% clay, and had a pH of 8.05, 0.0485 total N and 0.015% available P. This soil was air dried and passed through a 2 mm sieve. The soil to be used in the experiment consisted of a soil: sand mixture (3:1, v/v), which had been autoclaved for 20 minutes at 121 °C and 15psi. Each pot was filled with the sterilized soil:sand mixture to which 10% (w/w of soil) of the selected AM inoculum was added. *T. viride* inoculum containing 3.4×10^8 cfu gm^{-1} was added as per the treatment. The seeds were surface sterilized with 10% solution of sodium hypochlorite for 1–2 minutes and then washed thoroughly with distilled water. Before sowing seeds 10 ml of a liquid suspension of bacteria, with a density 10^8 cells/ml, was applied to each pot. Pots were watered once every two days, and fertilized with a nutrient solution after 15 days (Weaver and Fredrick 1982), which contained half the recommended level of phosphorus and no nitrogen. Pots were either treated with a single inoculum, a combined inoculum or no inoculum as outlined below:

1. Uninoculated i.e. autoclaved sterile sand: soil with no inoculum (control 1)
2. Uninoculated i.e. unsterilized sand: soil with no inoculum (control 2)
3. *Funneliformis mosseae* (F)
4. *Acaulospora laevis* (A)
5. *Trichoderma viride* (T)
6. F + T
7. A + T
8. F + A
9. F + A + T

Table 1 Combined effect of AM fungi, *Rhizobium* and *T. viride* on the growth of mung bean 120 days after inoculation.

Treatments	Plant height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	AM spore number / 10 g of soil	Root colonization (%)	Number of nodules / plant
C1	14.5 ± 1.25[f]	1.18 ± 0.05[g]	0.43 ± 0.003[g]	7.1 ± 0.55[g]	0.60 ± 0.004[f]	0.16 ± 0.006[f]	19.8 ± 2.86[g]	20.6 ± 2.40[h]	1.4 ± 0.54[f]
C2	19.3 ± 1.78[e]	3.01 ± 0.05[f]	0.59 ± 0.004[f]	8.3 ± 0.40[f]	0.62 ± 0.004[f]	0.20 ± 0.003[ef]	49.0 ± 2.64[f]	32.8 ± 1.92[g]	4.2 ± 0.83[e]
F	30.2 ± 2.38[b]	5.81 ± 0.12[c]	1.97 ± 0.008[c]	18.8 ± 1.92[b]	0.97 ± 0.041[b]	0.41 ± 0.014[b]	109 ± 2.54[b]	79.2 ± 1.92[b]	8.6 ± 0.89[bc]
A	20.6 ± 2.70[de]	3.13 ± 0.01[f]	0.62 ± 0.003[f]	9.8 ± 2.58[e]	0.72 ± 0.006[e]	0.29 ± 0.016[d]	81.2 ± 3.11[d]	53.8 ± 2.70[d]	6.0 ± 1.22[d]
T	25.8 ± 2.38[bc]	8.54 ± 0.05[a]	3.47 ± 0.014[a]	14.6 ± 2.40[c]	0.92 ± 0.005[c]	0.40 ± 0.008[b]	52.0 ± 1.58[ef]	37.4 ± 2.07[ef]	6.4 ± 1.51[d]
F + T	40.6 ± 2.40[a]	7.02 ± 0.01[b]	2.97 ± 0.009[b]	22.6 ± 2.40[a]	1.55 ± 0.060[a]	1.05 ± 0.090[a]	80.2 ± 2.38[d]	55.6 ± 2.70[d]	9.8 ± 1.48[a]
A + T	24.0 ± 1.58[c]	4.74 ± 0.01[d]	0.89 ± 0.003[d]	15.0 ± 1.58[c]	0.85 ± 0.005[d]	0.36 ± 0.020[c]	56.4 ± 1.94[e]	42.4 ± 1.81[e]	7.2 ± 1.78[c]
F + A	22.8 ± 1.92[cd]	4.12 ± 0.01[e]	0.80 ± 0.008[e]	10.6 ± 0.44[d]	0.69 ± 0.003[e]	0.23 ± 0.021[e]	119.2 ± 2.16[a]	89.8 ± 1.64[a]	7.6 ± 0.54[c]
F + A + T	28.2 ± 2.38[b]	6.98 ± 0.01[b]	2.14 ± 0.044[bc]	17.4 ± 2.70[b]	0.87 ± 0.002[c]	0.32 ± 0.023[cd]	94.8 ± 2.38[c]	62.2 ± 2.38[c]	9.4 ± 1.34[ab]
L.S.D (P ≤ 0.05)	3.0241***	0.5075***	0.57595***	3.1551***	0.0506***	0.0505***	6.4936***	5.3536***	1.6826***
ANOVA (F7,16)	45.098	156.879	26.920	21.802	262.79	228.58	228.588	138.32	19.820

Control1 (C1) – Un-inoculated sterile soil; Control 2 (C2) – Un-inoculated soil; F – *Funneliformis mosseae*; A – *Acaulospora laevis*; T – *Trichoderma viride*; F + T – *Funneliformis mosseae* + *Trichoderma viride*; A + T – *Acaulospora laevis* + *Trichoderma viride*; F + A – *Funneliformis mosseae* + *Acaulospora laevis*; F + A + T – *Funneliformis mosseae* + *Acaulospora laevis* + *Trichoderma viride*.
Values are means ± standard error, *** significant values.
Means were compared using the least significant difference (LSD) test (*P* ≤ 0.05). Data in each column followed by dissimilar letters differ significantly at *P* ≤ 0.05.

Table 2 Combined effect of AM fungi, *Rhizobium* and *T. viride* on physiological features of mung bean 120 days after inoculation.

Treatments	Chl. content (mg/100mg FW) Chl a	Chl b	Total Chl.	Phosphatase activity (IUg⁻¹FW) Acidic	alkaline	Stomatal conductance (mmol⁻² s⁻²) Morning	evening	No. of pods	Wt. of pods (gm)
C1	0.29 ± 0.01^g	0.23 ±0.02^f	0.53 ± 0.04^g	0.078 ±0.019^f	0.004 ± 0.002^d	L 115.70±1.63^h U 19.47 ±1.40^f	L 91.46 ±1.41^h U 12.50 ±1.52^e	4.6 ± 1.14^g	0.92±0.01^h
C2	0.47 ±0.03^f	0.33 ±0.02^e	0.81 ± 0.02^f	0.098± 0.03^f	0.006 ± 0.003^d	L 234.81±3.61^h U 23.04 ±2.06^e	L 95.48 ± 2.59^g U 13.34 ±0.55^e	7.8 ± 0.83^f	1.06 ± 0.006^g
F	1.04 ± 0.03^b	0.53 ±0.03^{bc}	1.59 ± 0.03^b	0.270 ±0.025^a	0.202 ± 0.028^a	L 453.54±2.19^a U 54.78 ± 1.95^b	L 286.05 ±3.13^c U 20.43 ±1.54^b	14.0 ±2.23^c	4.95±0.03^d
A	0.64 ± 0.02^e	0.48 ±0.02^c	1.12 ± 0.04^e	0.148 ±0.025^e	0.008 ± 0.001^d	L 310.96±3.11^d U 21.21 ±1.70^{ef}	L 256.37±2.56^d U 16.41 ±1.39^{cd}	10.4±1.14^d	3.94±0.02^e
T	0.75 ± 0.03^d	0.42 ±0.07^d	1.18 ±0.07^{de}	0.216±0.024^{cd}	0.146 ± 0.020^c	L 258.49 ±2.81^f U 64.26 ± 1.59 ^{a}	L 107.74 ±2.09^f U 15.52± 1.30^d	22.0±2.54^{ab}	6.28±0.04^b
F+T	0.83 ± 0.06^c	0.56 ±0.07^b	1.40 ± 0.09^c	0.250±0.022^{ab}	0.180 ±0.015^b	L 340.84 ±1.92^c U31.54 ± 1.54^c	L 317.98±2.05^a U 24.53 ± 1.64^a	24.2 ±1.92^a	7.80±0.01^a
A+T	0.74 ±0.03^{de}	0.37 ±0.02^e	1.17 ± 0.12^{de}	0.154 ± 0.020^e	0.009 ±0.001^d	L 303.36 ±2.82^e U 32.71 ± 1.95^c	L 245.51±2.49^e U18.09 ±1.26^c	9.4 ±1.14^{ef}	2.97±0.02^g
F+A	0.63 ± 0.02^e	0.58±0.02^b	1.21 ±0.03^d	0.230±0.015^{bc}	0.164 ± 0.020^{bc}	L 238.54±2.89^d U 26.31 ± 1.48^d	L105.36±2.92^f U17.02±2.02^{cd}	10.0±1.58^{de}	3.70±0.01^f
F+A+T	1.16±0.02^a	0.70 ±0.01^a	1.86 ±0.02^a	0.194±0.024^d	0.154 ± 0.020^c	L 371.68±2.86^b U32.03±1.89^c	L303.60±2.24^b U 17.28 ±1.45^{cd}	21.4±2.40^b	5.83±0.03^c
L.S.D(P<0.05)	0.0359***	0.0523***	0.0808***	0.0301***	0.0209***	L 3.4914*** U 2 .2438***	L 3.1317*** U 1.8747***	2.2665***	0.0356***
ANOVA (F 7,16)	356.04	61.169	194.441	40.664	142.886	L6211.873 U 390.647	L6211.873 U 30.842	79.907	34809.964

Control1 (C1) - Un-inoculated sterile soil; Control 2 (C2) - Un-inoculated soil; F- *Funniliformis mosseae*; A - *Acaulospora laevis*; T - *Trichoderma viride*; F+T - *Funniliformis mosseae* + *Trichoderma viride*; A+T - *Acaulospora laevis* +*Trichoderma viride*; F+A - *Funniliformis mosseae* + *Acaulospora laevis*; F+A+T – *Funniliformis mosseae* + *Acaulospora laevis* + *Trichoderma viride*.
Values are means ± standard values.
Values are means ± standard error, *** significant values.
Means were compared using the least significant difference (LSD) test ($P ≤ 0.05$). Data in each column followed by dissimilar letters differ significantly at $P ≤ 0.05$.

Plant harvest, growth and nutrient analysis

Plants were harvested after 120 days by uprooting them from the soil and various morphological and physiological features measured. For determining root and shoot fresh and dry weight, roots and shoots were harvested after 120 days, weighed and then, oven dried at 70 °C and weighed again. Amount of chlorophyll a, chlorophyll b and total chlorophyll was estimated using the method of Arnon (1949). Stomatal conductance (mmol⁻² sec⁻²) was measured using a Porometer (AP$_4$-DELTA-T DEVICES-Cambridge-U.K.). Phosphorus concentration were determined using the 'Vanado-molybdo-phosphoric yellow colour method' (Jackson 1973) and Nitrogen using the Kjeldahl method. Phosphatase activity was assayed using p-nitrophenyl phosphate (PNPP) as a substrate, which is hydrolyzed by the enzyme to p-nitrophenol (Tabatabi and Bremner 1969).

Identification and quantification of the number and colonization by AM spores

AM spores (*F. mosseae* and *A. laevis*) were identified by using the identification manual used by Walker (1983), Schenck and Perez (1990), Morton and Benny (1990) and Mukerji (1996). Quantification of the number of AM spores was done using the Adholeya and Gaur 'Grid Line Intersect Method' (1994). Mycorrhizal colonization of roots was determined using the 'Rapid Clearing and Staining Method' of Phillips and Hayman (1970). Percentage AM colonization of roots was: (Number of root segments colonized / number of root segments studied) × 100.

Statistical analysis

Data were subjected to analysis of variance and means separated using the least significant difference test in the Statistical Package for Social Sciences (ver.11.5, Chicago, IL, USA).

Results

It is evident from (Table 1) that inoculated plants were taller than the un-inoculated control plants. The increase in plant height was significantly greatest for those inoculated with both *F. mosseae* + *T. viride* followed by those inoculated only with *F. mosseae*. This indicates that mung bean benefited from the particular mycorrhizal symbioses used in this study.

Maximum increment in shoot biomass was recorded in single inoculation treatment with *T. viride* followed by dual inoculation with *F. mosseae* and *T. viride*. According to the results in Table 1 root biomass (fresh and dry) was also significantly greater in all the treatments than the control. After 120 days, the increase in root biomass was greatest in the dual treatment with *F. mosseae* and *T. viride* followed by that in the single treatment with *F. mosseae*.

The greatest root length was recorded for plants inoculated with both *F. mosseae* and *T. viride* followed by those inoculated only with *F. mosseae*.

The result indicates that the mycorrhizal colonization of roots and number of spores of AM recorded for the treated plants varied (Table 1). The maximum number of spores and percentage colonization of roots was recorded for plants treated with *F. mosseae* + *A. laevis* and *T. viride* and then those treated with a single inoculation of *F. mosseae*.

Chlorophyll content of all the treated plants was greater than that of the control. According to Table 2 the highest increment in total chlorophyll content was recorded for plants treated with *F. mosseae* + *A. laevis* + *T. viride* and then those treated with a single inoculation of *G. mosseae*.

According to Table 2 the phosphatase activity recorded for inoculated plants was considerably greater than that recorded for the control. This is due to the mycorrhizal fungal hyphae that secrete acid and alkaline phosphatases (APA and ALA) into the rhizosphere. The acidic phosphatase activity was greatest for the *F. mosseae* treated plants followed by those treated with *F. mosseae* + *T. viride*. In addition, the greatest alkaline activity was recorded for the treatment with *F. mosseae*. Acidic phosphatase activity was greater than that of alkaline phosphatase.

Mycorrhizal colonization of roots also influences the behaviour of the stomata of the leaves. Stomatal conductance (morning and evening) of plants treated with different combinations of AM fungi and *T. viride* increased (Table 2). Stomatal conductance was greatest in the morning in plants treated with *F. mosseae* and in the evening in those treated with *F. mosseae* + *T. viride*. The conductance recorded for the stomata on the lower surface of leaves was greater in the morning than the evening.

The maximum number of nodules was recorded for the dual inoculation treatment, i.e. with *F. mosseae* and *T. viride,* followed by the triple inoculation treatment with *F. mosseae*, *A. laevis* and *T. viride*. The inoculation of several different microbes appears to result in a synergistic interaction among them that substantially improves the nutrition available to the mung bean plants.

AM fungi improved the mineral nutrition of associated plants (Table 3). Mung bean plants inoculated with AM fungi and *T. viride* had increased N and P contents in roots and shoots compared with the control. Among all the treated plants those inoculated with *F. mosseae* showed the greatest percentage increase in shoot and root P content and those treated with both *F. mosseae* + *A. laevis* the greatest percentage in shoot and root N. This might be due to an increase in carbon supplied by the plant to the AM fungi, which increases the uptake and transfer of nutrients from fungi to plants. In the present study, more P than N was absorbed by all the AM inoculated mung bean plants. The increase in P content was

due to the AM fungal network that can take up P and convert it into polyphosphate granules in the extra radical hyphae and pass it to the arbuscules, which then make it available to plants. The increase in total N is attributed to the increased N_2 fixation by the AM hyphae.

Data on yield (Table 2) indicate that bioinoculation of mung bean plants with either AM or *T. viride* resulted in a significant increase in the weight of the pods and consequently higher yields compared to the control. Treatment with a mixture of *F. mosseae* and *T. viride* resulted in the greatest increase in yield in terms of the number of pods followed by those plants treated with *T. viride*. That is, the greatest yield was of plants inoculated with both AM fungi and *T. viride*, which operated together more effectively in supplying their host plants with their nutrient requirements than when either operated on its own.

Discussion

In the present investigation, dual inoculation or a mixed inoculation gave better results than either of the two inoculates on their own. The F + T treatment gave better results than the other treatments because *Trichoderma* might produce extra cellular metabolites that enhance the AM population in the soil. The increased height of mycorrhizal plants is attributed to a higher AM colonization, which is known to enhance plant growth by increasing nutrient uptake and plant growth promoting substances in the rhizosphere of mung bean. The beneficial effects of AM symbiosis are also known to be mediated by phytohormones,, which could be suitable candidates for signaling between plants and AM fungi

and important in the auto regulation of mycorrhization (Meixner et al. 2005). These observations accord with earlier reports of Nzanza et al. (2011) that *Solanum lycopersicum* inoculated with *Trichoderma harzianum* and AM fungi are taller than control plants. Vázquez et al. (2000) also found that inoculation with AM and other microbes resulted in taller maize plants. The better results obtained when unsterilized rather than sterilized soil is used may be due to the presence of natural micro-flora in the soil. This may be due to competition for space and nutrition between the diverse microflora in unsterilized soil.

Khan et al. (2008) also report a significant increase in shoot and root dry weight of *Medicago sativa* following inoculation with AM fungi. Similar results are also reported by Arumugam et al. (2010) for *Vigna unguiculata* inoculated with AM and *Rhizobium*. This may be due to the fact that there were no indigenous microbes to compete with the inoculated strain. Secondly, the greater reproduction of AM fungi in soil based inoculums, which sprouted rapidly from extracellular and intracellular hyphae, absorbsed more nutrients, even far from the surface of the roots, which enhanced the growth and biomass of the plants.

The better results obtained when only *F. mosseae* was inoculated may be due to better space and nutrition for its multiplication and survival in sterilized soil, and therefore it was able to absorb more nutrients from the soil resulting a better growth of mung bean. In addition, the mycorrhizal colonization of the soil in effect enabled the plants' root systems to extend deeper into the soil and invade nutrient depleted zones and enhance soil aggregation, which ultimately improves the access of the root system to soil water and nutrients. Galleguillos et

Table 3 Combined effect of AM fungi, *Rhizobium* and T. *viride* on the nitrogen and phosphorus content of mung bean 120 days after inoculation.

Treatments	Phosphorus content (%)		Nitrogen content (%)	
	Shoot	Root	Shoot	Root
C1	0.05 ± 0.024[f]	0.21 ± 0.022[e]	1.03 ± 0.022[g]	0.40 ± 0.024[f]
C2	0.16 ± 0.01[e]	0.22 ± 0.01[de]	1.34 ± 0.025[f]	0.51 ± 0.016[e]
F	0.30 ± 0.028[a]	0.36 ± 0.024[a]	1.62 ± 0.022[a]	0.67 ± 0.020[b]
A	0.22 ± 0.023[c]	0.27 ± 0.017[c]	1.41 ± 0.028[de]	0.54 ± 0.023[d]
T	0.21 ± 0.016[c]	0.25 ± 0.025[cd]	1.40 ± 0.015[e]	0.53 ± 0.028[de]
F + T	0.29 ± 0.019[ab]	0.35 ± 0.028[ab]	1.55 ± 0.019[b]	0.64 ± 0.020[b]
A + T	0.19 ± 0.019[d]	0.23 ± 0.020[de]	1.43 ± 0.023[d]	0.56 ± 0.020[cd]
F + A	0.26 ± 0.031[b]	0.33 ± 0.022[b]	1.64 ± 0.027[a]	0.74 ± 0.019[a]
F + A + T	0.20 ±0.024[d]	0.32 ± 0.020[b]	1.52 ± 0.031[c]	0.58 ± 0.020[c]
L.S.D (P < 0.05)	0.0317***	0.0295***	0.0314***	0.0282***
ANOVA (F7,16)	41.182	32.305	282.229	102.191

Control1 (C1) – Un-inoculated sterile soil; Control 2 (C2) – Un-inoculated soil; F – *Funniliformis mosseae*; A – *Acaulospora laevis*; T – *Trichoderma viride*; F + T – *Funniliformis mosseae + Trichoderma viride*; A + T – *Acaulospora laevis + Trichoderma viride*; F + A – *Funniliformis mosseae + Acaulospora laevis*; F + A + T – *Funniliformis mosseae + Acaulospora laevis + Trichoderma viride*.
Values are means ± standard error, *** significant values.
Means were compared using the least significant difference (LSD) test (*P* ≤ 0.05). Data in each column followed by dissimilar letters differ significantly at *P* ≤ 0.05.

al. (2000) record a significant increase in root length of plants inoculated with *G. mosseae* and Chen et al. (2006) that of *Trifolium repens* and *Coreopsis drummondii* growing in soil inoculated with an isolate of *G. mosseae*.

The mixed inoculum of F + A + T gave better results than the control, which may be due to their synergistic effect. Since the spatial distribution of soil hyphae can vary among fungal species, an increase in the overall hyphal density of AM mycelium around the roots resulting from colonization by several AM fungi may be greater than colonizations by a single species. Alternately, different AM fungi may produce different amounts of soil binding agents and the interactions of these agents within the aggregates may result in a synergistic effect on soil stability. *Rhizobium* species are also effective root colonizers as they produce secondary metabolites that enhance AMF growth and thus increase mycorrhizal spore number and colonization of mung bean. In addition, *T. viride* may behave as a mycorrhizal helper by promoting higher percentage colonization and AM spore number. The results of the present investigation are in agreement with the findings of Hemashenpagam and Selvaraj (2011) as *Solanum viarum* was also inoculated with AM fungus and plant growth promoting rhizobacteria (PGPR's).

The increase in chlorophyll content recorded in the present investigation may be due to an increase in stomatal conductance, photosynthesis, transpiration and enhanced plant growth. Increase in chlorophyll content in mycorrhizal treated plants indicates an increase in the rate of photosynthesis, which can be ascribed to an increase in the absorption of nutrients. This supports the hypothesis that soil micro-organisms produce phytohormones and a number of extra-cellular enzymes, which decompose the complex organic matter before it is absorbed as a source of energy, which stimulate plant growth (Richard et al. 2007). Several workers report more chlorophyll in the leaves of mycorrhizal plants than those of non-mycorrhizal plants (Karthikeyan et al. 2009; Arumugam et al. 2010).

The activity of acid phosphatase may be associated with the growth and development of the fungus within the tissue of the host and with phosphorus acquisition by the rhizosphere. In the present investigation, it was found that phosphatase activity (alkaline and acidic) in plants was greatest in those with the greatest mycorrhizal root colonization. Alkaline phosphatase activity is also closely linked with both the stimulation of mycorrhizal growth and arbuscular phase of the colonization. Studies have revealed that alkaline phosphatase activity is localized in the vacuoles of mature arbuscules (Gianiniazzi et al. 1979). These enzymes (APA and ALA) help in mineralization of bound phosphorus into a soluble form, which makes it more easily available to plants. The present findings are in agreement with numerous other reports (Dodd et al. 1987; Rubio et al. 1990; Abdel-Fattah 2001). Thus, phosphatase activity may be helpful in improving soil health and also act as an indicator of soil quality.

The progressive increase in stomatal conductance resulting from AM inoculation might be due to higher rates of photosynthesis and increase in the number of stomata on the lower surfaces of the leaves. Higher rates of stomatal conductance in mycorrhizal plants are also reported by Auge (2001). Fidelbus et al. (2001) also report that AM fungi have positive role in lowering the rate of leaf conductivity of citrus seedlings growing in very dry soils.

The greater number of nodules number of treated plants may be due to the mutual positive action of *Rhizobium* species and AM fungi strains in increasing the uptake of phosphorus and nitrogen from soil. The role of arbuscular mycorrhizae in improving nodulation and N_2 fixation is universally recognized. AM fungi are known to release an unidentified diffusional factor (myc factor) (Parniske 2008; Maillet et al. 2011), which activates the nodulation factor's inducible gene, involved in establishing the symbiosis with the nitrogen fixing rhizobial bacteria (Kosuta et al. 2003). It is also reported that effective mycorrhizal colonization can also increase nodulation and symbiotic nitrogen fixation in legumes (Hamel 2004). Moreover, when *Rhizobium* bacteria are present in the soil, mycorrhizal colonization is increased due to an increase in the concentration of chemical signals involved in the establishment of symbiosis (Hirsch and Kapulnik 1998).

The AM prompted nutrient uptake may be due to an increase in surface area of soil contact, increased movement of nutrients into mycorrhizae, a modification of the root environment and increased storage. The effect of the mycorrhizal infection of the host plant is an increase in the uptake of P, which is mainly due to the capacity of the mycorrhizal fungi to absorb phosphate from soil and transfer it to the host roots. Khan et al. (2008) also found an increase in both N and P followed by higher yield in *Medicago sativa* inoculated with *Gigaspora rosea* and *Glomus intraradices*. In addition, Safapour et al. (2011), report similar increases in yield of three varieties of red bean when inoculated with AM fungi and *Rhizobium* sp.

Conclusions

In conclusion, dominant arbuscular mycorrhizal fungi, either alone or in combination, can establish a symbiosis with mung bean, which results in this plant growing better and producing more beans under laboratory conditions. There is now a need to determine whether this technique can be used for increasing the growth and yield of mung bean under field conditions.

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