Acta Physiol Plant
DOI 10.1007/s11738-012-0950-x

ORIGINAL PAPER

Significance of arbuscular mycorrhizal and bacterial symbionts in a tripartite association with *Vigna radiata*

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Received: 18 August 2011 / Revised: 19 January 2012 / Accepted: 23 January 2012

Abstract Legumes as an important functional group of land plants are recognized to grow in water-deficient and low-nutrient environment because of their ability to form symbiosis with nitrogen fixing rhizobia and arbuscular mycorrhizal (AM) fungi, which improve nutrient acquisition from the soil and help plants to be well established. Aim of the present study was to evaluate the symbiotic potential of AM fungi, *Glomus intraradices* alone and/or in combination with two *Bradyrhizobium japonicum* strains MN-S and TAL-102 in *Vigna radiata.* Field experiment was conducted to investigate the influence of different microbial symbiotic associations on growth and yield of *V. radiata*. Dual inoculation of *G. intraradices* and both bacterial inoculants showed better potential of plant growth promotion over single inoculation of *G. intraradices* or bacterial inoculants. Both bacterial inoculants in combination with AM proved best with 3.78, 30.17 and 46.80 g plant^{-1} dry weight at 25, 45 and 90 days after sowing (DAS), respectively. Maximum grain yield of 1,506.87 kg ha^{-1} as well as phosphorus contents of 1.981 mg g^{-1} root, 3.830 mg g^{-1} shoot and 4.935 mg g^{-1} grain were observed with mix bacterial inoculants and AM at 90 DAS. The interactive effect of bacterial inoculants and AM was synergistically significant which improved the nitrogen contents by 68, 20 and 17% in root, shoot and grain, respectively, compared to uninoculated control at 90 DAS. The present study suggests the suitability of *G. intraradices* and *B. japonicum* having synergistic or additive interaction to be used as composite inoculum for enhancing crop production of *V. radiata*.

Keywords *Bradyrhizobium* · *Vigna radiata* · Arbuscular mycorrhizal fungi · Nitrogen · Phosphorus · Grain yield

Communicated by M. J. Reigosa.

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Introduction

Vigna radiata (mungbean) as a grain legume is considerably important in Asia and Africa for its nutritive values in vegetarian diets (Kumar et al. 2002; Rege 1981; Salunke et al. 2005) and good digestibility. It is not only a rich and economical source of protein, phosphorus, carbohydrate, minerals and provitamin A, but also is commonly used as fodder and green manure. *V. radiata* contains bioactive components (Ahmad et al. 2008; Madhujith et al. 2004) having, antioxidant, antimicrobial and insecticidal activities (Brounce 2002; Dubois et al. 1956; Kaprelynts et al. 2003). As availability of food as well as sufficient protein, micronutrient and vitamins is a rising problem affecting millions of people, particularly in the developing countries (Burchi et al. 2011) there is a need of innovative technologies to meet the growing challenges of scarcity of food and malnutrition in the poor and hungry world (Clugston 2002). To improve the quality and yield of economically important legume particularly *V. radiata* as well as fertility of rather poor fertile soils, application of environmentally friendly and potentially cost effective microbial biofertilizer could be a better solution (Mia and Shamsuddin 2010). Moreover, it would also help the low-income farmers by enhancing the crop productivity in their low-income land holdings.

Published online: 07 February 2012

Structural and physiological studies state that legumes form tripartite symbiotic associations with nodule forming rhizobia and arbuscular mycorrhizal (AM) fungi (Barea et al. 2005). The micro-symbionts in both associations are benefited by photoassimilates from the plant. The macro-symbiont obtains fixed nitrogen in case of bacterial symbiosis of root nodules (Brewin 1991; Crespi and Galvez 2000) and immobile nutrients especially phosphate in case of AM symbiosis (Miransari et al. 2009; Stract et al. 2003). As nitrogen availability as well as phosphorus (P) uptake from P fixing soils or from less soluble sources (Kwapata and Hall 1985; Satter et al. 2006) is an important issue, so the tripartite association of legumes with *Rhizobium* and AM in the broad sense have always been agronomically important.

There are some growing evidences that diverse microbial populations as well as the manipulation of these microbes in the rhizosphere play a significant role in agricultural sustainability (Barea et al. 2002). However, very little is known about the enormous diversity of soil microbes, their properties and behavior in the soil environment. The efforts have been taken under green house conditions to investigate the potential impact of these symbiotic associations, which might be of agricultural importance. However, the results obtained in green house experiment do not necessarily reflect the potential of micro-symbionts for plant growth in the field as the conditions in field environment are generally much stressful and complex than controlled environmental conditions. Moreover, the study of both fungal and bacterial symbionts together can be complex due to obligatory biotrophic nature of AM fungi (Gianinazzi-Pearson et al. 1995). Thus, it is essential to verify the symbiotic effectiveness and competitive ability of selected symbionts by considering the complexity of genetic variability of micro and macro-symbionts under field conditions. In view of these facts, different suitable combination of microbial symbionts were tested to evaluate the hypothesis that tripartite symbiotic association improves legume's productivity through increase in nodulation, biomass, nitrogen and phosphorus contents.

Materials and methods

Host plant and microsymbionts

V. radiata (Mungbean) var. NM-92 requiring 90–100 days of crop development (seed sowing to maturity/harvest) was selected for field trials in Faisalabad region of Punjab, Pakistan, with mean daily maximum day length of 14 h, mean daily maximum temperature of 40°C and mean daily minimum temperature of 29°C. *V. radiata* seeds obtained from mutation breeding division NIAB, Faisalabad, were inoculated by seed dressing with nodule forming and nitrogen fixing *B. japonicum* strains MN-S & TAL-102. The *B. japonicum* strain MN-S was obtained from BIRCEN culture collection of Plant Microbiology Division at NIBGE and *B. japonicum* Nif strain TAL-102 was obtained from TAL, Hawaii, USA. The bacterial inoculum of both strains was maintained on Yeast Extract Mannitol (YEM) agar plates (Somasegaran 1985) containing Congo Red at incubation temperature of 28 ± 2°C, pH 6.8 and autoclaved at 121°C for 20 min. Bacterial inoculum was prepared by culturing selected colonies in broth culture medium of YEM. The cultures were grown in 150 mL broth and incubated at 28 ± 2°C with constant shaking at 100 rpm until maximum bacterial cell growth of approximately 10^8 cells mL^{-1}.

AM fungal spores of *Glomus intraradices* showing hyphal connections were isolated by employing wet sieving and decanting method (Gerdemann and Nicolson 1963) from the rhizospheric soil samples collected from the crops growing in a field area of Faisalabad, Pakistan. Spores of *G. intraradices* were surface sterilized by immersing them in 2% (W/V) Chloramin T and 200 ppm Streptomycin for 15 min, followed by successive washing with sterilized distilled water until the removal of the sterilant. The sterile spores were used to infect *Allium cepa* (onion) seedlings grown in earthen pots filled with sterilized substrate (soil and sand 1:1 V/V). The root system was checked microscopically (Nikon Optiphot II fitted with a Leica DC 500 equipped with digital CCD camera) by staining with Trypan blue (Phillips and Hayman 1970) for uniform colonization at different time intervals (after 7 days). The roots system of well infected seedlings along with the adhering soil were finally chopped and used as starter inoculum. The bulk inoculum was produced by infecting fresh seeding raised in sterilized soil inoculated with 5–10% of starter inoculum as layer about two inches below the soil level.

Field experimental

The field experiments were carried out locally at NIBGE, Faisalabad, with soil pH 7.5, electric conductivity 685 μS cm^{-1}, total organic matter 0.6%, total soil phosphorus 5.30 mg kg^{-1} and total nitrogen of 500 mg kg^{-1} soil, in the cropping season of *V. radiata.* Randomized Complete Block Design (RCBD) based experiments were performed with eight inoculation treatments (Table 1) and three replications. Experimental unit was a plot of size, 12 m^2 with three rows. Seeds were planted at one inch depth from the surface soil, keeping distance of 5 inches among the seeds and 1foot among the rows. Phosphate fertilizer in the form of Diammonium phosphate (DAP) was added at 12 kg phosphorus per acre, as half the

Table 1 Effect of arbuscular mycorrhizae (*G. intraradices*) and bacteria (*Bradyrhizobium* MN-S & TAL-102) on nodule number, nodule fresh weight and dry weight plant^{-1} in *V. radiata* plants at first harvest (25 DAS) and second harvest (45 DAS)

Treatment	Nod. No. 25 DAS	Nod. No. 45 DAS	Nod. F. wt (mg) 25 DAS	Nod. D. wt (mg) 25 DAS	Nod. F. wt (mg) 45 DAS	Nod. D. wt (mg) 45 DAS
T1 = *B. japonicum* MN-S	22 b ± 3	27 e ± 3	5.89 d ± 0.66	3.53 bc ± 0.49	16.60 bc ± 2.37	3.92 cde ± 2.48
T2 = *B. japonicum* TAL-102	22 b ± 2	32 de ± 5	7.20 c ± 0.82	3.64 b ± 0.93	17.65 bc ± 3.58	4.26 bcde ± 0.52
T3 = *B. japonicum* MN-S + TAL-102	26 ab ± 3	42 c ± 2	8.20 ab ± 0.17	4.10 b ± 0.18	17.06 bc ± 0.47	5.08 bcd ± 0.76
T4 = *B. japonicum* MN-S +*G. intraradices*	25 ab ± 4	44 c ± 3	7.92 bc ± 0.20	3.81 b ± 0.16	20.23 b ± 1.64	5.93 bc ± 0.31
T5 = *B. japonicum* TAL-102 + *G. intraradices*	28 a ± 2	51 b ± 1	7.40 c ± 0.10	3.89 b ± 0.10	20.68 b ± 0.19	6.36 b ± 0.97
T6 = *B. japonicum* MN-S + TAL-102 + *G. intraradices*	29 a ± 2	64 a ± 3	8.73 a ± 0.15	4.96 a ± 0.11	25.90 a ± 3.27	8.61a ± 1.29
T7 = *G. intraradices*	22 b ± 4	34 d ± 6	3.97 e ± 0.46	2.83 c ± 0.30	13.57 c ± 1.98	3.07 de ± 0.02
T8 = Uninoculated control	14 c ± 3	14 f ± 1	2.77 f ± 0.23	1.69 d ± 0.28	3.16d d ± 1.67	2.67 e ± 0.98
	LSD	LSD	LSD	LSD	LSD	LSD
	0.05 = 4.996***	0.05 = 6.170***	0.05 = 0.745***	0.05 = 0.714***	0.05 = 3.809***	0.05 = 1.997***

Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance

Nod Nodule, *No.* number, *F. wt* Fresh weight, *D. wt* Dry weight, *DAS* Days after sowing

*** Highly significant

recommended dose of phosphorus in all the treatments including the control and no additional nitrogen fertilizer was applied. The plants were irrigated with canal water, as per requirement. *V. radiata* seeds were inoculated by seed dressing with the bacterial inoculants. The bacterial inoculum was maintained on Yeast Extract Mannitol (YEM) agar plates (Somasegaran 1985) containing Congo Red at incubation temperature of 28 ± 2°C, pH 6.8 and autoclaved at 121°C for 20 min. Bacterial inoculum was prepared by culturing selected colonies in broth culture medium of YEM. The cultures were grown with constant shaking at 100 rpm until maximum bacterial cell growth of approximately 10^8 cells mL^{-1}. AM fungal spores were isolated by employing wet sieving and decanting method (Gerdemann and Nicolson 1963) from the rhizospheric soil samples collected from the crops grown field areas of Faisalabad, Pakistan. AM spores were surface sterilized by immersing them in 2% (W/V) Chloramin T and 200 ppm Streptomycin for 15 min, followed by successive washing with sterilized distilled water until the removal of the sterilant. The sterile spores were used to infect *Allium cepa* (onion) seedlings grown in earthen pots filled with sterilized substrate (soil and sand 1:1 V/V). The roots system of well infected seedlings (85% infection) along with the adhering soil were finally chopped and used as starter inoculum. The bulk inoculum was produced by infecting fresh seedling raised in sterilized soil inoculated with 5–10% of starter inoculums and mixing in the soil bed before seed sowing.

The various treatments were (1) *B. japonicum* MN-S, (2) *B. japonicum* TAL-102, (3) *B. japonicum* MN-S + TAL-102, (4) *B. japonicum* MN-S + *G. intraradices*, (5) *B. japonicum* TAL-102 + *G. intraradices*, (6) *B. japonicum* MN-S + TAL-102 + *G. intraradices*, (7) AM alone and (8) Uninoculated control.

The parameters studied were nodule number plant^{-1}, nodule fresh and dry weight plant^{-1}, plant fresh and dry weight, nitrogen and phosphorus g^{-1} of plant sample and grain yield ha^{-1}. Nodulation data was collected at two harvesting stages i.e., 25 and 45 days after sowing. Grain yield ha^{-1}, total nitrogen, and phosphorus g^{-1} plant sample were taken at maturity i.e., 90 days after sowing.

At each harvest, three plants from each replicate of each treatment were selected randomly. The plants were separated into shoot, root and nodules and oven dried at 70°C for 3 days. For the observation of AM infection in root tissues through Light microscopy, root sub-sample from each treatment was also taken before oven drying the samples. The fresh mass of the sub-sample was recorded so that the dry mass of the sub-sample could be added to the total root dry mass. After the determination of dry mass, tissues were milled and analysed for total nitrogen (N) and phosphorus (P) concentrations. Sub-samples (0.5 g) were

digested with H_2SO_4 by wet digestion according to the microkjeldahl method (Bremner 1996) followed by standard colorimetric assays (Anderson and Ingram 1993) for N estimation. Phosphorus (P) content was determined by the Vanadomolybdate phosphoric acid yellow color method (Yoshida et al. 1976). All N and P measurements represented total elemental N and P (organic plus inorganic) present in plant tissues. The data regarding different plant characters under study were subjected to analysis of variance to determine significance of mean among the treatments (Steel and Torrie 1986) and comparison of treatment means accomplished by least significant difference (LSD) test at 0.05% level of significance.

Results

Nodulation

A considerable increase in nodulation in *V. radiata* was observed in the plants inoculated with nitrogen fixing bacterial and mycorrhizal inoculants. Furthermore, a significant increase in nodule number as well as nodule dry weight was noted especially in the plants inoculated with both bacterial inoculants (*B. japonicum* MN-S, TAL-102) plus mycorrhizae relative to the plants inoculated with single bacterial or arbuscular mycorrhizal (*G. intraradices*) inoculants. At first harvest (25 days after sowing), about two times more number of nodules plant^{-1} was observed in treatment T5 (*B. japonicum* TAL-102 + *G. intraradices*) with 28 ± 2 nodules plant^{-1} and in treatment T6 (*B. japonicum* MN-S + TAL-102 + *G. intraradices*) with 29 ± 2 nodules plant^{-1} as compared to that of uninoculated control 14 ± 3 nodules plant^{-1}. The dual inoculation of plants with bacteria and mycorrhizae proved better than single inoculum. Moreover, plants with dual inoculation showed 12, 21 and 11% increase in nodules number plant^{-1} in T4 (*B. japonicum* MN-S + *G. intraradices*), T5 (*B. japonicum* TAL-102 + *G. intraradices*) and T6 (*B. japonicum* MN-S + TAL-102 + *G. intraradices*), respectively over respective single bacterial inoculation. At second harvest (45 DAS), 39, 37 and 34% increase in nodules number plant^{-1} was calculated in T4 (*B. japonicum* MN-S + *G. intraradices*), T5 (*B. japonicum* TAL-102 + *G. intraradices*) and T6 (*B. japonicum* MN-S + TAL-102 + *G. intraradices*), respectively over respective single bacterial inoculation. The dual inoculation thus suggests that the nitrogen fixer (*B. japonicum* MN-S and TAL-102) and *G. intraradices* were compatible microbes, exhibiting a synergistic interaction amongst each other that contributes substantially by improving the nutrition of *V. radiata* plants. Maximum fresh weight of nodules with values of 8.73 and 25.90 mg plant^{-1} was observed in treatment T6 (*B. japonicum* MN-S + TAL-102 + *G. intraradices*) inoculated plants at 25 and 45 days after sowing (DAS), respectively. Similarly, more than three times increase in maximum nodule dry weight was also observed in treatment T5 (*B. japonicum* TAL-102 + *G. intraradices*) at both harvests as compared to that of control. As the field trial was conducted on the soil, which had previous cultivation history of various crops, hence, nodulation in uninoculated plants indicated the presence of indigenous rhizobial population in these soils. This could either be due to previous cultivation of the legumes, or from natural bacterial colonization of the soil. However, the number and size of the nodules was relatively lower in uninoculated control as compared to that of all other treatments. Effective nodulation with increased number, fresh and dry weight was observed on plants, co-inoculated with *B. japonicum* MN-S, TAL-102 and *G. intraradices* showing the beneficial effect and potential use as host plant inoculum.

Total biomass

Dual inoculation of bacteria and AM showed highest values of plant fresh and dry weight at all three harvesting stages i.e., 25, 45 and 90 days after sowing (Table 2). Highest values of fresh weight plant^{-1} i.e., 21.12, 174.64 and 110.61 g were observed in treatment T6 (*B. japonicum* MN-S + TAL-102 + *G. intraradices*) at 25, 45 and 90 days after sowing, respectively. Moreover, maximum difference among the treatment means was observed at 45 days after sowing among single bacterial inoculation and dual inoculation with mycorrhizae. Treatment means are comparable for both single bacterial inoculation and dual inoculation with *G. intraradices* at 45 and 90 days after sowing as well. Among the single bacterial inoculants *B. japonicum* MN-S was relatively less effective for fresh weight plant^{-1} at all three harvesting stages (25, 45 and 90 days after sowing) as compared to the *B. japonicum* TAL-102. Whereas, dual inoculation of *B. japonicum* MN-S + *G. intraradices* showed higher values of plant fresh and dry weight at all three harvesting stages compared to *B. japonicum* MN-S alone. Plant dry weight increased significantly due to co-inoculation of *G. intraradices* with bacterial inoculants. The dry weight plant^{-1} without inoculation was 2.39, 19.61 and 29.19 g at 25, 45 and 90 days after sowing, respectively. The fresh weight as well as dry weight plant^{-1} was at bottom in uninoculated control compared to all other treatments. Dual bacterial inoculants in combination with *G. intraradices* proved best with 3.78, 30.17 and 46.80 g plant^{-1} dry weight at 25, 45 and 90 days after sowing, respectively. Moreover, it was evident that dual inoculation of bacterial inoculants with *G. intraradices* was more effective than single bacterial inoculation.

Table 2 Effect of mycorrhizae (*G. intraradices*) and bacteria (*Bradyrhizobium* MN-S & TAL-102) on fresh weight and dry weight of *V. radiata* at first harvest (25 DAS), second harvest (45 DAS) and maturity (90 DAS)

Treatment	P. F. wt (g) 25 DAS	P. F. wt (g) 45 DAS	P. F. wt (g) at maturity	P. D. wt (g) 25 DAS	P. D. wt (g)45 DAS	P. D. wt (g) at maturity
T1 = *Bradyrhizobium* strain MN-S	13.29 c ± 1.46	74.13 f ± 4.35	84.12 b ± 3.75	2.65 bc ± 0.37	26.34 ab ± 2.83	37.66 cd ± 2.26
T2 = *Bradyrhizobium* strain TAL-102	14.17 c ± 2.30	94.13 de ± 3.80	86.39 b ± 6.25	2.86 bc ± 0.62	26.78 ab ± 0.90	40.60 bc ± 4.10
T3 = *Bradyrhizobium* strains MN-S + TAL-102	18.79 ab ± 2.01	104.18 cd ± 3.99	103.03 a ± 3.76	3.20 ab ± 0.20	27.55 ab ± 1.41	44.45 ab ± 2.03
T4 = *Bradyrhizobium* strain MN-S + *G. intraradices*	15.03 bc ± 1.59	112.69 bc ± 9.66	87.90 b ± 6.79	2.96 bc ± 0.08	27.89 ab ± 3.32	39.47 bcd ± 2.57
T5 = *Bradyrhizobium* strain TAL-102 + *G. intraradices*	15.38 bc ± 2.30	120.16 b ± 8.20	88.79 b ± 8.45	3.40 ab ± 0.10	28.04 ab ± 1.77	42.15 abc ± 1.46
T6 = *Bradyrhizobium* strains MN-S + TAL-102 + *G. intraradices*	21.12 a ± 3.80	174.64 a ± 3.20	110.61a ± 9.33	3.78 a ± 0.10	30.17 a ± 3.04	46.80 a ± 3.06
T7 = *G. intraradices*	14.31 c ± 1.06	87.74 e ± 9.76	72.40 c ± 3.66	3.28 ab ± 0.76	24.89 b ± 3.20	34.79 d ± 3.48
T8 = Uninoculated control	11.83 c ± 1.70	61.11 g ± 2.22	58.48 d ± 5.16	2.39 c ± 0.44	19.61 c ± 2.30	29.19 e ± 1.43
LSD	0.05 = 3.762**	LSD 0.05 = 10.949***	LSD 0.05 = 10.806***	LSD 0.05 = 0.702*	LSD 0.05 = 4.314***	LSD 0.05 = 4.671***

Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance

P. F. wt Plant fresh weight, *P. D. wt* Plant dry weight, *DAS* Days after sowing

* Significant, *** Highly significant

Nutrient uptake

Statistically significant difference for the nitrogen and phosphorus contents was observed among the treatments with higher values in the plants inoculated with dual inoculum of bacteria and *G. intraradices* (Table 3). Root, shoot and grain tissues showed 68, 20 and 17% increase over control for nitrogen contents, respectively. *B. japonicum* TAL-102 being nitrogen fixer was more effective compared to *B. japonicum* MN- S for total nitrogen contents in root, shoot and grain of *V. radiata* plant. However, co-inoculation of both bacterial inoculants with *G. intraradices* gave rather better nitrogen content in root, shoot and grain tissues than single bacterial inoculation with *G. intraradices*. Maximum values of phosphorus in the root, shoot and grain were again observed in the *B. japonicum* MN-S + TAL-102 + *G. intraradices* with 198.12, 383.06 and 493.56 mg P g^{-1} plant sample, respectively. Whereas, lowest values were observed in uninoculated control of all three tissues. Among the single bacterial inoculation *B. japonicum* TAL-102 was at better for phosphorus contents in root, shoot and grain tissues. However, phosphorus contents in all three tissues of *V. radiata* plants showed highest value when inoculated with both *B. japonicum* strains in the presence of *G. intraradices.*

Grain yield

Maximum grain yield (1,506.87 kg ha^{-1}) was recorded in treatment T6 (*B. japonicum* MN-S + TAL-102 + *G. intraradices*) followed by treatment T5 (*B. japonicum* TAL-102 + *G. intraradices*) with 68 and 60% increase over uninoculated control, respectively (Table 4). Marked increase in the grain yield was observed in the treatments where bacterial inoculants were applied in combination with *G. intraradices* as compared to bacterial inoculation alone. Moreover, nodulation, biomass, nitrogen and phosphorus contents as well as grain yield was higher in mycorrhizal plants compared to non-mycorrhizal plants.

Light microscopic (LM) studies

Arbuscular mycorrhizal infections localized in the root and nodules of the *V. radiata* were observed by simple staining with trypan blue under light microscope. Light microscopic studies revealed the presences of spores, vesicles and network of attached hyphae in the roots and nodules of the *V. radiata* plants. The presence of arbuscular mycorrhizal spores in the cortical tissues as well as in the close proximity of vascular bundle indicated the range of arbuscular mycorrhizal infection in all the tissues of the *V. radiata* roots (Fig. 1c, d). High level of mycorrhizal infection especially the arbuscules have been observed in

Table 3 Effect of mycorrhizae (*G. intraradices*) and bacteria (*Bradyrhizobium* MN-S & TAL-102) on % N and Phosphorus (mg g^{-100}) in root, shoot and grain of *V. radiata* at maturity (90 DAS)

Treatment	N in root (mg g^{-1})	N in shoot (mg g^{-1})	N in grain (mg g^{-1})	Phosphorus in root (mg g^{-1})	Phosphorus in shoot (mg g^{-1})	Phosphorus in grain (mg g^{-1})
T1 = *Bradyrhizobium* strain MN-S	6.33 bc ± 0.31	12.33 b ± 1.01	35.03 bc ± 0.67	1.08 e ± 0.70	3.02 b ± 3.10	3.36 d ± 0.03
T2 = *Bradyrhizobium* strain TAL-102	6.43 bc ± 0.21	12.60 ab ± 1.05	35.13 bc ± 0.99	1.20 de ± 7.68	3.20 b ± 8.43	3.55 cd ± 0.06
T3 = *Bradyrhizobium* strains MN-S + TAL-102	6.83 b ± 0.21	12.86 ab ± 0.31	35.53 bc ± 0.47	1.35 cd ± 2.74	3.41 ab ± 13.12	3.92 bc ± 0.03
T4 = *Bradyrhizobium* strain MN-S + AM	8.30 a ± 0.30	13.10 ab ± 0.36	36.56 ab ± 0.85	148 bc ± 8.17	3.71 a ± 6.14	4.32 b ± 1.93
T5 = *Bradyrhizobium* strain TAL-102 + AM	8.43 a ± 0.29	13.36 ab ± 0.41	37.96 a ± 0.15	1.55 b ± 2.53	3.63 a ± 2.36	3.66 cd ± 1.18
T6 = *Bradyrhizobium* strains MN-S + TAL-102 + AM	9.36 a ± 0.45	14.53 a ± 1.48	38.10 a ± 0.17	1.98 a ± 0.95	3.83 a ± 22.71	4.93 a ± 0.084
T7 = AM	6.13 bc ± 0.31	13.60 ab ± 1.10	34.66 c ± 0.75	1.26 d ± 5.26	3.63 a ± 15.24	3.94 bc ± 0.70
T8 = Uninoculated control	5.56 c ± 0.32	11.73 b ± 0.40	32.46 d ± 1.70	0.82 f ± 8.10	2.11 e ± 16.44	3.15 d ± 0.09
	LSD 0.05 = 1.025***	LSD 0.05 = 1.534*	LSD 0.05 = 1.483***	LSD 0.05 = 0.162***	LSD 0.05 = 0.384***	LSD 0.05 = 0.471***

Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance

DAS Days after sowing

* Significant, *** Highly significant

Table 4 Effect of mycorrhizae (*G. intraradices*) and bacteria (*Bradyrhizobium* MN-S & TAL-102) on yield (kg ha^{-1}) of *V. radiata* at maturity (90 DAS)

Treatment	Yield (kg ha^{-1})
T1 = *Bradyrhizobium* strain MN-S	1,262.71 e ± 24.69
T2 = *Bradyrhizobium* strain TAL-102	1,269.11 e ± 12.63
T3 = *Bradyrhizobium* strains MN-S + TAL-102	1,308.49 d ± 7.10
T4 = *Bradyrhizobium* strain MN-S + AM	1,365.61 c ± 8.23
T5 = *Bradyrhizobium* strain TAL-102 + AM	1,436.32 b ± 7.27
T6 = *Bradyrhizobium* strains MN-S + TAL-102 + AM	1,506.87 a ± 31.34
T7 = AM	1,182.85 f ± 22.29
T8 = Uninoculated control	898.59 g ± 25.33
	LSD 0.05 = 33.856***

Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance

*** Highly significant

the cortical region (Fig. 2a) and along the sides of vascular bundle (Fig. 2b) in the onion root tissues as well. As the arbuscules are considered to be the active site of nutrient exchange especially phosphorus with plant cells. Therefore, the presence of arbuscules shows metabolically active stage of nutrient exchange between onion roots and arbuscular mycorrhizal fungi.

Discussion

The role of arbuscular mycorrhizae in improving nodulation and N_2 fixation is universally recognized. Recent research has shown that AM fungi release an unidentified diffusional factor (myc factor) (Parniske 2008; Maillet et al. 2011), which activates the nodulation factor's inducible gene, involved in establishing symbiosis with the nitrogen fixing rhizobial bacteria (Kosuta et al. 2003). It has also been reported that effective mycorrhizal colonization can also increase the 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).

Symbiosis between nitrogen fixing bacteria and legumes, as major contributors to natural and biological nitrogen fixation, is a cheaper and more effective agronomic practice for ensuring an adequate supply of nitrogen than the application of nitrogen fertilizers (Mia and Shamsuddin 2010). Moreover, vesicular arbuscular

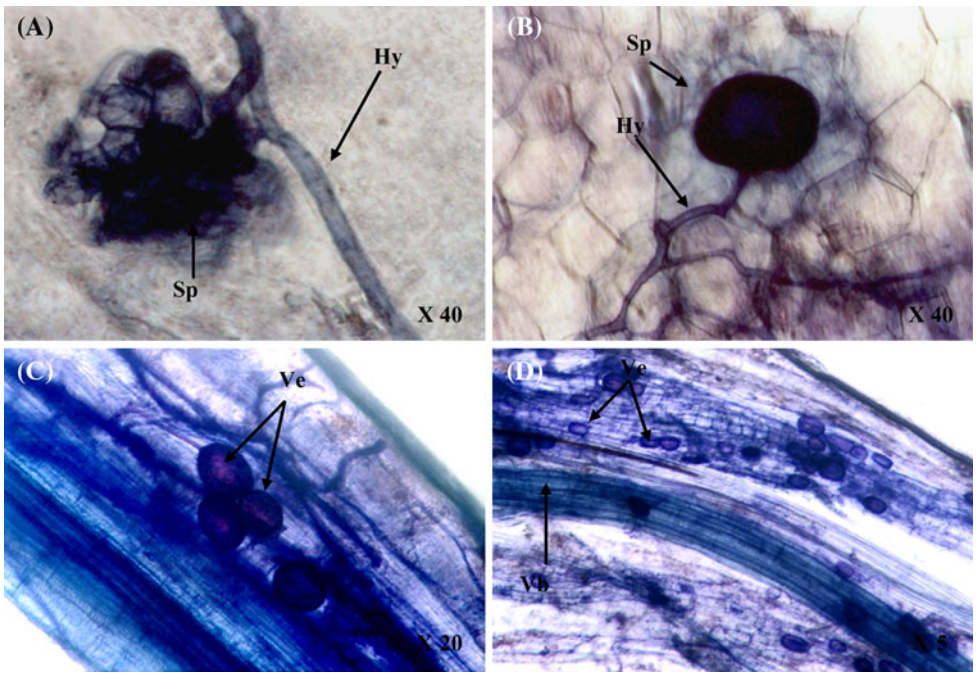

Fig. 1 Light microscopic micrograph of Trypane blue (in 0.05% lactoglycerole) stained *V. radiata* roots infected with arbuscular mycorrhizal fungi showing **a** a bunch of spores in the outer cortical region of root along with attached hypha, **b** nodule cells at higher magnification showing AM spores with attached hyphae (Hy), **c** vesicles of AM fungi dispersed in the inner cortical tissues of root along with hyphal network, **d** arbuscular mycorrhizal infection in the inner and outer cortical tissues especially along the side of vascular bundle (Vb)

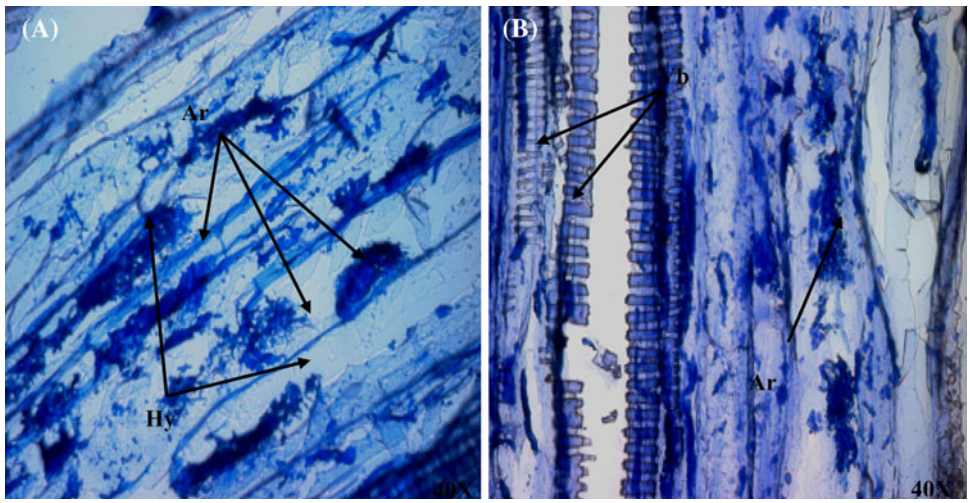

Fig. 2 Light microscopic micrographs of longitudinally sectioned *Allium cepa* roots applied as source of mycorrhizal inoculum, stained with trypan blue (in 0.05% lactoglycerole) showing **a** Presence of a large number of arbuscules (Ar) along with hyphal network (Hy), **b** presence of arbuscules (Ar) along the sides of vascular bundle (Vb)

mycorrhizae have also been found occurring widely with in a number of leguminous (Bargali 2011) and forage crops (Carrenho et al. 2001; Chen et al. 2005; Souchie et al. 2006) under different environmental conditions and its role in growth enhancement in different beans is well documented (Tarafdar et al. 1992, 1995). The legume plant productivity has been reported to be influenced due to tripartite symbioses with arbuscular mycorrhizal fungi (AMF) and rhizobia (Johnny 1999). Considering these property, in a field experiment, Treatment T1 (*B. japonicum* MN-S) and T2 (*B. japonicum* TAL-102) being IAA producer and N fixer (Hameed et al. 2004) were applied singly and as multi strain inocula with arbuscular mycorrhizal fungi, as biofertilizer to explore its potential for increased growth and grain yield. Significant effect of bacterial and mycorrhizal treatments was recorded for almost all of the growth parameters, in this study. Our results indicated remarkable increase biomass, nodulation, nitrogen, phosphorus contents and grain yield of *V. radiata* with the application of the consortia of these potential bacterial strains along with arbuscular mycorrhizae *G. intraradices.* These results are in accordance with that of reported by Tavasolee et al. (2011) and Chen et al. (2005) affirming that colonization of plant roots by arbuscular mycorrhizal fungi greatly increases the plant uptake of phosphorus and nitrogen. Moreover, the contribution of arbuscular mycorrhizal fungi to plant for phosphorus uptake is greater than nitrogen uptake (Khan et al. 2008).

As the plant is in its vegetative stage at 45 days after sowing, that's why higher values of fresh weight plant^{-1} were observed as compared with that of maturity (90 days after sowing), with pod formation completed and most of the leaves dehydrated moving fast towards the end of cropping season. In our study we found the dry weight of mycorrhizae (*G. intraradices*) inoculated plant alone as well as plants having dual inoculation of bacteria and mycorrhizae (*G. intraradices)* showed significantly higher dry weight compared to uninoculated control plants, that might be due to bacterial contribution for nitrogen fixation and growth hormone production coupled with the availability of nutrients through mycorrhizae.

The trial site in our experiment was low both in phosphorus and in nitrogen, which is rather a conducive environment for the establishment of mycorrhizal (Hayman 1986) and rhizobial symbiosis with plant roots. *V. radiata* crop grew very well in the presence of half recommended dose of DAP (diammonium phosphate) along with rhizobial inoculum responsible for nitrogen fixation and with no nitrogen added as fertilizer (Urea). Dual inoculation of mycorrhizae (*G. intraradices)* and bacteria significantly increased total nitrogen and phosphorus in root, shoot and grains of *V. radiata* plants. Moreover, the data regarding phosphorus uptake in *V. radiata* plant indicated the positive influence of mycorrhizae (phosphorus mobilizer) for the increase in phosphorus uptake. Mycorrhizal plants are thought to be more efficient than non-mycorrhizal inoculated plants for nutrient uptake (Stribley et al. 1980) as mycorrhizal plants usually transfer more assimilates to the roots than non-mycorrhizal plants (Eissenstat et al. 1993). Application of N_2 fixing bacteria and AM fungus in the present investigation exhibited an additive effect and significantly improved the nodulation, biomass production, nutrient uptake and grain yield of *V. radiata*. The inoculation of *V. radiata* with the fungus resulted in enhanced pant growth and eventual improvement in the yield. The growth increase might be potentially due to the fungus for improved water and phosphorus uptake as already been suggested by Atayese (2007).

In general, the beneficial effects are linked to the biological activity in the bacteria resulting from the expression of the symbiotic genes in nodules. However, it is suggested that inoculation simultaneously increases the degree of symbionts competition and this may in turn, modify host benefits from the relationship (Kiers et al. 2002). AM symbiosis is regarded as a key component of sustainable agriculture (Bethlenfalvay and Lindermann 1992; Jeffries and Barea 2001). Apart from agricultural systems (Kiers et al. 2002), the application of AM fungi has also been tested for the revegetation of desertified areas (Saito and Marumoto 2002) and in the cultivation of micropropagated plantlets (Yano-Melo et al. 1999). Therefore, major technological problems are the form of application of the AM inoculum (Saito and Marumoto 2002) and the combinations of AM inoculum with other microorganisms that are beneficial for plant growth (V'azquez et al. 2000, 2001; Vassilev et al. 2001). In our study, we found that onion roots colonized with arbuscular mycorrhizae are the good source of inoculum and its application in combination with *B. japonicum* MN-S & TAL-102 resulted improved growth and yield along with nutrient uptake in *V. radiata* plants. Improved growth of AM plants has partly been attributed to enhanced nutrient acquisition, especially better P nutrition (Plenchette and Dupponois 2005; Sharifi et al. 2007). The differences in nodulation, yield, nitrogen and phosphorus uptake in *V. radiata* plants upon inoculation with different *Bradyrhizobium* strains and AM shows different compatibilities of the microbes as recently been evaluated by Tajini et al. (2011). In conclusion, *B. japonicum* MN-S and TAL-102 in combination with arbuscular mycorrhizal fungi have a great potential for plant growth promotion. Synergistically significant effect on the nutrition and growth of *V. radiata* was obtained especially by dual inoculation. Moreover, the present results indicate that selected bacterial strains and mycorrhizae did promote the growth of *V. radiata* in ways that could be harnessed to practical benefit for farmers and consistent with sustainable agricultural practices in Pakistan.

Author contributions (1) Tahira Yasmeen: Dr. Yasmeen has major contribution in lab and field experiment. (2) Sohail Hameed: Dr. Hameed has designed and supervised the research project. (3) Mohsin Tariq: Mr. Tariq has contributed in data collection and some analysis. (4) Shafaqat Ali: Dr. Ali has helped in data analysis and manuscript preparation.

Acknowledgments We gratefully acknowledge the financial assistance from Higher education commission (HEC) of Pakistan and all the support of Microbial physiology group members especially technical support of Mr. Javed Iqbal and Mr. Asghar Ali.

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