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Original Article

Effect of Dual Inoculation of Arbuscular Mycorrhizal Fungi and Rhizobium on Grass Pea Under Pot Culture

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A pot experiment was carried out to study the effect of dual inoculation of arbuscular mycorrhizal (AM) fungi and *Rhizobium* (R) on grass pea at Soil Science Division, BARI, Joydebpur, Gazipur during 2006-07 and 2007-08. The experiment was designed in CRD with eight treatments and four replications. Grass pea variety BARI khesari-2 was used as a test crop. Peat based rhizobial inoculum (RLs-10) was used in this experiment. Soil based AM inoculum containing about 200 spore and infected root pieces of the host plant was used at the rate of 50g pot^{-1}. The treatments were Control, *Rhizobium*, AM, *Rhizobium* + AM, NP, NP + AM, NP + *Rhizobium* and NP + *Rhizobium* + AM. Dual inoculation of grass pea increased nodule number and nodule weight compared to single inoculation. Other growth parameters were also influenced significantly by dual inoculation. Further, dual inoculation in presence of N and P fertilizers showed better performance than that of dual inoculation alone in respect of growth and yield of grasspea.

Key words : Arbuscular mycorrhiza, Rhizobium, Grass pea

Introduction

Many tropical legumes are in fact highly mycorrhizal dependent. The leguminous plants can form two types of symbiotic association with microorganisms. One with *Rhizobium* involved in atmospheric nitrogen fixation and another with arbuscular mycorrhizal (AM) fungi concerned with the uptake of phosphorus and other nutrients[1]. Inoculation of AM fungi increased root colonization and spore population significantly when soil was inoculated.

Generally, legumes exhibited a higher percentage of AM fungal colonization. A high level of colonization was reported in the members of Leguminosae family and no colonization in Amaranthaceae, Chenopodiaceae and Cruciferae[2]. No colonization by AM fungi in *Leucaena leucocephala* was detected in 12 uninoculated soils at 25 days after planting and increased linearly in the different farm soils to values of 33-65% at 69 days after planting[3]. Arbuscular mycorrhizal inoculation also increased root colonization in *Sesbania grandiflora*. Soil inoculation with arbuscular mycorrhiza (*Glomus mosseae*) and seed inoculation with *Rhizobium* with different doses of P fertilizer (0, 30 or 60 kg/ha) on soybean increased root colonization[4].

The legume crop, grass pea can fix atmospheric N_2 but needs adequate supply of phosphorus for the same. Mycorrhizal infection might help this crop to obtain the required phosphorus for nodulation. The root system of grass pea can be infected by arbuscular endomycorrhizal fungi and by nitrogen fixing bacteria. These two microorganisms are beneficial to the plant and the possibility of a direct interaction between the fungus and bacterium was considered. It would be better if maximum supply of N and P for crop production can be obtained biologically from rhizobial inocula and mycorrhizal fungi respectively. But no attempt has so far been made on the dual inoculation of AM and *Rhizobium* on grasspea. Therefore, it is essential to determine the effect of dual inoculation of AM fungi and *Rhizobium* on the symbiotic nitrogen fixation and N and P nutrition in grasspea. Keeping these views in mind, the present investigation was taken to study the role of AM fungi and *Rhizobium* on the growth, yield and nutrient status of grasspea.

Materials and Methods

A pot experiment was carried out in the net house of Soil Science Division of Bangladesh Agricultural Research Institute (BARI) Joydebpur, Gazipur during 2006-07 and 2007-08 with a view to study the effect of dual inoculation of AM and *Rhizobium* on grass pea variety, BARI Khesari-2. The experiment was designed in CRD with eight treatments and four replications. Sandy clay loam soils collected from the bank of Turag river at Kodda, Gazipur was mixed with cow dung at 5:1 ratio and was used as growth media. Ten (10) kg of these mixtures was used per pot. The pH of cow dung was 6.2 and the nutrient contents were organic matter 10.1%, N 0.8%, P 0.4%, K 0.6%, Ca 0.4%, Mg 0.3%, S 0.15% and Mn 0.6%. The physical and chemical properties of the soil are presented in Table 1. The soil contained 30 AM (g^{-100} soil) spores of indigenous mixed AM fungal species and the experiment was conducted under unsterilized soil condition. Peat based rhizobial inoculum (RLs-10) was used containing 10^8 cell g^{-1} inoculum. The initial rhizobial population of the experimental soil was 10^3 cells g^{-1} soil. 10^8 cell g^{-1} inoculum. The initial rhizobial population of the experimental soil was 10^3 cells g^{-1} soil.

Chemical fertilizers were applied according to the treatments at the rate of 50 kg nitrogen ha^{-1} from urea and 22 kg

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Table 1. *Physical and chemical properties of the soil used as potting media*

Soil variable	Content	Critical level
Texture	Sandy clay loam	
pH	7.4	
Organic matter (%)	0.53	
Total N (%)	0.03	
Avaiable P (ppm)	11.0	14
Exchangeable K (meq/100g)	0.15	0.2
Exchangeable Ca (meq/100g)	3.80	2.0
Exchangeable Mg (meq/100g)	1.10	0.8
Available Zn (ppm)	2.50	2.0
Available B (ppm)	0.14	0.2
Available Cu (ppm)	2.10	1.0
Available Fe (ppm)	35.0	10
Available Mn (ppm)	11.0	5.0

phosphors ha^{-1} from triple super phosphate were used as per treatments. Also used at the rate of potassium 44 kg ha^{-1} from muriate of potash, sulphur 20 kg ha^{-1} from gypsum, zinc 5 kg ha^{-1} from zinc oxide and boron 1 kg ha^{-1} from boric acid were applied as basal dose. Treatments were done as control (T_1), *Rhizobium* (T_2), AM (T_3) *Rhizobium* + AM (T_4), NP (T_5), NP + AM (T_6), NP + *Rhizobium* (T_7) and NP + *Rhizobium* + AM (T_8). Soil based AM inoculum containing about 200 spores and infected root pieces of sorghum plant was used at the rate of 50 g pot^{-1}. A layer of AM inoculum was first placed in each pot and was covered with a thin soil layer of 1 cm in which *Rhizobium* inoculated seeds were sown. Seeds were sown in the soil layer above the AM inoculum to ensure the penetration of the roots the inoculum layer immediately after germination. The seedlings were thinned out within a week of emergence keeping ten uniform seedlings per pot. Watering and other intercultural operations were done as and when necessary. The pots were carefully observed regularly to record any change of plant growth. No insecticide was used during the growth period. The plants were free from insects and diseases. At 50% flowering stage five plants pot^{-1} were uprooted carefully for recording data on number of leaves, plant height, collar diameter, nodule number, nodule weight and shoot weight plant^{-1}. Another five plants pot^{-1} was allowed to grow up to yield. After harvesting, stover yield and seed yield plant^{-1}, and 100-seed weight were recorded.

An estimation of percentage of mycorrhiza formation was made for each root system at 50% flowering stage. To assess AM root colonization, the roots were processed after Koske and Gemma (1989) and observed under a compound microscope [5]. Presence of fungal bodies (mycelium, spores, arbuscule, and vesicles) in the root tissues were considered as positive for infection. Percent root colonization was calculated as follows:

$$\% \text{ root colonization} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments observed}} \times 100$$

Soil samples from the pot were collected after harvesting of the crop for counting AM spore population. Assessment of spore population was done by following the wet Sieving and Decanting Method[6]. Spores were observed under stereomicroscope and the numbers of spores were counted and the result was expressed as number g^{-100} of dry soil basis.

Results and Discussion

A. Effect of different combinations of AM-Rhizobium on nodulation and growth parameters of grass pea

At 50% flowering stage, the highest number of nodule (13.88 plant^{-1}) and nodule weight (17.75 mg plant^{-1}) were recorded with dual inoculation (*Rhizobium* + AM) which were significantly higher over all other treatments (Table 2). The 2nd highest nodule number (10.50 plant^{-1}) and nodule weight (14.25 mg plant^{-1}) were obtained with only *Rhizobium* treatment which were also significantly higher over rest of the treatments. Among the different combinations of AM, *Rhizobium* and NP, the lowest nodule number (8.13 plant^{-1}) and nodule weight (10.50 mg plant^{-1}) were obtained with NP. As the soil was unsterilized some nodules were obtained with other treatments including control. The highest nodule number and nodule weight recorded with *Rhizobium* + AM may be attributed to greater availability of P through AM, which is crucial for nodulation[7-8]. Further, dual inoculation produced significantly higher nodule number as compared to single inoculation. This is likely because dual inoculation helped plant in increased P uptake which is essential for nodulation[8]. The results are in good agreement with others who reported increased nodulation due to dual inoculation as compared to single inoculation with *Rhizobium*[9-10]. Data presented in Table 2 also indicate that single or dual inoculation along with fertilizer N (NP+R and NP+R+AM) displayed less number of nodules compared to inoculation of *Rhizobium* without N. This less nodulation may be attributed to inactivity of rhizobia in presence of fertilizer nitrogen[11]. Similar trend was observed in the nodulation by native rhizobia with NP and NP+AM treatments. Hernandez and Hernandez (1996)[12] recorded significantly increased nodule number and nodule weight of soybean at flowering stage with AM - *Rhizobium* inoculation in absence of added N.

The highest number of leaf (21.33 leaf plant^{-1}) was recorded with the treatment R+AM which differed significantly only with control, *Rhizobium*, AM, NP and NP+R treatments. The maximum plant height was obtained with R+AM, AM and NP+AM among all the treatments but no significant differences were observed with R+NP (Table 2). Dual inoculation along with NP (NP+R+AM) showed significant increase in plant height compared to *Rhizobium* alone. This might be due to the application of nitrogenous and phosphors fertilizer that enhanced vegetative growth. There are records of maximum plant height in greengram by the dual inoculation of AM - *Rhizobium* along with 50 per cent recommended nitrogen and phosphorus fertilizers[13]. The highest collar diameter (1.75 mm) was recorded with NP+AM. But this treatment did not differ significantly with the treatments NP+R+AM. The highest shoot weight was recorded with the treatments NP+R+AM which was significantly higher over all the treatments except R+AM.

Table 2. *Effect of dual inoculation of AM and Rhizobium on growth parameters of grass pea*

Treatment	Nodule no. plant^{-1}	Nodule weight mg plant^{-1}	No. of leaf plant^{-1}	Plant height (cm)	Collar diameter (mm)	Shoot weight (g plant^{-1})
Control	6.88d	11.25cd	11.00c	17.17d	1.31d	0.22c
Rhizobium	10.50b	14.25b	15.00b	21.83c	1.51bc	0.36b
AM	9.75bc	12.50bcd	15.42b	25.25ab	1.53bc	0.28c
R+ AM	13.88a	17.75a	21.33a	27.08a	1.54bc	0.42ab
NP	8.13cd	10.50d	13.08bc	22.67bc	1.42cd	0.27c
NP+AM	10.38b	14.00b	19.58a	25.50ab	1.75a	0.40b
NP+R	9.13bc	13.25bc	12.92bc	21.08c	1.53bc	0.22c
NP+R+AM	9.00bc	12.75bcd	19.83a	24.33b	1.67ab	0.48a
CV (%)	13.23	10.08	12.76	8.17	6.85	12.76

Data presented in Table 3 show that dual inoculation (R+AM) significantly increased stover yield (3.96 g plant^{-1}) compared to chemical fertilizer (NP), which was supported by other studies[14-15]. The highest stover yield (4.82 g plant^{-1}) was recorded with NP+R among all the treatments but it was statistically identical to NP+R+AM treatment. In case of seed yield same trend was observed in both the years. Dual inoculated plants along with chemical fertilizers (NP+R+AM) produced the highest seed yield plant^{-1} (1.11 and 1.10 g) in the year 2006-07 and 2007-08, respectably. But it was statistically similar to most of the treatments except control and single inoculation *i.e. Rhizobium* and AM. Singh [16] (1996) also observed significantly increased grain yields of pigeon pea due to *Rhizobium* and AM inoculation when the plants were grown in pots especially fertilized with 50 kg P_2O_5 ha^{-1}. The same treatment (NP+R+AM) also showed the highest increase in seed yield (83%) over control compared to other treatments. The highest weight of 100 seeds (3.80 g) was recorded with the treatment AM but it was identical to NP, NP+AM and NP+R+AM treatments.

Table 3. *Effect of dual inoculation of AM and Rhizobium on yield contributing characters of grass pea*

Treatment	Stover yield (g plant^{-1})	100 seed weight (g)	Seed yield (g plant^{-1})		Increase seed yield over control (%)
			2006-07	2007-08	
Control	2.93d	2.08d	0.63 d	0.60e	-
Rhizobium	3.96b	2.85c	0.92 bc	0.88d	47
AM	3.76bc	3.80a	0.87 c	0.84d	40
R+AM	3.96b	3.11bc	1.01 ab	0.98bc	63
NP	3.68c	3.52ab	1.00 ab	0.92cd	53
NP+AM	3.85bc	3.20abc	1.05 a	1.05ab	75
NP+R	4.82a	2.83c	1.06 a	1.03ab	72
NP+R+AM	4.75a	3.73ab	1.11 a	1.10a	83
CV (%)	5.76	12.92	8.5	6.37	-

B. Mycorrhizal development

Root colonization and spore population of arbuscular mycorrhiza are presented in Table 4. The extent of AM colonization (per cent root colonization) was 45% in AM inoculated grasspea, which was increased to 60% when inoculated with AM-*Rhizobium*. The increased per cent root colonization by dual inoculation was also reported by Naqvi and Mukerji (1998) [17]. In this study, R+AM and NP+R+AM recorded the highest percentage (60%) of root colonization compared to other treatments. But there was no significant difference among the treatments R+AM, NP+AM, and NP+R+AM. The highest spore population (58.3 100 g^{-1} soil) was recorded with R+AM treatment which was statistically significant over the treatments. Higher percentage of root colonization and spore population at 50% flowering stage in chickpea by dual inoculation has also been reported[18].

Table 4. *Effect of dual inoculation of AM and Rhizobium on root colonization by AM fungi and spore number in rhizosphere soils of grasspea during 2006-07 and 2007-08*

Treatments	Root colonization (%)		Spore number (100 g^{-1} soil)	
	2006-07	2007-08	2006-07	2007-08
Control	45.0 c	25.0d	23.3	26.0c
Rhizobium	50.0 bc	42.5bc	25.9	33.3bc
AM	57.5 abc	45.0b	28.0	29.0bc
R+AM	65.0 a	60.0a	29.7	58.3a
NP	47.5 bc	32.5cd	26.7	31.3bc
NP+AM	60.0 ab	52.5ab	29.7	30.8bc
NP+R	50.0 bc	47.5b	26.7	35.8bc
NP+R+AM	57.5 abc	60.0a	30.3	38.8b
CV (%)	15.2	16.8	18.1	18.7

C. Nutrient uptake

Uptake of different major and micronutrients by grass pea at 50% flowering stage was found to be greatly influenced by dual inoculation of AM and *Rhizobium*. Like yield and stover yield, uptake of different major and micronutrients by grass pea at 50% flowering stage was also higher with dual inoculation of AM and *Rhizobium* (Tables 5 and 6).

Table 5. *Uptake of major nutrients by grasspea at 50% flowering stage with dual inoculation during rabi season of 2007-08.*

Treatment	Uptake of major nutrients (mg/ plant)					
	N	P	K	Ca	Mg	S
Control	8.20e	0.99e	3.55e	3.32d	1.09e	1.09e
Rhizobium	17.74a	1.96ab	6.99ab	3.66bc	1.91b	2.31a
AM	10.61c	1.16e	4.46c	3.14c	1.21c	1.58b
R+ AM	18.46a	2.13a	7.71a	5.16a	2.28a	2.60a
NP	10.31c	1.25c	4.50c	3.19c	1.18c	1.39bc
NP+AM	14.06b	1.77b	6.11b	3.29c	1.65b	1.61b
NP+R	8.30c	0.98c	3.81c	1.80d	0.99c	1.19bc
NP+R+AM	14.65b	1.69b	6.41b	4.21b	1.86b	2.32a
CV (%)	13.37	15.36	14.73	16.12	13.41	17.03

Table 6. *Uptake of micro-nutrients by grasspea at 50% flowering stage with dual inoculation during rabi season of 2007-08.*

Treatment	Uptake of micro nutrients (μg/ plant)				
	Cu	B	Zn	Mn	Fe
Control	1.87e	10.34e	9.72e	21.83c	129.2c
Rhizobium	3.56ab	22.95abc	20.43ab	44.36a	260.2a
AM	2.19de	17.33cd	13.34d	27.64c	166.4c
R+ AM	3.99a	26.27ab	22.12a	47.59a	282.9a
NP	2.56cd	17.89cd	12.84de	26.92c	158.4c
NP+AM	2.93bc	21.38bc	16.53c	35.88b	211.8b
NP+R	2.01de	13.35de	10.23de	23.70c	139.8c
NP+R+AM	3.39ab	27.72a	17.89bc	42.21ab	252.3a
CV (%)	15.30	20.13	13.56	14.13	13.77

Conclusion

Dual inoculation of AM - *Rhizobium* greatly influenced the nodulation, growth and yield of grass pea. Percent root colonization and spore population indicated mycorrhizal development. Nutrient uptake was also influenced by dual inoculation.

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