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INFLUENCE OF PHOSPHORUS AND INOCULATION WITH *RHIZOBIUM* AND AM FUNGI ON GROWTH AND DRY MATTER YIELD OF CHICKPEA

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Abstract

The response of three chickpea (*Cicer arietinum* L.) varieties namely. BARI Chola-3, BARI Chola-4, BARI Chola-5 grown in sterile soil was studied against dual inoculation with *Rhizobium* (R) strain Rca-201 and arbuscular mycorrhiza (AM) in presence and absence of phosphorus (P). Roots of sorghum (*Sorghum vulgare* L.) with rhizosphere soil propagules were used as AM inoculants. The performance of *Rhizobium* inoculant alone was superior to control in all the parameters of the crop. The highest number and dry weight of nodule and shoot were obtained by inoculating BARI Chola-3 with *Rhizobium* and AM in presence of P. BARI Chola-4 in combination with *Rhizobium,* AM and P recorded the highest plant height, root length, root colonization, spore population of AM and mycorrhizal dependency but the effect of BARI Chola-3 was comparable to BARI Chola-4 in these parameters of the crop. BARI Chola-3, BARI Chola-4 and BARI Chola-5 showed 31.3, 35.1 and 30.6% mycorrhizal dependency. From the view point of nodulation, dry matter production, root colonization and spore population of AM, growth and mycorrhizal dependency, dual inoculation of BARI Chola-3 with *Rhizobium* and AM in presence of P was considered to be the balanced combination.

Key words: Chickpea, *Rhizobium*, arbuscular mycorrhiza, phosphorus

Introduction

Chickpea (*Cicer arietinum* L.) is one of the protein rich legume crops and has occupied sixth position in production and acreage in Bangladesh (Anon. 2004). Although predominantly consumed as a pulse, dry chickpea is also used in preparing various snack foods, sweets and condiments (Saxena 1987). After the rice harvest in Bangladesh, there is often enough stored soil moisture, along with any subsequent rainfall, to support a following, short-duration crop (Rahman *et al.* 1995). For a number of reasons, legumes are a particularly attractive choice in this regard and Johansen *et al.* (2000) recently reviewed the constraints and opportunities for legumes in rotation with rice. Better utilization of rice fallows by cultivating legumes should improve soil organic matter and fertility status, thereby contributing to the long-term sustainability of rice cropping

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(Kumar Rao *et al.* 1998). Chickpea has proved to be particularly suitable for growing after rice in this system because of its strong rooting characteristics and of the availability of new shorter-duration improved varieties, as compared with traditional local landraces (Rahman *et al.* 2000). Chickpea, like most other legumes, is capable of fixing atmospheric nitrogen for its growth, enriching the nitrogen fertility of the soil if properly inoculated with the right strain of nodule bacteria. Two types of symbiotic association can form with microorganisms and the leguminous crops in which *Rhizobium* is involved in atmospheric N fixation and AM fungi is concerned with the uptake of P (Abdel- Fattah 1997). A good number of literature says that *Rhizobium* inoculation works favorably in respect to nodulation and biological nitrogen fixation in chickpea (Solaiman *et al.* 2007) and pea (Solaiman and Rabbani 2005, Ahmed *et al.* 2007). Arbuscular mycorrhizal fungi increase uptake of immobile nutrients, especially phosphorus and micronutrients (Douds and Miller 1999). Normally legume crops have less extensive root systems and are dependent on colonization by native AM fungi for their nutritional needs (Benthlenfalvay and Newton 1991). The root system of chickpea can be colonized by AM fungi and nodulated by N fixing bacteria. These two microbes are beneficial to the legume crop and the possibility of a direct interaction between the fungus and bacteria was considered to supply a good amount of N and P.

Mycorrhizal dependence has been defined by Gerdeman (1975) as "the degree to which a host relies on the mycorrhizal condition to produce maximum growth at a given level of soil fertility." The importance of arbuscular mycorrhizal fungi associations in agricultural crops and their significance in nodulating nitrogen fixing plants are well documented (Barea and Azcon-Angular 1983). Khalil *et al.* (1994) studied the mycorrhizal dependency and nutrient uptake by improved and unimproved corn and soybean cultivars and showed that soybean had higher mycorrhizal dependency than corn. Considerable variations occurred within soybean cultivars where the relative growth of improved cultivars was less enhanced with mycorrhizal colonization than the unimproved ones. Differences in the relative mycorrhizal dependency between crop species or even cultivars are also related to other plant factors such as root structure, plant growth rates and microorganisms in the rhizosphere which could affect the demand for P (Xie *et al.* 1995).

A scanty research (Solaiman *et al.* 2005, 2006) on the response of chickpea to inoculation with *Rhizobium* and AM was conducted in respect of nodulation, growth and yield, and AM colonization and spore formation. Keeping these facts in mind, the present experiment was carried out to assess the response of three varieties of chickpea to inoculation with *Rhizobium* and arbuscular mycorrhiza in presence and absence of phosphorus.

Materials and Methods

A pot experiment was conducted at the Bangabandhu Sheikh Mujbur Rahman Agricultural University, Gazipur, Bangladesh using three chickpea varieties *viz.* BARI Chola-3, BARI Chola-4 and BARI Chola-5 as the test crop. The soil was silty clay loam having organic carbon 0.93%, pH 6.80, CEC 15.30 meq per 100 g soil, total nitrogen 0.07%, available P 11.30 mg/kg, available K 0.31 meq per 100 g soil, exchangeable Ca 6.60 meq per 100 g soil, exchangeable K 7.32 meq per 100 g soil, exchangeable Mg 3.10 meq per 100 g soil, exchangeable Na 0.73 meq per 100 g soil, and number of *Rhizobium* 4.1×10^6 per g soil. The collected soil samples were autoclaved at 121°C for 2 hours before use. Five kg autoclaved soil was put in each earthen pot (25 cm × 22 cm). Phosphorus at the rate of 2.40 g per pot in the form of triple super phosphate, and inoculant containing arbuscular mycorrhizae at the rate of 10 g per pot were applied according to the treatments assigned. Potassium, sulphur and zinc at the rate of 1.3, 2.4 and 0.06 g per pot were applied as basal dose in the form of muriate of potash, zypsum and zinc sulphate, respectively. Roots of sorghum (*Sorghum vulgare* L.) with rhizosphere soil propagules were used as AM inoculant and strain Rca-201 was used as *Rhizobium* inoculant which was collected from Soil Microbiology Section, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh. Counts of viable rhizobia in the *Rhizobium* inoculant were taken following the Drop Plate Method of Miles and Misra (1938). An amount of 0.40 g *Rhizobium* inoculant containing 1.3×10^9 cells per g was mixed with 24 g seeds with the help of gum arabic. The experiment was laid out in a factorial randomized complete block design (RCBD) with three replications. There were seven treatment combinations, *viz.* T_1: Control, T_2: Phosphorus (P), T_3: Arbuscular mycorrhiza (AM), T_4: AM + P, T_5: *Rhizobium* (R), T_6: AM + R, T_7: AM + R + P. Six seeds of chickpea treated with mercuric chloride for surface sterilization were sown in each pot. A layer of AM inoculant was first placed in each pot filled with sterilized soil and was covered with a thin soil layer of 2 cm in which seeds were sown. Pots were watered up to saturation to allow the soil and inoculant to settle down in the pots. Two healthy seedlings were allowed to grow in each pot up to 50 per cent flowering stage of the crop. To maintain soil moisture pots were watered when needed. Weeding and mulching were done as and when needed. With minimum disturbance of roots, the plants were carefully uprooted at 50 per cent flowering stage so that no nodules were left in the soil. The roots were washed with tap water and finally rinsed with distilled water. Plant height and root length were measured using meter scale. The nodules from the roots were separated and then nodule number and weight were recorded. The root, nodules and shoot were first air-dried and then oven-dried at 65°C for 72 hours. According to Koske and Gemma (1989), one hundred root segments of 1 cm long were stained for assessing AM colonization. The percentage of AM root colonization was estimated by Root Slide Technique (Read *et al.*

1976) and spore population was determined following the wet sieving and decanting method (Gerdermann and Nicolson 1963). The mycorrhizal dependency or response to mycorrhizal colonization was calculated by using the following formula (Plenchette *et al.* 1983):

$$MD = \frac{(\text{Dry weight of mycorrhizal plants} - \text{Dry weight of non mycorrhizal plants}) \times 100}{\text{Dry weight of mycorrhizal plants}}$$

Data were analyzed in the computer using MSTAT program.

Results and Discussion

Plant height

The highest plant height of 45.3 cm was obtained through interaction of arbuscular mycorrhiza, *Rhizobium* and phosphorus in treatment T_7 (AM + R + P) for the variety BARI Chola-4 which was statistically similar to BARI Chola-3 × T_5, BARI Chola-3 × T_6, BARI Chola-3 × T_7, BARI Chola-4 × T_5, BARI Chola-4 × T_6 and BARI Chola-5 × T_7 treatments (Table 1). The lowest height was recorded in uninoculated control. Balachandar and Nagarajan (1999) found the highest shoot length in greengram by dual inoculation of *Rhizobium* and AM along with 50% recommended N and P fertilizers.

Root length

A significant variation was observed in root length of different varieties of chickpea (Table 1). BARI Chola-4 in treatment T_7 (AM + R+ P) recorded the highest root length of 27.5 cm which was statistically similar to BARI Chola-3 × T_7 and BARI Chola-4 × T_6. This might be due to the adequate supply of N through fixation by *Rhizobium* and proper supply of P. Results of the present experiment is in agreement with the findings of Balachandar and Nagarajan (1999). All treatments recorded significantly higher root length over uninoculated control.

Dry weight of shoot

The effects of different treatments on dry weight of shoot of different varieties of chickpea were found significant (Table 1). Treatment T_7 (AM + R + P) along with the variety BARI Chola-3 recorded the highest dry weight of shoot of 6.95 g/plant which was statistically similar to BARI Chola-3 × T_6 and BARI Chola-4 × T_7. Solaiman *et al.* (2006) concluded that dual inoculation with *Rhizobium* and AM in presence of P performed the best in case of chickpea. In this study, the lowest dry weight of shoot was obtained in uninoculated control.

Table 1. Effects of phosphorus and inoculation with arbuscular mycorrhiza and *Rhizobium* on plant height, root length and dry weight of shoot on three varieties of chickpea grown in sterile soil.

Treatment	Plant height (cm)			Root length (cm)			Dry weight of shoot (g plant^{-1})		
	BARI Chola-3	BARI Chola-4	BARI Chola-5	BARI Chola-3	BARI Chola-4	BARI Chola-5	BARI Chola-3	BARI Chola-4	BARI Chola-5
T_1. Control	23.2 fg*	29.0 efg	22.6 g	15.1 lm	16.3 j-m	14.1 m	2.71 d-g	2.24 fg	2.00 g
T_2. Phosphorus (P)	31.7 def	33.0 cde	29.4 efg	17.7 h-l	18.2 g-k	16.0 klm	3.54 c-g	2.95 d-g	2.59 efg
T_3. Arbuscular mycorrhiza (AM)	33.3 b-e	34.0 b-e	30.3 d-g	19.1 f-j	19.3 f-j	17.0 i-m	3,95 cde	3.45 c-g	2.88 d-g
T_4. AM + P	35.0 b-e	35.3 b-e	32.3 cde	20.0 e-i	21.0 c-g	18.5 g-k	4.25 bcd	3.83 cde	3.31c-g
T_5. *Rhizobium* (R)	37.0 a-e	38.0 a-e	34.3 b-e	21.7 c-f	23.0 b-e	20.7 d-h	4.82 bc	4.02 cde	3.61 c-f
T_6. AM+ R	39.0 a-d	40.7 abc	35.6 b-e	23.3 bcd	25.1 ab	22.0 c-f	5.75 ab	4.62 bc	4.07 cde
T_7. AM + R + P	42.2 ab	45.3 a	37.5 a-e	25.0 ab	27.5 a	24.0 bc	6.95 a	6.37 a	4.55 bc
CV (%)	13.03	13.03	13.03	7.59	7.59	7.59	19.65	19.65	19.65

*Means followed by a common letter(s) in a column are not significantly different at 5 % level by DMRT.

Number of nodules

The highest number of nodules of 82.50/plant was recorded in association with BARI Chola-3 in treatment T_7 (AM+ R+ P) (Table 2). The second highest number of nodules/ plant was observed in the treatment T_7 (AM + R + P) in association with BARI Chola-4 and T_7 (AM + R + P) in association with BARI Chola-5 (Table 2). The lowest number of nodules/plant was recorded in uninoculated control. The maximum number of nodules obtained with AM + R + P that might be attributed to greater availability of P, which was crucial for nodulation (Hayman 1986). This is likely as dual inoculation might help plant in increased P uptake which was essential for nodulation. Dual inoculation produced significantly higher number and dry weight of nodule as compared to single inoculation. Solaiman *et al.* (2006) stated that dual inoculation with *Rhizobium* and AM in presence of P performed the best in recording number of nodule of chickpea.

Table 2. Effects of phosphorus and inoculation with arbuscular mycorrhiza and *Rhizobium* on number and dry weight of nodule on three varieties of chickpea grown in sterile soil.

Treatment	Number of nodule/plant			Dry weight of nodule (g/ plant)		
	BARI Chola-3	BARI Chola-4	BARI Chola-5	BARI Chola-3	BARI Chola-4	BARI Chola-5
T_1. Control	8.50 i*	7.83 i	8.17 i	0.11 de	0.10 e	0.19 b-e
T_2. Phosphorus (P)	18.50 ghi	16.00 hi	12.50 hi	0.16 b-e	0.15 cde	0.25 abc
T_3. Arbuscular mycorrhiza (AM)	19.83 f-i	18.33 ghi	14.17 hi	0.18 b-e	0.17 b-e	0.16 b-e
T_4. AM + P	32.33 ef	26.17 e-h	25.17 e-h	0.21 bcd	0.19 b-e	0.19 b-e
T_5. *Rhizobium* (R)	46.33 cd	38.50 de	31.83 efg	0.23 abc	0.21 b-e	0.21 bcd
T_6. AM+ R	65.00 b	46.75 cd	37.67 de	0.24 abc	0.22 abc	0.22 abc
T_7. AM + R + P	82.50 a	62.17 b	53.50 bc	0.33 a	0.26 ab	0.26 ab
CV (%)	11.74	11.74	11.74	18.73	18.73	18.73

*Means followed by a common letter(s) in a column are not significantly different at 5 % level by DMRT.

Dry weight of nodule

BARI Chola-3 showed the highest dry weight of nodules of 0.33 g/plant in treatment T_7 (AM + R + P) which was statistically similar to BARI Chola-3 × T_5, BARI Chola-3 × T_6, BARI Chola-4 × T_6, BARI Chola-4 × T_7, BARI Chola-5 × T_2, BARI Chola-5 × T_6 and BARI Chola-5 × T_7 (Table 2). The lowest dry weight of nodules was recorded in uninoculated control. Solaiman *et al.* (2006) stated that dual inoculation with *Rhizobium* and AM in presence of P performed the best in recording dry weight of nodule of chickpea.

Root colonization

The highest root colonization (66.67%) was recorded by the treatment T_7 (AM + R + P) in association with BARI Chola-4 which was statistically similar to BARI Chola-3 and BARI Chola-5 (Table 3). The lowest colonization was found in control in all the varieties. Percent root colonization was increased significantly by dual inoculation than single inoculation. Similar results were reported by Nwoko and Sanginga (1999) who reported increased root colonization in soybean by dual inoculation of *Rhizobium* and AM. In this study dual inoculation was more effective when P fertilizer was applied. Colonization percentage of AM was higher with dual inoculation and increased further due to addition of P.

Table 3. Effects of phosphorus and inoculation with arbuscular mycorrhiza and *Rhizobium* on per cent root colonization and number of spore on three varieties of chickpea grown in sterile soil.

Treatment	Per cent root colonization			Number of spore 100/ g soil		
	BARI Chola-3	BARI Chola-4	BARI Chola-5	BARI Chola-3	BARI Chola-4	BARI Chola-5
T_1 Control	0.00 j*	0.00 j	0.00 j	7.00 i	8.00 i	7.00 i
T_2 Phosphorus (P)	30.00 h	33.33 h	26.67 h	35.00 hi	38.67 h	33.67 ij
T_3 Arbuscular mycorrhiza (AM)	43.33 fg	46.67 efg	40.00 g	52.00 g	55.00 fg	51.33 g
T_4 AM + P	50.00 def	53.33 cde	46.67 efg	60.00 de	63.67 d	58.33 ef
T_5 *Rhizobium* (R)	16.67 i	20.00 i	16.67 i	30.00 jk	31.33 ij	27.00 k
T_6 AM+ R	56.67 bcd	60.00 abc	53.33 cde	69.00 c	71.00 c	68.00 c
T_7 AM + R + P	63.33 ab	66.67 a	60.00 abc	81.33 ab	83.67 a	79.33 b
CV (%)	10.04	10.04	10.04	14.51	14.51	14.51

*Means followed by a common letter(s) in a column are not significantly different at 5 % level by DMRT.

Number of spore

Arbuscular mycorrhiza, either individually or in combination with *Rhizobium* or dual inoculation of chickpea along with P fertilizers significantly increased spore population over control (Table 3). The highest number of spore (83.67 100/g) was recorded by the treatment T_7 (AM + R + P) with BARI Chola-4 whose effect was statistically similar to BARI Chola-3. The lowest number of spore was found in control in all the varieties. Tarafdar and Rao (1997) reported that dual inoculation produced maximum number of spore than the number of AM spores in the rhizosphere soil of clusterbean, mungbean and mouthbean upon AM inoculation. Poor number of spores was obtained by the application of *Rhizobium* in presence or absence of P fertilizers but higher number of spores was found by the application of AM and P fertilizers. This might me due to the decisive influence of P and N supplied by *Rhizobium* on AM colonization.

Mycorrhizal dependency

Mycorrhizal dependency ranged from 30.6 to 35.1 per cent (Figure 1). Among the varieties BARI Chola-4 recorded the highest mycorrhizal dependency of 35.1 and BARI Chola-3 of 31.3, BARI Chola-5 of 30.6 per cent. According to mycorrhizal dependency all three varieties of chickpea were highly dependent plants.

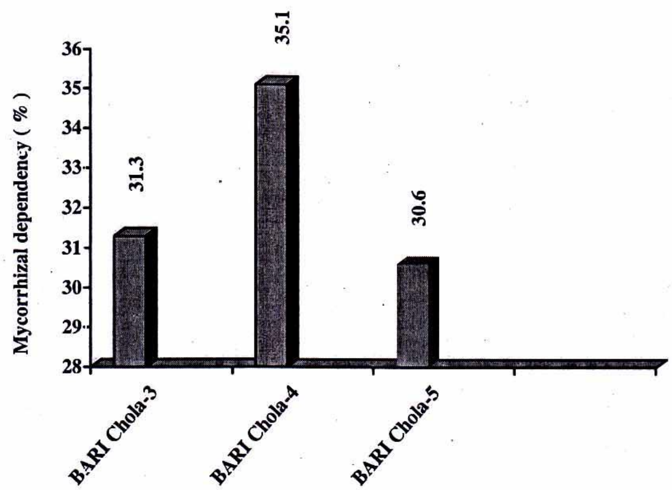

Figure 1. Mycorrhizal dependency of chickpea.

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