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INTERACTION OF ARBUSCULAR MYCORRHIZAL FUNGUS *GLOMUS MOSSEAE* AND PHOSPHORUS ON GROWTH AND NUTRIENT UPTAKE OF MAIZE PLANTS GROWN UNDER DIFFERENT SOIL CONDITIONS

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Abstract

Effects of inoculation with arbuscular-mycorrhizal fungi and phosphorus fertilization on growth and uptake of N and P by maize plants were studied in pots in a net house in sterile and non- sterile soil under drought-stressed and unstressed conditions. The inoculation with *Glomus mosseae* (40 g inoculum per 3 kg soils) alone and with P (120 kg P/ha) increased height, dry weight of the shoot and root, and N and P content of maize plants. Phosphorus fertilization reduced the degree of infection without affecting plant growth and uptake of N and P.

Introduction

The improvement of phosphorus uptake and plant growth by arbuscular-mycorrhizae (AM) fungi has been documented in crop plants (Mosse 1973, Smith and Gianinazzi-Pearson 1988, Rahman and Parsons 1997). Maize which is known to be a mycorrhizal crop has considerably benefitted from mycorrhizal association (Mosse 1977, Omar 1995). Mycorrrhizal fungi have an important role in plant community, nutrient cycling and maintenance of soil structure (Miller and Jastrow 1994). The main effects of agricultural practices on the formation of mycorrhizas relate to changes in plant species and phosphate fertilizer application (Abbott and Robson 1994). There is increasing evidence to show that the mycorrhizal symbiosis has a very important role in plant water relations. The effects of the soil moisture status on AM colonization of crop plants have been investigated because it was suggested that colonization by AM fungi improved the drought resistance of the plants (Ellis *et al.* 1985, Sylvia *et al.* 1993). Farming in Bangladesh is intensive, diverse and dynamic. With the support of modern agricultural technology using biofertilizer *viz.* AM fungi and HYV seeds economization in foodgrain production can be achieved under different climatic and edaphic conditions. Scanty information is available on the role of AM fungi on the growth and nutrient uptake of maize especially in soils of Bangladesh which are generally phosphorus deficient or have an insoluble form during drought when the moisture availability is scarce.

In view of this, present experiment was carried out to evaluate the effect of inoculation with *G. mosseae,* an AM fungi, and phosphorus fertilization on the growth of and N and P uptake by maize plant grown in sterile and non-sterile soil under drought-stressed and unstressed conditions.

Materials and Methods

Soil: Surface soil (0 to 20 cm depth) was collected from the charlands of the river Padma at the village Kartikpur under Dohar Upazila in Dhaka district, air- dried, sieved (< 3 mm) and stored in polyethylene bags. The physicochemical properties of the soil were determined following standard methods (Table 1). A portion of soil was autoclaved at 121ºC in a Pyrex beaker for 3h. The sequence of sterilization was 1 h autoclaving and 24 h cooling.

[1]East West Seed (Bangladesh) Ltd., Bashon, Gazipur, Bangladesh.

Pot experiment: Three kg of soil was taken into each sterilized earthen pot (22.5 cm diameter × 18.0 cm height). The pots and saucers were sterilized with 20 % sodium hypochlorite solution. Basal dressings of nitrogen (80 kg/ha) and potassium (50 kg/ha) were added as urea and muriate of potash, respectvely. Forty gram crude inoculum of *G. mosseae* (fragments of heavily infected maize roots, soil, hyphae etc.) were applied on the surface of 2.9 kg soils in pots as a thin layer and then 100 g soil was spread over the surface of the inoculum. *G. mosseae* inoculum was obtained from the Department of Plant and Soil Science, University of Aberdeen, Scotland. Equivalent amount of soil was added in pots where no AM was inoculated. Phosphorus (triple super phosphate) alone and in association with AM was applied separately in sterile and non- sterile soil. Treatments with three replicates were as follows: M1. Control (without AM and P), M2. *G. mosseae*, M3. 30 kg P/ha, M4. 60 kg P/ha, M5. 120 kg P/ha, M6. *G. mosseae* plus 30 kg P/ha, M7. *G. mosseae* plus 60 kg P/ha and M8. *G. mosseae* plus 120 kg P/ha.

Table 1. Some physicochemical characteristics of the soil used in the pot experiment.

Parameters	Values	Parameters	Values
pH (1: 2.5 w/v H_2O)	8.0	Total K (%)	0.65
Organic carbon (%)[a]	0.20	Cation exchange capacity (meq/100g)[f]	18.9
Available N (mg/100g soil)[b]	4.04	Field capacity (%)	31.1
Total N (%)[c]	0.07	**Particle size (%)[g]**	
C/N ratio	2.86	Sand	4.8
Available P (µ/g)[d]	4.0	Silt	76.61
Total P (%)	0.04	Clay	18.95
Exchangeable K[e]	12.0	Texture	Silt loam

[a]Wet-oxidation method (Jackson 1958), [b]Extractable in 2*M* KCl (Marr and Cresser 1983), [c]by Kjeldahl extraction (Marr and Cresser 1983), [d]by ascorbic acid blue colour method (Murphy and Riley 1962), [e]extractable in 1*M* ammonium acetate (pH 7.0), [f]leaching tube technique (Tinsley 1967), [g]hydrometer method (Piper 1944)

Two sets of pots were arranged separately under drought-stressed and unstressed conditions (control). The individual pots were put randomly in the net house of Soil, Water and Environment Department, Dhaka University. Five water soaked seeds of maize were sown in each pot and after five days of emergence only three seedlings were allowed to grow. Drought-stressed condition (60 % field capacity) was maintained gravimetrically and pots under unstressed condition received water daily in the morning. The height of the individual plant was measured from the soil level to the tip of the leaflet.

Harvesting and analysis: Six-week-old plants were harvested and separated into shoots and roots. Roots were washed and fine roots were kept in small vials in 50% ethanol solution for assessing the fungal infection. Shoots and roots were air-dried, oven-dried (65ºC) for 72 h, weighed, ground (< 1 mm) in a mechanical grinder and stored in air-tight polyethylene bags (16 cm × 10 cm). For total N analysis, 0.1 g ground shoot was digested with 5 ml concentrated sulphuric acid and 2 ml 4 % (v/v) solution of perchloric acid (62 %) in concentrated sulfuric acid. The digest was cooled and diluted to 100 ml with distilled water (Cresser and Parsons

1979). The uptake of N by shoot was determined using Kjeldahl semi micro steam distillation apparatus. The uptake of P by shoot was determined by the ammonium (acid molybdate-ascorbic acid-potassium antimony tartrate) molybdate blue colour method in a Cecil spectrophotometer (Murphy and Riley 1962).

For mycorrhizal colonization assessment, root pieces (1.5 cm long) were cleared in 0.5 *M* KOH for 30 min at 90ºC in a water bath, then rinsed in water and soaked in 0.06 *M* HCl for 24 h. After soaking, the roots were stained in an acidic glycerol solution containing 0.05% trypan blue for 30 min at 90ºC in a water bath. The roots were destained and stored in acidic glycerol (Koske and Gemma 1989). The stained root pieces were mounted on sterile membrane filters on a microscopic slide and a cover slip was placed on the top. The mounted root pieces were observed under a light microscope. The presence or absence of infection in the root pieces was recorded and then percentage infection was calculated (Giovannetti and Mosse 1980). Statistical analyses of the results were carried out and respective LSDs are indicated.

Results and Discussion

Effects on growth: Plant growth was assessed in terms of plant height and dry matter production. Plant heights varied significantly ($p < 0.5$). Plant heights (Table 2) were greater (except M4-60 kg P/ha) in sterile soil under both drought-stressed and unstressed conditions. The maximum height recorded in plants were grown in stressed and unstressed soils with 120 kg P/ha. While for non-sterile condition, the maximum heights obtained were under stressed and unstressed conditions at 60 kg P/ha application (Table 2). Heights generally increased due to addition of *G. mosseae* and *G. mosseae* plus phosphorus e.g. M2 (20.2,11.1,23.3 and 7.7%), M6 (29.6, 24.7, 15.5 and 13.3 %), M7 (20.0, 8.6, 16.3 and 20.2%) and M8 (27.7, 25.5, 25.3 and 14.4%) in sterile and non-sterile soils under drought stressed and unstressed conditions, respectively over control (M1) treatment. Addition of phosphorus possibly helped to increase the efficiency of *G. mosseae* and thereby height of plants.

Table 2. Effect of *G. mosseae* inoculation and phosphorus fertilization on the height (cm) of maize plants grown in sterile and non-sterile soil under drought-stressed and unstressed conditions.

Treatments	Drought-stressed condition		Unstressed condition	
	Sterile soil	Non-sterile soil	Sterile soil	Non-sterile soil
M1. Control (without AM and P)	61.3	58.7	65.2	60.9
M2. *G. mosseae*	73.7	65.2	80.4	65.6
M3. 30 kg P/ha	66.9	65.6	80.8	72.8
M4. 60 kg P/ha	76.2	77.0	74.5	77.9
M5. 120 kg P/ha	80.4	63.9	86.4	73.6
M6. *G. mosseae* + 30 kg P/ha	79.5	73.2	75.3	69.0
M7. *G. mosseae* + 60 kg P/ha	73.6	63.9	75.8	73.2
M8. *G. mosseae* + 120 kg P/ha	78.3	73.7	81.7	69.7
LSD at 5%	13.11	9.38	14.34	9.14

The dry weight of shoots and roots are presented in Table 3. The differences in values for shoot and root dry matter production varied significantly. Under drought-stressed condition, the shoot dry matter yields were recorded higher in plants grown in sterile soil (except M1. control) and similar trend were observed under unstressed condition. The highest values of shoot dry matter yield were 3.27, 2.71, 9.47 and 6.09 g/pot in M5, M8, M5 and M5 treatments in sterile and non-sterile soil under drought- stressed and unstressed conditions, respectively. The highest quantity of root dry matter were 1.68 in M8, 2.06 in M8, 5.08 in M5 and 3.51 g/pot in M5 in sterile and non- sterile soil under drought-stressed and unstressed conditions, respectively (Table 3). Generally shoot and root yields were also higher in *G. mosseae* plus phosphorus (M6, M7 and M8) over *G.mosseae* (M2) or control (M1) treatment. Application of both AM and P had positive effects on shoot and root dry matter production but the effect was more pronounced when P was added alone (M5) in a sterile soil under unstressed condition (Table 3). Kasarawa *et al*. (2000) reported that the effect of inoculation on maize growth and P uptake was distinct in dry soil in which AM colonization of roots occurred significantly more frequently in inoculated than in non-inoculated soil.

Table 3. Effect of *G. mosseae* inoculation and phosphorus fertilization on the dry weight of shoots (g/pot) and roots (g/pot) of maize plants grown in sterile and non- sterile soil under drought-stressed and unstressed conditions.

Treatments	Drought-stressed condition				Unstressed condition			
	Sterile soil		Non-sterile soil		Sterile soil		Non-sterile soil	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
M1. Control (without AM and P)	1.29	0.54	1.36	0.98	6.88	3.46	3.70	2.10
M2. *G. mosseae*	2.33	1.30	1.81	1.23	7.91	3.94	4.10	2.44
M3. 30 kg P/ha	1.91	1.15	1.79	1.19	8.58	4.19	5.11	2.91
M4. 60 kg P/ha	2.14	1.21	2.36	1.43	9.00	4.52	5.57	3.07
M5. 120 kg P/ha	3.27	1.48	2.51	1.58	9.47	5.08	6.09	3.51
M6. *G. mosseae* + 30 kg P/ha	2.69	1.41	2.08	1.76	7.97	4.03	4.42	2.55
M7. *G. mosseae* + 60 kg P/ha	2.74	1.48	2.32	1.79	8.18	4.24	4.81	2.77
M8. *G. mosseae* + 120 kg P/ha	2.85	1.68	2.71	2.06	8.41	4.61	5.43	3.37
LSD at 5%	0.56	0.75	0.69	0.56	1.58	0.97	1.03	0.52

Effects on N and P uptake: The uptake of N and P in shoots and roots are presented in Tables 4 and 5, respectively. In both the cases the variation, between the treatment values were significant. The highest amount of N uptake by shoots were 17.98, 29.54, 59.77 and 56.81 mg/pot in M5, M8,

Table 4. Effect of *G. mosseae* inoculation and phosphorus fertilization on the uptake of N (mg/pot) by the hoots of maize plants grown in sterile and non-sterile soil under drought-stressed and unstressed conditions.

Treatments	Drought-stressed condition		Unstressed condition	
	Sterile soil	Non- sterile soil	Sterile soil	Non- sterile soil
M1. Control (without AM and P)	9.67	14.82	46.48	39.59
M2. *G. mosseae*	14.21	13.57	48.25	38.95
M3. 30 kg P/ha	11.65	17.00	47.20	44.97
M4. 60 kg P/ha	8.77	25.72	36.90	56.81
M5. 120 kg P/ha	17.98	23.84	52.08	54.20

M6. *G. mosseae* + 30 kg P /ha	14.79	24.13	59.77	42.00
M7. *G. mosseae* + 60 kg P/ha	13.15	25.29	45.00	52.43
M8. *G. mosseae* + 120 kg P/ha	15.67	29.54	57.20	45.07
LSD at 5%	4.24	6.47	1.38	3.02

M6 and M4 and the highest P uptake by shoots were 5.56, 5.15, 16.2 and 10.96 mg/pot in M5, M8, M4 and M5 treatments in sterile and non-sterile soil under drought-stressed and unstressed conditions, respectively. These results indicate that the uptake of N and P were higher in *G. mosseae* plus phosphorus (M6, M7 and M8) over *G. mosseae* (M2) or control M1) treatment.

Assessment of mycorrhizal colonization: Mycorrhizal infection was significantly higher in *G. mosseae* treatment alone (Table 6). In sterile soil no infections were observed in plants that were not inoculated with *G.mosseae*. The degree of infection did not correlate well with growth, and N

Table 5. Effect of *G. mosseae* inoculation and phosphorus fertilization on the uptake of P (mg/pot) by the shoots of maize plants grown in sterile and non-sterile soil under drought-stressed and unstressed conditions.

Treatments	Drought-stressed condition		Unstressed condition	
	Sterile soil	Non-sterile soil	Sterile soil	Non-sterile soil
M1. Control (without AM and P)	2.06	2.18	11.0	5.92
M2. *G. mosseae*	3.96	3.08	14.24	7.38
M3. 30 kg P/ha	3.05	2.86	15.44	7.66
M4. 60 kg P/ha	3.42	4.25	16.20	9.47
M5. 120 kg P/ha	5.56	4.52	16.10	10.96
M6. *G. mosseae* + 30 kg P/ha	4.84	3.54	15.14	7.95
M7. *G. mosseae* + 60 kg P/ha	5.21	4.41	15.54	9.62
M8. *G. mosseae* + 120 kg P/ha	4.84	5.15	15.98	10.32
LSD at 5%	1.82	0.71	2.15	2.66

and P uptake. In this experiment, AM colonization was higher under drought-stressed condition than unstressed condition (Table 6). Soil moisture may reduce the efficiency of AM colonization in roots. Furthermore, a comparison of the N and P uptake values between drought-stressed and unstressed conditions showed that the influence of the AM inoculum on the N and P uptake was

Table 6. Effect of *G. mosseae* inoculation and phosphorus fertilization on the percentage AM colonization of roots grown in sterile and non- sterile soil under drought-stressed and unstressed conditions.

Treatments	Drought-stressed condition % root piece infection		Unstressed condition % root piece infection	
	Sterile soil	Non-sterile soil	Sterile soil	Non-sterile soil
M1. Control (without AM and P)	0.0	2.3	0.0	2.0
M2. *G. mosseae*	18.4	20.80	16.8	17.3

M3. 30 kg P/ha	0.0	2.7	0.0	1.8
M4. 60 kg P/ha	0.0	2.1	0.0	1.7
M5. 120 kg P/ha	0.0	1.9	0.0	1.5
M6. *G. mosseae* + 30 kg P/ha	16.7	18.1	16.3	17.2
M7. *G. mosseae* + 60 kg P/ha	13.6	14.7	13.2	14.0
M8. *G. mosseae* + 120 kg P/ha	10.5	11.8	8.4	10.6
LSD at 5%	0.72	0.99	0.90	1.24

attenuated with the increase in the soil moisture status (Tables 4 and 5). Soil moisture is known to affect the P uptake by maize (Olsen *et al.* 1961), as a low moisture status reduces P diffusion through the soil to the root surface (Hira and Singh 1977). Osonubi (1994) suggested that AM inoculation enhanced plant growth through the improvement of drought resistance as well as P nutrition in low **-** P soil under dry condition. The soil moisture level is markedly deficient in winter compared to ordinary water stress in Bangladesh. Further study are required to clarify the influence of the soil moisture status on soil N and P availability and efficiency of AM colonization of maize roots.

The experiment revealed that *G. mosseae* has positive effect on the growth of and N and P uptake by maize plants and can be regarded as an additional strategy for a more rational agricultural program.

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