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Interactive Effects of Endomycorrhizal Fungus *Glomus etunicatum* and Phosphorous Fertilization on Growth and Metabolic Activities of Broad Bean Plants under Drought Stress Conditions

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Abstract: The influence of arbuscular mycorrhizal fungus (*Glomus etunicatum*) and phosphorous fertilization was studied on growth and metabolic changes in broad bean plants (*Vicia faba*) grown under drought conditions. Addition of phosphate to the soil caused an increase in the growth of mycorrhizal and non-mycorrhizal plants. The applied drought stress reduced growth responses, net assimilation rate, mycorrhizal infection and nodulation of broad bean plants. Arbuscular mycorrhizal inoculation significantly increased dry matter production, photosynthetic pigments, phosphorous content, relative water content and alkaline phosphatase of broad bean compared to non-mycorrhizal plants, but these beneficial effects were significantly reduced with increasing soil phosphorous. In most instances, drought-stressed mycorrhizal plants had significant higher shoot dry weight, leaf area, nodule number, relative water content, acid and alkaline phosphatases and total soluble sugars relative to non-mycorrhizal plants particularly in P-deficient soil. Most of these parameters were unaffected by phosphorous fertilization, and therefore phosphorous had no effect on drought resistance of broad bean plants. So, *G. etunicatum* may be beneficial to the growth of broad bean plants exposed to soil water deficit.

Key words: Mycorrhiza, drought, phosphorous, broad bean, RWC, phosphatase, *Glomus etunicatum*

Introduction

Broad bean is one of the most potential food seed crops in Egypt. Reduction of plant growth is one of the most conspicuous effects of water restriction on the plant growth and is mainly caused by inhibition of leaf and stem elongation when water potential decreases below a threshold, which differs among species (Pelleschi *et al*., 1997, Younis *et al*., 2000). Root growth is less sensitive to a decrease in soil water potential than stem growth (Creelman *et al*., 1990), and this leads to the increase in the root/shoot ratio that is commonly observed in plants exposed to water deficit (Davies *et al*., 1993).

Several studies indicate that colonization of root system by arbuscular mycorrhizal fungi affords host plants greater resistance to drought stress (Nelsen and Safir, 1982; Graham *et al*., 1987; Bethlenfalvay *et al*., 1987; Davies *et al*., 1993; El-Tohamy *et al*., 1999; Auge, 2001, Davies *et al*., 2002). Possible mechanisms for improved drought resistance of VA-mycorrhizal plants include increased root hydraulic conductivity (Safir *et al*., 1972; Reichenback and Schonbeck, 1995; Meddich *et al*., 2000) stomatal regulation or transpiration rate (Allen *et al*., 1982; Allen and Boosalis, 1983), enhanced water uptake at low soil moisture levels due to extraradical hyphae (Reid, 1978; Hardie, 1985; Fagbola *et al*., 2001) osmotic adjustment that promotes turgor maintenance even at low tissue water potential (Auge *et al*., 1986), cell wall elasticity changes (Auge *et al*., 1987), increased photosynthetic activity, proline and carbohydrate accumulation (Azcon *et al*., 1996; Schellenbaum *et al*., 1999) and increased nutritional status in mycorrhizal plants (Azcon *et al*., 1996; Subramanian and Christiane, 1999). These mechanisms may be important in adaptation by the mycorrhizal plants to drought conditions. Conversely, mycorrhizal colonization did not affect osmotic adjustment, plant water status, water use efficiency or water uptake in safflower and wheat plant, and therefore had no effect on drought resistance (Bryla and Duniway, 1998). Increased drought resistance of mycorrhizal plants was in part attributed to drought induced colonization of mycorrhizae and the ability of the mycorrhizal plants to maintain high transpiration rates as a result of greater lateral root formation and lower shoot mass and a higher root/shoot ratio (Davies *et al*., 1996; Levy *et al*., 1983).

The objective of this study was to investigate the effects of interaction between arbuscular mycorrhizal fungi and phosphorous fertilization on growth and metabolic activities of broad bean plants subjected to drought stress.

Materials and Methods

Seeds of broad bean (*Vicia faba* var Giza 2, obtained from the Egyptian Ministry of Agriculture) were surface sterilized in 7% calcium hypochlorite for 15 minutes, subsequently rinsed with sterilized water and left to germinate for 4 days in moist sterilized filter paper. Uniform seedlings were planted (one plant/pot) in plastic pots (25 cm diameter) containing 1500 g autoclaved clay:sand (1:1, v/v) substrate. Both substrate components were collected from the Dakhlia province, Egypt. Soil characteristics were: pH (water) 7.8; 23 mg^{-1} available phosphorous kg^{-1}; 17 mg available nitrogen kg^{-1} ; 28 mg potassium kg^{-1} and 34 mg magnesium kg^{-1}) . Half of the pots received a mycorrhizal inoculum consisted of soil, spores and chopped roots of leek (*Allium porrum* L.) colonized by stock cultures of *G etunicatum*. The mycorrhizal fungus was originally isolated from Egyptian desert. The inoculum was placed 3 cm below the surface of the soil before sowing to produce mycorrhizal plants. The non-mycorrhizal treatment received filter leachates (1 ml $plant^{-1}$) from infected roots and an equal amount of sterilized soil inoculum to provide the same microflora without mycorrhizal fungi. Phosphorous applied in the form of K_2HPO_4 at 0 and 0.5 g kg^{-1} soil and then thoroughly mixed with the soil. All plants were inoculated with *Rhizobium leguminosarum* biovar *viciae*, watered regularly to near field capacity with tap water and fed with 32 mg S (K_2SO_4 solution) per plant at 4 weeks. The plants were grown in a greenhouse under controlled conditions (photon irradiance, 400 μmol $m^{-2}s^{-1}$; air temperature, 22/18°C; 16 h photoperiod; relative humidity, 70%). Forty five days after planting, half the plants were subjected to drought stress by withholding water for 16 days, while the control plants were irrigated to field capacity. The soil surface in each pot was covered with polythene beads to prevent evaporation. Eight replicates from each treatment were harvested after 8 and 16 days from withholding water.

Measurements: Fresh and dry weights of shoots and roots, shoot height, number of leaves, flowers and nodules were immediately determined. Leaf area was measured by Digital Planimeter KP-90 N (PLAKOM).

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined in the third upper leaf according to the method described by Harborne (1984).

Shoots and roots for each treatment were dried at 90°C for 48 h and then ground separately for phosphorous and total soluble

sugars estimation. Total phosphorous was analyzed by the vanado-molybdate colorimetric method in nitric acid (John, 1970). Total soluble sugars were determined by the anthrone method as described by Riazi *et al.* (1985).

Immediately after harvest, part of the root system was washed carefully in ice cold distilled water to remove the adhering soil particles and then extracted by macerating the detached roots and leaves in a pre-cooled mortar at 4°C using 0.1M borate buffer (pH 8.3) plus 0.1% glutathionate. The macerate was centrifuged at 48,000 g for 30 minutes and the soluble acid and alkaline phosphatases were determined according to Gianinazzi-Pearson and Gianinazzi (1976).

The remainder of the root system was cut (0.5-1.0 cm segments), cleared in 10% potassium hydroxide, stained with trypan blue in lactophenol (Phillips and Hayman, 1970) and examined microscopically for mycorrhizal colonization using the method of Trouvelot *et al.* (1986).

Soil water content and leaf relative water content were estimated as recommended by Ritchie *et al.* (1990). Net assimilation rate was calculated according to the following equation:

$$(W_2\text{-}W_1)\ (\ln S_2\text{-}\ln S_1)/\ S_2\text{-}S_1)\ (T_2\text{-}T_1)$$

where S_1 and S_2 are the assimilatory areas at times T_1 and T_2 respectively, and W_1 and W_2 are plant dry weights at times T_1 and T_2 respectively as recorded by Beadle (1993).

All results were analyzed by ANOVA and Duncan's multiple range test to determine the significance of differences among them. The correlation coefficients were estimated by using SPSS programme.

Results

Soil moisture content: Soil moisture contents of mycorrhizal and non-mycorrhizal broad bean plants were decreased as the duration of the drought stress period increased (Fig. 1). Under well watered conditions (control), soil moisture content of mycorrhizal plants were significantly greater than those in equivalent non-mycorrhizal plants. On the other hand, no significant differences in soil moisture content was observed between mycorrhizal and non-mycorrhizal plants in soil fertilized with phosphorous.

Under drought stress conditions, soil moisture content of mycorrhizal plants was significantly higher than non-mycorrhizal plants either in presence or absence of phosphorous treatments. Such increase in these contents in response to mycorrhizal effects was related to the levels of mycorrhizal infection.

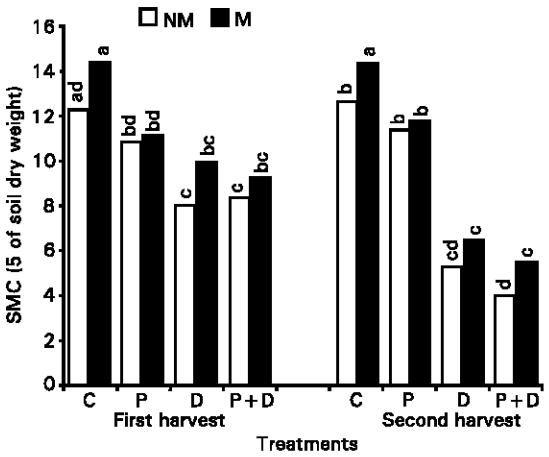

Fig. 1: Effect of drought stress on soil moisture content of mycorrhizal bean with and without addition of phosphorous. Bars at each harvest labeled with the same letter are not significantly different at P = 0.05. C, control; P, phosphorous; D, drought; SMC, soil moisture content.

Plant growth: Under well watered conditions, mycorrhiza significantly increased shoot biomass, leaf area, root nodules and flower number of broad bean plants compared with non-mycorrhizal plants when grown under low soil phosphorous (Table 1). Addition of phosphate to the soil increased the growth of all plants, while the beneficial role of mycorrhizal fungus in broad bean plants was not pronounced.

Drought stress significantly reduced shoot dry weight, net assimilation ratio, root nodules and leaf area of broad bean plants. This reduction was greatly offset by *G. etunicatum* stimulation of growth response as compared to non-mycorrhizal plants (Table 1). Root growth, in most cases, was not significantly affected by water stress, but mycorrhizal inoculation markedly decreased root:shoot ratio of stressed broad bean plants. At the same time, P addition mitigated to some extent the effect of water stress on growth criteria of mycorrhizal plants. No significant differences in root nodules were observed between mycorrhizal and non mycorrhizal stressed plants grown in soil amended with phosphorous at all harvests.

Table 1: Effect of mycorrhizal inoculation with *Glomus etunicatum* and soil phosphorous fertilization on growth criteria of broad bean plants grown under well watered and drought conditions

Days from withholding water	Treatments	Mycorrhizal colonization	Shoot d.wt.(g)	Root d.wt.(g)	Root /Shoot ratio	Shoot height (cm)	Leaves no./plant	leaf area (cm^2)	Nodule no./plant	Flowers no./plant	Netassimilation rate($g\ m^{-2}d^{-1}$
	Control	NM	1.0^{a}	0.56^{a}	0.56^{a}	30.90^{a}	9.33^{ad}	163.26^{a}	45.33^{ad}	0.00	-
		M	1.36^{b}	0.55^{a}	0.44^{a}	35.03^{a}	12.00^{b}	247.50^{b}	102.33^{b}	0.00	-
	Phosphorous	NM	1.63^{b}	0.77^{bd}	0.47^{a}	36.23^{b}	13.66^{c}	305.90^{c}	55.66^{d}	0.00	-
		M	1.58^{b}	0.68^{d}	0.43^{a}	33.73^{a}	12.66^{b}	204.50^{d}	74.33^{c}	0.00	-
8	Drought stress	NM	0.96^{a}	0.82^{b}	0.85^{b}	25.40^{c}	8.33^{a}	133.0^{e}	41.66^{ad}	0.00	-
		M	1.22^{b}	0.54^{ac}	0.44^{a}	28.33^{ac}	9.66^{a}	194.33^{d}	46.33^{ad}	0.00	-
	Drought stress +	NM	1.16^{ab}	0.50^{ac}	0.43^{a}	35.50^{ab}	10.33^{d}	254.86^{b}	40.66^{a}	0.00	-
	phosphorous	M	1.41^{b}	0.52^{ac}	0.37^{c}	29.13^{ac}	10.33^{d}	219.23^{d}	40.00^{a}	0.00	-
	Control	NM	1.87^{a}	0.82^{a}	0.43^{a}	46.00^{a}	11.33^{ac}	327.70^{ad}	93.66^{a}	7.33^{a}	3.68^{a}
		M	2.55^{b}	0.74^{a}	0.29^{bc}	47.33^{a}	13.00^{a}	378.56^{a}	111.66^{b}	15.66^{b}	3.80^{a}
	Phosphorous	NM	3.46^{c}	0.73^{a}	0.21^{b}	51.66^{a}	18.00^{b}	489.80^{b}	133.33^{c}	11.66^{c}	4.62^{b}
		M	3.21^{c}	0.73^{a}	0.22^{b}	49.00^{a}	13.00^{a}	377.70^{a}	138.66^{c}	15.00^{b}	5.76^{c}
16	Drought stress	NM	1.17^{d}	0.43^{b}	0.37^{ac}	30.33^{c}	10.33^{c}	135.50^{c}	55.33^{d}	3.00^{d}	1.66^{d}
		M	1.57^{ad}	0.46^{b}	0.29^{bc}	35.66^{b}	13.33^{a}	261.13^{d}	84.00^{a}	8.33^{a}	2.97^{a}
	Drought stress +	NM	1.79^{ad}	0.70^{a}	0.39^{ac}	38.33^{a}	11.00^{c}	231.56^{d}	69.00^{e}	3.66^{d}	2.56^{d}
	phosphorous	M	1.87^{a}	0.66^{a}	0.35^{ac}	39.66a	12.00^{c}	216.16^{d}	65.66^{e}	8.66^{a}	2.27^{d}

M, vesicular-arbuscular mycorrhiza; NM, non vesicular arbuscular mycorrhiza.

Values at each harvest, in each column and labeled with the same letter are not significantly different at P = 0.05.

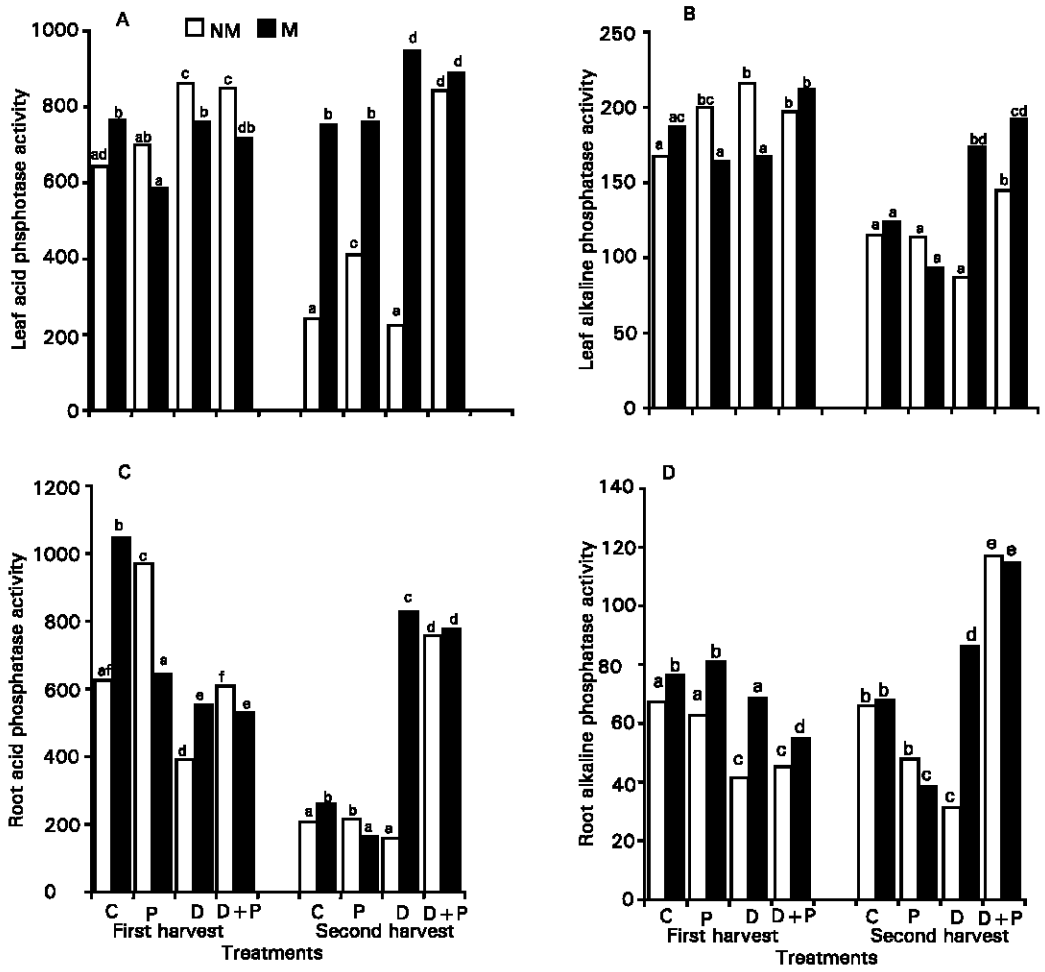

Fig. 2: Effect of *Glomus etunicatum* and soil phosphorous fertilization on acid and alkaline phosphatases (μmole PNP + min^{-1} + m^{-1}) of leaf (A,B) and root (C,D) of broad bean grown under well watered and drought stress conditions. control; P, phosphorous; D, drought stress. Bars at each harvest labeled with the same letter are not significantly different at P = 0.05.

Under drought stress, mycorrhizal fungus stimulated greater growth criteria compared with their non-mycorrhizal plants. Such increases in these criteria in response to mycorrhizal effect were positively correlated (r = 0.77 for shoot dry weight; r = 0.96 for total leaf area; r = 0.94 for nodules number and r = 0.96 for net assimilation ratio) with respective levels of mycorrhizal infection (Table 4). On the contrary, mycorrhizal infection was negatively correlated (r = -0.99) with mycorrhizal response for net assimilation rate of broad bean plants grown under well watered conditions.

Quantitative changes in phosphatase activities: In all treatments, soluble acid phosphatase activity was much higher than alkaline phosphatase and these activities were highly affected by mycorrhizal inoculation, phosphorous fertilization and drought stress, (Fig. 2). In most cases, total soluble acid and alkaline phosphatase activities in leaf (Fig. 2A and B) and root (Fig. 2C and D) extracts were significantly higher in mycorrhizal plants than non-mycorrhizal ones.

Phosphorous addition to soil, in general, significantly increase leaf and root acid phosphatase activities of non-mycorrhizal plants than mycorrhizal ones, whereas its effect on the alkaline phosphatase was not consistent. Drought stress significantly increased the acid and alkaline phosphatase activities in leaf extracts of non-mycorrhizal plants. On the other hand, mycorrhizal colonization greatly increased phosphatase activities in root extracts of droughted bean plants compared with non-mycorrhizal plants and the effect was more pronounced at 16 days from withholding water. Such increases of their activities in droughted root extracts in response to mycorrhizal effect were positively correlated (r = 0.97 for acid phosphatase, r = 0.82 alkaline phosphatase) with the degree of mycorrhizal infection. Generally, acid and alkaline phosphatase of mycorrhizal and non-mycorrhizal droughted plants were not affected by phosphorous fertilization particularly after 16 days from withholding water.

Relative water content: Under well-watered conditions, relative water content of broad bean plants was generally unaffected by mycorrhizal inoculation and phosphorous treatments (Table 2). On contrast, drought stress greatly reduced relative water content of both mycorrhizal and non-mycorrhizal bean plants and this reduction was more pronounced with increasing the stress period. However, mycorrhizal colonization appeared to improve relative water content of droughted bean plants compared with non-mycorrhizal plants at both harvests. Increases in water content of stress plants in response to the beneficial effects of the mycorrhizal fungus were positively correlated (r = 0.71), (Table 4) with mycorrhizal infection.

Table 2: Effect of *Glomus etunicatum* and soil phosphorous fertilization on relative water content, photosynthetic pigments, total soluble sugars and shoot phosphorous of broad bean plants grown under well watered and drought conditions

Days from withholding water	Treatments	Mycorrhizal colonization	RWC (%)	Photosynthetic pigments (mg g^{-1} d.wt.)				Total soluble sugars (mg g^{-1} d.wt.)			Shoot P (mg g^{-1} d.wt.)
				Chl. a	Chl. b	Carot.	Total chl/carot.	Leaf	Stem	Root	
	Control	NM	95.22^{a}	9.24^{a}	4.63^{a}	2.79^{a}	4.64^{ad}	40.16^{a}	15.71^{a}	15.30^{a}	1.83^{a}
		M	93.82^{a}	9.52^{b}	5.56^{b}	3.35^{b}	4.50^{a}	79.08^{b}	24.70^{b}	21.91^{b}	2.31^{b}
	Phosphorous	NM	94.42^{a}	11.11^{c}	5.92^{c}	3.22^{c}	5.29^{b}	52.79^{c}	20.08^{c}	17.20^{a}	5.92^{c}
8		M	86.85^{a}	8.30^{d}	4.68^{a}	2.91^{a}	4.46^{a}	64.58^{d}	37.71^{d}	22.64^{b}	6.20^{c}
	Drought stress	NM	82.85^{b}	7.18^{e}	3.99^{d}	2.66^{d}	4.20^{c}	59.62^{c}	29.79^{e}	18.30^{a}	1.25^{d}
		M	89.82^{ab}	9.85^{f}	5.58^{b}	3.00^{e}	5.14^{b}	64.03^{d}	32.81^{cd}	23.16^{b}	1.84^{a}
	Drought stress + phosphorous	NM	69.35^{c}	8.21^{d}	4.29^{e}	2.58^{d}	4.84^{d}	69.40^{e}	24.26^{b}	26.42^{c}	4.51^{e}
		M	72.22^{c}	9.80^{f}	4.99^{f}	3.25^{bc}	4.55^{a}	65.75^{de}	36.83^{d}	30.97^{d}	5.11^{f}
	Control	NM	88.25^{a}	7.26^{a}	4.05^{a}	1.72^{a}	6.58^{a}	40.87^{a}	18.40^{a}	15.75^{a}	1.35^{a}
		M	88.67^{a}	9.52^{b}	4.79^{b}	2.70^{b}	5.30^{b}	41.54^{a}	19.22^{a}	19.66^{b}	1.78^{b}
	Phosphorous	NM	88.03^{a}	7.99^{c}	4.43^{c}	2.07^{a}	6.00^{ad}	54.12^{b}	21.22^{a}	18.25^{b}	3.22^{c}
		M	93.75^{a}	7.14^{a}	3.59^{d}	1.94^{ad}	5.53^{bd}	52.46^{b}	25.04^{a}	26.10^{c}	3.15^{c}
16	Drought stress	NM	48.93^{b}	4.51^{d}	2.54^{e}	2.35^{c}	3.00^{c}	51.33^{b}	54.33^{b}	17.66^{b}	0.71^{d}
		M	58.78^{c}	6.44^{e}	3.51^{d}	2.23^{cd}	4.46^{c}	52.25^{b}	78.96^{ce}	47.00^{d}	1.30^{a}
	Drought stress + phosphorous	NM	49.25^{d}	7.56^{f}	4.60^{b}	1.92^{a}	6.33^{a}	50.77^{b}	74.63^{c}	34.20^{e}	2.54^{e}
		M	55.21^{d}	4.67^{d}	2.65^{e}	1.63^{a}	4.49^{c}	68.25^{c}	83.87^{de}	49.07^{d}	2.80^{e}

RWC, relative water content; Chl a, chlorophyll a; chl b, chlorophyll b; carot, carotenoids ;TSS, total soluble sugars.
Values at each harvest, in each column, and labeled with the same letter are not significantly different at P = 0.05.

Table 3: Effect of drought stress on levels of mycorrhizal infection of broad bean plants grown in soil with or without soluble phosphate added

		Days from withholding water					
		8 days			16 days		
Treatments	Mycorrhiza	F (%)	M (%)	A (%)	F (%)	M (%)	A (%)
Control	NM	00.0^{a}	00.0^{a}	00.0^{a}	00.0^{a}	00.0^{a}	00.0^{a}
	M	78.1^{b}	50.1^{b}	42.8^{b}	83.3^{b}	58.8^{b}	43.6^{b}
Phosphorous	NM	00.0^{a}	00.0^{a}	00.0^{a}	00.0^{a}	00.0^{a}	00.0^{a}
	M	31.3^{c}	19.3^{c}	17.0^{c}	39.2^{c}	21.2^{c}	18.8^{c}
Drought stress	NM	00.0^{a}	00.0^{a}	00.0^{a}	00.0^{a}	00.0^{a}	00.0^{a}
	M	47.7^{d}	29.3^{d}	22.6^{d}	65.8^{d}	43.0^{d}	29.6^{d}
Drought + Phosphorous	NM	00.0^{a}	00.0^{a}	00.0^{a}	00.0^{a}	00.0^{a}	00.0^{a}
	M	43.0^{d}	22.9^{c}	20.2^{cd}	50.6^{e}	24.9^{e}	20.1^{c}

F, frequency of mycorrhizal infection. M, intensity of cortical root colonization. A, extent of arbuscular development.
Values in each column labeled with the same letter are not significantly different at P = 0.05.

Table 4: Values of r for correlations between intensity of mycorrhizal infection (M%) and mycorrhizal growth response (MR[a] for several parameters of broad bean plants grown in soil with or without drought conditions

Parameters	-Drought	+Drought
Shoot f.wt.	0.75*	0.56
Shoot d.wt.	0.97**	0.74*
Root f.wt.	0.69	0.21
Root d.wt	-0.07	0.18
Shoot length	0.79*	0.70*
Leaves number	0.84**	-0.41
Total leaf area	0.83**	0.96**
Net assimilation rate	- 0.98**	0.77*
Nodules number	0.49	0.94**
RWC	- 0.065	0.71*
Total chlorophylls/carotenoids	-0.39	0.88**
Leaf total soluble sugars	0.23	- 0.61
Stem total soluble sugars	-0.45	0.25
Root total soluble sugars	-0.33	0.98**
Leaf acid phosphatase	0.57	0.70*
Leaf alkaline phosphatase	0.95**	0.74*
Root acid phosphatase	0.74*	0.97**
Root alkaline phosphatase	0.10	0.82**

MR = (M-NM)/ NM ×100, where M is the parameter value of mycorrhizal, plants and NM is parameter value of non-mycorrhizal plants.
* P = 0.05 ** P = 0.01

Total soluble sugars: Under well watered conditions, mycorrhizal bean plants had significantly higher leaf, stem and root total soluble sugars than non-mycorrhizal plants in soil amended with or without phosphorous particularly after 8 days from withholding water (Table 2).

Compared to well watered plants, drought stress significantly increased total soluble sugars of mycorrhizal and non-mycorrhizal plant tissue. Mycorrhizal colonization increased these contents of total soluble sugars than stressed non-mycorrhizal plants regardless of soil phosphorous fertilizations. Such increases in these contents of droughted bean root in response to mycorrhizal effect were significantly positively correlated (r =0.98) with degree of mycorrhizal infection. At the same time, total soluble sugars in mycorrhizal and non-mycorrhizal droughted plant tissues were generally increased by phosphorous fertilization in relation to other treatments.

Photosynthetic pigments: In general, the contents of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in leaves of mycorrhizal plants were significantly greater than those in non-mycorrhizal plants under well watered conditions (Table 2). In addition, phosphorous fertilization appeared to increase chlorophyll a and b contents to some extent especially in non-mycorrhizal plants, but reduced that of the mycorrhizal plants.

Drought stress significantly reduced Chlorophyll a and b contents of bean plants and the effect was more elicited at 16 days from withholding water. On the other hand, carotenoid content was not significantly affected by water stress treatments. In most cases, these contents were significantly increased in mycorrhizal plants grown in P deficient soil compared with non mycorrhizal plants. The increase in total chlorophylls/carotenoids concentrations of droughted plants in response to mycorrhizal effects was positively correlated (r =0.88) with respective levels of mycorrhizal infection.

In this connection, mycorrhizal colonization appeared to stimulate these sugars, in most cases, in plant organs of droughted bean plants compared with non-mycorrhizal plants, this effect was more pronounced at 16 days from withholding water.

Phosphorous content: Addition of phosphorous to the soil increased shoot phosphorous content of mycorrhizal and non-mycorrhizal plants grown under well watered or drought stress conditions (Table 2). Under the same conditions, shoot P content of mycorrhizal and non-mycorrhizal plants, in most cases, were not affected by phosphorous fertilization.

Compared to well-watered plants, drought stress reduced P content in mycorrhizal and non-mycorrhizal plants. However, phosphorous content of mycorrhizal plants was significantly greater than non-mycorrhizal plants grown in soil either subjected or not to drought stress. Such increases in the nutrient content were positively related with intensity of mycorrhizal infection.

Assessment of mycorrhizal root colonization: Drought stress and phosphorous fertilization significantly reduced the frequency of infection, intensity of mycorrhizal cortical root infection and arbuscular development within roots of bean plants with relative to well-watered mycorrhizal plants (Table 3). However, reduction in these levels of mycorrhizal infection was the most pronounced with phosphorous fertilization alone or compared with other treatments.

With phosphorous fertilization, no significant differences in the intensity of cortical colonization (8 days from withholding water) and the extent of arbuscular development (16 days from withholding water) in the root tissues were observed between droughted and well watered bean plants. No mycorrhizal colonization was observed in root segments from non-mycorrhizal plants.

Discussion

Drought stress significantly reduced growth biomass of broad bean plants, and the effect was most pronounced after 16 days from water holding. Water stress is known to induce loss of turgor which affects the rate of cell expansion and ultimately cell size and consequently it decreases growth rate, stem elongation, leaf expansion and stomatal aperture (Hale and Orcutt, 1987). The reduction of dry matter production under water stress conditions was reported to be mainly due to the reduction of leaf area and net photosynthesis and the increase in the rate of photorespiration (Younis *et al.*, 2000).

Under drought conditions, broad bean plants inoculated with *Glomus etunicatum* showed increased growth biomass, leaf area, photosynthetic pigments, net assimilation rate and root nodules compared to non-mycorrhizal plants. Such increases were related to the degree of mycorrhizal infection. These observations are in agreement with other previous investigators with different plants (Nelson and Safir, 1982; Levy *et al.*, 1983; McGraw and Miller, 1986; Udaiyan *et al.*, 1997; El-Tohamy *et al.*, 1999) who found that mycorrhizal plants were more drought tolerant than non-mycorrhizal plants, when exposed to several periods of drought stress. In this connection, Graham *et al.* (1987) suggested that higher transpiration rates of mycorrhizal infected plants may have depleted soil water more quickly than the non-mycorrhizal plants and resulted in more severe water stress conditions during drought periods. On the contrary, growth and yield of water-stressed plants were unaffected by mycorrhizal inoculation (Hetrik *et al.*, 1986b; Schellenbaum *et al.*, 1999).

The root/shoot ratio of droughted plants inoculated with mycorrhizal fungus was significantly decreased as compared with non-mycorrhizal plants, and this indicates the enhanced host drought tolerance by *G. etunicatum*. In this connection, Davies *et al.* (1996) reported that mycorrhizae tended to alter root morphology and carbon allocation patterns of shoots and roots. Increased drought resistance of mycorrhizal plants was in part attributed to drought-induced colonization by mycorrhizae and the ability of the mycorrhizal plants to maintain higher transpiration rates (Levye *et al.*, 1983) as a result of greater lateral root formation, lower shoot mass and leaf abscission.

Our results show that shoot phosphorous content of mycorrhizal plants was significantly greater than those of non-mycorrhizal plants particularly in deficient P soil. The interaction of mycorrhiza and drought stress showed that mycorrhiza was more beneficial to P uptake of stressed plants compared with non-stressed plants. These data support the hypothesis that enhanced water relations of mycorrhizal plants resulted from improved P uptake (Nelson and Safir, 1982; Bethlenfalvay *et al.*, 1987). In addition, Graham *et al.* (1987) concluded that the increased P status of mycorrhizal plants is responsible for changes in stomatal conductance of citrus plants. The reduction in stomatal resistance was attributed to altered hormone balance or other physiological response to the mycorrhizal fungus (Allen and Boosalis, 1983). In other studies, mycorrhiza increased P nutrition of plants and enhanced water conductivity were attributed at least in part to increased surface area for water uptake provided by mycorrhizal hyphae in soil (Allen *et al.*, 1981). Hyphae may also bridge the gap between soil and root that occurs when dry soil and root shrink away from each other. In addition, Davies *et al.* (1993) concluded that drought resistance in VAM droughted plants was not attributable to leaf P concentration. More extraradical hyphae developed on VAM droughted plants which have facilitated water uptake during high stress.

In our study, leaf relative water content and soil water extraction of stressed plants were enhanced by *G. etunicatum* particularly in deficient P soil. This data supports the hypothesis that mycorrhiza significantly enhance water relations of plants under the drought stress conditions (Levi *et al.*,1983, Busse and Ellis, 1985, Auge *et al.*, 1986b; El-Tohamy *et al.*, 1999; Meddich *et al.*, 2000). In contrast to these results, Graham *et al.* (1987) reported that under drought stress conditions, water relations of citrus plants were unaffected by mycorrhizal colonizations. In the same pattern, mycorrhizal colonization did not affect osmotic adjustment, plant water status, water use efficiency or water uptake in safflower and wheat plants and therefore had no effect on drought resistance (Bryla and Duniway, 1998). The major difference between the present study and those cited above perhaps is due to these fungi were not originally isolated from drought native soil.

It is evident from the present study that mycorrhizal stressed plants had higher total soluble sugars than non-mycorrhizal plants regardless of soil phosphorous. Similarly, Schellenbaum *et al.* (1999) found that the content of fungal disaccharide was greatly increased in the roots of all mycorrhizal plants upon exposure to drought. The accumulation of these solutes in mycorrhizal plants may serve as osmoregulators, which is an important adaptation of plants to drought stress.

Drought stress significantly decreased mycorrhizal colonization, and a significant interaction between drought and P effects on the reduction of the mycorrhizal colonization was observed. In this connection, drought caused a reduced elongation rate of parent roots (Fagbola *et al.*, 2001). On the contrary, water stress and P fertilization did not affect root infection (Nelson and Safir, 1988) but decreased fungal reproduction as determined by number of spores in the soil.

A significant decrease in the levels of mycorrhizal infection and also their efficiency in bean plants were observed with phosphorous amendment. These results agree with the previous report (Amijee *et al.*, 1982) or decrease in the density of internal infection (Gianinazzi-Pearson and Gianinazzi, 1986).

Under drought stress conditions, acid and alkaline phosphatase activities were significantly higher in mycorrhizal than non-mycorrhizal root extracts of broad bean plants grown in P-deficient soil. These were in agreement with those of Ezawa and Yoshida (1994) and Abdel-Fattah (2000) who found that infection specific phosphatase was detected only in the mycorrhizal root extracts and there is strong evidence that it is of fungal origin. The close relation between mycorrhizal specific phosphatase activity

and the amplitude of the mycorrhizal growth response supports the hypothesis that this enzyme is somehow involved in the assimilation of phosphorous by arbuscular mycorrhizal fungi (Abdel-Fattah, 1997). On the other hand, in most cases, no significant differences were detected in phosphatase activities between mycorrhizal and non-mycorrhizal stressed and fertilized plants. These observations are in agreement with other previous studies (Mitchell and Read, 1981; Abdel-Fattah, 1997) who demonstrated a consistent inverse relationship between phosphorous concentration and mycorrhizal infection and their phosphatase activities in associated plants.

Improved drought tolerance is critical to successful crop productivity when water is a limiting factor. The present results concluded that colonization of broad bean root system by *G. etunicatum* affords the host plants greater resistance to drought stress. Mycorrhizal plants may tolerate drought through enhanced water uptake and improved phosphorous nutrition of stressed plants.

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