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Influence of arbuscular mycorrhiza and phosphorus fertilization on the gas exchange, growth and phosphatase activity of soybean (*Glycine max* L.) plants

G.M. ABDEL-FATTAH[*,**,+], A.A. ASRAR[*], S.M. AL-AMRI[***], and E.M. ABDEL-SALAM[*]

Plant Production Department, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia[*]
Botany Department, Faculty of Science, Mansoura University, Egypt[**]
Department of Biology, College of Science and Art, Shaqra University, Saudi Arabia[***]

Abstract

We studied the effect of arbuscular mycorrhizal (AM) fungus, *Glomus constrictum* (Trappe), and soil phosphorus (P) on gas-exchange parameters, growth, and nutrition of soybean plants grown in pots with sterilized soil. Two contrasting concentrations of KH_2PO_4, *i.e.* no added and 0.5 g(P) kg^{-1}(soil), were used. Addition of soluble phosphate increased all growth parameters, P and N concentrations, and most of the studied photosynthetic parameters of both the mycorrhizal and nonmycorrhizal plants. The mycorrhizal inoculation significantly increased plant growth responses, P and N concentrations in shoot and root tissues, acid and alkaline phosphatase activities, and total soluble proteins in root tissues compared with the nonmycorrhizal plants. The stimulations were related to the level of the mycorrhizal colonization in the root tissues. The mycorrhizal plants showed significantly higher net photosynthetic rate, stomatal conductance, and transpiration rate than those of nonmycorrhizal plants, especially in soil without added P. The phosphate addition to soil reduced generally the percentage of the mycorrhizal colonization in the root tissues, and consequently the mycorrhizal benefits. In general, growth, nutrition, and photosynthetic parameters of the soybean plants showed a high degree of dependency on the mycorrhizal fungus in nonfertilized soil when compared with the soil fertilized with P. This study confirmed that AM colonization could improve growth and nutrition of the soybean plant through increasing photosynthesis in leaves, particularly at low P in soil.

Additional key words: arbuscular mycorrhizal symbiosis; depletion zone; inoculum; root/shoot ratio.

Introduction

Phosphorus (P) is an extremely important mineral for plant nutrition and its deficiency can greatly limit plant growth. For many plants, arbuscular mycorrhizal (AM) associations are one way of acquiring adequate supplies of P from soils (Gianinazzi *et al.* 1983, Abdel-Fattah 2001, Al-Amri *et al.* 2013, Sheng *et al.* 2013). AM symbiosis is particularly important for plants growing in soils with low available P (Smith *et al*. 1986, Abdel-Fattah 1997, Ibrahim *et al.* 2011). It increased the rate of plant growth and P concentration in plant tissues (Blal *et al.* 1990, Abdel-Fattah and Asrar 2012, Sheng *et al.* 2013). Alkaline phosphatase (ALP) has been identified as an active enzyme for AM by using gel electrophoresis (Abdel-Fattah 2001) and ultracytochemistry (Gianinazzi-Pearson and Gianinazzi 1978). It is generally admitted that ALP is involved in P acquisition by mycorrhizal plants, as demonstrated by its presence in the vacuoles of mature arbuscules revealed by ultracytochemical studies (Abdel-Fattah 2001).

Soybean (*Glycine max* L.) is one of the potential oil seed crops in Saudi Arabia (Al-Kahtani 1989). Raising soybean yield through increasing the productivity per unit of area, as well as expanding the cultivated area in newly

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[+]Corresponding author; tel.: (966) 502001457, fax: (966) 014678467, e-mail: Abdelfattaham@yahoo.com
Abbreviations: P_N – net photosynthetic rate; ALP – alkaline phosphatase; AM – arbuscular mycorrhizal; AMF – arbuscular mycorrhizal fungi; AMR – arbuscular mycorrhizal response; DM – dry mass; E – transpiration rate; g_s – stomatal conductance; LA – leaf area; MSPase – mycorrhizal-specific phosphatase, NAMF – nonarbuscular mycorrhizal fungi; PNPP – p-nitrophenol phosphate; R/S – the ratio of root to shoot.
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reclaimed lands, is one of the major important goals. Increased growth and yield of soybean in the presence of AM fungi (AMF) has been attributed mainly to the enhanced uptake of P (Smith *et al.* 2004, Arriagada *et al.* 2007, Smith *et al.* 2011, Abdel-Fattah and Asrar 2012). Improving plant acquisition of phosphate by AMF depends on the particular plant-fungus combination, symbiotic phosphate uptake may partially participate or dominate over all phosphate acquisition (Smith *et al*. 2003). In addition to highly reduced mobility of phosphate in the soil, rapid phosphate uptake into the root leads to development of a phosphate depletion zone, causing a new pool of soluble phosphate (Abdel-Fattah 1997, Arriagada *et al.* 2007). Whereas in nonmycorrhizal roots, the phosphate depletion zone greatly exceeds the root hair cylinder. This indicates that phosphate is not directly available to the plant. In fact, the external hyphae of AMF can absorb phosphate beyond the depletion zones around the root hairs and transport it to the root tissues (Smith and Gianinazzi-Pearson 1988, Abdel-Fattah 1997, Wu *et al.* 2010). AMF are also strongly implicated in transferring N from one plant to another (He *et al.* 2003) or taking up N directly and transferring it to the host roots (Ibrahim *et al.* 2011), increasing also the utilization of different N forms (Ames *et al.* 1983, Hodge *et al.* 2001).

AMF induced changes in whole-plant and root hydraulic conductance (Asrar *et al.* 2012). Colonization of plant roots and soils by AMF is often accompanied by increases in stomatal conductance (g_s) and transpiration rate (E) in host plants (Auge 2001, Cho *et al.* 2006, Auge *et al.* 2008). The activity of acid and alkaline phosphatase, enzymes catalyzing the hydrolysis of phosphate complex forms, can be enhanced by AMF colonization (Gianinazzi-Pearson and Gianinazzi 1978, Abdel-Fattah and El-Katony 1996). Furthermore, Ezawa and Yoshida (1994) stated that mycorrhizal-specific phosphatase (MSPase) was detected only in the mycorrhizal root extract, and there was strong evidence that it was of fungal origin (Graham and Syvertsen 1984, Abdel-Fattah 1997, Daei *et al.* 2009). Phosphate fertilizers are routinely used in intensive agricultural practices to maintain soil P fertility level. Thus, phosphate fertilizers make a significant contribution to current global food production and security. However, residual P from repeated applications of high rates of mineral P to soil increases soil P saturation concentration and general P fertility, which in turn inhibits AMF development (Smith *et al.* 2011, Vaseghmanesh *et al.* 2013).

Although the role of mycorrhizal fungi in improving growth and some metabolic processes of plants is well studied, few studies are available on the functional activity of AMF regarding the gas-exchange status of plants. Therefore, the aim of this work was to study the effects of the AMF, *G. constrictum* (Trappe), on growth, P and N concentrations, acid and alkaline phosphatases, and gas-exchange parameters of soybean plants grown under two concentrations of soil P. The changes in gas exchange (E, g_s, and P_N) in soybean leaves due to efficiency of AMF and their stimulation in plant growth and nutrition were also discussed.

Materials and methods

Experimental design: The experiment with a 2 × 2 factorial design was carried out. The experimental treatments consisted of two AMF treatments – *G. constrictum* inoculated (AMF) and noninoculated (NAMF) plants both grown either with added phosphate [P0.5, 0.5 g(P) kg^{-1}(soil)] or without (P0). Each of the four treatments was replicated ten times (one plant per pot) to give a total of 40 pots.

AMF inoculum preparation: The AMF inoculum, consisting of spores, soil, hyphae, and infected root fragments of onion plants (*Allium cepa* L.) from a stock culture of *G. constrictum* (Trappe), was provided by the stock mycorrhizal cultures of the Experimental Station of Plant Production Department, College of Food and Agriculture Sciences, King Saud University. The AMF inoculum consisting of 20 g of rhizosphere soil (approx. 950 spores) and 0.5 g of infected onion root fragments with an infection level of 75.5% were inoculated to each pot.

Plant and growth conditions: Seeds of soybean (*Glycine max* L.) were surface sterilized in 7% sodium hypochlorite for 10 min, subsequently rinsed with sterilized distilled water, and left to germinate for 48 h on moistened sterilized filter paper in the dark at 25ºC. Uniform germinated seedlings were transplanted (one plant per pot) into plastic pots (25 cm in diameter) containing 3.5 kg of autoclaved sandy-loam soil (70% sand, 20% silt, and 10% clay). Soil characteristics were: pH 7.61; organic matter of 0.43%; available N of 25.0 mg kg^{-1}; available P of 7.02 mg kg^{-1}; potassium of 52 mg kg^{-1}; magnesium of 85 mg kg^{-1}, and electrical conductivity of 0.35 dS m^{-1}. Pots were adjusted to two different P concentrations described before. KH_2PO_4 was thoroughly mixed with the soil during planting. Half of the pots were inoculated with the AMF *G. constrictum* (20 g of soil stock culture and 0.5 g of infected onion root per pot). The AMF inoculum was placed at 3-cm-depth in the soybean seedling medium at planting time. The NAMF-treated plants were supplied with filtered washings of an equal amount of the mycorrhizal soil inoculum to provide the same associated microorganisms other than mycorrhizal propagules. Pots were arranged in a complete randomized design in a greenhouse under controlled conditions with light intensity of 450 µmol(photon) m^{-2} s^{-1}, 25/20ºC day/ night temperature, 70–80% relative humidity, and 16-h photoperiod. All plants were regularly irrigated as needed with tap water. Four weeks after planting, all plants received 32 mg of K_2SO_4 per pot as a nutrient solution. All plants received a

standard inoculum of *Rhizobium japonicum*, strain G3, after four weeks from planting. Ten plants (five plants for functional and chemical analysis and the other five for mycorrhizal assessment) from each treatment were harvested ten weeks after planting.

Growth parameters: At harvest, shoot height per plant was recorded. Shoots and roots were oven dried at 80ºC for 24 h and weighted and root/shoot (R/S) ratios were determined. Leaf area was measured using a leaf area meter (*Li-Cor*, Lincoln, NE, USA). AMF growth responses (AMR) was defined as a percentage of a plant growth subjected to AMF application and calculated for every studied parameter using the formula of Menge *et al.* (1978) and modified by Son and Smith (1988) as follows: $(DM_m - DM_{nm}/DM_{nm}) \times 100$, where DM_m represents the dry mass of mycorrhizal plants and DM_{nm} the dry mass of nonmycorrhizal plants.

Photosynthesis, transpiration, and stomatal conductance: After 40 days from planting, P_N, E, and g_s of the fully expanded, third leaves were measured using *Li-Cor 6400XT* (*Li-Cor*, Lincoln, NE, USA). A leaf was fitted into a 6-cm^2 leaf chamber. Gas-exchange measurements were made in the growth chamber under saturated light conditions. All measurements were made at light intensity of 1,800 μmol(photon) m^{-2} s^{-1}, a leaf temperature of 27°C, and a leaf-to-air vapor pressure deficit of 1.1 ± 0.1 kPa. Five replicates were used for each treatment.

Determination of P and N: Shoot and root samples from the five randomly chosen plants were oven-dried, ground and sieved through a 0.5-mm sieve. A known mass of the grounded material was digested in a digestion flask containing a triple acid mixture [HNO_3:H_2SO_4:HCl (60%), with a ratio of 10:1:4, respectively] to analyze the total P. P was analyzed using the vanadate-molybdate colorimetric method (Jackson 1973). Total N was measured in samples of 0.1 g dry mass using Kjeldahl method (Nelson and Sommers 1973). Each sample was heated in a digestion tube for at least 8 h with concentrated sulfuric acid (98.8%). Distillation of the completely digested samples was carried out using an aqueous solution of sodium hydroxide (40%). The extracted ammonium was dissolved in 15 ml of boric acid and then automatically titrated with 25 mmol L^{-1} sulfuric acid using bromocresol green-methyl red mixture as an indicator.

AMF colonization: After harvest, 30 root segments of 0.5–1 cm were cut from five plants per treatment. Root samples were cleared with 10% KOH solution and stained with 0.05% Trypan blue in lactophenol (Phillips and Hayman 1970). The stained segments were placed on slides and were observed with a microscope (*Carl Zeiss*, Italy) at 40× magnification. They were visually allocated to six classes of AMF colonization (from 0 to 5, depending on the occurrence of AMF structure in the root segment) and to four levels of AMF abundance (from A0 to A3). Mycorrhizal colonization levels of the stained roots were estimated by the method of Trouvelot *et al.* (1986) according to the frequency of mycorrhizal colonization (F [%]), the intensity of colonization (M [%]), and the rate of arbuscular development (A [%]) using the *Mycocalc* software (http://www.dijon.inra.fr/mychintec/ mycocalc-prg/download.html).

Acid and alkaline phosphatases and total soluble protein: Immediately after harvest, a 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 in pre-cooled mortar at 4ºC using 0.1 M borate buffer (pH 8.3) with 0.1 ml of glutathione. The macerate was centrifuged at 73,000 × g for 30 min and the soluble acid and alkaline phosphatases were determined according to the method of Gianinazzi-Pearson and Gianinazzi (1976). Activities of phosphatases were expressed as the unit of enzyme activity which transforms 1 μmol of the substrate under specific experimental conditions. Total soluble protein in root extract was determined by the method of Bradford (1976). Protein concentration was determined against a standard curve with bovine serum albumin (*Sigma-Aldrich Chemie*, Steinheim, Germany).

Statistical analysis: Data were subjected to statistical analysis using two way factorial analysis of variance (*ANOVA*). Means were separated by *Duncan*'s multiple range tests ($P \leq 0.05$) method using the *Costat* software (*Cohort*, Berkeley, CA, USA). All the measurements were performed five times for each treatment as the ten harvested plants divided into two sets of five plants each (as mentioned above), and the means and calculated standard error (SE) were reported.

Results

Changes in growth responses: Addition of P improved growth parameters (shoot and root DM, LA, and shoot height) of both AMF and NAMF soybean plants (Table 1). Inoculation of the plants increased significantly the growth parameters regardless of the P0.5 plants as compared with the NAMF plants. However, the stimulations in growth parameters due to the AMF colonization (AMR) were more pronounced in the plants grown in P0 soil (control). The R/S DM ratio of AMF plants was significantly lower than that of the NAMF ones grown in P0 soil (Table 1). No significant differences in R/S ratio were observed between AMF and NAMF soybean plants grown in soil with P0.5.

Gas-exchange parameters: In general, g_s, P_N, and E in

leaves of the AMF plants were significantly higher than those in the NAMF plants grown either in P0.5 or P0 soil (Table 2), and the effects were more remarked in P0 soil. Such stimulations in gas-exchange parameters were linked to the degree of the mycorrhizal colonization for each treatment. The differences in g_s, P_N, and E were not significant between the AMF plants grown in P0.5 and P0 soil.

Nutrient contents: Generally, P and N concentrations in shoots and roots of the AMF and NAMF soybean plants increased with P addition to the soil. However, the presence of AMF significantly increased P and N concentrations in shoots and roots compared with those of NAMF plants, particularly in P0 soil (Table 3). Such increases in nutrient contents in response to the mycorrhizal effects were highly associated, respectively, with the intensity of the mycorrhizal colonization in each treatment.

Mycorrhizal colonization levels: Both the intensity of the mycorrhizal colonization (M) and the arbuscular frequency (A) significantly decreased in soybean root tissues with addition of P to the soil (Table 4). However, no significant differences were observed in the frequency of mycorrhizal colonization (F) between AMF plants grown either in P0.5 soil or P0. No mycorrhizal colonization was observed in the noninoculated soybean plants.

Acid and alkaline phosphatase activities: In all treatments, soluble acid phosphatase activity was much higher than alkaline phosphatase, and those activities were elevated, in most cases, by the mycorrhizal inoculation (Table 5). Addition of soluble phosphate to soil decreased the activities of acid and alkaline phosphatase in mycorrhizal and nonmycorrhizal soybean root extracts. Regardless of P concentration, soluble acid and alkaline phosphatase activities in root extracts were significantly higher in mycorrhizal soybean than in nonmycorrhizal ones. Such increases in activities in response to mycorrhizal fungi were parallel to the degree of mycorrhizal colonization. This effect was more pronounced in mycorrhizal plants grown in P0 soil. On the other hand, no significant differences were observed in alkaline phosphatase activity between the root extracts of mycorrhizal and nonmycorrhizal plants grown in P0.5 soil.

Changes in total soluble protein content: Total soluble protein of the mycorrhizal and nonmycorrhizal root extracts of soybean plants were greatly reduced with

Table 1. Effect of phosphate fertilization and mycorrhizal inoculation on the growth response of soybean plants grown in sterilized soil. Values in each column (except AMR) followed by *the same letter(s)* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test). Each value represents the mean of five replicates ± SE. AMF – arbuscular mycorrhizal fungi, NAMF – nonmycorrhizal fungi, AMR – arbuscular mycorrhizal response, DM – dry matter, R/S DM – root/shoot dry matter, SH – shoot height, and LA – leaf area.

KH_2PO_4 [g kg^{-1}(soil)]	AMF inoculation	shoot DM [g per plant]	root DM [g per plant]	R/S DM [%]	SH [cm per plant]	LA [mm^2 per plant]
0.0	NAMF	3.24 ± 0.56^{C}	1.48 ± 0.19^{B}	0.456 ± 0.02^{A}	50.4 ± 3.50^{C}	255 ± 8.05^{C}
	AMF	5.65 ± 0.62^{B}	2.25 ± 0.37^{A}	0.398 ± 0.03^{B}	66.2 ± 5.60^{B}	374 ± 9.11^{B}
	AMR [%]	74.4 ± 2.37	52.0 ± 2.05	-	31.4 ± 0.83	46.7 ± 1.95
0.5	NAMF	4.91 ± 0.59^{B}	1.85 ± 0.39^{B}	0.377 ± 0.01^{BC}	67.9 ± 5.80^{B}	380 ± 7.23^{B}
	AMF	6.37 ± 0.65^{A}	2.55 ± 0.16^{A}	0.400 ± 0.01^{B}	76.5 ± 5.95^{A}	445 ± 9.20^{A}
	AMR [%]	29.7 ± 1.94	37.8 ± 1.80	-	12.7 ± 0.75	17.1 ± 0.66
LSD (0.05)		0.650	0.305	0.040	6.550	15.50

Table 2. Photosynthetic rate (P_N), transpiration rate (E) and stomatal conductance (g_s) in leaves of mycorrhizal (AMF) and nonmycorrhizal (NAMF) soybean plants grown in sterilized soil with or without phosphorus fertilizer. Values in each column (except AMR) followed by *the same letter(s)* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test). Each value represents the mean of five replicates ± SE. AMR – arbuscular mycorrhizal response.

KH_2PO_4 [g kg^{-1}(soil)]	AMF inoculation	P_N [µmol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]
0.0	NAMF	7.99 ± 0.50^{C}	5.054 ± 0.40^{C}	0.124 ± 0.024^{B}
	AMF	10.61 ± 0.45^{A}	7.240 ± 0.55^{A}	0.192 ± 0.03^{A}
	AMR [%]	32.79 ± 2.07	43.25 ± 1.88	54.84 ± 2.48
0.5	NAMF	08.61 ± 0.68^{B}	5.362 ± 0.48^{B}	0.130 ± 0.022^{B}
	AMF	10.56 ± 0.65^{A}	7.121 ± 0.60^{A}	0.188 ± 0.037^{A}
	AMR [%]	22.65 ± 1.44	32.81 ± 1.95	44.62 ± 1.980
LSD (0.05)		0.950	0.205	0.050

Table 3. Phosphorus (P) and nitrogen (N) concentrations in shoots and roots of mycorrhizal (AMF) and nonmycorrhizal (NAMF) soybean plants grown in sterilized soil with or without phosphorus fertilizer. Values in each column (except AMR) followed by *the same letter(s)* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test). Each value represents the mean of five replicates ± SE. AMR – arbuscular mycorrhizal response.

Treatments		P [%]			N [%]		
KH_2PO_4 [g kg^{-1}(soil)]	AMF inoculation	Shoot	Root	Total	Shoot	Root	Total
0.0	NAMF	0.17 ± 0.005^{C}	0.14 ± 0.005^{C}	0.31 ± 0.015^{D}	2.80 ± 0.25^{C}	1.84 ± 0.105^{C}	4.64 ± 0.08^{C}
	AMF	0.30 ± 0.008^{A}	0.23 ± 0.006^{B}	0.53 ± 0.022^{B}	3.15 ± 0.20^{A}	2.35 ± 0.188^{A}	5.50 ± 0.33^{AB}
	AMR [%]	76.5 ± 2.55	64.3 ± 1.00	71.0 ± 1.95	12.5 ± 0.98	27.7 ± 0.88	18.5 ± 0.18
0.5	NAMF	0.22 ± 0.005^{B}	0.21 ± 0.006^{B}	0.43 ± 0.025^{C}	2.96 ± 0.25^{B}	2.19 ± 0.245^{B}	5.15 ± 0.23^{B}
	AMF	0.32 ± 0.007^{A}	0.27 ± 0.007^{A}	0.59 ± 0.037^{A}	3.28 ± 0.28^{A}	2.47 ± 0.205^{A}	5.75 ± 0.20^{A}
	AMR [%]	45.5 ± 1.82	28.6 ± 0.84	37.2 ± 1.05	10.8 ± 0.47	12.8 ± 0.29	11.7 ± 0.28
LSD (0.05)		0.025	0.020	0.040	0.250	0.120	0.540

Table 4. Frequency of mycorrhizal colonization (F [%]), intensity of mycorrhizal colonization (M [%]), and arbuscular frequency (A [%]) in the root tissues of mycorrhizal (AMF) and nonmycorrhizal (NAMF) soybean plants grown in sterilized soil with or without phosphorus fertilizer. Values in each column followed by *the same letter(s)* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test). Each value represents the mean of five replicates ± SE.

KH_2PO_4 [g kg^{-1}(soil)]	AMF inoculation	F [%]	M [%]	A [%]
0.0	NAMF	0.0^{B}	0.0^{C}	0.0^{C}
	AMF	97.5 ± 8.5^{A}	70.7 ± 7.2^{A}	50.3 ± 4.8^{A}
0.5	NAMF	0.0^{B}	0.0^{C}	0.0^{C}
	AMF	88.0 ± 9.1^{A}	56.0 ± 6.5^{B}	38.0 ± 4.0^{B}
LSD (0.05)		9.50	7.22	5.50

addition of P to the soil (Table 5). However, colonization of the soybean plants with AMF significantly increased the total soluble protein in root extracts than in NAMF plants regardless of P treatments, and this beneficial effect was highly pronounced in plants grown in P0 soil. No significant differences in total soluble protein contents in root extracts were observed between the nonmycorrhizal plants grown either in P0 or P0.5 soil.

Discussion

Phosphorus is a critical nutrient for plant growth and makes up about 0.2% of a plant DM (Smith *et al*. 2011), but it is of the most difficult nutrients for plants to acquire. In soil, P may be present in a relatively large amount, but much of it is poorly available to plants due to its low mobility in the soil. In consequence, uptake of the phosphate by root hairs (the direct pathway) leads to lowering of phosphate concentrations in the rhizosphere (called depletion zones) because replacement does not keep pace with uptake. Accordingly, plants have evolved a range of strategies that increase either P uptake capacity or availability of P in the soil (Cheng *et al*. 2011). The most common of these strategies is AM symbiosis (Abdel-Fattah 1997, Liu *et al*. 2003, Smith *et al*. 2011, Sheng *et al*. 2013). In the present study, the AM inoculation significantly increased growth parameters (DM, LA, and shoot height) of soybean plants grown in soil either with or without P comparing to nonmycorrhizal plants. Such increases in growth parameters of plants resulted from the mycorrhizal colonization and were directly proportional to the respective level of the mycorrhizal colonization. The rate of growth in response to the mycorrhizal colonization was more pronounced in P-deficient soils. Enhanced growth of mycorrhizal plants is often related to improved essential nutrition due to hyphal networks leading to plants. This pattern of response to the mycorrhizal infection in low P soils is entirely consistent with previous studies (Liu *et al*. 2003, Smith *et al*. 2011, Abdel-Fattah and Asrar 2012, Sheng *et al.* 2013). Addition of soluble phosphate to soil significantly reduced the mycorrhizal growth response in associated soybean plants as compared with the nonfertilized soil. This result supports the previous findings which indicated that adding P to soil generally reduced AMF development and consequently the mycorrhizal benefits (Smith and Gianinazzi-Pearson 1990, Cavagnaro *et al*. 2005, Sheng *et al*. 2013).

P and N concentrations in shoots and roots of the mycorrhizal and the nonmycorrhizal soybean plants increased significantly with phosphate addition to the soil. However, the mycorrhizal plants had higher contents of P in both roots and shoots than those in the nonmycorrhizal plants, particularly in the P0 soil. Such increases in nutrient concentrations in response to the mycorrhizal effects were highly associated with the level of the mycorrhizal

Table 5. Quantitative changes in protein [μg g^{-1}(root FM)], soluble acid and alkaline phosphatase activities of mycorrhizal (AMF) and nonmycorrhizal (NAMF) soybean plants grown in sterilized soil with or without phosphorus fertilizer. Values in each column (except AMR) followed by *the same letter(s)* are not significantly different at $P \leq 0.05$ (*Duncan*'s multiple range test). Each value represents the mean of five replicates ± SE. AMR – arbuscular mycorrhizal response.

KH_2PO_4 [g kg^{-1}(soil)]	AMF inoculation	Protein [μg g^{-1}]	Phosphatase activities [mU ml^{-1}]		
			Acid	Alkaline	Total
0.0	NAMF	450 ± 5.80^{C}	750 ± 11.5^{B}	220 ± 1.07^{B}	970 ± 0.80^{B}
	AMF	662 ± 8.05^{A}	810 ± 10.8^{A}	260 ± 1.09^{A}	1070 ± 0.85^{A}
	AMR [%]	47.1 ± 1.05	8.00 ± 0.86	18.2 ± 0.15	10.3 ± 0.08
0.5	NAMF	430 ± 6.85^{C}	725 ± 8.50^{C}	195 ± 1.03^{C}	920 ± 0.50^{C}
	AMF	580 ± 2.95^{B}	755 ± 8.55^{B}	205 ± 1.05^{C}	960 ± 0.75^{B}
	AMR [%]	34.9 ± 0.85	4.13 ± 0.35	5.13 ± 0.05	4.34 ± 0.04
LSD (0.05)		25.40	22.50	15.00	30.55

infection (Fattah 2013, Sheng *et al*. 2013). No significant differences in R/S DM yield ratio were found between the mycorrhizal and the nonmycorrhizal soybean plants grown in P-fertilized soil. In contrast, the R/S DM ratio of the mycorrhizal plants was significantly lower than that of the nonmycorrhizal plants grown in P-deficient soils, presumably due to the greater shoot growth of the mycorrhizal plants. These results are in agreement with the most previous studies (Smith *et al*. 1986, Abdel-Fattah 1997, Smith *et al.* 2011).

It is evident from the present study that g_s, P_N, and E were significantly higher in the mycorrhizal than in the nonmycorrhizal plants grown in soil with or without P. The increases were related to the degree of mycorrhizal colonization. These data are in agreement with those found by Auge *et al*. (2008). Abdel-Fattah *et al.* (2013) reported increased E and g_s in AM plants under amply watered and drought conditions. A higher E in leaves of the mycorrhizal plants would therefore be consistent with the higher rates of the g_s which often accompany the mycorrhizal symbiosis, and are presumed to be necessary to supply the carbon needs of the fungal symbiont (Maggio *et al*. 2000, Auge 2001, Cho *et al*. 2006). A significant increase in P and its transport by hyphae have been observed or computed in instances in which the AM symbiosis has also affected the stomatal behavior (Allen 1982, Ruiz-Lozano and Azcon 1995, Shu *et al*. 2014). Moreover, the mycorrhizal soybean plants in this study produced more shoot and root DM than the nonmycorrhizal plants. This might partially explain why the mycorrhizal plants had higher gas-exchange parameters than the nonmycorrhizal plants. The ability of the AMF to increase leaf number, leaf area, and root density is consistent with earlier investigations (Berta *et al.* 1993, Al-Karaki and Al-Raddad 1997, Abdel-Fattah *et al.* 2013, Srinivasan and Govindasamy 2014).

Reductions in AMF colonization levels in root tissues of soybean plants with increasing amount of P in soil are consistent with the previous field and greenhouse studies (Abdel-Fattah 1997, Smith *et al*. 2011, Shu *et al*. 2014). In contrast, the previous studies have shown that the percentage of root colonized by AM fungi was not affected by increasing the P fertilizer in soils (Smith and Gianinazzi-Pearson 1988, Auge 2001, Li *et al*. 2013). In this study, we also observed that the beneficial effects of the AMF on growth and biomass of the soybean plants were highly pronounced in P-deficient soil (Table 1). The positive effects of the mycorrhizal fungi are likely attributed to the improvement of the P and N contents (Smith *et al*. 2011), enhanced gas-exchange parameters (Auge 2001), and the increase of root length density (Bryla and Duniway 1997, Al-Qarawi 2010).

Acid and alkaline phosphatase activities were significantly higher in mycorrhizal than in nonmycorrhizal root extracts of the soybean plants grown either in soil with P0.5 or P0. Increase in phosphatase activities and growth responses of the soybean plants to AMF symbiosis were directly proportional to the levels of the mycorrhizal colonization. These results were in agreement with the previous observations (Abdel-Fattah and El-Katony 1996, Ibrahim *et al*. 2011, Smith *et al*. 2011, Gosling *et al*. 2013) who stated that mycorrhiza-specific phosphatase (MPSase) was detected only in the root extract colonized with mycorrhizal fungi as compared to nonmycorrhizal root extract. The close relationship between the mycorrhizal growth responses and the intensity of the mycorrhizal colonization support the hypothesis that the phosphatase enzymes are somehow involved in the assimilation of P by AMF (Gianinazzi *et* al. 1979, Abdel-Fattah 2001). Addition of soluble phosphate to soil significantly decreased the levels of the mycorrhizal colonization in root tissues of the soybean plants and therefore reduced the activities of acid and alkaline phosphatase in the mycorrhizal root extracts of the plants. Similar results have been reported in soybean and other plant species (Krishna *et al*. 1983, Shu *et al*. 2014).

AM inoculated soybean plants had higher contents of protein in root extracts than NAMF plants grown either in P0 or P0.5. The observed increase in protein contents in response to mycorrhizal colonization was highly pronounced in P-deficient soil. These data were in agreement

with the results of other researchers (Smith *et al*. 2011, Abdel-Fattah and Asrar 2012, Shu *et al*. 2014). In connection, the total soluble protein of the mycorrhizal and nonmycorrhizal root extracts of the soybean plants were generally reduced with the P addition to the soil. This result is in a good conformity with the results obtained by Lekberg *et al*. (2008) who stated that increased soil P inhibited AMP development and then decreased the beneficial effects of AMF symbiosis.

Conclusion: Arbuscular mycorrhizal inoculation significantly improved growth of soybean plants grown in a sandy loam soil not only through the increasing P and N concentrations, but also *via* stimulating gas-exchange parameters, and some metabolic aspects of the soybean plants particularly in P-deficient soil. However, these benefits in response to the mycorrhizal inoculation generally decreased when the P fertilizer was added to the soil, suggesting that P reduced AMF function and effects.

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