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EFFICIENCY OF ARBUSCULAR MYCORRHIZAL (AM) FUNGI FOR IMPROVING GROWTH, ROOT SYSTEM ARCHITECTURE, NUTRIENT UPTAKE, LEAF HYDRAULIC CONDUCTANCE AND PHOTOSYNTHETIC PIGMENTS OF MAIZE AND PEA PLANTS

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ABSTRACT

A greenhouse pot experiment was conducted to investigate the effect of colonization of arbuscular mycorrhizal fungi (AMF) on the growth, root system, nutrient uptake, leaf hydraulic conductance and photosynthetic pigments of maize (monocotyledon) and pea (dicotolyledon) plants over 9 weeks of planting. AMF colonization significantly increased growth parameters (biomass of roots and stems, shoot height, no. of leaves and leaf area), phosphorus and nitrogen uptake, leaf hydraulic conductance and photosynthetic pigments than those non-mycorrhizal pea and maize plants. Such increases in these parameters were related with the degree of mycorrhizal colonization for each plant. Pea plants inoculated with mycorrhizal fungi reached flowery 6 days earlier than those non-inoculated plants. In general, AM colonization significantly increased the total root length, root volume, root surface area, root diameter and tap root length of maize and pea compared to non-mycorrhizal plants. A great variation in mycorrhizal colonization dependency was observed among the two plants. In most cases, the beneficial roles of mycorrhizal fungi were significantly higher in pea when than maize plants. The greater formation of arbuscules could contribute to the improvement of shoot and root growth and consequently the other studied parameters particularly in pea plants

Key words: *Arbuscular mycorrhizal fungi, growth responses, root system architecture, maize, pea, leaf hydraulic conductance*

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are the widespread root fungal symbionts and associated with the vast majority of higher plants (Smith and Read, 1997; Abdel-Fattah et al., 2009), especially the agriculturally important crop and vegetables plants. AMF were shown to improve soil structure (Miller and Jastrow, 2000; Sensoy et al., 2007) and increased plant growth (Jaizme-Vega and Azcon, 1995; Asghari et al., 2005; Kaya et al., 2009) and yield (Talukdar and Germida, 1994; Mena-Violante et al., 2006) by improving plant nutrient uptake (Abdel-Fattah, 1997; Liu et al., 1998; Charron et al., 2001). Arbuscular mycorrhizal fungi were shown to enhance the uptake of phosphorus and nitrogen (Ames et al., 1983; Ba and Guissou, 1996; Abdel-

Fattah and Mohamedin, 2000) and other immobile nutrient elements, namely copper and Zinc (Tee Boon et al., 1997; Abdel-Fattah, 2001). In addition, mycorrhizal colonization, increased chlorophyll contents in mungbean (Rabie, 2005) and pepper (Kaya et al., 2009) plants grown at high salinity.

Maize (Zea mays) and pea (Pisum sativum) are important crop and vegetable plants in Saudi Arabia and in the world (Asmah, 1995). Colonization of plant roots and soils by arbuscular mycorrhizal (AM) fungi is often accompanied by changes in stomatal conductance and transpiration in the host plants (Cho et al., 2006; Auge et al., 2008). There are some reports of AM-induced changes in whole-plant and root hydraulic conductance (Allen et al., 1981; Auge, 2001). Although, these fungal endophytes are obligate symbionts and their agricultural use remains limited due to the large quantities of inocula required (Waterer and Coltman, 1988).

Root system architecture is an important morphological feature and plays a significant role in acquiring nutrients from soil volume (Lynch, 1995). In response to environmental stresses (drought, salinity, heavy metals and nutrient limitation in particular), root system architecture can be modified to promote the nutrient-acquiring capacity (Al-Qarawi, 2002; Sorgona et al., 2007) Atkinson et al. (1994) reviewed the literature on the influence of AM fungi on root system architecture and indicated that the modification of root system architecture by AM fungi can contribute to the increased nutrient uptake from soil and pathogen resistance of host (Abdel-Fattah and Shabana, 2002; Yao et al., 2009). Therefore, the present study was performed to investigate the effects of arbuscular mycorrhizal (AM) fungi on growth, nutrient uptake, leaf hydraulic conductance, photosynthetic pigments and root system architecture of maize (mono-cotyledon) and pea (di-cotolydon) plants grown in greenhouse over 9 weeks of planting.

MATERIALS AND METHODS

Mycorrhizal inoculum

Inoculum of Mycorrhizal fungi tested in this study consisted of spores and infected roots of Glomus mosseae (Nicol. & Gerd.) Gerdemann & Trappe and Glomus etunicatum (Becker & Gerdemann). Tested mycorrhizal fungi obtained from Durab farm, Collage of Food and Agricultural Sciences, King Saud University, Saudi Arabia. The isolates were propagated on maize seedlings in greenhouse for 12 weeks on a sand medium and watered as needed with tap water. Each seedling was inoculated with both 30 g soil containing approximately 390 spores and 3 g fresh infected roots from a pure standard culture. Spores of the mycorrhizal fungi were collected from stock maize soil by a procedure of decanting and wet sieving (Gerdemann and Nicolson, 1963) through a series of 250, 150 and 5^{o} μm. The inoculum was placed 3 cm below the surface of the soil before planting to produce mycorrhizal plants. Non-mycorrhizal plants received a soil inoculum free of arbuscular mycorrhizal (AM) propagules to equilibrate soil microbiota between mycorrhizal and non-mycorrhizal treatments.

Plants and growth conditions :

Seeds of maize (Zea mays L.) and pea (Pisum sativum L.) were surface sterilized for 15 min in 3% sodium hypochlorite, rinsed with sterile water and germinated for 5 days in moist sterilized filter paper in Petri dishes. Uniform seedlings (when the radicals had appeared) were planted into sterilized plastic tubes (one plant for each tube) containing clay sandy soil collected from Durab farm. Half of the tubes for each plant species received the mycorrhizal inoculum by placing inoculum 3 cm deep in 10-cm diameter holes in the center of the tube prior to planting to serve as mycorrhizal treatments. Non-mycorrhizal (Sterilized soil) plants received a filtered washings of the inoculum to include microorganisms associated with the mycorrhizal inoculum. Mycorrhizal and non-mycorrhizal tubes were arranged in a completely randomized design in a growth chamber (Research Station of the College of Food and Agricultural Sciences) at temperature 22±2^{o}C with 16 h fluorescent illumination and relative humidity (70%) and watered regularly to near field capacity with tap water. All plants received 10% Hoagland solution minus phosphorus (Hoagland and Arnon, 1950). Eight plants for each treatment were harvested after 9 weeks from planting.

Measurements :

After harvest, shoots and roots were separated immediately, fresh weights of shoots and roots were recorded. Leaf area, no. of leaves and shoot height for each plant were determined. Tissues were then dried at 80^{o}C for 24 h and their dry weights were recorded. Dried plant material was digested using sulphuric / perchloric acid digestion procedure (Cresser and Parsons, 1979). Phosphorus in the digests of shoots and roots was measured using the vanadate-molybdate yellow procedure (Kacar, 1984). Total nitrogen content in shoots and roots of each plants were measured by the Kjeldahl method.

Plant photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in the leaves of each plant were determined according to the spectrophotomteric method described by Porra et al. (1989). A known fresh weight of leaves was extracted in 5ml of di-methylformamide and left overnight in refrigerator. The filtered extract was measured against a blank of pure solvent at 3 wave lengths 664, 644 and 452 using Ultraospec 2000 - UV / Visible spectrophotometer. Leaf hydraulic conductance normalized to leaf area was measured in nmol m-2s-1, following the electrical analogue approach using the procedures of Franks (2006).

Roots were carefully cleared of soil with tap water and further rinsed with distilled water and then carefully arranged for the image capture using a scanner, followed by the analysis of root system architecture with the WinRhizo image analysis system (V4 - 1C, Regent Instruments, Quebec, Canada) (Rillig et al., 2008). Using this system, total root length, root surface area, root volume, average root diameters of different size were analyzed.

Randomly chosen root segments (0.5 - 1.0 cm) colonized with AM fungi were cleared with 10% (w/v) KOH and stained with 0.05% (w/v) trypan blue in lactophenol as described by Phillips and Hayman (1970), and examined microscopically for mycorrhizal colonization using the method of Trouvelot et al. (1986).

All data were statistically analyzed using SAS program, with variance analysis (one - way ANOVA). Differences between treatments were determined using Duncan's Multiple Range Test (SAS, 2005).

RESULTS

Growth responses :

Fresh & dry biomass, shoot length, number of leaves and leaf area of maize and pea plants were significantly increased with mycorrhizal inoculation when compared to the non-mycorrhizal control plants (Table, 1 and Plates 1 & 2). The appearance of a positive growth response varied between the two plants depending upon the degree of mycorrhizal root colonization and arbuscular frequency for each plant. Root : shoot dry weight ratios of mycorrhizal pea plants were significantly decreased than that of non-mycorrhizal plants. On other hand, no significant differences in these ratios were observed between mycorrhizal and non-mycorrhizal maize plants.

Table 1. Influence of arbuscular mycorrhizal (AM) inoculation on the growth response of maize and pea plants grown in growth chamber.

Parameters	Maize		Pea	
	- AM	**+AM**	**- AM**	**+ AM**
Shoot fresh weight (g / plant)	**1.52 b**	**2.20 a**	**0.697 b**	**1.407 a**
Root fresh weight (g / plant)	**0.871 b**	**1.087 a**	**0.219 b**	**0.392 a**
Shoot dry weight (g / plant)	**0.122 b**	**0.189 a**	**0.056 b**	**0.173 a**
Root dry weight (g / plant)	**0.075 b**	**0.128 a**	**0.018 b**	**0.026 a**
Root / shoot ratio	**0.615 a**	**0.677 a**	**0.321 a**	**0.150 b**
Shoot height (cm)	**34.42 b**	**49.08 a**	**10.85 b**	**14.97 a**
Number of leaves	**4.5 b**	**6.0 a**	**07.25 b**	**09.00 a**
Leaf area (cm^2 / plant))	**68.58 b**	**100.9 a**	**22.21 b**	**50.64 a**
Arbuscular frequency (%)	**0.0**	**20.8**	**0.0**	**50.64 a**

Values with different letters within line for each plant are significantly different as determined by the Duncan's test (*P* = 0.05) Where: -AM, non-mycorrhizal treatments, +AM, mycorrhizal treatments.

Plate 1. Growth of maize plants with arbuscular mycorrhizal fungi (Right, +AM) or without arbuscular mycorrhizal inoculation (Left, -AM).

It is interesting to note that arbusculr mycorrhizal colonization stimulated early fruit of pea plants compared to uninoculated control plants (Plate 2).

Leaf hydraulic conductance :

In general, Arbuscular mycorrhizal (AM) plants often have higher leaf hydraulic conductance than non AM plants (Figure 1). Leaf hydraulic conductance of mycorrhizal pea plants were significantly higher than non-mycorrhizal plants. On the other hand, there was no significant difference between mycorrhizal and non-mycorrhizal leaf hydraulic conductance of maize plants.

Root system architecture :

Table (2) indicated that arbuscular mycorrhizal inoculation tended to increase tap root length, root surface area, root volume, average root diameter and tap root length of maize and pea plants when compared to non-mycorrhizal plants. In this connection, these parameters of mycorrhizal pea plants were significantly higher than that of non-mycorrhizal plants. However, there was no significant difference in root system development between mycorrhizal and non-mycorrhizal maize plants.

Plate 2. Growth of pea plants with arbuscular mycorrhizal fungi (Right, +AM) or without arbuscular mycorrhizal inoculation (Left, -AM). Note the flower appearance in the mycorrhizal treatment.

Table 2. Influence of arbuscular mycorrhizal (AM) inoculation on the root system architecture of maize and pea plants grown in growth chamber.

parameters	Maize		Pea	
	- AM	**+AM**	**- AM**	**+ AM**
Total root length (cm / plant)	**554.8 a**	**629.5 a**	**108.9 b**	**225.7 a**
Root surface area (cm^2 / plant)	**85.71 a**	**99.85 a**	**18.99 b**	**35.45 a**
Root volume (cm^3 / plant)	**1.052 a**	**1.220 a**	**0.270 b**	**0.442 a**
Root diameter (mm / plant)	**0.459 a**	**0.483 a**	**0.488 a**	**0.509 a**
Tap root length (cm / plant)	**1307 a**	**1818 a**	**267 b**	**901 a**

Values with different letters within line for each plant are significantly different as determined by the Duncan's test (*P* = 0.05), Where: -AM, non-mycorrhizal treatments, +AM, mycorrhizal treatments.

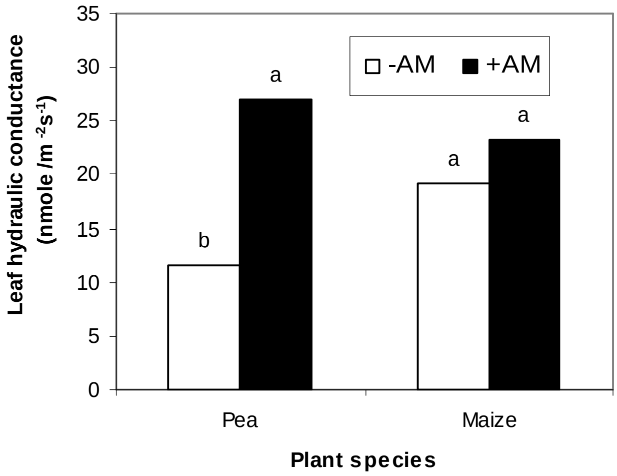

Figure 1. Influence of arbuscular mycorrhizal (AM) inoculation on leaf hydraulic conductance of maize and pea plants grown in growth chamber. Bars for each parameter of each plant topped by the same letter are not significantly different according to Duncan's multiple range test. Where: -AM, non-mycorrhizal treatments, +AM, mycorrhizal treatments.

Mycorrhizal root colonization :

The frequency of root colonization (F%), intensity of root cortex colonization (M%) and arbuscule development (A%) by mycorrhizal tested fungi were affected by the type of plant species (Table 3). Roots of pea plants were significantly higher to the levels of colonization by arbuscular mycorrhizal fungi than maize plants. No mycorrhizal root colonization was observed in non-mycorrhizal plants of the two plant species

Photosynthetic pigments :

The concentrations of photosynthetic pigments (chloropyll a, chloropyll b and carotenoid) in leaves were significantly affected both by mycorrhizal inoculation and type of plant species (Figure 2). In general, arbuscular mycorrhizal fungi increased these concentrations in the two plant species to a certain extent. The increases in their content was related to degree of mycorrhizal root colonization. In this connection, photosynthetic pigments of pea mycorrhizal plants were significantly greater than those of the non-mycorrhizal plants. In contrast, no significant differences in photosynthetic pigments contents were observed between mycorrhizal and non-mycorrhizal maize plants.

Phosphorus and nitrogen contents :

Arbuscular mycorrhizal colonization significantly increased the contents of phosphorus and nitrogen in shoots, roots and whole maize and pea plants relative to the equavelent non-mycorrhizal control plants (Table 4). Such increases in these contents were related to the degree of mycorrhizal colonization for each plant. Furthermore, there was a significant correlation between P & N contents of maize and pea plant tissues and the percent of arbuscular frequency (data not shown). Results in Table (4) indicate that arbuscular mycorrhizal pea plants had higher P and N contents in their tissues than mycorrhizal maize plants.

Table 3. Levels of mycorrhizal infection of maize and pea plants grown in growth chamber.

parameters	Maize		Pea	
	- AM	**+AM**	**- AM**	**+AM**
Frequency of mycorrhizal infection (F %)	**0.0 c**	**80.3 b**	**0.0 c**	**93.0 a**
Intensity of root cortical infection (M %)	**0.0 c**	**50.2 b**	**0.0 c**	**68.6 a**
Frequency of arbuscular development in roots (A %)	**0.0 c**	**20.8 b**	**0.0 c**	**35.3 a**

Values with different letters within line are significantly different as determined by the Duncan's test (*P* = 0.05), Where: -AM, non-mycorrhizal treatments, +AM, mycorrhizal treatments.

Table 4 Influence of arbuscular mycorrhizal (AM) inoculation on the total phosphorus and nitrogen concentration (%) of maize and pea plants grown in growth chamber.

parameters	Maize		Pea	
	- AM	**+AM**	**- AM**	**+ AM**
Phosphorus (%)				
Shoot	**0.86 b**	**1.85 a**	**0.98 b**	**2.38 a**
Root	**0.80 b**	**1.53 a**	**0.89 b**	**2.04 a**
Total	**1.66 b**	**3.38 a**	**1.87 b**	**4.42 a**
Nitrogen (%)				
Shoot	**9.51 b**	**23.16 a**	**9.61 b**	**26.62 a**
Root	**4.38 b**	**10.86 a**	**10.04 b**	**22.30 a**
Total	**13.89 b**	**34.02 a**	**19.65 b**	**48.92 a**

Values with different letters within line for each plant are significantly different as determined by the Duncan's test (*P* = 0.05) Where: -AM, non-mycorrhizal treatments, +AM, mycorrhizal treatments.

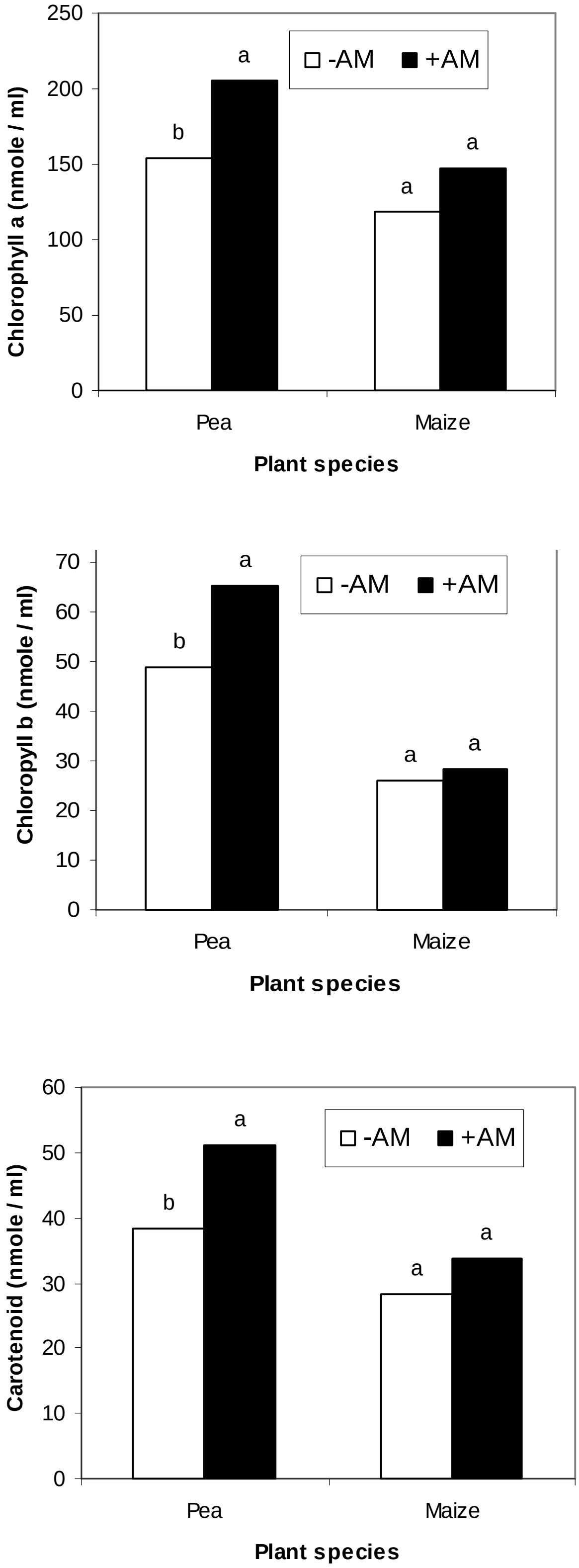

Figure 2. **Influence of arbuscular mycorrhizal (AM) inoculation on the concentration of photosynthetic pigments of maize and pea plants grown in growth chamber. Bars for each parameter of each plant topped by the same letter are not significantly different according to Duncan's multiple range test. Where: -AM, non-mycorrhizal treatments, +AM, mycorrhizal treatments.**

DISCUSSION

Arbuscular mycorrhizal (AM) inoculation increased fresh & dry biomass, shoot length, number of leaves, leaf area and photosynthetic pigments of maize (monocotyledon) and pea (dicotyledon) plants compared with the non-inoculated control plants. The appearance of a positive growth response among two plant species varied depending upon the level of mycorrhizal colonization in the root of each plant. Despite the influence of AM fungi on growth and yield of most vegetable and crop plants as documented in many reports (Jaizme-Vega and Azcon, 1995; Asghari et al., 2005; Mena-Violante et al., 2006; Kaya et al., 2009), little is known about the potential of AMF for improving root system architecture (tap root length, total surface length, surface area, root volume and root diameter) of pea and maize plants. The results clearly demonstrated the positive impact of arbuscular mycorrhizal fungi on root system architecture in addition to photosynthetic pigments, nutrient contents and leaf hydraulic conductance of pea and maize plants grown in growth chamber under controlled conditions.

It is noticed from the present study that phosphorus and nitrogen contents of pea and maize shoots and roots increased in response to inoculation with arbuscular mycorrhizal fungi. The results from this experiment are in agreement with the findings obtained by Guissou et al. (1999) who showed higher growth and nutrient uptake by Zizyphus mauritiana given the rock phosphate and AM inoculation. Such increases in the nutrients content were related to the arbuscular frequency in the root of pea and maize plants. These results are in agreement with those reported by Abdel-Fattah (2001) and Rillig et al. (2008) also observed a positive correlation between the P content of soybean and Solanum lycopersicum plants and colonized root length of AM fungi. Such relationships are expected because they reflect both the importance of AMF to P uptake by plants and effect of an adequate P supply on biomass production.

It is interesting to note from the present results that arbuscular mycorrhizal (AM) fungi accelerates the flowering of pea plants compared to non-mycorrhizal plants. These results are supported by Fan et al. (2008) who concluded that AM colonization shortened the ripening of strawberry by 6 days compared with non-inoculated ones. Furthermore, AM inoculation of chile ancho (Capsicum annuum L.) produced fruits that were 13% wider and 15% longer than non-inoculated treatment (Mena-Violante et al., 2006).

Increased leaf hydraulic and stomatal conductance rates in AM plants have been recorded under amply watered conductance during drought (Auge, 2001) and after exposure to NaCl stress (Cho et al., 2006). The size of the AM induced increased in unstressed plants is often 50% depending on host species and experimental conditions (Auge et al., 2008). These findings support our data show that AM pea plants had significantly higher leaf hydraulic conductance than non AM plants. However, the AM inoculation did not cause significant increase in this parameter of maize plants when compared to non AM plants. The difference in the relative efficacy of AM inoculation in the two plants might be explained by functional differences at the level of the host-fungus interface. Pea root plants significantly higher levels of mycorrhizal colonization compared to zea plants. Abdel-Fattah (2001) noted that arbuscules are the principle AM fungal structures that function in nutrient transfer between the host and fungus, and predicated that arbuscule development by AMF may reflect its relative benefit to the host crop. The present results support this hypothesis.

Throughout the study, the results strongly demonstrated that AM inoculation significantly increased the total root length, total surface area, root volume and root surface area of pea plants than that of non-inoculated plants. In contrast, no significant differences in parameters of root system architecture were observed between mycorrhizal and non-mycorrhizal maize plants. The altertions of root system characteristics by AM fungal colonization have been intensively reported, with inconsistent results for different plant and / or fungal species (Yao et al., 2009). A decrease in total root length was recorded for the herbaceous rice plants inoculated with AM fungi (Herdlera et al., 2008) but an increase was recorded for woody species of Garcnia mangostanal L. (Masri and Azizah, 1998). This inconsistence probably reflects the difference between herbaceous and woody plants, whose root anatomy and carbon distribution between shoots and roots are of different pattern (Zai et al., 2007).

Mycorrhizal inoculation increased, in general, root biomass and root volume of tested plants when compared to non-mycorrhizal ones. The external hyphae can extend up to several centimeters beyond the infected root surface and can increase root surface area and the absorption zone for exploration of greater soil volume for nutrient and moisture uptake (Satter et al., 2006). The rate of nutrient uptake by mycorrhizal roots is also faster than that by non-mycorrhizal roots (Son and Smith, 1988). All these factors enabled mycorrhizal corn and pea plants in the present study to absorb more nutrients (P and N) compared to non-mycorrhizal plants enhancing them to produce more root biomass.

CONCLUSION

From the results obtained here, it is evident that inoculation with Arbuscular mycorrhizal (AM) fungi increased plant growth, nutrient uptake, photosynthetic pigments, leaf hydraulic conductance and root system architecture of maize and pea plants. The positive impact of AM fungi could also be explained by different colonization levels in roots of each plant. The high percentage of arbuscules (sites of nutrient exchange between host and fungus) in the AMF treatment indicated a very active symbiosis. Greater formation of arbuscules could contribute to increase growth and root system particularly in pea plants. The improved growth and nutrient uptake could be due to direct effects of mycorrhizal fungi on plant nutrient uptake and also indirect effects via mycorrhizal induced changes in the root system architecture. Other important benefits of AMF symbiosis, such as drought and salinity tolerance, heavy metals alleviation and disease resistance may also be under recognized in current practices.

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الملخص العربى

كفاءة فطريات الجذور التكافلية الشجيرية فى تحسين كل من معدلات النمو وصفات المجموع الجذرى والجهد التوصيلى المائى للأوراق والمحتوى الصبغى والغذائى لنباتى الذرة والبسلة

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فى تجربة أجريت فى غرف النمو لمدة ٩ أسابيع أستهدفت التعرف عن الدور الحيوى لفطريات الجذور الشجيرية فى تحسين كل من معدلات النمو وصفات المجموع الجذرى والجهد التوصيلى المائى للأوراق والمحتوى الصبغى والغذائى لنباتى الذرة (أحادى الفلقة) والبسلة (ثنائى الفلقة) وتم التوصل إلى النتائج التالية :-

١- بصفة عامة أظهرت الدراسة أن تحوصل جذور نباتى الذرة والبسلة بفطريات الجذور التكافلية الشجيرية أدى إلى زيادة معنوية فى كل من معدلات الوزن الرطب والجاف للسيقان والجذور ومساحة وطول الساق والجهد التوصيلى المائى للأوراق بالإضافة إلى كل من المحتوى الغذائى (الفسفور والنيتروچين) والصيغى للأوراق (كلوروفيل أ، ب والكاروتينات) بالمقارنة بنظائرها من النباتات الغير متحوصلة بهذه الفطريات، وكانت معدلات الزيادة فى هذه الصفات لهذه النباتات مرتبطة بدرجة كبيرة بدرجة إصابتها بهذه الفطريات.

٢- أوضحت النتائج أن نباتات البسلة المتحوصلة بفطريات الجذور التكافلية الشجيرية أزهرت مبكراً (حوالى ٦ أيام) بالمقارنة بالنباتات الغير متحوصلة بهذه الفطريات.

٣- بينت النتائج أن لفطريات الجذور الشجيرية دوراً واضحاً فى زيادة صفات المجموع الجذرى (عدد القمم النامية، مساحة وطول وحجم الجذور) لنباتى البسلة والذرة بالمقارنة بالنباتات الغير متحوصلة بهذه الفطريات، وكانت هذه الزيادات معنوية بدرجة كبيرة فى نباتات البسلة، على الجانب الآخر، لم تظهر النتائج أى فروق معنوية فى معظم الصفات بين نباتات الذرة المتحوصلة والغير متحوصلة بهذه الفطريات المتكافلة.

٤- أكدت النتائج التى تم التوصل إليها أن لفطريات الجذور التكافلية الشجيرية دوراً حيوياً فى تحسين نمو نباتى الذرة والبسلة بطرق مباشرة (زيادة محتواها الغذائى) أو غير مباشرة (تحسين معدلات نمو المجموع الجذرى)، وأظهرت نباتات البسلة (أحادى الفلقة) إستجابة كبيرة للدور الإيجابى لهذه الفطريات بالمقارنة بنباتات الذرة (أحادى الفلقة)، وقد تناسبت هذه الاستجابات تناسباً طردياً مع تحوصل جذور نباتى البسلة والذرة بالتركيبات الشجيرية (مراكز تبادل العناصر الغذائية بين الفطر وخلايا العائل) لهذه الفطريات.

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