Using of Arbuscular Mycorrhizal Fungi to Reduce the Deficiency Effect of Phosphorous Fertilization on Maize Plants (*Zea mays* L.)

Almagrabi O. A.[1] and Abdelmoneim T. S.[1&2*]

[1]Biology Department, Faculty of Science, King AbdulAziz University, P.O. Box 15758, Jeddah 21454, Saudi Arabia.
[2] Suez Canal University, Faculty of Agriculture, Department of Agricultural Botany, P.O. Box 41522, Ismailia, Egypt.

tmabrouk@kau.edu.sa

Abstract: A greenhouse study was conducted to investigate the effect of three species of arbuscular mycorrhizal fungi (AMF) *Glomus mosseae*, *G. etunicatum* and *G. clarum* at two levels of phosphorus (P) fertilization (*zero* and *60 μg* P/g) on plant growth parameters and physiological character of maize plants (*Zea mays* L.) at seedling stage. The results showed that the best values of all plant growth parameters were recorded at P level (60 *μg* P/g) and inoculation with *G. etunicatium* and *G. mosseae* (The increase by rate 34.5%; 32.9% and 32.5%; 32.2% respectively) comparing with untreated plants. Also the highest value of plant dry weight was recorded in the presence of inoculation with *G. clarum* by increasing rate 55.7% comparing with control. On contrast the treatment with P causing decreased in all values of root/shoot ratios comparing with the same treatment in the absent of P fertilization. The highest values of plant P uptake were recorded in the presence of P with inoculation by *G. etunicatium*, then *G. clarum* followed by *G. mosseae* comparing with untreated plants. All treatments in zero P were decreased in values of protein content comparing with P level 60 *μg*P/g, and increases in proline values. The highly values of plant chlorophyll content were recorded in the presence of P fertilization and inoculation by *G. clarum*, then *G. etunicatum* (8.306, 7.840 unit respectively). On the other hand AMF root colonization% was affected by phosphorus fertilization levels. The highest values of AMF root colonization% (50.5%, 80.3%) were found when plants inoculation with *G. etunicatum* at both level of P (*zero*, 60 *μg* P/g respectively) followed by *G. clarum* then *G. mosseae*. The same results were observed in number of AMF spores/100g of soil. The AMF specie *G. etunicatum* was recorded as a highest spores numbers (252, 320 spores /100g of soil) at two levels of P followed by *G. clarum* then *G. mosseae.* In generally AMF inoculation can be used as biofertilizer to reduce the deficiency effect of phosphorous fertilization on *Zea mays* L.

[Almagrabi O. A. and Abdelmoneim T. S. **Using of Arbuscular Mycorrhizal Fungi to Reduce the Deficiency Effect of Phosphorous Fertilization on Maize Plants (*Zea mays* L.)**. Life Sci J 2012; 9(4):1648-1654]. (ISSN:1097-8135).http://www.lifesciencesite.com. 253

Keywords: Maize, P-deficiency, Morphology, Mycorrhiza , Low-P, Physiology.

1. Introduction

The availability of Phosphorus (P) is one of the most significant determinants in plant growth (Wang *et al.*, 1998). Plants depend almost exclusively on P absorbed from soil. Total P in soil is abundant, but it is largely unavailable (Liu *et al.*, 1994). As many other plants, maize is sensitive to P and faces the dilemma of "P-deficiency in heredity" (Usuda and Kousuke, 1991). It is reported that P deficiency had a detrimental effect on morphogenesis and physiological mechanism in maize, and P deficiency symptoms and biomass have been known as indicative traits of maize in response to low P stress (Hajabbasi and Schumacher, 1994; Duan *et al.*, 2002; Liu *et al.*, 2003; Ortas *et al.*, 2011). The plant nutrition has been estimated that nearly 30 million tons of P based fertilizers (in terms of P_2O_5) are applied worldwide every year. However, the use efficiency of applied P is generally very low, ranging from 10% to 30% in the year applied (Mc.Laughlin *et al.*, 1991). Continuous application of P fertilizers also increases the risk of P loss from soil to water, causing toxic algal blooms in water bodies (Sharpley *et al.*, 2000). Phosphate is present in the soil in the form of inorganic orthophosphate (Pi) and is readily sequestered by cations especially in acidic conditions, of which the most abundant are iron, aluminium and calcium. The mobility of sequestered phosphate is reduced (Bucher, 2007). The arbuscular mycorrhizae (AM) fungal hyphae extend beyond the host root system to promote physiological responses in the host, such as root branching and phosphatase secretion that indirectly promote phosphate uptake (Ezawa *et al.*, 2005). Following fungal uptake phosphate is transferred to the fungal vacuole where it is polymerized to form polyphosphate chains (Ezawa *et al.*, 2001). Poly-phosphate is translocated through the vacuolar compartment to the intraradical hyphae (Ohtomo and Saito, 2005). The mechanism of poly phosphate breakdown has not been characterized but is hypothesized to require the action of fungal phosphatase enzymes present in the arbuscule (Javot *et al.*, 2007). Nye (1977) found that the increase plant uptake P due to mycorrhization results mainly from

the increased soil volume exploited by the mycorrhizal root system. The external AMF hyphae extend to soil volumes beyond the depletion zone around the roots (Sanders and Tinker, 1971), and to smaller soil pores and closer to the surfaces of soil particles than do the roots and root hairs (O'Keefe and Sylvia, 1992). Besides that, effective acquisition by external hyphae is related to rapid formation of polyphosphates in the hyphae which maintain a low internal concentration of inorganic phosphates (Callow *et al.,* 1978). A greater effect caused by an increased conversion of inorganic to organic phosphate in the leaves of mycorrhizal plants (Allen *et al.,* 1981) and a greater affinity of the absorbing sites for $H_2 PO_4$ in mycorrhizal roots have also been suggested (Cress *et al.,* 1979). Some studies have also been carried out to screen and improve the tolerance to P-deficiency in maize, most of which focused on maize in hybrid lines (Gong *et al.,* 2002; Li *et al.,* 2003; Wang *et al.,* 2003). In this study, we need to investigate the effects of plant inoculation with mycorrhizal fungi to reduce the P deficiency effect on the morphological and some physiological traits of maize plants under greenhouse condition. In addition to study the effectively of mycorrhizae on uptake of soil P depend extraradical mycelium and mobility of the nutrients themselves in soil.

2. Material and Methods

2.1. Preparation of mycorrhizal inoculums

The arbuscular mycorrhizal fungi (AMF) isolates used in this study are *Glomus mosseae* (Nicolson and Gerdemann), *G. etunicatum* (Becker and Gerdemann), *G. clarum* (Nicolson and Schenck) supplied from microbiology Lab. Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. The three AMF species were multiplied under onion plant root in sterilized soil. After 40 days of growth, plant shoots were removed and the substrate containing hyphae, spores and root was air dried and used as the inoculum. The inoculum was calculated based on number of spores present in 10 g dry roots (500 spores/10gm)

2.2. Inoculation and experimental design

Maize seeds (*Zea Mays* L.) were surface sterilized with sodium hypochloride (1% available chlorine) for 10 minutes, rinsed three times in sterilized distilled water, and then left to germinate for 3-4 days at 29 ±2° C rolled in sterilized filter paper. Germinated seedlings were planting in plastic pots (25 cm diameter and 30cm depth) each pot was filled with 1.5 Kg sterilized Peat moss soil (pH 5.4 the soil had 33.4±3.2 mg kg^{-1} extractable N, 6.2±0.45 mg kg^{-1} extractable P, and 44.6±5.6 mg kg^{-1} extractable K). Each pot was planted with 5 seeds and then thinned to three plants after 14 days. The individual plant was inoculated with various mycorrhizal fungi species separately. Ten grams of AMF inoculum (500 spores) were added in a deep truck around the plant stem into each pot. The treatments were distributed in randomized complete block design with three replicates. Two treatments with different phosphorus applications levels of zero and 60 µg P/g of soil added as a rock phosphate, each block including eight treatments were replicated three times as follows: 1-Plants were treated with two level of phosphorus fertilization rate. 2-Plants were inoculated by three AM fungi species individually as500 spores/plant. 3-Plants were inoculated by AM fungi at the above mentioned rates of phosphorous fertilization. 4-Plants were left free to serve as a check. The plants were grown in greenhouse at 23-29° C and a relative humidity of 70-85%, with a 16h day and 8h dark photoperiod. The pots were irrigated regularly to near field capacity with tap water.

2.3. Plant sampling and biomass measurement

The plants were harvested 45 days after seedling. Root systems were separated from shoots and fresh root system was weighed immediately as well as plant shoots. Half of each root sample was fixed in FAA (37% Formaldehyde- Glacial Acetic Acid -95 Ethanol, 9:0.5:0.5, *V:V:V*) for quantification of AM fungal colonization and vesicular numbers. The remaining half of each samples (for root and shoot) were oven dried (80 °C for 48h) and used for measurement of P concentration.

2.4. Measurments of mycorrhizae, and P

Arbuscular mycorrhizal fungi colonization of roots was quantified using a dissection microscope (20-40×) after cleaning the roots in 10% KOH (w/v) and staining them in trypan-blue. A variation of the gridline intersection method, developed by Giovannetti and Mosse, (1980), was used to determine the proportion of root length in which arbuscules, vesicles or hyphae occurred.

Shoot P concentration was determined by the molybdate blue ascorbic acid method according to Murphy and Riley, (1962) after the plant material was digested by nitric acid and perchloric acid.

2.5. Determination of physiological characteristics

Soluble protein content and proline content were determined by extraction method as described by Zhang, (1990). The youngest leaf was collected from the plant sampled and samples were stored at -4 °C prior to analysis. The following method was used for extraction. Each sample with a weight of 1g was homogenized with a chilled mortar and pestle in 10ml of 100mg ml^{-1} trichloroacetic acid buffer (pH 8.0). Homogenates of samples were centrifuged at 4000 rpm for 10 min. Top aqueous layer was then transferred into 5ml tubes which were incubated in a boiling water bath and quickly placed in an iced-

water bath for 5 min, then centrifuged again. Two ml thiobarbituric acid reagent was added to 2 ml of extracted supernatant. The supernatant was spectrophotometrically determined by measuring the absorbances at different wavelength. The chlorophyll content in leaves were measured by Chlorophyll Content Meter model CL-01 Co. Hansatech Instruments

2.6. Data analysis

Data was analyzed using ANOVA by using SAS statistical software (SAS Institute, Cary, NC, USA). The significance of differences within treatments was separated by using Least Significant Difference test at 5%.

3. Results

3.1. Effect of P- deficiency on plant growth parameters

Data illustrated in Fig. 1 show the effect of three species of arbuscular mycorrhizal fungi (AMF) on maize plant growth parameters in the presence or absent phosphorus (P) fertilization (zero, 60µg P/g of soil or with P, without P respectively). The beast values of all plant growth parameters were detected in the presence of P fertilization level (with P) comparing with untreated P (without P) in all deferent treatments. The highest values of plant height and plant stem length were recorded in the presence of P fertilization and inoculation with AMF species *Glomus etunicatium* and *G. mosseae* (increase by rate 34.5%; 32.9% and 32.5%; 32.2% respectively) comparing with control plants. While the values of plant roots length and plant fresh weight were increased by rate 40%; 46.3% and 37.1%; 46.3% respectively, when maize plants inoculated with *G. clarum* and *G. etunicatum* in the presence of P comparing with values of control plants (Fig. 2). Also the highest value of plant dry weight was recorded in the case of inoculation with AMF specie *G. clarum* by increasing rate 55.7% comparing with control. On contrast the treatment with P causing decreased in all values of root/shoot ratios comparing with the same treatment in the absent of P. The largest decline values at two level of P were recorded in the plant treated with AMF specie *G. mosseae*, then *G. clarum* and *G. etunicatum* followed by control treatment.

3.2. Effects of P- deficiency on some physiological traits for maize plants

Data in Table (1) showed values of P uptake, soluble protein, proline and leaves chlorophyll content for all treatments under sufficiency and deficiency of P. The P uptake values in all treatments were significantly under P sufficiency (60 μg P) than that under P deficiency (zero μg P). The highest values of plant P uptake were recorded in the presence of P and inoculation with AMF specie *Glomus etunicatium*, then *G. clarum* followed by *G. mosseae* comparing with untreated plants. Also all treatments of maize plants in zero level of P were observed a decrease in values of protein content comparing with P fertilization level 60 µg, while increases in proline values were found in all plants at zero level of P comparing with other level. The highly values of plant chlorophyll content (8.306, 7.840 unit) were recorded in the presence of P fertilization and inoculation with AMF species especially *G. clarum* and *G. etunicatum* respectively.

3.3. Mycorrhizae root colonization and spores numbers

No mycorrhizae were found in all the treatments without mycorrhizal inoculation. The mycorrhizae root colonization% was affected by phosphorus fertilization levels. The rate of fertilization 60.0µg P/g of soil was more suitable for AM colonization in plant roots than zero level of P. The highest values of AMF root colonization% (50.5%, 80.3%) were found when plants inoculation with AMF specie *G. etunicatum* at both level of P treatments (zero, 60µg p/pot respectively), then *G. clarum* followed by *G. mosseae*. The same results were observed in number of AMF spores/ 100g of soil. The specie of *G. etunicatum* recorded the highest value of spores (252, 320 spores /100g of soil) at two levels of P fertilization, then *G. clarum* followed by *G. mosseae* (Figs. 3, 4).

4. Discussion

In general, P starvation induced a wide array of metabolic effects that modify plant growth. The presented data provided the strong preliminary evidence for the effects of low P stress on the morphology and physiology of maize plants, which exhibited a reduction in biomass. The inoculation maize plants (*Zea mays* L.) with three arbuscular mycorrhizal fungi (AMF) species are causing significant increase in plant growth parameters, and some physiological traits of maize plants under greenhouse condition compared to control plant. The similar result has been shown by Jakobsen *et al.* (1992); Nurlaeny *et al.*, (1996) and Ortas, (2003, 2009). The large differences in crop growth due to AMF inculation under low fertility soil conditions have been shown in similar studies (Jackson *et al.*, 2002; Martin and Stutz, 2004; Ortas *et al.*, 2011). Also under P deficient condition the density of root hairs was increase in plant roots as symptoms of P starvation. These symptoms are limited in the inculation with AMF without treatment with P. This result was agreement with Baylis, (1970) who found that density of root hairs of maize inbred lines decreased with an increase in soil P. The increase in density of root hairs, contributing to P uptake as

confirmed, appeared to be a response by the plant to low P stress. P is an essential nutrient for plant and associates with many physiological processes. In plant body, such as soluble protein, proline and chlorophyll content are usually recognized as indicative factors under stress conditions. Liang *et al.*, (2005) reported that P deficiency increased the free proline and decreased in protein and chlorophyll content. On the other hand the higher AMF root colonization% was found in the higher level of P addition than in zero level. As well as AMF spores forming in soil increased by increasing of P fertilization. These results are agreement with Asimi *et al.*, (1980), Koide and Li (1990), Koide (1991), Toro *et al.*, (1997) and Ortas *et al.*, (2011) they studied the dramatic effects of infection by mycorrhizal fungi on the host plant, which increase in phosphorus fertilization rate, which mainly due to the capacity of the mycorrhizal fungi to absorb phosphate from soil and transfer it to the host roots.

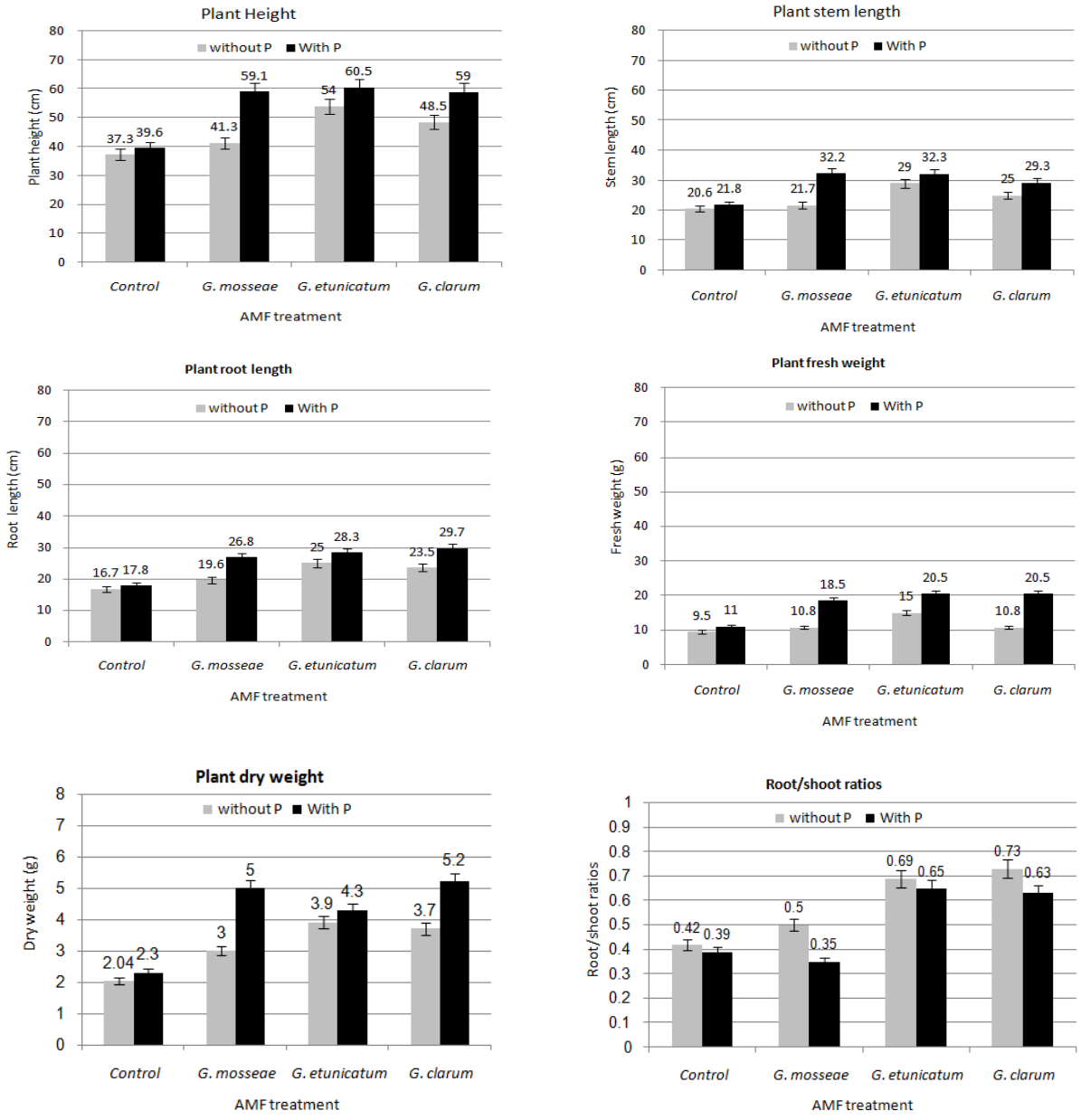

Figure 1: The effect of three AMF species on *Zea mays* L. plant growth parameters at two levels of phosphorus fertilization (without P zero μg, with P 60 μg) after 45 days from inoculation.

Figure 2: Effect of inoculation with three AM fungi species on maize plant (*Zea mays* L.) growth. A: Plants suffering deficiency of P which observing as a purple color on the back side of plant leaves and high density of root hairs (arrows). B: Plants treated with *Glomus mosseae*. C: Plants treated with *G. clarum*. D: Plants treated with *G. etunicatum*.

Table 1: Phosphorus uptake, soluble protein, proline and leaf chlorophyll of maize plant (*Zea mays* L.) at different inoculation with three AMF species in the presence two level of P

Treatment		Plant physiological parameters			
AMF species	Phosphate ($\mu g\ g^{-1}$)	P uptake ($mg\ g^{-1}$)	Soluble protein ($mg\ g^{-1}$)	Proline content ($\mu g\ g^{-1}$)	chlorophyll content (Unit)
Glomus mosseae	Zero	$0.245 \pm 0.03^{*}$	10.338 ± 0.731	$26.571 \pm 0.413^{*}$	4.726 ± 0.662
	60.0	0.698 ± 0.06	13.078 ± 0.464	19.703 ± 0.391	$6.920 \pm 0.548^{*}$
G. etunicatum	Zero	$0.203 \pm 0.05^{*}$	$12.609 \pm 0.461^{*}$	$21.304 \pm 0.562^{*}$	$6.110 \pm 0.806^{*}$
	60.0	0.774 ± 0.06	13.396 ± 0.478	19.703 ± 0.391	$7.840 \pm 0.631^{*}$
G. clarum	Zero	$0.203 \pm 0.05^{*}$	$11.268 \pm 0.475^{*}$	$25.726 \pm 0.562^{*}$	4.876 ± 0.856
	60.0	0.765 ± 0.04	13.632 ± 0.505	19.483 ± 0.435	$8.306 \pm 0.581^{*}$
Untreated	Zero	0.157 ± 0.03	10.278 ± 0.539	27.464 ± 0.496	4.733 ± 0.595
	60.0	0.665 ± 0.05	13.110 ± 0.369	20.325 ± 0.444	5.563 ± 0.190

-Mean of three replication and ± is standard error. - $^{(*)}$ significant level at 5%

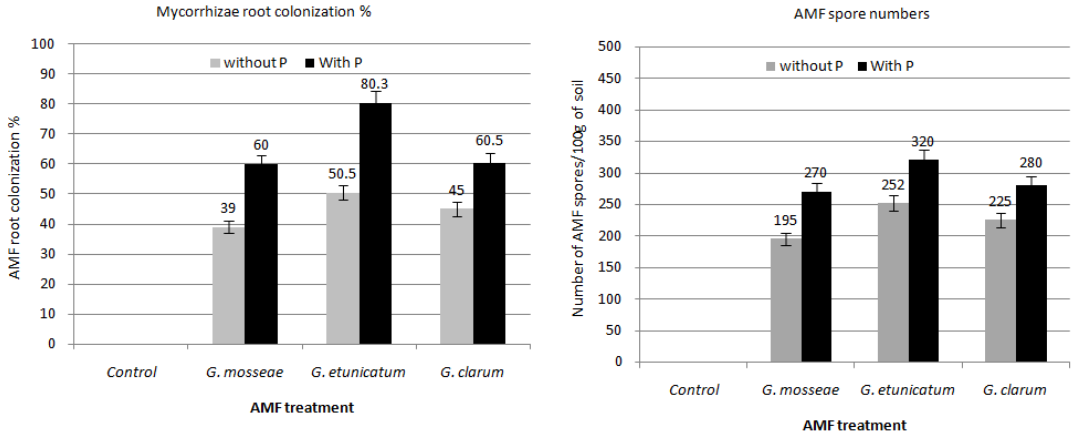

Figure 3: The AMF root colonization%, and spores numbers on maize plant roots (*Zea mays* L.) at inoculation with different AMF species in the presence two level of P

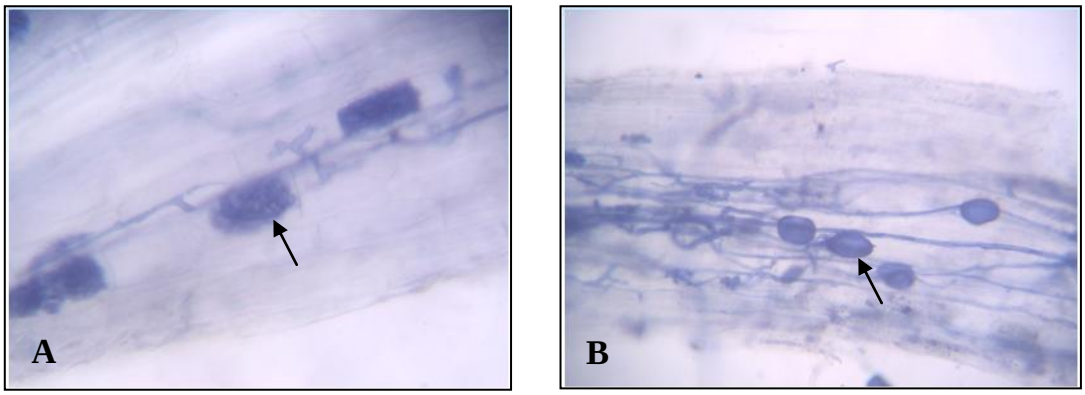

Figure 4: Photomicrographs for arbusculer mycorrhizal fungi (AMF) structures in *Zea mays* L. roots after clearing and staining (200×). The arrows showing a typical arbusculer of AMF(A) and typical vesicle was formed by AMF in the root cortex of maize plants (B).

***Corresponding author**

Abdelmoneim T. S.[1&2*]

[1]Biology Department, Faculty of Science, King AbdulAziz University, P.O. Box 15758, Jeddah 21454, Saudi Arabia.
[2]Suez Canal University, Faculty of Agriculture, Department of Agricultural Botany, P.O. Box 41522, Ismailia, Egypt.
tmabrouk@kau.edu.sa – t.shawky@agr.suez.edu.eg

5. References

Allen, M. F., J. C. Sexton, T. S. Moore and M. Christensen. 1981. Influence of phosphate source on vesicular arbuscular mycorrhizae of Bouteloua gracilis. New Phytologist. 87: 687.

Asimi, S., V. Gianinazzi-Pearson and S. Gianinazzi. 1980. Influence of increasing soil phosphorus levels on interactions between vesicular-arbuscular mycorrhizae and Rhizobium in soybeans. Canadian Journal of Botany 58:2200-2205

Baylis, G. S. 1970. Root hairs and phycomycetous mycorrhizas in phosphorus deficient soil. Plant Soil 33:713-716.

Bucher, M. 2007. Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. New Phytologist. 173: 11–26.

Callow J. A, L. C. M. Capaccio, G. Parish and P. B Tinker. 1978. Detection and estimation of polyphosphate in vesicular-arbuscular mycorrhizas. New Phytologist. 80: 125.

Cress W. A., G. O. Throneberry and D. L. Lindsey. 1979. Kinetics of phosphorus absorption by mycorrhizal and nonmycorrhizal tomato roots. Plant Physiology. 64: 484-487.

Duan, H. Y., F. S. Xu and H. Y. Wang. 2002. Study on difference of phosphorus allocation and accumulation among different cultivars of Brassica napus. Chinese Journal of Oil Crop Sciences 24:46-49.

Ezawa, T., M. Hayatsu and M. Saito. 2005. A new hypothesis on the strategy for acquisition of phosphorus in arbuscular mycorrhiza: up-regulation of secreted acid phosphatase gene in the host plant. Molecular Plant-Microbe Interactions. 18: 1046–1053.

Ezawa, T., S. E. Smith, and F. A. Smith. 2001. Differentiation of polyphosphate metabolism between the extra- and intraradical hyphae of arbuscular mycorrhizal fungi. New Phytologist. 149: 555–563.

Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist. 84:498-500.

Gong, J., L. C. Li, C. L. Xia, J. Wang and S. M. Li. 2002. Selection of inbred for high phosphate efficiency in low phosphate stress. Xinjiang Agricultural Sciences. 39:77-81.

Hajabbasi, M. A. and T. E. Schumacher. 1994. Phosphorus effects on root growth and development in two maize genotypes. Plant and Soil. 158:39-46.

Jackson, L. E., D. Miller and S. E. Smith. 2002. Arbuscular mycorrhizal colonization and growth of wild and cultivated lettuce in response to nitrogen and phosphorus Scientia Horticulturae. 94:205-218.

Jakobsen, L., L. K. Abbett and A. D. Robson. 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with Trifolium subtenaneum L. Hyphal uptake of 32p at detined distances from roots. New Phytologist. 120: 509-516.

Javot, H., R. V. Penmetsa , N. Terzaghi, D. R. Cook and M. J. Harrison. 2007. A Medicago truncatula phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. Proceedings of

the National Academy of Science of the United States of America. 104: 1720–1725.

Koide, R. T. 1991. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. New Phytologist 117:365-386

Koide, R. T. and Li, M. 1990. On host regulation of the vesicular-arbuscular mycorrhizal symbiosis. New Phytologist 114:59-65

Li, S. C., J. Gong and J. Wang. 2003. Screening maize inbred lines seedling for tolerance to low phosphate stress. Journal of Maize Sciences. 11:85-89.

Liang, X.L., Y.C. Lin, H. Nian and L. X. Xie. 2005. The effects of low phosphorus stress on main physiological traits of different maize genotypes. Acta Agronomica Sinica 31: 667-669.

Liu, J. Z., Z. S. Li and J. Y. Li. 1994. Utilization of plant potentialities to enhance the bio-efficiency of phosphorus in soil. Ecoagriculture Research.2:16-23.

Liu, X. S., F. J. Chen, J. L. Song and G. H. Mi. 2003. Genotypic difference of maize inbred lines in tolerance to phosphorus deficiency. Journal of Maize Sciences. 11:23-27.

Martin, C. A. and J. C. Stutz. 2004. Interactive effects of temperature and arbuscular mycrrhizal fungi on growth. P uptake and root respiration of Capsicum annuum L. Mycorrhiza 14(4):241-244.

Mc.Laughlin, M. J., J. R. Fillery and A. R. Till. 1991. Operation of the phosphorus, sulphur and nitrogen cycles. In: Gifford R. M., Barson M. M. Australia's Renewable Resources. Sustainability and Global Change. Canberra. Australia: Bureau of Resources. 67-116

Murphy, J. and J. Riley. 1962. A modified single solution method for the determination of phosphate in natural wasters. Analytic chimica Acta. 27:31-36.

Nurlaeny, N., H. Marschner and E. George. 1996. Effects of liming and mycorrhizal colonization on soil phosphate depletion and phosphate uptake by maize (Zea mays L.) and soybean (Glycine max L.) grown in two tropical acid soils. Plant Soil. 181:275-285.

Nye, P. B. 1977. The rate-limiting step in plant nutrient absorption from soil. Soil Sciences. 123:292-297.

O'Keefe, D. M. and D. M. Sylvia. 1992. Chronology and mechanisms of P-uptake by mycorrhizal sweet potato plants. New Phytologist. 122: 651-659.

Ohtomo, R., and M. Saito. 2005. Polyphosphate dynamics in mycorrhizal roots during colonization of an arbuscular mycorrhizal fungus. New Phytologist. 167: 571–578.

Ortas, I. 2003. Effect of selected mycorrhizal inoculation on phosphorus sustainability in sterile and non-sterile soils in the Harran Plain in South Anatolia. Journal of Plant Nutration. 26:1-17

Ortas, I. 2009. Mycorrhizae application in horticultural production on plant growth. Healthy planets and healthy human. In: XVI International Plant Nutrition Colloquium: Plant Nutrition for Sustainable Development and Global Health. August 26th -30th 2009. Sacramento, California, USA.

Ortas, I., S. Nebahat, C. Akpinar and Y. Halit. 2011. Screening mycorrhiza species for plant growth, P and Zn uptake in pepper seedling grown under greenhouse conditions. Scientia Horticulturae. 128:92-98.

Sanders, F. E. and P. B. Tinker. 1971. Mechanism of absorption of phoshate from soil by Endogone mycorrhizas. Nature 233: 278-279.

SAS Institute, 1988. SAS/STAT User's Guide. Release 6.03 Edition-6th edition. SAS institute Inc., North Carolina, Cary. Inc. pp.1028, 1988.

Sharpley, A. N., B. H. Foy, and P. J. A. Withers. 2000. Practical and innovative measures for the control of agricultural phosphorus losses to water: An overview. Journal of Environmental Quality.29: 1-9

Toro, M., Azcon, R. and Barea, J. 1997. Improvement of arbuscular mycorrhizae development by inoculation of soil with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability (32P) and nutrient cycling. Applied and Environmental Microbiology. Pp. 4408-4412.

Usuda, H. and S. Kousuke. 1991. Phosphate deficiency in maize. Leaf phosphate status, growth, photosynthesis and carbon partitioning. Plant cell Physiology. 32:497-504.

Wang, Q. R., Y. J. Li and Z. S. Li. 1998. Dynamics and prospect on study of high acquisition of soil unavailable phosphorus by plant. Plant Nutrition and Fertilizer Science 4:107-116.

Wang, Y., J. Sun, R. P. Wang, W. Y. Wang, L. S. Zheng and F. S. Zhang. 2003. Selection of genotypic difference in phosphorous efficiency of maize inbred lines. Journal of Shanxi Agricultural Sciences. 31:7-10.

Zhang, Z. L. 1990. Plant Physiology Experiment Manual. 2nd ed. High Educational Press, Beijing. Pp. 141-158.