Egypt. J. Bot. **Vol. 62**, No. 1, pp. 131-148 (2022)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Influence of Local Isolates of Arbuscular Mycorrhizal Fungi on Growth and Physiological Performance in *Zea mays* Grown in Phosphorus-deficient Calcareous Soil

Amel Tammam[(1)#], Weam EL Aggan[(1)], Hala Badry[(2)], RedaAbou-Shanab[(3)], Soha El-Sawy[(1)]

[(1)]Department of Botany and Microbiology, Faculty of Science, Alexandria University, Alexandria, Egypt; [(2)]Department of Soil and Water Science, Faculty of Agriculture, Alexandria University, Alexandria, Egypt; [(3)]Department of Environmental Biotechnology, City of Scientific Research and Technology Applications, New Borg El Arab City, 2193 Alexandria, Egypt.

ARBUSCULAR mycorrhizal fungi (AMF) are fungi that form symbiotic relationships with the roots of higher plants. AMF can potentially be applied to enhance plant tolerance to stress and thus minimize the deleterious effects of abiotic stress. Deficiencies in phosphorus, an essential macronutrient for plants, can have negative effects. Our target was to determine the mitigation effect of local AMF inoculum on the growth and metabolic activity of *Zea mays* at different phosphorus levels (from 0 to 120mg P kg^{-1} soil). Phosphorus deficiency disturbed physiological performance; however, AMF mitigated associated negative effects, enhanced dry weight significantly ($P< 0.05$), and increased P and alkaline phosphatase levels in calcareous soil compared with non-inoculated controls. The maximum H^+-ATPase activity was 28.13µmoL Pi^{-1} ng P^{-1} min in the leaves of *Z. mays* in AMF-inoculated soil treated with 60mg P kg^{-1} soil. AMF enhanced the activities of the antioxidant enzymes superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase together with their substrates glutathione and ascorbic acid, with concurrent reductions in lipid peroxidation and hydrogen peroxide content. The highest mycorrhizal colonization (88%) was recorded in maize grown with 60 mg P kg^{-1} soil. Established data on morphological characteristics revealed that the local AMF isolates contained four native spores related to the genera *Glomus, Acaulospora, Scutellospora,* and *Entrophospora*. Analysis of genetic material confirmed that the spores were related to *Glomus mosseae* and *Acaulospora spinose*. These findings demonstrate that root colonization via local AMF inoculum could ameliorate phosphorus deficiency in calcareous soils from the northwestern coast of Egypt.

Keywords: Alkaline phosphatase, AMF, Antioxidants, Calcareous soil, DNA, Lipid Peroxidation, *Zea mays*.

Introduction

Under the Egyptian National Project for the Reclamation and Development of 1.5 million Feddans, which started in 2016, attention has been focused on the southwestern coast of Egypt, in the Nubaria and Borg El-Arab regions. According to Ministry of Agriculture estimations, calcareous soils constitute 25%–30% of the total area of Egypt. The highly calcareous soils in the northwestern coastal zone offer great possibilities for land reclamation and development to help solve the problem of food production shortages given the available rainfall in that area (Taalab et al., 2019).

Maize (*Zea mays* L.) is one of the most important cereal crops grown principally during the summer season in Egypt. It ranks third among cereal crops in Egypt after wheat and rice, both

#Corresponding author email: amel_tammam@yahoo.com Telephone: +2035936157/ 00201281651183
Fax number: +3911794 0000-0003-4952-1432
Received 12/02/ 2021; Accepted 02/08/ 2021
DOI: 10.21608/ejbo.2021.58373.1612
Edited by: Prof. Dr. Hamada Abdelgawad, Department of Biology, University of Antwerp, Belgium.

in terms of area and production (Khaffagy et al., 2020). Maize is also important in the national economy of Egypt as it is a multipurpose crop; it provides human food, poultry and animal feed, raw materials for some industries (such as the starch industry), and is used in the preparation of other products. Maize bread is a staple food in most urban and rural areas of Egypt (Mohamed, 2020). In 2018, the total cultivated area of maize was about 2.5 million Feddans (Agricultural Statistics, 2019). Its production is predicted to increase by 161 million tons to 1.2 billion tons by 2027 (OECD-FAO, 2018).

Phosphorus (P) is one of the 17 essential nutrients required for plant growth and function; it forms an integral part of the chemical structures of adenosine triphosphate, phospholipids, and nucleic acids. It is required in regulatory metabolic pathways, including energy transfer, amino acid synthesis, and protein activation (Dixon et al., 2020).

Phosphorus is the least accessible macronutrient and the most frequently deficient nutrient in most agricultural soils because of its low bioavailability, especially in calcareous soil. The low availability of P to plants is attributed to the fact that the bulk of soil P is present in non-soluble forms, and plants take up only soluble forms of P, i.e., monobasic ($H_2PO_4^-$) and/or dibasic (HPO_4^{2-}) forms (Kalayu, 2019).

Plant roots have a high degree of phenotypic plasticity and their morphology is affected by numerous factors, including soil microorganisms, which play an important role in mediating the forms of P available to plant roots. Phosphate-solubilizing microorganisms prevail in soils; these microorganisms exude phosphatases and organic acids, which facilitate the conversion of unavailable forms of P into available forms (Kalayu, 2019). Phosphatases are good indicators of soil fertility; they play a vital role in soil structure and are responsible for the production of anhydrides of phosphoric acid and the hydrolysis of esters, which increases P availability for plants (Cardoso et al., 2013).

To overcome P deficiency, about 80% of plant species establish symbioses with arbuscular mycorrhizal fungi (AMF) (Andrino et al., 2021). AMF are among the most important endophytic fungi that form arbuscular mycorrhizas with plant roots. AMF can absorb mineral nutrients for plant partners through extensive networks of hyphae in the soil in exchange for photosynthates and lipids (Wu et al., 2019). Among AMF, *Glomus spp.* (local isolates) have been recorded in many agricultural soils. These fungi play a clear role in protecting plants from stress and the harmful effects of pollution (Huang et al., 2020).

AMF have the potential to improve soil characteristics, thereby promoting plant growth in normal and stressful environments and enhancing plant tolerance of biotic and/or abiotic stresses (Hashem et al., 2018). AMF perform critical roles in osmatic adjustment, improve respiration, increase photosynthetic efficiency, and regulate the hydraulic conductance of roots (Sharma et al., 2017).

Phosphorus deficiency can negatively impact plant development and severe deficiency can lead to death. Such deficiencies can impact plant metabolic processes, which may trigger oxidative stress and the production of harmful reactive oxygen species (ROS), including superoxide anions ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydroxyl radicals ($HO^{\bullet}$), and hydrogen peroxide (H_2O_2) (Hashem et al., 2018). If not scavenged, these ROS can easily damage the functional and structural integrity of many important macromolecules, including proteins, enzymes, phospholipids, and nucleic acids (Dixon et al., 2020). To counteract these harmful effects and exert close control over ROS production, plants utilize enzymes, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), together with their non-enzymatic substrates glutathione (GSH) and ascorbic acid (AA), in antioxidant defense systems. These defense systems catalyze redox reactions, which control levels of ROS in cell membranes and prevent lipid peroxidation (Miret & Munné-Bosch, 2015). H^+-ATPase, a "master enzyme" of the plant plasma membrane, is involved in the correlation between nutrient uptake and environmental stresses. Its activity is closely linked with various essential processes related to plant growth and development, including cellular expansion, ion uptake and homeostasis, changes in intracellular pH, and stomatal responses (Liu et al., 2016).

Due to the increasing Egyptian population, the need to produce larger quantities of quality

cereals is currently an emergent challenge. Thus, optimization of the use of P fertilizer is becoming a significant topic for study. There is also a need for extensive and consistent efforts to increase cereal cultivation particularly along the coast of Egypt, where the soils are calcareous. Thus, the present study was conducted to evaluate the influence of selected local isolates of AMF inoculum (containing *Glomus mosseae* and *Acaulospora spinose)* on the growth, physiological performance, and biochemical attributes of maize plants and the protective role of AMF colonization against the deleterious effects of low P availability in calcareous soil.

Materials and Methods

Soil sampling, analysis, and preparation

Samples of calcareous soil were collected from the northwestern coastal region of Alexandria, in Borg El-Arab city, Egypt. Soils were mixed in a large container, air-dried, ground, and then bulked up to obtain composite samples. The chemical and biological properties of the soil were determined at the Soil Testing Laboratory, Faculty of Agriculture, Alexandria University, Egypt. The chemical properties of the soil are outlined in Table 1.

TABLE 1. Biological and chemical properties of soil used in this study

Soluble cations & anions (meq/L) in soil paste	
E.C (dS/m) in soil paste	1.1
Ca^{2+}	3.0
Mg^{2+}	0.1
Na^{+}	11
K^{+}	1.3
HCO_3^{-}	4.4
Cl^{-}	0.4
SO_4^{-}	0.7
Number of spore (spore/gm soil)	200
pH	8.55
Available phosphorus (mg/kg)	7.00
Available potassium (mg/kg)	240
Available nitrogen (mg/kg)	
NH_4^{+} - N	119
NO_3^{-} - N	119
Total carbonate %	32.5
Organic matter%	0.82

Isolation of AMF spores and inoculum preparation

Spores were isolated from soil using wet filtering and decanting techniques according to Pacioni (1992). The AMF spores were multiplied on onion roots grown in sterilized calcareous soil for 45 d. Soil samples containing hyphae, spores, and root residue were dried in air for use as a source of AMF spores (inoculum). Inoculum was analyzed based on the number of spores per g of air-dried soil.

Plant cultivation and experimental design

The pure grain variety of *Z. mays* L. cv Sakha 131 was kindly provided by the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Maize grains were carefully chosen for viability and homogeneity of size and form. Grains were sterilized by immersion in sodium hypochloride (1% available chlorine) for 10min, then washed several times with sterilized water. The experiment was conducted in a greenhouse under normal light intensity, a temperature of about 30°C±2°C, a cycle of 14/10h light/dark, and 85% relative air humidity.

Experiments were conducted in 40 plastic pots with three holes each at the bottom that were covered with two layers of filter paper before the pots were filled with prepared calcareous soil. Seven maize grains were sown in each pot and left to germinate for 7 d, then the plants were thinned to four seedlings. A completely randomized block design with four replicates for each treatment was used. The experiment combined two treatments: a non-mycorrhizal control and *Glomus mosseae* and *Acaulospora spinose* inoculated at five P levels (0, 15, 30, 60, and 120mg P kg^{-1} soil, added as KH_2PO_4). The soil was sterilized at a high temperature to ensure elimination of AMF, and the same quantity of soil was used for each treatment. AMF inoculation was achieved by thoroughly mixing 50 g of inoculum consisting of sand, spores, hyphal fragments, and pieces of mycorrhizal roots with the soil in each pot.

Plants were given 250mL of half-strength Hoagland solution (Hoagland & Arnon, 1950) per pot every week and 100mL distilled water per pot every 2 d. After 45 d, *Z. mays* plants were harvested. Two plants from each pot were used to determine fresh and dry weights and the other two were used to measure physiological parameters. Fresh roots and shoots were harvested separately. Dry weights were obtained by drying samples at 70°C in an oven until they reached a constant weight.

Half of the root samples were used to determine the mycorrhizal colonization percentage. The collected samples were rinsed thoroughly in running water, cut into 1 cm pieces, then stained with Trypan blue according to Phillips & Hayman (1970). Stained root segments were examined using a digital computerized microscope (model DP-72, Olympus) at 20× magnification.

Percentage root colonization was determined using the following equation:

$$\text{Mycorrhizal colonization \%} = \frac{\text{no of root segments colonized}}{\text{no of root segments observed}} \times 100$$

Physiological parameters and plant analyses

Determination of phosphorus

Phosphorus was estimated using vanadium phosphomolybdate method by digestion with an acid mixture (di-acid or tri-acid). Phosphorus content of plant sample is converted into orthophosphate according to (United state Environmental Protection Agency, EPA, 1983).

Oxidative damage signals

Lipid peroxidation expressed as the amount of malondialdehyde (MDA) produced by the thioburbituric acid (TBA) reaction (Wang et al., 2009). Hydrogen peroxide production decided according to Velikova et al. (2000).

Estimation of alkaline phosphatase activity, ALP (EC 3.1.3.1)

Plant sample (5.0g fresh weight) were broken using a frozen mortar with acid - washed sand and 20mL of chilled 0.05M sodium carbonate buffer (pH 10). Filter the homogenate through dual levels of cheesecloth then centrifuged at 20.000g for 20min. The supernatant was used as the source of the crude enzyme. Alkaline phosphatase activity assayed using modified method described by Bowers & McComb (1966).

Estimation of H^+- ATPase activity (EC 3. 6. 1. 35)

Plasma membranes separated from maize plant (roots or leaves) as modified method for high purity plasma membrane isolation by Faraday & Spanswick (1992). H^+-ATPase activity assessed according to the method described by Wu &Seliskar (1998).

Estimation of antioxidant enzymes activity

Enzyme extract for superoxide dismutase (SOD, EC 1.15.1.1) activity was prepared from sample of fresh shoots or roots (1g), homogenized in 8mL potassium phosphate buffer (50mM, pH 7.8) containing 0.1 mM Na_2 EDTA and 1% polyvinylpyrrolidone (PVP) with a chilled pestle and mortar. The homogenate centrifuged at 20.000 xg for 20min. The supernatant used for SOD assay following the method of Beyer & Fridovich (1987).

Preparation of enzyme extract for estimation catalase, ascorbate peroxidase and glutathione reductase activities were determined as described by Gossett et al. (1994); samples homogenate, centrifuged at 10.000g for 10min at 4°C and supernatants were collected. Catalase (CAT, EC1.16.1.6) activity assessed by determining the rate of H_2O_2 disappearance using spectrophotometer at 25°C (Patterson et al., 1984). Ascorbate peroxidase (APX, EC 1.11.1.11) activity assayed as described by Nakano & Asada (1981). Glutathione reductase (GR, EC 1.8.1.7) activity calculated by method of Smith et al. (1988).

Estimation of non-enzymatic antioxidants

Samples of approximately 0.1g leaf fresh weight were extracted by grinding in liquid nitrogen and 1ml of 5% (w/v) trichloroacetic acid was added. Centrifuged at 12.000 xg for 10min at 4°C, the supernatant used for determination the water-soluble antioxidant contents (GSH and AA content). Reduced AA and oxidized DHA was determined according to Pinto et al. (1999). Reduced GSH and oxidized GSSG glutathione levels decided according to Griffith (1980).

Identification of AMF spores

Morphological identification of spores was carried out according to Walker & Schüßler (2004) and culture database established by International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM, 2014).

Molecular identification of arbuscular mycorrhizal fungi

A preliminary rough molecular characterization of AMF inoculum (propagules) using as a first step the genus specific primers VAGLO, and VAACAU in conjunction with VANS1 (Simon et al., 1993) using to confirm the morphological identification of local AMF inoculum. Genomic DNA extracted from spores of AMF using SDS/CTAB lysis protocol and phenol /chloroform extraction method. The extracted DNA was purified by Gene JET Genomic DNA Purification kit. PCR amplification

reactions were performed with VAACAU primer (5'-TGATTCACCAATGGGAAACCC-3') and VAGLO primer (5'-CAAGGGAATCGGTTGCCCGAT-3') which as specific to the arbuscular mycorrhiza (Schüßler et al*.,* 2001). VAACAU primer is considered specific to classify AM belonging to the *Enterophospora* or *Acaulospora* whereas, VAGLO primer used to detect AM belonging to *Glomus*, *Gigaspora* and *Scutellospora.* PCR was carried out using 25ng of genomic DNA and 0.5 µM of each primer (there is no primer paring) add to the master mix (DreamTaq Green PCR Master Mix (2X), K1081) to final volume of 25µL. The cycling profile was as follows: 35 amplification cycles at 94°C for 60sec, 50°C for 45min and 72°C for 1min go along by a final extension step, 10 min at 72°C in the thermocycler (Creacon, Holand). PCR products of SSU rRNA gene were run on a 1.5% agarose gel at 80V for 100min and imagined by staining with ethidium bromide (Simon et al*.,* 1993). PCR amplified product of interest was excised from gel and was purified by using gel extraction kit (PROMEGA, USA). After purification, PCR product was sequenced using sequencing primers (specific primer for each amplicon) on an ABI PRISM 3730 × l DNA Analyzer System at Macrogen (Seoul, Korea). Sequence data were supported in CLUSTAL W (1.8) program. The DNA sequences generated from the sample was compared to sequence in the public database using basic local alignment search tool (BLAST) on the national center for biotechnology information (NCBI) website. Homology of the sequence of the isolate was analyzed by using BLAST program (Thompson et al., 1997).

Statistical analysis

Statistical analyses were conducted according to Duncan's multiple range tests. Data were analyzed by two-way ANOVA and the LSD at $P \leq 0.01$ was established after system of Sokal & Rohlf (1995).

Results

The chemical and biological properties of soil were recorded; the soil pH was 8.55 and the electrical conductivity of soil paste was 1.1 dS/m. The levels of available soluble cations (Ca^{2+}, Mg^{2+}, and K^{+}) and anions (HCO_3^-, Cl^-, and SO_4^-) in the soil as well as soil organic matter were recorded (Table 1).

Effect of AMF on growth of Z. mays plants

The effects of both external P levels and local AMF inoculum (*G. mosseae* and *A. spinosa*) on the growth of *Z. mays* plants, as measured in terms of plant height and shoot and root traits, are shown in Table 2. The highest value for each parameter was detected in the presence and absence of mycorrhizal treatments for all P treatments.

The highest values of these parameters were recorded in the presence of 60 and 120 mg P kg^{-1} in both mycorrhizal treatments. A reduction all values was recorded at phosphate levels below 60 mg P kg^{-1}. All tested traits showed higher values in mycorrhizal plants than in the corresponding controls at all P levels tested. Application of local isolates AMF inoculum (*G. mosseae* and *A. spinosa*) under P deficiency resulted in a 20% and 26% increase in root and shoot dry weight, respectively.

P treatment caused a gradual decrease in root/shoot ratio dependent on P concentration. This ratio decreased with increasing P concentrations, reaching 0.81 at P deficiency and 0.58 at 60 mg P kg^{-1}. Insignificant differences in root/shoot ratios between different P treatments and their corresponding controls were recorded, indicating that under P stress the root system of *Z. mays* spreads out in the soil.

Colonization with AMF

As shown in Fig. 1, there was an approximately constant percentage of mycorrhizal root colonization (about 29%–31%) in maize plants under P treatments without external mycorrhizal inoculation. The highest percentage of mycorrhizal root colonization was about 88% in inoculated plants at 60mg P kg^{-1}. As the P concentration increased to 120mg K^{-1}, the colonization percentage decreased by about 31%.

Effects of AMF on the status of the biochemical reactions of Z. mays

In general, maize H^+-ATPase activity was higher in leaves than in roots under all treatment conditions. Under P deficiency and mycorrhizal treatment, maize leaves exhibited 1.2-fold higher H^+-ATPase activity than roots. Under P sufficiency and mycorrhizal treatment, maize leaves exhibited 1.4-fold higher H^+–ATPase activity than roots.

TABLE 2. Effect of five phosphorus levels (0, 15, 30, 60, and 120mg P kg^{-1} soil) on plant height (cm), fresh weight (g plant^{-1}) and dry weight (g plant^{-1}) of *Zea mays* plant in presence (+M) and absence (-M) of mycorrhizal inoculum

Treatments	Plant height (cm)	Fresh weight (g plant^{-1})		Dry weight (g plant^{-1})	
		Shoot system	Root system	Shoot system	Root system
-P-M	20.67 ^{e}±1.274	6.51^{h} ±0.248	4.11^{f}±0.291	1.5^{g}± 0.115	1.31^{g}± 0.114
+P+M	20.83^{e}±1.305	8.04^{g} ±0.438	4.33^{f}±0.379	1.95^{f}± 0.217	1.57^{f}± 0.295
+15mg P kg^{-1} soil-M	23.83^{d} ±0.572	11.54^{f}±0.435	5.85^{e}±0200	2.68^{d,e}± 0.324	1.76^{f}± 0.242
+15mg P kg^{-1} soil+M	24.33^{d} ±0.281	11.90^{f}± 0.200	6.76^{d}±0.226	2.57^{e}± 0.268	1.83^{e,f}± 0.266
+30mg P kg^{-1} soil-M	24.83^{d} ±0.285	14.34^{e} ±0.485	9.73^{b}±0.580	3.19^{c,d}± 0.493	2.00 ^{e}±0.382
+30mg P kg^{-1} soil+M	25.50^{c} ± 0.791	15.22^{d}± 0.621	9.02^{c}±0.531	3.41c± 0.501	2.32^{c,d}±0.487
+60mg P kg^{-1} soil-M	26.33^{c}±0.563	18.15^{c}±0.637	10.07^{b}± 0.527	4.29^{b}±0.4334	2.45^{b,c}±0.392
+60 mg P kg^{-1} soil+M	28.00^{b} ± 0.432	20.90^{b}±0.406	10.81^{a} ±0.440	4.91^{a}± 0.284	2.83^{a,b}±0.276
+120mg P kg^{-1} soil-M	31.00^{a} ±1.216	23.19^{a}± 0.477	11.31^{a} ±0.252	5.12^{a}± 0.355	2.95^{a,b}±0.168
+120mg P kg^{-1} soil+M	31.17^{a} ± 0.175	23.70^{a} ±0624	11.40^{a}± 0529	5.35^{a}± 0.132	3.07^{a}± 0.061

Data are the means of four replicates (±SD).
Means indexed by the same superscript are not significantly different from control as evaluated by Duncan's multiple comparison test.

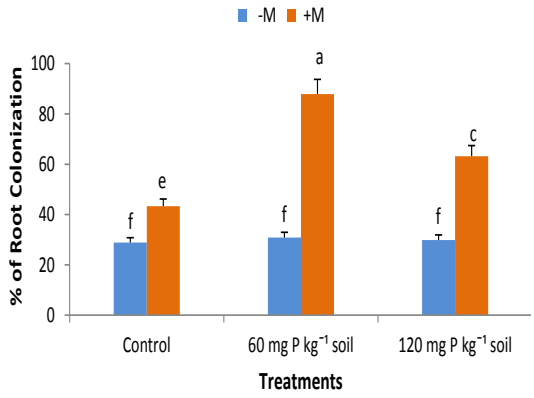


Fig. 1. Effect of phosphorus levels on root colonization percentage of *Zea mays* plant in presence (+M) and absence (-M) of mycorrhizal inoculum [Data are the means of four replicates (±SD). Means indexed by the same superscript are not significantly different from control as evaluated by Duncan's multiple comparison test]

With AMF inoculation, H^{+}–ATPase activity was higher than in the corresponding controls. The H^{+}-ATPase activity in maize leaves at 0 and 60 mg P kg^{-1} was significantly increased by about 28.4% and 18.7%, respectively, compared to controls. The corresponding values for roots were 15.9% and 30.7%, respectively (Fig. 2).

Effects of AMF on the P content and alkaline phosphatase activity of Z.mays

Under control conditions at all P concentrations, maize showed higher P content in its roots than its shoots. As the concentration of P increased, intracellular P was significantly increased in the root and shoot systems in the presence and absence of AMP (Fig. 3A).

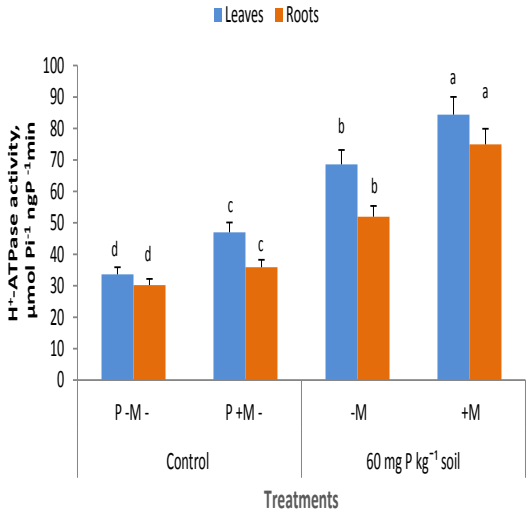


Fig. 2. Effect of P-sufficient (60 mg P kg^{-1} soil) and P-deficient (0mg P kg^{-1} soil) conditions on activity of H+-ATPase (µmol Pi^{-1}ngP^{-1}min) of *Zea mays* grown in calcareous soils in the presence (+M) and absence (-M) of mycorrhizal inoculum [Data are the means of four replicates (±SD). Means indexed by the same superscript are not significantly different from control as evaluated by Duncan's multiple comparison test]

The influence of phosphate fertilization on alkaline phosphatase (ALP) activity was investigated as a prospective indicator of efficiency of AMF symbiosis. In general, inoculated plants exhibited higher ALP activity than control plants regardless of P availability. Under P deficiency, ALP activity in the leaves and roots of non-mycorrhizal plants reduced by 12.2% and 11.1%, respectively, compared to mycorrhizal plants (Fig. 3B). The ALP activity in mycorrhizal roots at 60 mg P kg^{-1} was 2.6-fold higher than that under P deficiency. Maize exhibited elevated ALP activity in the roots compared to the leaves at all P concentrations studied.

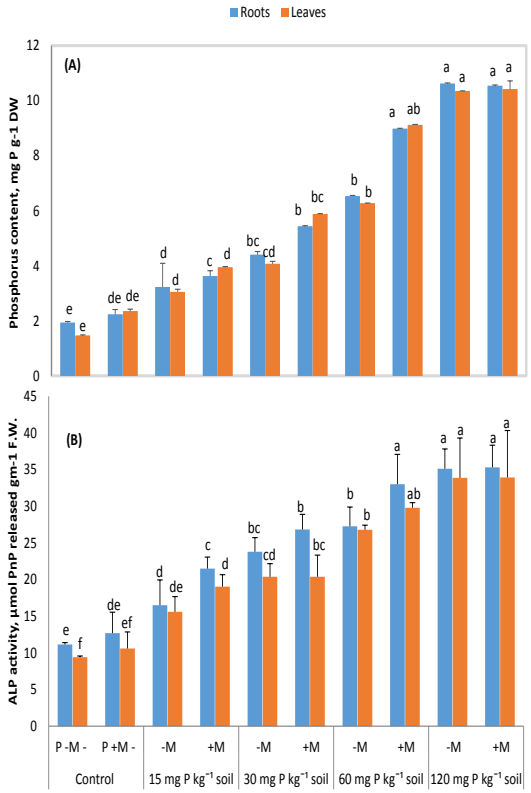


Fig. 3. Effect of different phosphorus levels on phosphorus contents (mg g^{-1} DW) and alkaline phosphatase activity (ALP), µmol PnP released gm^{-1} F.W. of *Zea mays* plant grown in calcareous soils in the presence (+M) and absence (-M) of mycorrhizal inoculum [Data are the means of four replicates (±SD). Means indexed by the same superscript are not significantly different from control]

Effects of AMF on oxidative damage signals in Z. mays

Oxidative damage was measured based on MDA content, and was higher in non-inoculated than inoculated plants at all P levels. Results also indicated that P deficiency caused a significant increase in MDA content (Fig. 4A). The MDA level was decreased significantly by increasing P concentration; at 60mg P kg^{-1}, mycorrhizal treatment led to 44.4% and 53.7% decreases in MDA content in leaves and roots, respectively, compared to 30mg P kg^{-1}.

Analysis of the production of H_2O_2 in maize leaves and roots (Fig. 4B) showed that mycorrhizal plants exhibited lower production of H_2O_2 than non-mycorrhizal plants. Under P deficiency, mycorrhizal maize leaves exhibited a 26% decrease in H_2O_2 production compared to controls. At 30mg P kg^{1}, the H_2O_2 content in mycorrhizal leaves and roots was decreased by 37% and 35%, respectively, relative to controls. Hydrogen peroxide production was significantly decreased as P increased to 60 or 120 mg P kg^{-1} with a particularly remarkable decrease in mycorrhizal plants (Fig. 4B).

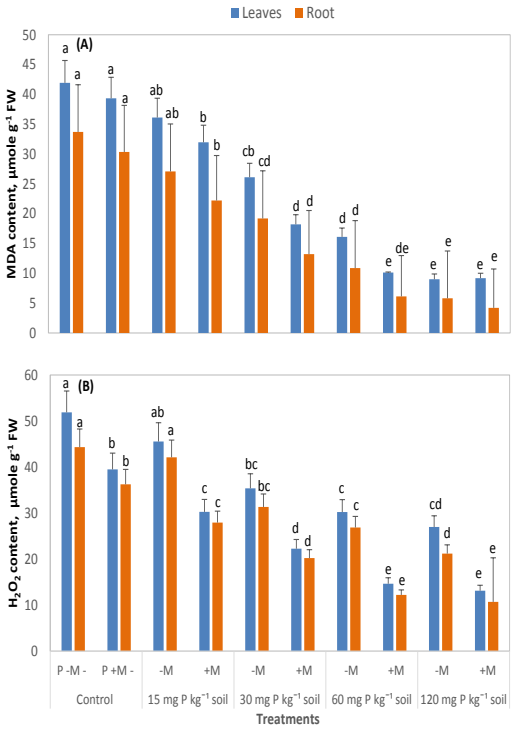


Fig. 4. Effect of different phosphorus levels on (A) Malondialdehyde (MDA) concentrations and (B) Hydrogen peroxide (µmole g^{-1} FW) production of *Zea mays* plant grown in calcareous soils in the presence (+M) and absence (-M) of mycorrhizal inoculum [Data are the means of four replicates (±SD). Means indexed by the same superscript are not significantly different from control as evaluated by Duncan's multiple comparison test]

Effects of AMF on the activity of antioxidant enzymes in Z. mays

The SOD activity in both the roots and leaves of inoculated plants was significantly higher than that in non-inoculated plants under P deficiency. Mycorrhizal treatment under P deficiency decreased SOD activity by 12.4% and 7.0% in *Z. mays* roots and leaves, respectively, compared to corresponding controls. Meanwhile, the leaves and roots of non-inoculated plants exhibited higher SOD activity than those of inoculated plants under moderate or sufficient P conditions (Fig. 5A).

Significant reductions in the SOD activity of maize roots and leaves were associated with increased P concentrations. SOD activity in mycorrhizal maize leaves was significantly decreased by about 14% and 37% at 15 and 30mg P kg^{-1}, respectively, compared to that in P-deficient plants. The same trend was observed for mycorrhizal maize roots, in which the corresponding values were 26.6% and 60.8%, respectively. The SOD activity of non-mycorrhizal leaves and roots decreased with the addition of P to the soil. The percentage decrease in the SOD activity of inoculated plants was clearly higher than that in non-inoculated plants.

The CAT activity of non-inoculated maize was lower than that of inoculated plants under sufficient P conditions. Under P stress, the CAT activity of inoculated plants was low compared to that of non-inoculated plants. At all studied P concentrations, CAT activity in both roots and leaves showed insignificant differences between mycorrhizal treatments. At the lowest and moderate P concentrations used, the CAT activities of mycorrhizal *Z. mays* leaves were 1.1-fold higher than those of the corresponding controls. Also, at high P concentrations (60 and 120mg P kg^{-1}), CAT activities were about 1.1- and 1.2-fold higher than those of the corresponding controls. CAT activity in leaves decreased significantly with increasing P concentrations under both mycorrhizal treatments. This decrease in activity was observed even at the maximum P level used in non-mycorrhizal plants. The lowest CAT activity was found at 60 and 120mg P kg^{-1} in mycorrhizal plants (Fig. 5B).

APX activity was always higher in inoculated than in non-inoculated maize roots and leaves. In mycorrhizal plants, APX activity was lower in roots than in leaves at all P levels. Phosphorus stress increased the APX activity of leaves in the presence and absence of mycorrhizal treatments compared with sufficient P conditions (Fig. 5C).

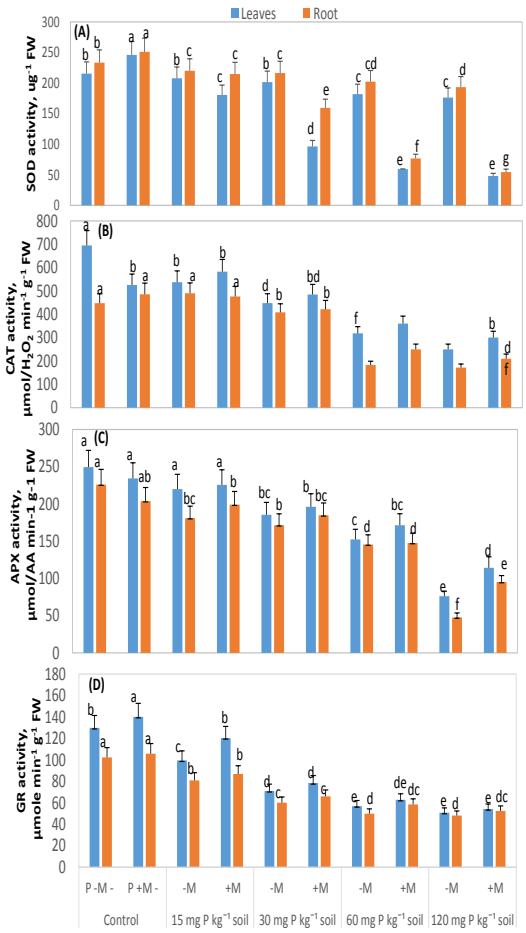


Fig. 5. Effect of different phosphorus levels on (A) Superoxide dismutase (SOD), (B) Catalase (CAT), (C) Ascorbate peroxidase (APX) and (D) Glutathione reductase (GR) of *Zea mays* plant grown in calcareous soils in the presence (+M) and absence (-M) of mycorrhizal inoculum [Data are the means of four replicates (±SD). Means indexed by the same superscript are not significantly different from control as evaluated by Duncan's multiple comparison test.]

Under P deficiency, APX activities in the roots and leaves of inoculated plants decreased by about 95% and 6%, respectively, compared to corresponding controls. Treatment with 15 and 30mg P kg^{-1} decreased APX activity significantly in both roots and leaves by different degrees (Fig. 5C). APX activity sharply decreased when maize

plants were supplemented with sufficient P under both mycorrhizal treatments.

GR activity was higher in inoculated than in non-inoculated maize at all P levels used (Fig. 5D). GR activity in the leaves of maize plants under mycorrhizal treatment and P deficiency was 7% higher than in non-mycorrhizal treatment. GR activity was highest under P deficiency, but a significant decline in GR activity was observed as P concentrations increased. With mycorrhizal treatment, GR activity in the roots of maize at 15 and 30mg P kg^{-1} increased by about 7% and 9%, respectively, relative to corresponding controls (Fig. 5D). At the highest P concentration used, GR activity decreased by about 61% in mycorrhizal maize leaves relative to P-deficient leaves. These values were 50% and 53% in the roots of inoculated and non-inoculated plants, respectively.

Effects of AMF on the non-enzymatic antioxidant status of Z. mays

GSH content in plant leaves and roots decreased as P concentrations increased in the presence and absence of mycorrhizal treatments. At all P levels tested, GSH contents were higher in non-inoculated plants than in inoculated plants. Under P deficiency, GSH accounted for 68.8% and 56.6% of the total glutathione pool in leaves and roots, respectively, in non-mycorrhizal maize. The corresponding values at P sufficiency were 50% and 38.5% (Fig. 6A). The concentration of GSSG remained largely unchanged in both mycorrhizal treatments (Fig. 6B). The ratio GHS/GSSG (redox state) decreased with increasing P levels, but the rate of increase in both leaves and roots in non-inoculated plants was greater than that in inoculated plants (Fig. 6C).

Contents of ascorbic acid in its reduced (AA) and oxidized (DHA) forms as well as the AA/DHA ratio of maize at all P levels in the presence and absence of AMF are represented in Fig. 7A, B, and C. Mycorrhizal maize leaves and roots contained higher amounts of AA under P deficiency than under P sufficiency. As P concentration increased, AA content decreased in both leaves and roots in the presence and absence of AMF. Under P deficiency, AA accounted for 48.6% and 45.0% of the total ascorbate pool of leaves and roots, respectively, in non-mycorrhizal plants. The equivalent values at P sufficiency were 32.8% and 29.2%. Meanwhile, the DHA concentration remained largely unchanged in maize leaves under both mycorrhizal treatments (Fig. 7B). The ratio of AA/DHA (redox state) decreased with increasing P levels; the rate of increase in both the leaves and roots of non-inoculated plants was greater than that in AMF-inoculated plants.

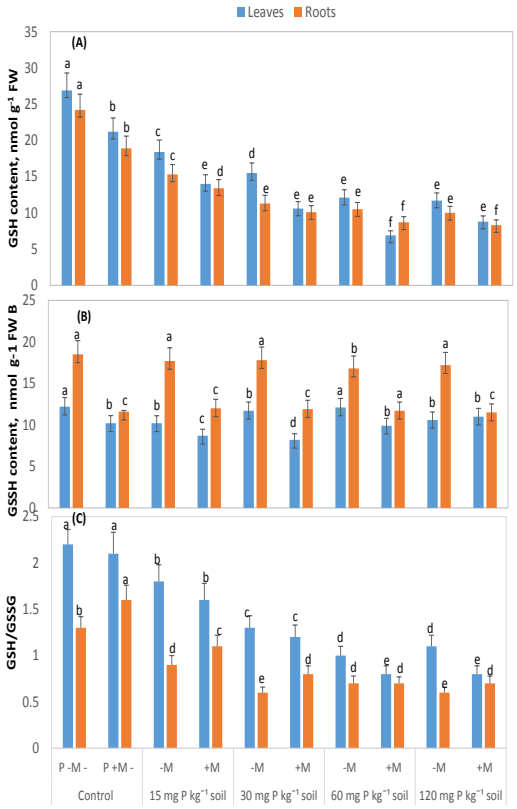


Fig. 6. Effect of different phosphorus levels on (A) Reduced glutathione (GSH), (B) Oxidized (GSSG), and (C) The ratio of GSH/GSSG (nmol g^{-1} FW) of *Zea mays* grown in calcareous soils in the presence (+M) and absence (-M) of mycorrhizal inoculum [Data are the means of four replicates (±SD). Means indexed by the same superscript are not significantly different from control as evaluated by Duncan's multiple comparison test.]

Morphological and molecular identification of AMF

Morphological variation among spores was observed depending on the species. Four native dominant AMF genera were separated from the rhizosphere of *Z. mays* grown on calcareous soils. Based on the morphological features of the spores (size, color, and cell wall structure), these genera were identified as *Glomus*, *Acaulospora*, *Scutellospora,* and *Entrophospora,* belonging to the families Glomaceae, Acaulosporaceae, and Gigasporaceae, respectively (Fig. 8).

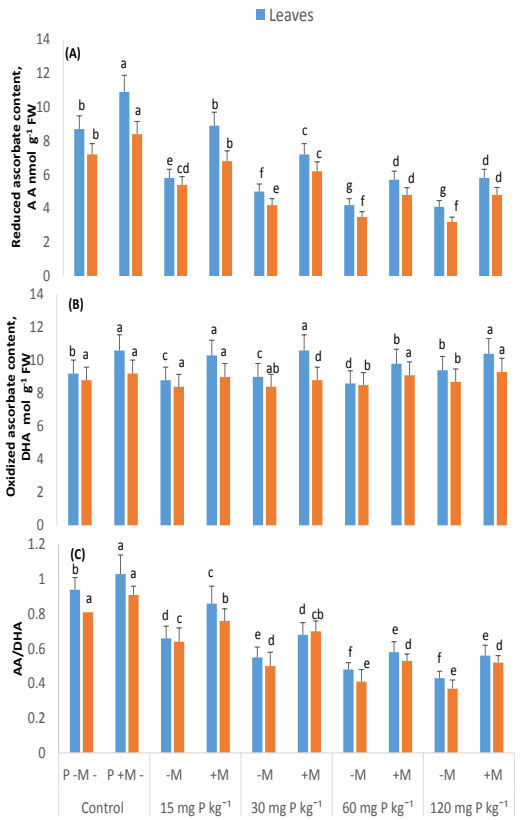
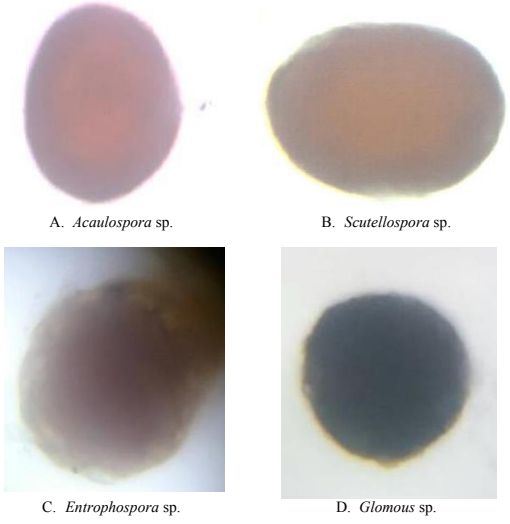


Fig. 7. Effect of different phosphorus levels on (A) Reduced (AA), (B) Oxidized (DHA) ascorbic acid contents and (C) The ratio of AA/ DHA of *Zea mays* grown in calcareous soils in the presence (+M) and absence (-M) of mycorrhizal inoculum [Data are the means of four replicates (±SD). Means indexed by the same superscript are not significantly different from control as evaluated by Duncan's multiple comparison test.]



A. *Acaulospora* sp. B. *Scutellospora* sp.

C. *Entrophospora* sp. D. *Glomous* sp.

Fig. 8. Different spors seperated from the calcareous soil in Borg EL-Arab

Molecular identification of family-specific amplifications

Fungal identification using two taxon-specific primers (VAACAU and VAGLO) generated two different PCR products of 150 and 200 bp, which corresponded to different AMF families. The presence or absence of an amplified fragment in the two separate amplification reactions performed could be used to discriminate between Glomaceae, Acaulosporaceae, and Gigasporaceae. Positive PCR results for Glomaceae and Acaulosporaceae were obtained in the tested sample. No PCR product bands were generated for Gigasporaceae. Purified PCR products of the expected size were sequenced with the same primer (VAACAU, VAGLO) using an Applied Biosystems 3730X-1DNA Analyzer (Fast Smack Inc., Division DNA Synthesis, Kanagawa, Japan).

Sequence analysis of PCR products

A search for sequences similar to found in the isolate was performed with the BLAST tool (http://www.ncbi.nlm.nih.gov/webcite) provided by GenBank. A homology sequence of the isolate was analyzed using the BLAST program. Sequences with high similarity and a wide selection of AMF taxa, including representatives of the major clades, were analyzed. The obtained sequence of 184 bp scored 97% identity with *G. mosseae* isolate BEG99. Meanwhile, the sequence of 195 bp scored 97% identity with *A. spinose* strain FO316.

Sequence of *Acaulospora spinose*

```
gataagtaat gtgaattgca gaattccgtg aatcatcaaa tctttgaacg caaattgcac      60
tctttggtat tccgaagagt atgcttgctt gagggttgtt caaataaatc gtaaattttt     120
tgcggacctg agttttaaaa ttttggtaac taaggtaacg attttaaatt taagttttcc     180
aatctttgaa aatgt                                                     195
```

Sequence of *Glomus mosseae*

```
gtcacgggaa atcaaccttt tgagttcgtg ggtttgaaga gtttcaaagc cttcggattt      60
gtgagattgg gatctcttgg tGaagtgtta tagcctttgg tagatgtgat gtttgagacc     120
gaggattgca acggttaccc ttcagggcta ttcgtctgat ctctgatacg ttgccttgat     180
gttg                                                                 184
```

Discussion

Phosphorus is a main constituent element of many biologically important compounds, such as nucleic acids and phospholipids. It is also required for photosynthesis, energy storage and transfer, carbohydrate transport, and regulation of the activity of some enzymes (Dixon et al., 2020). AMF symbiosis is a stoichiometric relationship between the roots of vascular plants and fungi of the phylum Glomeromycota. A key feature of this symbiosis is bidirectional exchange of carbohydrates and nutrients, whereby phosphates

and possibly other nutrients are transported through the fungal hyphae into plant roots (Gaude et al., 2015). The data obtained in the present study show a reduction in the maize traits tested (height and fresh and dry weights of shoots and roots) as phosphate levels decreased. This may be due to iron deficiency in calcareous soils (Ferreira et al., 2019), as iron is important in photosynthesis and carbohydrate synthesis. Phosphorus stress causes a reduction in stomatal conductance and non-stomatal limitations, which leads to severely reduced leaf growth (Singh et al., 2013). Phosphorus stress also increases root biomass, which causes substantial alterations in root morphology and architecture, including total root system size and lateral root number and length, leading to an altered root/shoot ratio (Li et al., 2012**)**.

We also noticed that values for the above traits were higher in inoculated than in non-inoculated plants, suggesting the formation of external mycelium around maize roots by AMF, which would increase the absorption and utilization of phosphate. These results may support the theory that AMF symbiosis evolved to balance nutrient levels in both mycorrhizal fungi and plants with mutualistic C, N, and P exchange. This is because of the following findings: (a) At low water potential, inoculated roots induced higher hydraulic conductivity (Kapoor et al., 2008); (b) Demand for transpiration is increased by higher stomatal conductance (Sheng et al., 2008); (c) AMF accumulate solutes and improve osmotic adjustment processes (Abdel Latef & Chaoxing, 2014). Therefore, mycorrhizal colonization improves the ability of the host plant to use water more efficiently.

In the present study, root/shoot ratios decreased with increasing P concentrations. Mycorrhizal treatment had no obvious effect on this trait. This growth retardation in maize could partially be ascribed to severely reduced leaf growth under P stress, which is a result of diminished leaf assimilates and translocation of photosynthetic metabolites to the root system to prioritize root growth. This improves the ability of stressed plants to take up more P from the surrounding environment in response to P stress. Our result is comparable to the observations of Nguyen & Stangoulis (2019), who reported differences in root morphology and structure in two wheat genotypes as a response to P deficiency. They attributed these differences to changes in root/shoot ratios.

Earlier studies revealed that AMF colonization decreased in soils with high phosphate concentrations (Kaeppler et al., 2000). Here, we recorded highest colonization at 60mg p kg^{-1}, and then significantly reduced colonization at 120mg p kg^{-1}. Therefore, the amount of available phosphate could be the major factor influencing mycorrhizal colonization in the calcareous soil used in the current study. This result demonstrates the inhibitory effect of P on AMF colonization of maize roots at high P concentrations. It is possible that plants are able to weigh up the costs of providing assimilates to fungi vs. the benefits that the fungi will provide (P supply) in a symbiotic relationship; if the balance is unfavorable, then formation of fungal colonization will be inhibited in the plant roots. With a high exogenous phosphate supply (120mg P kg^{-1}), maize plants can obtain sufficient P directly from the surrounding environment without the aid of fungi. Under these conditions, it could be considered a good strategy for the plant to conserve its own energy by inhibiting AMF colonization. Breuillin et al. (2010) reported that high P concentration markedly inhibits symbiotic P transporters and some symbiosis-linked genes, and concluded that P transporters and the effect of high P levels may participate, at least in part, in some overlapping plant processes.

The data in the present study indicate that P levels in maize plants (shoots and roots) under both mycorrhizal treatments increased significantly when P was applied to the soil. Higher uptake of P was recorded in mycorrhizal maize compared to the control. This may be ascribed to the release of inorganic phosphate from AMF to plant root tissues causing high phosphatase activity (Fig. 2), which eventually leads to enhanced growth rates (Table 2). Smith et al. (2011) explained that improvements in the stress tolerance of mycorrhizal maize plant come from direct phosphorus supply by external fungal hyphae, enhanced nutrient status, and improved antioxidant levels in plants. After mycorrhizal colonization of plant roots, ALP is usually present, and this is an indicator of the symbiotic efficiency of root colonization **(**Hu et al., 2015**)**.

The present results reveal that the activities of ALP were higher in mycorrhizal maize compared

with non-mycorrhizal maize at the P levels examined. Given that ALP activity increased as the concentration of P increased, it may be concluded that ALP participates in some way in P assimilation by AMF, which ultimately results in an enhanced growth response in *Z. mays*. Abdel-Fattah et al. (2014) recorded higher ALP activities in mycorrhizal root extracts than in non-mycorrhizal root extracts when soybean plants were grown either in the presence of absence of P.

Plasma membrane H^+-ATPase plays an essential role in plant responses to nutrient and environmental stresses. Nutrient transport through the plant cell membrane is driven by an electrochemical proton gradient, which is established by the plasma membrane H^+-ATPase proton pump (Wang et al., 2014). The present study shows that plants inoculated with *G. mosseae* and *A. spinosa* exhibited better H^+-ATPase activity than non-inoculated plants. Lower H^+-ATPase activity under P starvation may be explained by the fact that P is a key factor in plasma membrane H^+-ATPase, where it enhances nutrient uptake and transport and thereby increases growth. We propose that nutrient uptake and H^+-ATPase activity are closely associated in maize. Smith & Read (2008) reported that H^+-ATPases facilitate active P uptake by activating plant membranes surrounding intracellular fungal structures.

The peroxidation of phospholipids in plant plasma membranes leads to the production of MDA, which affects most physiological processes that are associated with producing ROS under stress conditions **(**Bernstein et al., 2010**)**. The present results indicate that P deficiency led to significant increases in the MDA content of maize (leaves and roots) due to oxidative damage. This can pose a threat to cells by affecting cell membrane permeability and integrity. The data also indicate that inoculation with AMF under P deficiency decreased MDA content. We suggested that AMF symbiosis could ameliorate the negative effects of P deficiency in maize and improve membrane stability by enhancing P uptake and increasing antioxidant production. Beltrano et al. **(**2013) reported an increase in the cell membrane integrity of pepper with AMF treatments under low P levels and salinity stress.

Hydrogen peroxide is continuously produced as a product of oxidative metabolism during plant anabolism. It plays a role in the regulation of plant metabolism and cellular signaling in response to environmental stresses **(**Ślesak et al., 2007). The present data shows that H_2O_2 levels were increased by P stress both in the presence and absence of mycorrhizal treatments, but were significantly higher in the absence of mycorrhizal treatment. This shows that AMF can lower plant membrane permeability, alleviate damage to the cell membrane caused by P stress, improve uptake of mineral nutrients from the soil under phosphorus stress, and reduce adverse stress responses. Our results also showed a correlation between CAT activity and the production of H_2O_2 and MDA in maize plants under P stress, indicating enhancement of oxidative stress. H_2O_2 levels were lower in roots than in leaves under all treatments, which implies that the antioxidant mechanism of *Z. mays* roots quenches H_2O_2 more efficiently. Yang et al. (2015) recorded higher H_2O_2 production in non-mycorrhizal *Robinia pseudoacacia* L. plants than in mycorrhizal plants.

Oxidative stress modifies the activity of antioxidant enzymes, which reflects a strategy for overcoming deleterious effects in plants subjected to salt stress (Ahmad et al., 2010). The current study shows elevated SOD activity in the leaves and roots of non-mycorrhizal *Z. mays* plants compared to mycorrhizal plants under P deficiency. This possibly reflects the capacity of plants to scavenge superoxide radicals. Therefore, SOD is important for tolerating P stress and could be considered essential for mycorrhizal colonization of maize plants grown in calcareous soil. These results corroborate the observations of Singh et al. (2018), who found enhanced SOD activity under P deficiency in wheat (*Triticum aestivum* L.) cultures. Singh (2015) reported that SOD activity in non-mycorrhizal tomato plants was higher than in mycorrhizal plants cultivated under appropriate water levels. In contrast, Giannakoula et al. (2010) found that SOD activity in inoculated maize leaves was higher than in non-inoculated leaves under phosphorus levels.

Similarly, CAT and APX are important for detoxifying H_2O_2 (Gill & Tuteja, 2010). The results of the present study show that CAT and APX activities in both roots and leaves were significantly higher in inoculated than in non-inoculated maize at the P levels tested. A considerable decline in CAT activity was also recorded with increasing P content in AMF-treated plants. These results suggest that CAT and

APX play an essential role in providing different degrees of protection against oxidative stress caused by P deficiency in maize. Induction of SOD activity coincided with an increase in the H_2O_2-scavenging activity of CAT and APX (Fig. 5). This is of special interest due to the increase in H_2O_2 production resulting from higher SOD activity, necessitating a rise in the enzymatic decomposition of H_2O_2. The increase in APX activity of maize roots and leaves with P decrease was lower than the increase in CAT activity, suggesting that catalase is more efficient under the prevailing experimental conditions. These results agree with those of Singh (2015), who stated that CAT activity in non-inoculated tomato plants was lower than in inoculated plants. Rahmaty & Khara (2011) reported a slight induction of APX activity in inoculated maize roots compared to non-inoculated roots. Kumar et al. (2009) stated that transient induction of CAT and APX in maize plants colonized with *Piriformo spora* indicates a defense mechanism against stress during symbiosis development.

GR is required for the reduction of GSSG to GSH by NADPH (Ahmad et al., 2010). The results of the current study show an increase in GR activity in AMF-inoculated maize compared to non-inoculated plants under P deficiency stress; this increase may be connected to the preservation of intracellular GSH required for scavenging H_2O_2 in the ascorbate-glutathione cycle. Jiang et al. (2016) reported that GR activity was markedly higher when *Lonicera japonica* plants were inoculated with *G. versiforme*. Several studies have reported that mycorrhizal association helps plants to overcome the deleterious effects of high salinity by improving the activity of antioxidant enzymes (Hajiboland et al., 2010; Tian et al., 2013; Singh, 2015).

The oxidized form of GSSG is reduced to GSH, which acts as an antioxidant agent **(**Shu et al., 2011**)**. AA is an important antioxidant that gives an electron to APX for H_2O_2 detoxification. AA provides electrons to photosystem II in the thylakoid membrane to protect the system from damage when electron flow from water is disturbed (Liu et al., 2007). The results obtained in the present study suggest the function of GSH and AA in the defense against P deficiency in maize plants. This conclusion was based on the following observations: (a) A greater increase in glutathione pool concomitant with the formation of GSSG in maize plants (leaves and roots) when maize grown under P deficiency, (b) An increase in GR activity resulting in stimulation of GSH regeneration, leading to an increase in the glutathione pool, (c) Increases in AA concentration in P-deficient maize (leaves and roots) could result from increased synthesis of AA, and (d) AA content was higher in maize inoculated with *G. mosseae* and *A. spinose* than in non-inoculated maize, while GSH was lower in AMF-inoculated than in non-inoculated maize at the P levels tested. The enhancement of AA contents in mycorrhizal plants suggests that mycorrhizal colonization helped to relieve oxidative stress in maize plants. This result agrees with the findings of Jiang et al. (2016), who observed higher AA contents and lower GSH in inoculated mycorrhizal *L. japonica* plants than in non-inoculated plants. Wu & Zou (2009) reported higher AA and GSH contents in the leaves and roots of mycorrhizal grafted citrus trees than in non-mycorrhizal citrus trees under drought stress. Kandlbinder et al. (2004) reported that AA and GSH levels in *Arabidopsis thaliana* leaves increased under P deficiency.

Conclusion

The results of the present study indicate that local isolates of *G. mosseae* and *A. spinose* could increase the tolerance of maize plants to P deficiency. AMF colonization appeared to have strongly synergistic effects on the ability of maize to withstand P deficiency because of morphological and architectural changes, including alterations to the root/shoot ratio and total biomass, as well as physiological changes such as antioxidant production and membrane transport by H^+-ATPase; therefore, enhanced nutrient uptake enabled the maize plants to survive under P stress. AMF inoculation showed promising effects in ameliorating the deleterious effects of ROS metabolism in phosphate-stressed maize plants, related mainly to increased activity of certain antioxidant enzymes (SOD, CAT, APX, and GR) and increased levels of non-enzymatic antioxidants (GSH and AA) that provided protection to maize cell membranes under P deficiency by lowering MDA and ROS. This may result in greater membrane stability, maintenance of ion balance, and increased photochemical reactions, which all enhance P stress tolerance in maize plants grown in calcareous soil. This study strongly suggests that local isolates of AMF inoculum containing *G. mosseae* and *A. spinosa*

are an effective and environmentally friendly biofertilizer that could improve maize cultivation in calcareous soils (Fig. 9).

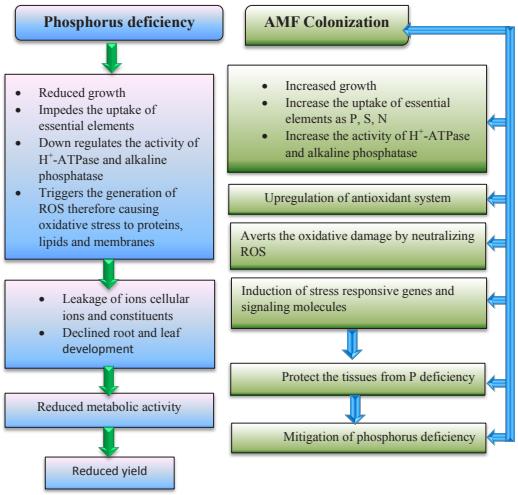


Fig. 9. Diagrammatic representation of main mycorrhizal functions to regulate P deficiency in maize plant

Acknowledgments The authors thank Dr. Niven Allam Associate professor in Botany and Microbiology Department, Assuit University for his kind help in morphological identification of spores.

Conflict of interests: The authors declare no conflict of interest.

Authors contribution: Professor Amel tammam was: Author of research concept and design, critical revision of article, final approval of article. Professor Weam Elaggan was: Author that involved in putting idea, critical revision of article. Dr. Hala Badry: Shared in isolation and identification of mycorrhiza. Dr. Reda Abu Shanab was: The author that shared in molecular part. Soha El Sawy: MSc student, Faculty of Science, Department of Botany and Microbiology, Alexandria University, Alexandria, Egypt.

Ethical approval: Not applicable

References

Abdel Latef, A.A., Chaoxing, H. (2014) Does the inoculation with *Glomus mosseae* improve salt tolerance in pepper plants? *Journal of Plant Growth Regulation*, **33**, 644-653.

Abdel-Fattah, A., Asrar, S., Amiri, A.L., Abdel-Alam, E.M. (2014) Influence of arbuscular mycorrhiza and phosphorus fertilization on the gas exchange, growth and phosphatase activity of soybean (*Glycine max* L.) plants. *Photosynthetica*, **52**(4), 581-588.

Agricultural, Statistics (2019) "*Ministry of Agricultural and Land Reclamation. Economic Affairs*", Sector. Summer Crops, **3**, 52.

Ahmad, P., Jaleel, C., Sharma, S. (2010) Antioxidant defense system, lipid peroxidation, proline-metabolizing enzymes, and biochemical activities in Two *Morus alba* genotypes subjected to NaCl Stress. *Russian Journal of Plant Physiology*, **57**(4), 509-517.

Andrino, A., Guggenberger, G., Sauheitl, L., Burkart S., Boy, J. (2021) Carbon investment into mobilization of mineral and organic phosphorus by arbuscular mycorrhizal. *Biology and Fertility of Soils*, **57**, 47–64.

Beltrano, J., Ruscitti, M., Arango, M., Ronco, M. (2013) Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and P levels. *Journal of Soil Science and Plant Nutritionis*, **13**(1), 123-141.

Bernstein, N., Shoresh, M., Xu, Y., Huang, B. (2010) Involvement of the plant antioxidative response in the differential growth sensitivity to salinity of leaves vs roots during cell development. *Free Radical Biology and Medicine,* **49**(7), 1161-1171.

Beyer, W.F., Fridovich, I. (1987) Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. *Analytical Biochemistry*, **161**, 559–566.

Bowers, G.N., McComb, R.B. (1966) A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase. *Clinical Chemistry*, **12**(2), 70–89.

Breuillin, F., Schramm, J., Hajirezaei, M., Ahkami, A., Favre, P., et al. (2010) Phosphate systemically inhibits development of arbuscular mycorrhiza in *Petunia hybrida* and represses genes involved in mycorrhizal functioning. *Plant Journal*, **64**, 1002–1017.

Cardoso, E.J.B., Vasconcellos, R.L.F., Bini, D., Miyauchi, M.Y.H., Santos, C.A., et al. (2013) Soil health: Looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? *Scientia Agricola,* **70**(4), 274-289.

Dixon, M., Simonne, E., Obreza, T., Guodong Liu G. (2020) Crop response to low phosphorus bioavailability with a focus on tomato. *Agronomy*, **10**, 617.

Faraday, C.D., Spanswick, R.M. (1992) Maize root plasma membranes isolated by aqueous polymer two-phase partitioning: assessment of residual tonoplast ATPase and pyrophosphatase activities H^+-ATPase. *Journal of Experimental Botany*, **43**, 1583–1590.

Ferreira, C.M.H., López-Rayo, S., Lucena, J.J., Soares, E.V., Soares, H.M.V.M. (2019) Evaluation of the efficacy of two new biotechnological-based freeze-dried fertilizers for sustainable Fe deficiency correction of soybean plants grown in calcareous soils. *Frontiers in Plant Science,* **10**, 1335.

Gaude, N., Bortfeld, S., Erban, A., Kopka, J., Krajinski, F. (2015) Symbiosis dependent accumulation of primary metabolites in arbuscule-containing cells. *BMC Plant Biology*, **15**, 234.

Giannakoula, A., Moustakas, M., Syros, T. and Yupsanis, T. (2010) Aluminum stress induces up-regulation of an efficient antioxidant system in the Al-tolerant maize line but not in the Al-sensitive line. *Environmental and Experimental Botany,* **67**(3), 487–494.

Gill, S., Tuteja, N. (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, **48**, 909-930.

Gossett, D.R., Millhollon, E.P., Lucas, M.C. (1994) Antioxidant response to NaCl stress in salt tolerant and salt sensitive cultivars of cotton. *Crop Science,* **34**, 706–714.

Griffith, O.W. (1980) Determination of glutathione and glutathione disulphide using glutathione reductase and 2-vinyl pyridone. *Analytical Biochemistry,* **106**, 207–212.

Hajiboland, R., Aliasgharzadeh, N., Laiegh, S.F., Poschenreider, C. (2010) Colonization with arbuscular mycorrhizal fungi improve salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. *Plant Soil*, **331**, 313–327.

Hashem, A., Alqarawi , A., Radhakrishnan , R., Al-Arjani, A., Aldehaish, H., Egamberdieva, D., Abd_Allah, E. (2018) Arbuscular mycorrhizal fungi regulate the oxidative system, hormones and ionic equilibrium to trigger salt stress tolerance in *Cucumis sativus* L. *Saudi Journal of Biological Sciences,* **25**, 1102–1114.

Hoagland, D.R., Arnon, D.I. (1950) The water-culture for growing plants without soil. *California Agricultural Experiment Station Circ*., **347**.

Hu, J., Yang, A., Zhu, Wang, J., Dai, J., Wong, M., Lin, X. (2015) Arbuscular mycorrhizal fungal diversity, root colonization, and soil alkaline phosphatase activity in response to maize-wheat rotation and no-tillage in North China. *Journal of Microbiology,* **53**(7), 454–461.

Huang, G.M., Zou, Y.N., Wu, Q.S., Xu, Y.J., Kuča, K. (2020) Mycorrhizal roles in plant growth, gas exchange, root morphology, and nutrient uptake of walnuts. *Plant, Soil and Environment*, **66**(6), 295–302.

INVAM (2014) International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi. West Virginia University, Morgantown, West Virginia. URL: http://invam.wvu.edu/the- fungi/species-descriptions.

Jiang, Q., Zhuo, F., Long, S., Zhao, H., Yang, D., Ye, Z., et al. (2016) Can arbuscular mycorrhizal fungi reduce Cd uptake and alleviate Cd toxicity of *Lonicera japonica* grown in Cd-added soils? *Scientific Reports*, **6**, 21805 DOI: 10.1038/srep21805

Kaeppler, S.M., Parke, J.L., Mueller, S.M., Senior, L., Stuber, C., Tracy, W.F. (2000) Variation among maize inbred lines and detection of quantitative trait loci for growth at low phosphorus and responsiveness to arbuscular mycorrhizal fungi. *Crop Science,* **40**, 358-364.

Kalayu, G. (2019) Phosphate solubilizing microorganisms: Promising approach as biofertilizers. *International Journal of Agronomy*, Article ID 4917256, 7 pages

Kandlbinder, A., Finkemeier, I., Wormuth, D., Hanitzsch, M., Dietz, K.J. (2004) The antioxidant status of photosynthesizing leaves under nutrient deficiency: redox regulation, gene expression and antioxidant activity in *Arabidopsis thaliana*. *Physiologia Plantarum*, **120**, 63–73.

Kapoor, R., Sharma, D., Bhatnagar, A.K. (2008) Arbuscular mycorrhizae in micropropagation systems and their potential applications. *Scientia Horticulturae,* **116**, 227–239.

Khaffagy, A., El-Hassan, R., Sharshar, A. (2020) Improving the efficiency of herbicides by adding mineral oil on maize (*Zea mays* L.) crop and associated weeds. *Egyptian Journal of Agronomy,* **42**(2), 151-162.

Kumar, M., Yada, Y., Tuteja, N., Johri, A. (2009) Antioxidant enzyme activities in maize plants colonized with *Piriformo spora* indica. *Microbiology*, **155**, 780–790.

Li, T., Hu, Y.J., Hao, Z., Li, H., Wang, Y., Chen, B. (2012) First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist,* **197***,* 617-630.

Liu, J., Liu, J., Chen, A., Ji, M., Chen, J., Yang, X., et al. (2016) Analysis of tomato plasma membrane H^+-ATPase gene family suggests a mycorrhiza-mediated regulatory mechanism conserved in diverse plant species. *Mycorrhiza*, **26**(7), 645-656.

Liu, Y., Xin Wang, X., Zeng, G., Qu, D., Gu, J., et al. (2007) Cadmium-induced oxidative stress and response of the ascorbate–glutathione cycle in *Bechmeria nivea* (L.) Gaud. *Chemosphere*, **69**, 99–107.

Miret, J.A., Munné-Bosch, S. (2015) Redox signaling and stress tolerance in plants: a focus on vitamin E. *Annals of the New York Academy of Sciences*, doi: 10.1111/nyas.12639.

Mohamed, A. (2020) Diallel analysis of maize inbreds for grain yield, protein and tryptophan content. *Egyptian Journal of Agronomy,* **42**(1), 1-17.

Nakano, Y., Asada, K. (1981) Hydrogen peroxide is scavenged by ascorbate- specific peroxidase in spinach chloroplasts*. Plant & Cell Physiology*, **22**, 867–880.

Nguyen, V.L., Stangoulis, J. (2019) Variation in root system architecture and morphology of two wheat genotypes is a predictor of their tolerance to phosphorus deficiency. *Acta Physiologiae Plantarum,* **41**, 109.

OECD-FAO (Organisation for Economic Co-operation and Development) (2018) OECD-FAO Agricultural Outlook 2018-2027. OECD Publishing.

Pacioni, G. (1992) Wet-Sieving and decanting techniques for the extraction of spores of vascular-arbuscular fungi. In: "*Methods in Microbiology*", Vol. 24: Techniques for the study of Mycorrhiza, Norris, J.R., D.J. Read and A.K. Varma (Eds.). Chapter 16, Academic Press, San Diego, CA, USA, ISBN: 978-0-12-521524-4, pp. 317-322.

Patterson, B., Payne, L., Chen, Y., Graham, D. (1984) An inhibitor of catalase induced by cold chilling-sensitive plants. *Plant Physiology*, **76**, 1014–1018.

Phillips, J.M., Hayman, D.S. (1970) Improved procedures for clearing roots and staining parasitic vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society,* **55**, 158-161.

Pinto, M., de Francis, D., Gara, L. (1999) The redox state of asscorbate- dehydroascorbate pairs a specific sensor of cell division in tobacco BY-Z cells. *Protoplasma*, **209**, 90-97 .

Rahmaty, R., Khara, J. (2011) Effects of vesicular arbuscular mycorrhiza *Glomus intraradices* on photosynthetic pigments, antioxidant enzymes, lipid peroxidation, and chromium accumulation in maize plants treated with chromium. *Turkish Journal of Biology,* **35**(1), 51-58.

Schüßler, A., Schwarzott, D., Walker, C. (2001) A new fungal phylum, the Glomeromycota: Phylogeny and evolution. *Mycological Research*, **105**, 1413-21.

Sharma, S., Anand, G., Singh, N., Kapoor, R. (2017) Arbuscular mycorrhiza augments arsenic tolerance in wheat (*Triticum aestivum* L.) by strengthening antioxidant defense system and thiol metabolism. *Frontiers in Plant Science*, **8**, 906–927.

Sheng, M., Tang, M., Chan, H., Yang, B., Zhang, F., Huang, Y. (2008) Influence of arbuscular mycorrhizae on photosynthesis and water status

of maize plants under salt stress. *Mycorrhiza*, **18**, 287-296.

Shu, D., Wang, L., Duan, B., Deng, Y., Meng, Q. (2011) Antisense- mediated depletion of tomato chloroplast glutathione reductase enhances susceptibility to chilling stress. *Plant Physiology and Biochemistry,* **49**, 1228-1237.

Simon, L., Levesque, R.C., Lalonde, M. (1993) Identification of endomycorrhizal fungi colonizing roots by fluorescent single-strand conformation polymorphism-polymerase chain reaction. *Applied and Environmental Microbiology,* **59**, 4211–4215.

Singh, P.K. (2015) Arbuscular mycorrhizal fungi influences oxidative stress in tomato plants in drought stress. *Journal of Botanical Sciences*, **4**(3), 46-50.

Singh, P., Jaiswal, S., Sunita Sheokand, S., Duhan, S. (2018) Morpho-physiological and oxidative responses of nitrogen and phosphorus deficiency in wheat (*Triticum aestivum* L.). *Indian Journal of Agricultural Research*, **52**(1), 40-45.

Singh, S.K., Badgujar, G.B., Reddy, V.R., Fleisher, D.H., Timlin, D.J. (2013) Effect of phosphorus nutrition on growth and physiology of cotton under ambient and elevated carbon dioxide. *Journal of Agronomy and Crop Science,* **199**, 436–448.

Ślesak, I., Libik, M., Karpinska, B., Karpinski, S., Miszalski, Z. (2007) The role of hydrogen peroxide in regulation of plant metabolism and cellular signalling in response to environmental stresses. *Acta Biochimica Polonica,* **54**(1), 39-50.

Smith, S., Read, D. (2008) "*Mycorrhizal Symbiosis*", 3rd ed. New York: Academic Press.

Smith, I.K., Vierheller, T.L., Thorne, C.A. (1988) Assay of glutathione reductase in crude tissue homogenates using 5, 5-dithiobis-(2-nitrobenzoic Acid). *Analytical Biochemistry,* **175**, 408-413.

Smith, S.E., Jakobsen, I., Gronlund, M., Smith, F.A. (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition; Interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiology*, **156**, 1050-1057.

Sokal, R.R., Rohlf, F.J. (1995) "*Biometry: The Principles and Practice of Statistics in Biological Research*", 3rd ed. New York: W.H. Freeman, pp. 321-356.

Taalab, A.S., Ageeb, G. W., Hanan, S. S., Safaa, A.M. (2019) Some characteristics of calcareous soils. A review. *Middle East Journal of Agriculture Research,* **8**(1), 96-105.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G. (1997) The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25**(24), 4876-4882.

Tian, Y.H., Lei, Y.B., Zheng, Y.L., Cai, Z.Q. (2013) Synergistic effect of colonization with arbuscular mycorrhizal fungi improves growth and drought tolerance of Plukenetia volubilis seedlings. *Acta Physiologiae Plantarum,* **35**, 687-696.

United State Environmental Protection Agency. (1983) Phosphorus, All Forms. Method 365.1 (Colorimetric, Automated, Ascorbic Acid). 365-1.1 -- 365-1.7. In Methods for Chemical Analysis of Water and Wastes, EPA-600/ 4-79-020. U.S.E.P.A., Cincinnati, Ohio, USA.

Velikova, V., Yordanov, I., Edreva, A. (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. *Plant Science*, **151**, 59–66.

Walker, C., Schüßler, A. (2004) Nomenclatural clarifications and new taxa in the Glomeromycota. *Mycological Research*, **108**, 979–82

Wang, C., Zhang, S.H., Wang, P.F., Hou, J., Zhang, W.J., et al. (2009) The effect of excess Zn on mineral nutrition and antioxidative response in rapeseed seedlings. *Chememosphere*, **75**, 1468-1476.

Wang, E., Yu, N., Bano, A., Liu, C., Miller, A., Cousins, D., et al. (2014) A H^+-ATPase that energizes nutrient uptake during Mycorrhizal symbioses in rice and *Medicago truncatula*. *Plant Cell*, **26**, 1818–1830.

Wu, J., Seliskar, D. (1998) Salinity adaptation of plasma membrane H^+-ATPase in the marsh plant *Spartina patens*: ATP hydrolysis and enzymatic kinetics. *Journal of Experimental Botany*, **49**, 1005–1013.

Wu, Q.S., Zou, Y.N. (2009) Mycorrhiza has a direct effect on reactive oxygen metabolism of drought-stressed citrus. *Plant, Soil and Environment,* **55**(10), 436–442.

Wu, Q.S., He, J.D., Srivastava, A.K., Zhang, F., Zou Y.N. (2019) Development of propagation technique of indigenous AMF and their inoculation response in citrus. *Indian Journal of Agricultural Sciences,* **89**, 1190–1194.

Yang, Y., Han, X., Liang, Y., Ghosh, A., Chen, J., Tang, M. (2015) The combined effects of arbuscular mycorrhizal fungi (AMF) and Lead (Pb) stress on Pb accumulation, plant growth parameters, photosynthesis, and antioxidant enzymes in *Robinia pseudoacacia* L. *PLOS ONE,* doi:10.1371 /journal. pone. 0145726-1-24.

تأثير العزلات المحلية من الفطريات على النمو والأداء الفسيولوجي في نبات الذرة في تربة الجيرية بها نقص عنصر الفوسفور

أمل تمام[1]، ونام العجان[1]، هالة بدري[2]، رضا أبو شنب[3]، سها الصاوي[1]

[1]قسم النبات والميكروبيولوجي- كلية العلوم- جامعة الإسكندرية- الإسكندرية- مصر، [2]قسم علوم الاراضي والمياه- كلية الزراعة- جامعة الإسكندرية- الإسكندرية- مصر، [3]قسم التقنية الحيوية البيئية مدينة البحث العلمي والتطبيقات التكنولوجية الجديدة ببرج العرب - الإسكندرية - مصر.

يعد فطر الميكوريزا من الفطريات الأكثر انتشارا في التربة الزراعية، حيث يكون علاقة تبادلية مع جذور معظم فصائل النباتات. هذه الفطريات تحفز قدرة النبات في التخلص من الاثار السلبية للاجهاد الاحيائي وذلك بالمساعدة علي زيادة امتصاص المياه والمواد الغذائية ومن خلال تحفيز النبات على إمتصاص الفوسفور. لذا كان الهدف من البحث هو دراسة التأثير الإيجابي لفطر الميكوريزا في تقليل الأثر السلبي لعدم قدرة نبات اقتصادي هام وهو نبات الذرة في امتصاص عنصر الفوسفور من التربة الجيرية الفقيرة بهذا العنصر والموجودة بالساحل الشمالي غرب مدينة الأسكندرية . وقد تضمن هذا البحث استخدام فطر الميكوريزا المستخلص من هذه التربة الجيرية . وعرفت الاجناس المتواجدة في هذه التربة بدراسة الشكل الظاهري لجراثيم الفطر والتي أظهرت تواجد أربع اجناس مختلفة من الفطر وهي (*Entrophospora- Scutellospora– Glomus – Acaulospora*) كما بينت فحص العينات جزيئيا أن الجنس السائد هو *Glomus* وأكدت التحاليل الجزيئية أن نوع هذا الجنس هو *Glomus mosseae*.

أظهرت النتائج أن نقص عنصر الفوسفور في النبات والذي يؤدي إلى اختلال في الأداء الفسيولوجي، أمكن تقليل الأثار السلبية له بإضافة فطر الميكورايزا للتربة الجيريه حيث ازداد الوزن الجاف معنويا وكذلك زيادة في نشاط انزيم الكالين فوسفاتيز وذلك بالمقارنة بالتركيز الحاكم .

أسفرت النتائج على قيم لنشاط إنزيم H^+-ATPase في المعاملات الملقحه بفطر الميكوريزا نسبة إلى المعاملات المقارنه والتي لم يتم تلقيحها بالميكوريزا. وقد سجلت أعلى قيم لنشاط انزيم H^+-ATPase في وجود الفوسفور والتلقيح بفطر الميكوريزا في كلا من الأوراق والجذور، وتباينت هذه القيم من 28.13 و24.98 $\mu mol\ Pi^{-1}ngP^{-1}min^{-1}$ على التوالي.

أظهرت نتائج قياس نشاط خمس إنزيمات مضادة للأكسدة (سوبر أكسيد ديسميوتاز، كتالاز، جوايكول بيرأكسيديز، أسكوربات بير أكسيديز، جلوتاثيون ريدكتيز) تحت تأثير التركيزات المختلفة من الفوسفور وفي وجود أو غياب التلقيح بفطر الميكوريزا أن السوبر أكسيد ديسميوتاز الذي يعمل على تنظيم تركيز (فوق أكسيد الهيدروجين) يقل نشاطه بصوره واضحة في كلا من الأوراق والجذور وذلك مرتبطا بزياده نسبه تركيز الفوسفور.

ساهم إنزيم الكاتالاز وأسكوربات بير أكسيديز في التخلص من كمية كبيرة من فوق اكسيد الهيدروجين والذي تكون كمية منه بمساعدة إنزيم السوبر أكسيد ديسميوتاز. حيث أن أنزيم أسكوربات بير أكسيديز يمتلك قدرة عالية على الإرتباط بفوق اكسيد الهيروجين لوجوده في أماكن مختلفة تحت خلوية والذي لا يمكن الوصول إليه عن طريق الكتاليز. كما حدث زيادة في نشاط إنزيم الجوايكول بير أكسيدز في وجود الميكوريزا مقارنة بالنباتات التي لم يتم معاملتها بالميكوريزا وذلك تحت نقص عنصر الفوسفور. إرتبط إنخفاض ملحوظ في نشاط الجوايكول بير أكسيدز في كلا من الجذور والأوراق مع زياده تركيزات الفوسفور في التربة.

يحفز إنزيم الجلوتاثيون ريدكتيز إختزال الجلوتاثيون المؤكسد (GSSG) إلى الجلوتاثون المختزل(GSH) مصاحبا بإختزال (NADPH). ويعد هذا الإنزيم مكونا هاما في إزالة السموم الناتجة من عناصر الأكسجين النشطة في النباتات.وقد أظهرت النتائج وجود إختلاف معنوي في نشاط إنزيم الجلوتاثيون ريدكتيز بين النباتات الملقحة والغير ملقحة بفطر الميكوريزا. حيث زاد نشاط هذا الإنزيم في حالة النباتات الملقحة بالمقارنة بالنباتات الغير ملقحة وذلك تحت كل تركيزات الفسفور المختبرة. أظهرت النتائج أن أعلى نسبة إصابة فطرية بجذور نبات الذرة (88%) سجلت عند تركيز 60 mg P kg^{-1}soil.