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Effect of Arbuscular Mycorrhizal Fungi on the Drought tolerance of Sorghum bicolor.

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ABSTRACT

In order to study the role of FAM on water stress tolerance of sorghum plants. Factorial experiment under greenhouse conditions was conducted with four levels of water potential (-0.04, -0.2, -0.4 and -0.8 Map) and two levels of phosphorus (0 and 25 mgkg$^{-1)}$. The results demonstrate that there were no roots colonized with AMF in non-inoculated plants. In inoculated plants roots colonized with AMF percentage increased with increased of water stress and decreased with phosphorus addition and the higher value was 86% in plants grow under water potential -0.8 MPa and received 0 phosphorus while the lowest value was 46% in plants grow under water potential -0.04 MPa and received 25 phosphorus. Inoculation with AMF increased plant fresh weight, roots, shoots dry matter, and plant phosphorus uptake. The results show AMF have protected sorghum plants against drought stress and it is clear that the effects of AMF on plant growth parameters was higher than the effects of phosphorus addition specially in drought stress conditions hence we can suggested that there are addition ways than phosphorus nutrition improvement that AMF can increase host plant drought tolerance.

Keywords: Arbuscular Mycorrhaza Fungi, Drought Tolerance, Sorghum

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تأثير فطريات المايكورايزا الشجيرية على مقاومة الذرة البيضاء للجفاف

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الملخص

لدراسة تاثير فطريات المايكورايزا الشجيريه على مقاومة محصول الذره البيضاء للجفاف اجريت تجربه عاملية تحت ظروف البيت الزجاجي باستخدام اربع مسنويات من الشد الرطوبي (–٠.٠٠٤، –٠.٠٢، –٠.٠٤، –٠.٠٨ ميكاباسكال) ومستويين من الفسفور(٠ و ٢٥ ملغم/كغم). اوضحت النتائج انه لم تظهر اصابه بفطريات المايكورايزا الشجيريه بالنباتات غير الملقحة . ازدادت نسبة الاصابه في النباتات الملقحه بزيادة الشد الرطوبي بينما انخفضت نسبة الاصاب باضافة الفسفور وكانت اعلى نسبة اصابه ٨٦% في النباتات الناميه تحت شد رطوبي (–٠.٠٨ ميكابسكال) والغير معامله بالفسفور بينما كانت اقل نسبة اصابه ٤٦% في النباتات الناميه تحت شد رطوبي (–٠.٠٠٤ ميكاباسكال) والمعامله ب ٢٥ ملغم /كغم فسفور. التلقيح بفطريا المايكورايزا الشجيرية ادى الى زيادة وزن النبات الرطب ووزن المجموع الخضري والجذري الجاف والفسفور الممتص تحت كل مستويات الشد الرطوبي والفسفور. تاثير فطريات المايكورايزا على معايير النمو كان اعلى من تاثير الفسفور وخاصة في النباتات الناميه تحت الشد الرطوبي. نتائج هذه الدراسه اوضحت ان فطريات المايكورايزا حمت نباتات الذره البيضاء من الجفاف واقترحت الدراسه ان هناك طرق اضافية من غير تحسين تغذية النبات بالفسفور تستخدمها فطريات المايكورايزا الشجيريه لزيادة مقاومة النبات العائل للجفاف.

الكلمات الدالة: فطريات المايكورايزا الشجيريه، مقاومة الجفاف، الذرة البيضاء

1. INTRODUCTION

Water availability is considered to be one of the major limiting factors for plant growth and yield in many areas [1]. Due to the world population growth and long-term trends in global climate change and precipitation will significantly reduce [2], water shortages worldwide are increasingly becoming a concern. The predicted warmer and drier climate conditions will significantly affect the crops productivity and the expansion of crops production in drought-prone regions. Thus, it is important to adopt appropriate management techniques and the development of drought-tolerant crop varieties is of high importance in order to maintain crop production under the more severe drought conditions. Whole-plant-level responses to drought stress include reduced leaf, silk, stem, root, and expansion of grain kerne, stomatal closur, decreased photosynthesis and respiration; reduced assimilate flux to growing organs, as a result of cellular-level responses to water stress include abscisic acid (ABA) accumulation, decreased cell expansion and division, osmotic adjustment, accumulation of proline, photooxidation of chlorophyll , and reduced activity of enzyme . It has been suggested that Arbuscular Mycorhizal Fungi (AMF) will play a pivotal role in sustainable agriculture as they improve plant water relations, thus increasing the drought resistance of plants [3] [4], they improve disease control [5], and they enhance mineral uptake, which reduces the use of fertilizers [6], [7]. The mechanisms that AM relationship can enhance host plant drought tolerance generally were concluded by several earlier studies that enhanced drought resistance results from increased P nutrition [8]. Several further studies have revealed the existence of other mechanisms; some of which are correlated with plant nutrition and size, while others are uncorrelated [9]. Some of these mechanisms include: plant gas exchange and leaf hydration, changes in plant hydraulic, enhancement in soil properties, increase in roots surfaces areas of host plants, improvement in efficiency of water absorption **as** well as protection against oxidative stress facilitated by drought; [10], [11]. The objective of the present work is to determine the role of AMF on sorghum plnts drought tolerance.

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2. Material and methods

2-1 Experimental design

A 2x2x4 factorial randomized complete block design was used with three treatments. Inoculation, inoculated with *(Glomus leptoticum)* (+M) and non-mycorrhizal (-M). Two levels of phosphorus addition: 0 and 25 mg Kg^{-1} (P0, P1) respectively as a superphosphate and four level of soil water potential, the plants watered to field capacity where the soil water potential reach (0.04, 0.1, 0.4 and 0.8) megapascal (MPa) W1, W2, W3 and W4 respectively and each treatment replicate three times.

2-2 Inoculum preparation

Inoculum was produced in sterile soil having *Zea mays* as a host plant, for a period of four months in pot culture. 25 grams of Arbuscular Mycorrhizal Fungus AMF inoculum *Glomus leptoticum* (mixture of soil with spores and colonized roots) was placed 2-3 cm bellow the seeds.

2-3 Growth Conditions

Clay loam soil was Collected from surface layer (0- 20cm), sieved (2mm), and sterilized by the steam-sterilized (121 C for 30 min) and air-dried. The soil had a pH of 7.9 (1:2.5 soil water suspension) ; 2.73 % organic matter by rapid titration method; 55 (meq /100 g soil) CEC, by the sodium acetate-method; 8.11(mg kg1) available phosphorus concentrations (NaHCO3 extractable); 0.32(meq/L) K; 0.58(meq/L Na by flame photometer; 2.1 (meq/L) Ca; 0.12(meq/L) Mg by titration with EDTA.Texture was made up of 33.81% sand, 28.16% silt, and 38.03% clay. The experiment was carried out in greenhouse conditions. Sorghum (*Sorghum bicolor*) seeds were sterilized in 5% H2O2 for 8 minutes, and then washed three times with water. Three seeds were sown into plastic pots contained 4 kg of sterile sieved soil. Plants were grown under well irrigation condition. Water was supplied daily to maintain at a soil water potential of 0.04MPa (close to field capacity) for the first 4 weeks then the plants were subjected to drought stress conditions as follows w1,w2,w3,w4.

W1. The plants continued watered daily (well irrigation condition) to maintain at a soil water potential of 0.04MPa

W2.The plants watered to field capacity where the soil water potential reaches 0.2, MPa

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W3. The plants watered to field capacity where the soil water potential reaches 0.4 MPa
W4. The plants watered to field capacity where the soil water potential reaches 0.8 Mpa
Plants were harvested 90 days after planting.

2-4 Soil water potential control

Soil water potential was determined by pressure plate device, while soil moisture was determined by weighing the samples pre- and post-drying at 110 C for 24h and then determining the volumetric soil moisture. In order to manage the levels of drought stress, the soil moisture in pot was measured daily with the HH2 Moisture meter, ThetaProbe ML2, (measures volumetric soil moisture content) and the amount of water lost was added to each pot to return soil water content to the desired soil moisture.

2-5 Measurements

The plants fresh weights were recorded post harvesting. The root system for each plant was then separated from the shoot. The shoots and roots dry weights were determined after they were dried for 24 hrs. at 70°C. The dried plant parts were digested using nitro-perchloric. The phosphorus concentration in plant shoots and roots were determined using the molybdate blue ascorbic acid method according to Murphy and Riley [12]. Phosphorus uptake was calculated for each pot as the sum of phosphorus content of shoots and roots for 3 plants (P concentration x shoots or roots dry weight). The presence of an AM fungus infection was determined visually by clearing washed roots in 10%KOH and staining the preparation with 0.05%(vol/vol) trypan blue in lactophenol as described by [13]. The calculation of AMF colonization % was estimated for each sample by examination about one hundred pieces of roots (1cm long) under microscopic

2-6 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and followed by Least Significant Difference (LSD) to compare between means at $p < 0.05$. Percentage values were arcsintrans formed before statistical analysis.

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3. Results and Discussion

3-1 AM Colonization Rate

The results in Fig. (1) demonstrates that in inoculated plants, the AM colonization percentage significantly increased with increasing water stress and decreased with phosphorus addition. The highest value of colonization was 86% in plants grown under water potential -0.8 MPa and received 0 mg Kg^{-1} phosphorus while the lowest value was in plants grown under water potential -0.04 MPa and received 25 mg Kg^{-1} phosphorus. An earlier study that reported similar findings concluded that AM colonization percentage was increased by drought stress elevation [14]. Moreover, the decreasing AM colonization with increasing water stress may be due to decreasing of available phosphorus concentration in soil with the decrease of soil moisture [15].

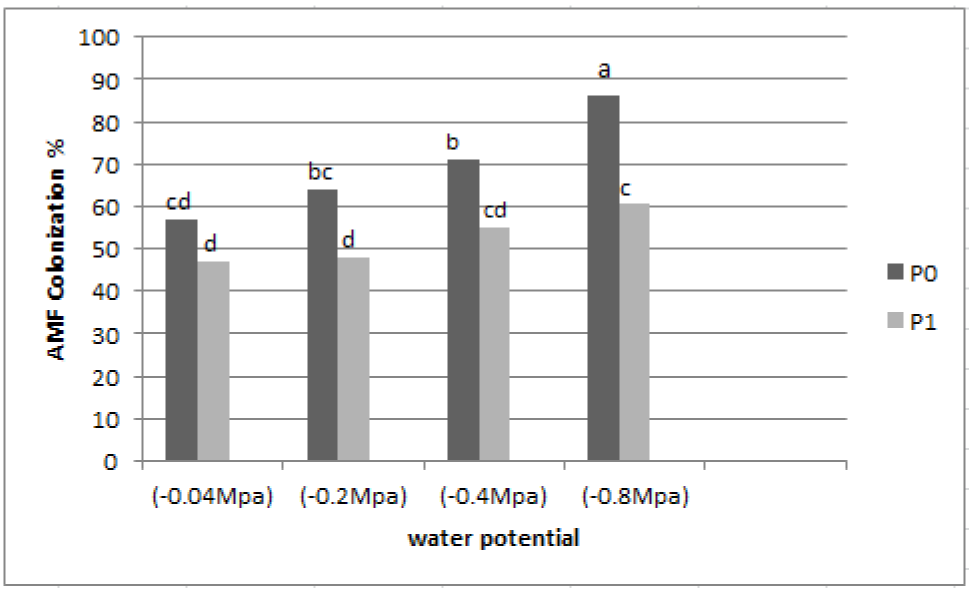

Fig. (1): Effect of water potential and phosphorus on sorghum root colonization by AMF

P0= 0 phosphorus addition

P1= 25 mg kg-1 phosphorus addition

*Different letters above the bars show significant differences (P<0.05)

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3-2 Plant growth parameters

Fig. (2) shows in general that the increase drought stress (decreases of water potential) causing decrease fresh plant weight, shoots and roots dry matter, in both mycorrhizal and non mycorrhizal plants comparing with control -0.04 MPa. Inoculation with AMF was recorded a significant increase in fresh plant weight shoots and roots dry matter in all water potential and phosphorus addition levels, shown in figures 2, 3, and 4. Our results show the effect of AMF on plant growth was higher in plants grow under drought stress comparing with plants grow under well watered condition and these results are consistent with [16]. Inoculation with AMF increased shoots dry matter 185% under well water condition while the inoculation increase shoots dry matter 300% under drought stress (water potential -8 MPa) comparing with non-inoculated plants grow under the same condition. In general addition of phosphorus significantly increased fresh plant weight, shoots and roots dry matter and its effect was higher in non mycorrhizal plants, Figures (2), (3), and (4)

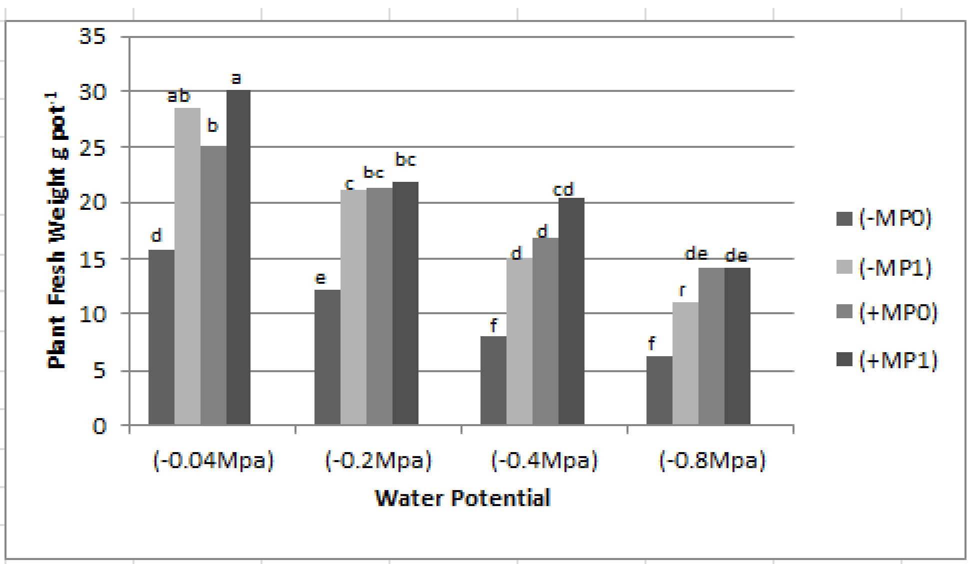

Fig. (2): Effect of AMF, water potential and phosphorus on plant fresh weight

-M= non-inoculated with AMF P0= 0 phosphorus addition

+M= inoculated with AMF P1= 25 mg kg^{-1} phosphorus addition

*Different letters above the bars show significant differences (P<0.05)

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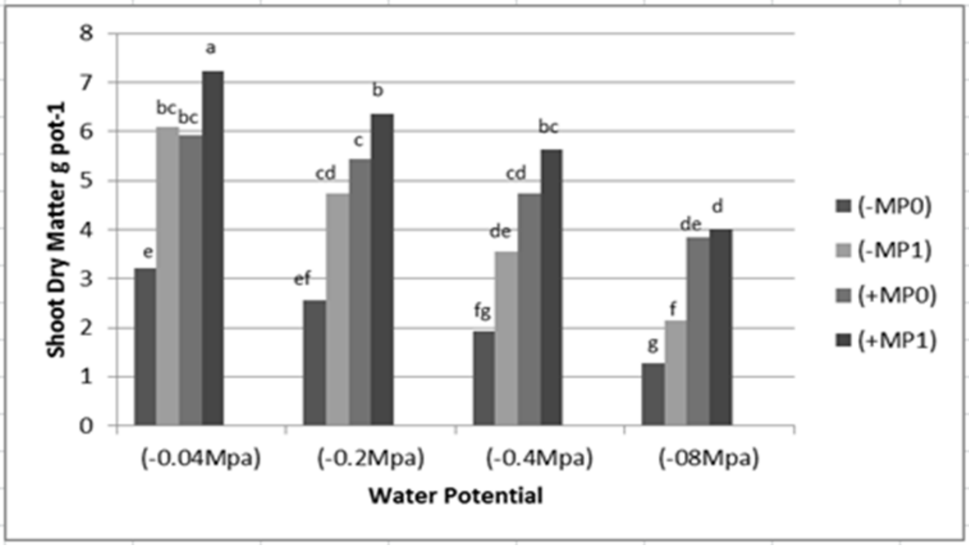

Fig. (3): Effect of AMF, water potential and phosphorus on shoots dry matter

-M= non-inoculated with AMF P0= 0 phosphorus addition

+M= inoculated with AMF P1= 25 mg kg^{-1} phosphorus addition

*Different letters above the bars show significant differences (P<0.05)

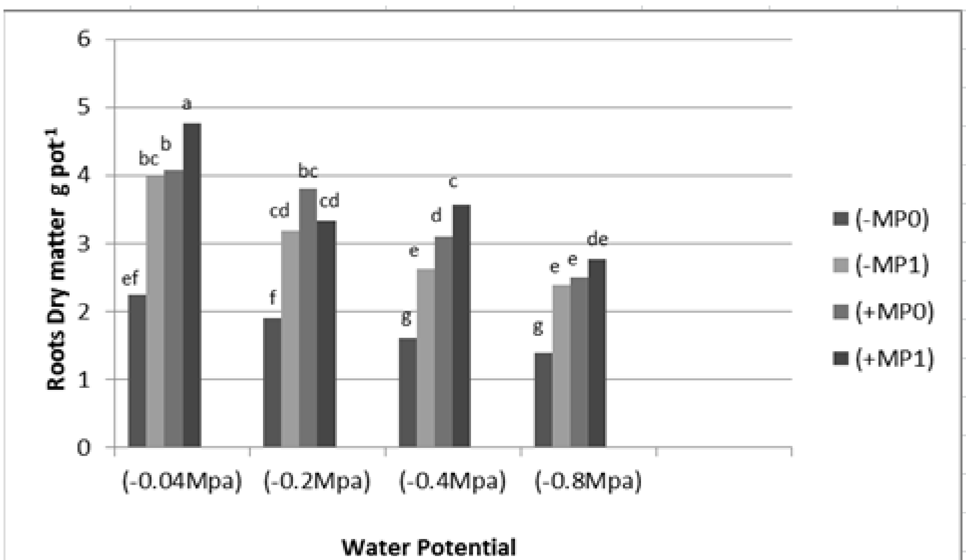

Fig. (4): Effect of AMF, water potential and phosphorus on roots dry matter

-M= non-inoculated with AMF P0= 0 phosphorus addition

+M= inoculated with AMF P1= 25 mg kg^{-1} phosphorus addition

*Different letters above the bars show significant differences (P<0.05)

The results show AMF have protected sorghum plants against drought stress and it is clear that the effects of AMF on plant growth parameters was higher than the effects of phosphorus addition, especially under drought stress conditions. Hence, we can conclude that there are mechanisms, other than phosphorus nutrition improvement, by which AMF can enhance host plant drought tolerance. Ruiz-Lozano have demonstrated that the mechanisms by which AM symbiosis enhances plant drought tolerance as a result of combination of nutritional, physiological, physical and cellular influences [17]. Some other mechanisms have been suggested to explain this phenomenon including improved osmotic control resulting in leaf hydration and postponed drop in leaf water potential in the course of drought stress. Other mechanisms may include improved hydraulic conductivity; elevated AM water uptake, adjusted plant metabolism [18], [19], [20], [21], [22]. This phenomenon can also be related to the reduced oxidative stress damage caused by the reactive oxygen species (ROS) generated during drought stress [23], [24], [25].

3-3 Phosphorus concentration and uptake

In general the inoculation with AMF and addition of phosphorus cause a significant increase in shoots and roots phosphorus concentration and uptake, Figures (5), (6), (7), and (8). The elevation of drought stress (decrease of water potential) caused a decreased phosphorus uptake in shoot and roots. The highest value of phosphorus uptake was in mycorrhizal plants which received 25 (mgKg^{-1}) phosphorus and grow under well watered condition. The lowest value was in non mycorrhizal plants which received 0 (mgKg^{-1}) phosphorus and grow under water potential (-0.8 Mpa).

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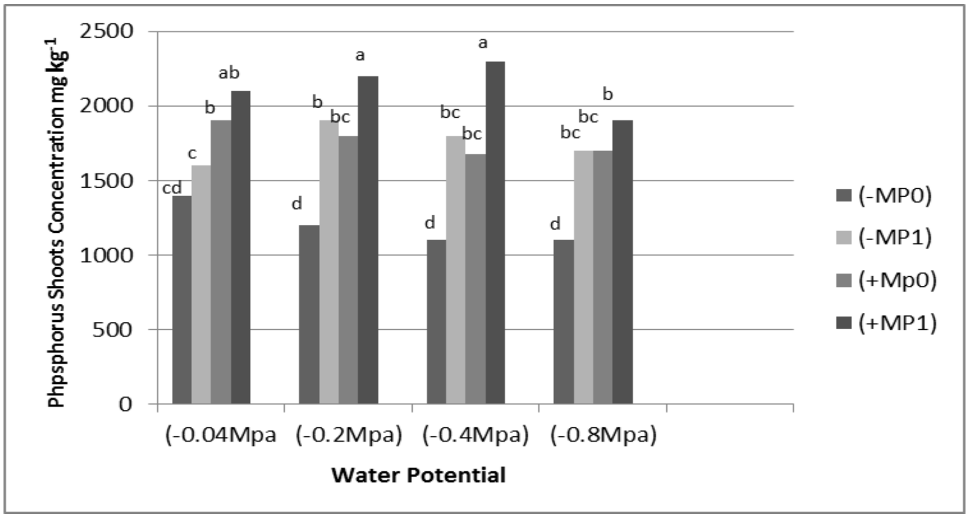

Fig. (5): Effect of AMF, water potential and phosphorus on shoots Phosphorus concentration

-M= non-inoculated with AMF P0= 0 phosphorus addition

+M= inoculated with AMF P1= 25 mg kg^{-1} phosphorus addition

*Different letters above the bars show significant differences ($P<0.05$)

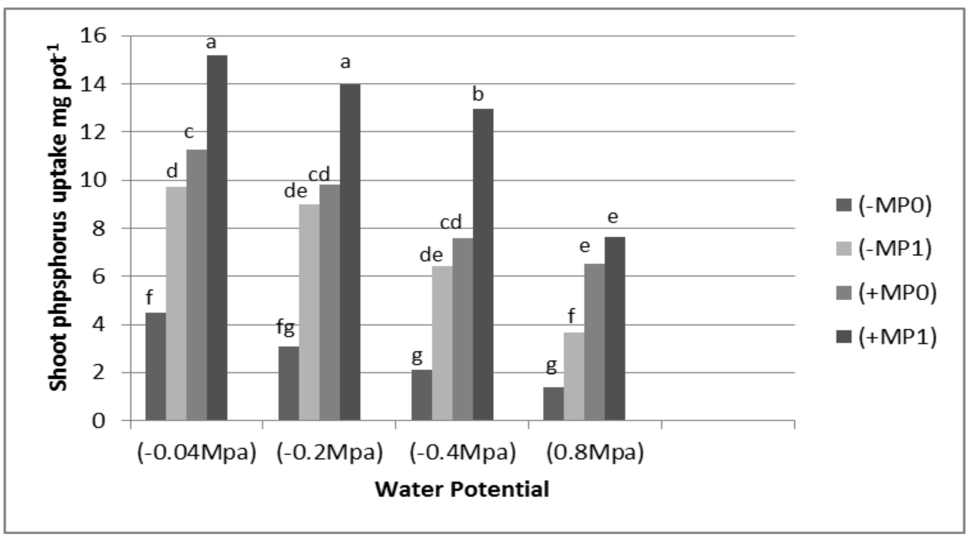

Fig. (6): Effect of AMF, water potential and phosphorus on shoots Phosphorus uptake

-M= non-inoculated with AMF P0= 0 phosphorus addition

+M= inoculated with AMF P1= 25 mg kg^{-1} phosphorus addition

*Different letters above the bars show significant differences ($P<0.05$)

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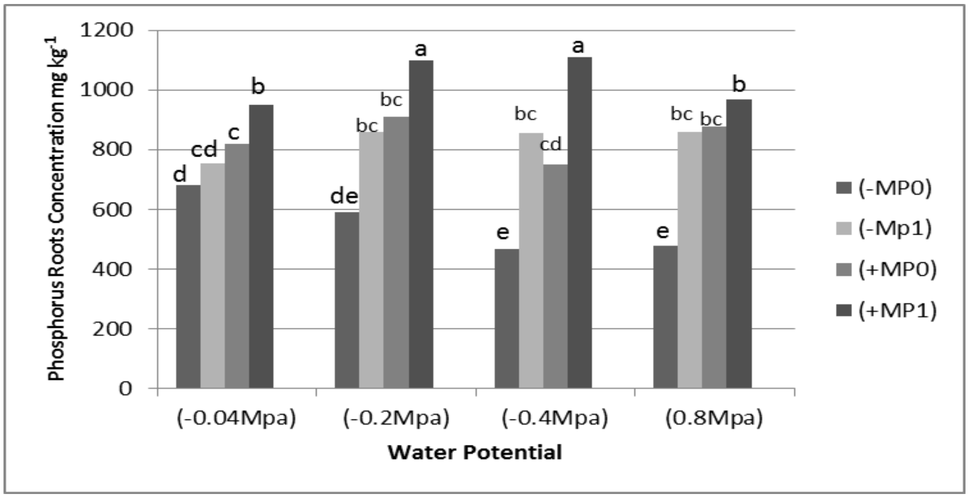

Fig. (7): Effect of AMF, water potential and phosphorus on root phosphorus Concentration

-M= non-inoculated with AMF P0= 0 phosphorus addition

+M= inoculated with AMF P1= 25 mg kg^{-1} phosphorus addition

*Different letters above the bars show significant differences (P<0.05

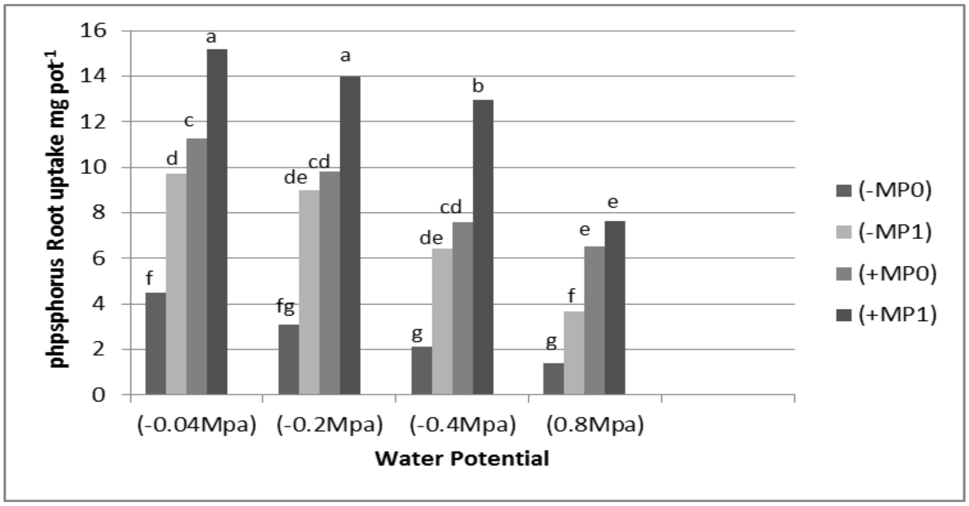

Fig. (8): Effect of AMF, water potential and phosphorus on roots phosphorus uptake

-M= non-inoculated with AMF P0= 0 phosphorus addition

+M= inoculated with AMF P1= 25 mg kg^{-1} phosphorus addition

*Different letters above the bars show significant differences (P<0.05)

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