

MORPHOLOGICAL AND PHYSIOLOGICAL PERFORMANCE AND DROUGHT RESISTANCE IMPROVEMENT OF POMEGRANATE SEEDLINGS BY MYCORRHIZAL INOCULATION

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ABSTRACT

The present investigation was conducted during 2014 and 2015 growing seasons in order to study the effects different drought stress levels in particular; 100%, 50% and 25% of the soil field capacity (FC) as well as the effects of arbuscular mycorrhizal (AM) inoculation on morphological and physiological performance and drought resistance of pomegranate seedlings. Drought stress decreased arbuscular mycorrhizal (AM) colonization. In both the AM and non AM, growth rate, leaf area, trunk cross sectional area and total dry weight decreased with increasing drought stress levels. AM colonization significantly stimulated plant growth indices and biomass production regardless of water status. Increasing drought stress levels tended to increase the proline accumulation, while the chlorophyll content, leaf total carbohydrates, and catalase activity (CAT) decreased. By contrast, AM colonization increased chlorophyll content, proline, total carbohydrates and CAT activity. A gradual decrease in leaf water potential (Ψ) and relative water content was evident with increasing drought stress levels. However, the seedlings inoculated with AM fungus had significantly higher leaf water potential (- 1.5MPa) compared to the non-inoculated seedlings (- 2.0MPa).

Keywords: drought, mycorrhizae, growth indices, catalase, leaf water potential

INTRODUCTION

Drought is considered the single most devastating environmental stress, which decreases crop productivity more than any other environmental stress (Lambers *et al.* 2008). Water scarcity will continue to be a major factor on agricultural land (Bates *et al.*, 2008). About 70% of the total water use worldwide is consumed for the irrigation in the agriculture (FAO, 2015) and other sectors such as industry and domestic consumption will put large pressures on the availability of irrigation water for horticultural crops. The horticultural crops embody 43% of the total value of crops in 2002 and 35% in 2007 in the USA (USDA, 2009), and 39% in Europe in 2007–2008 (European Commission, 2009). Consequently, assessing the effects of water-wise cultivation systems such as deficit irrigation and enhanced drought tolerance in horticultural crops is essential. Deficit water supply at any growth stage poses detrimental effects on morphological, physiological, and biochemical processes in plants, which include reduced rate of cell division and expansion, leaf area, stem elongation and root size and depth, and disturbed stomatal formation, plant water and nutrient relations with decreased crop

productivity, and water use efficiency (Li *et al.*, 2009). Drought stress can increase production of reactive oxygen species (ROS). These toxic molecules can cause oxidative damage to lipids, proteins and DNA (Miller *et al.*, 2010).

Pomegranate plants (*Punica granatum* L.) have been grown as a common backyard crop for decades in the South Asia. In recent years, there has been an increased interest in the commercial production of the fruit in Egypt and surrounding regions. This production increase is largely in response to increased demand for the fruit by the consumer. Global production has increased substantially in the past decade, and pomegranate is being consumed not only as a fresh fruit, but also as juice or as a freshly prepared product. The fruit is also being utilized in numerous consumer products, including tea and juice blends (MacLean *et al.*, 2014). Pomegranates are drought-tolerant, but they require normal watering to produce good fruit crops (Khattab *et al.*, 2011) and to avoid dehydration during the hottest season (particularly after transplant). Moreover, adequate soil moisture will result in a substantial improvement in plant vigor and fruit yield. Furthermore, providing adequate water throughout drought periods will minimize the amount of fruit splitting when the rain returns. In this study, pomegranate plants were chosen as a model to study the importance of the application of AM to the production of semiligneous seedlings under drought conditions.

Apart from to the intrinsic protective systems of plants against stress, plants grow associated with a number of soil microorganisms that can alleviate the stress symptoms. Arbuscular mycorrhizal (AM) fungi are widespread microorganisms able to establish a symbiotic association with the roots of most terrestrial plants. Mycorrhizal plants are able to grow much better under drought conditions compared with non-mycorrhizal plants. Several studies have demonstrated that many plants inoculated with AMF increases the drought tolerance (Augé, 2001). Early studies examining the effects of AM symbiosis on plant water relations generally concluded that improved drought tolerance results from enhanced P nutrition (Nelsen and Safir, 1982). It is currently accepted that the contribution of AM symbiosis to plant drought tolerance is the result of accumulative physical, nutritional, physiological, and cellular effects (Ruiz-Lozano *et al.*, 2012). The underlying mechanisms include increased absorbing surface caused by soil-growing hyphae combined with the fungal capability to take up water from soils with low water potential (Lehto and Zwiazek, 2011). AM fungi enhanced osmotic adjustment and leaf hydration or reduced oxidative damage caused by the reactive oxygen species (ROS) generated during drought (Ruiz-Lozano, 2003). AM plants had postponed declines in leaf water potential (Ψ) during drought stress (Davies *et al.*, 1992 and El-Tohamy *et al.*, 1999) and leaf water potential returned to non-AM level more quickly in AM than non-AM plants after the relief of drought (Subramanian *et al.*, 1997). Therefore, the objective of this investigation was to evaluate the effect of the inoculation of AM on the morphology, physiology and water status of pomegranate seedlings grown under different drought stress levels.

MATERIALS AND METHODS

Plant material and growth conditions

Experiments were performed during 2014 and 2015 growing seasons at experimental plot in Faculty of Agriculture, Alexandria University, Egypt. One-year-old Wonderful pomegranate (*Punica granatum*, L.) seedlings were used. The experimental plants were singly planted in black polyethylene bags filled with about 6 kilograms of sandy soil. The chemical composition and physical characteristics of the experimental soil are shown in Table 1. On the 25th March in each experimental season, 30 pomegranates seedlings were chosen for this study. The experimental seedlings were divided equally into two groups each of 15 seedlings. First group of plants was inoculated by mycorrhizae, *Glomus intraradices*, while the second was left without mycorrhizal inoculation; as control. Inoculation was achieved by adding 10 g/plant of the inoculum in the soil under the seedlings. The mycorrhizal strain *G. intraradices*, isolated from the Experimental Station of Alexandria University at Abies, (Aboul-Nasr, 1993), was used in both experimental seasons. The inoculum consists of expanded clay aggregates (2–4mm in diameter, Leca), which contain chlamydo spores and fungus mycelium. The mycelium had been produced on *Tagetes erecta* L. (Aboul-Nasr, 2004). The control plants received the same amount of heat sterilized expanded clay. Thereafter, the plants were carefully handled for about two months before the commencement of the drought treatments. During this period of adaptation; all plants started new growth and seemed healthy, vigorous and well established. For initiating the soil moisture treatments, each group with or without mycorrhizal inoculation, was divided into three subgroups; each of five seedlings. The soil moisture in these subgroups reached 100%, 50% and 25% of the soil field capacity by adding 780ml, 390ml, and 195ml of water for each pot, respectively, at the beginning of the experiment. Thereafter, different amounts of water were weekly added to the pots of each subgroup to maintain the soil moisture at these soil field capacity levels. The different amounts of water added to the pots were calculated after the determination of the field capacity of the experimental soil. The soil field capacity was determined directly by pressure Cooker method at 1/3 atm, as described by Israelsen and Hansen (1962). The amounts of water added to the pots were estimated by weighing the pots of each treatment periodically, at weekly intervals. The experimental plants were arranged in Randomized Complete Block Design (RCBD), with five replicates in each treatment, with a single plant for each replicate (3 levels of soil field capacity, 2 mycorrhizae treatments (with or without AM) x 5 replicates = 30 seedlings in each experimental season).

Plant growth and assessment of mycorrhizal colonization

The total growth of the experimental seedlings was estimated by measuring the length of each seedling at the beginning and at the termination of the experiment. The growth rate of each seedling was calculated using the following equation: Growth rate = final length - Initial length / Initial length. The trunk circumference and leaf area was estimated. The trunk circumference of

each replicate was measured at the soil surface, and the trunk cross-sectional area of each seedling was calculated. For leaf area determination, five mature leaves from each seedling were collected at the end of the experiment and their areas were measured using a planimeter.

After 90 days of beginning water treatments, at the termination of the experiment, the seedlings were carefully excavated from polyethylene bags. The leaves, stem and roots were separated and dried at 70 °C for 48 h, and the dry weights of the leaves, stem and roots of each seedling were recorded. A fraction of roots were used to estimate the percentage of mycorrhizal root colonization by the observation of cleared and stained 1-cm root segments under a research microscope for every treatment. The procedure was done according to the method described by Gianinazzi (2004).

Table 1. Physical and chemical analysis of soil used at experimental seedlings.

Characteristics:-	
<u>Physical properties</u>	
Texture	Loamysand
Field capacity%	16.25
Wilting point%	3.12
pH	8.15
EC (mmohs/cm)	1.31
<u>Chemical properties:</u>	
(meq/100gm)	
Sodium (Na ⁺)	5.20
Potassium (K ⁺)	0.36
Calcium (Ca ⁺)	1.65
Magnesium (Mg ⁺⁺)	4.98
Bicarbonates (HCO ₃)	3.60
Chloride (Cl ⁻)	3.69
Sulfates (SO ₄)	3.12
Organic matter (%)	0.14

EC= electrical conductivity

Chlorophyll determination

Leaf total chlorophyll content was determined in the fresh leaf samples according to the method described by Yadava (1986), using a Minolta SPAD chlorophyllmeter. Five readings were taken for each plant at the end of either season. The results were expressed as SPAD.

Carbohydrate determination

The total carbohydrates were extracted from half gram of dry materials of the leaf of each replicate. The total carbohydrates estimations were done by Nelson-Somogyi method using oven dried samples as described by Thimmaiah (2004).

Proline determination

The determination of leaf free proline was done according Bates *et al.* (1973). A 0.1 gm from the dry ground materials of each replicate was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and filtered through 2 filter papers. Two milliliter of the filtrate stands to react with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 hour at 100 °C.

The reaction was terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously for 15-20 seconds. The chromophore containing toluene was aspirated from the aqueous phase and the absorbance was read at 50 nm against a proline stand. Leaf proline content was expressed as mg/g dry weight of leaf tissues.

Leaf catalase (CAT) activity

For the determination of leaf catalase (CAT) and peroxidase (POX) activity, a fresh leaf sample composed of 5 mature leaves was taken from each experimental seedling. The leaf catalase activity was assayed as described by Kar and Mishra (1976), analyzed according to KMnO₄ titration method and expressed as μ mole H₂O₂ reduced g⁻¹ FW min⁻¹ (μ mole H₂O₂ / g FW / min).

Leaf water potential (Ψ) and relative water content (RWC) determination

Measurements of leaf water potential and relative water content were conducted at midday using the fully expanded leaves from the middle part of shoots of these seedlings. Relative water content was determined as described by Smart and Bingham (1974). The leaves of each replicate, were cleaned with tissue paper and their fresh weight (FW) was recorded. The turgid weights (TW) of the leaves were recorded after floating in water in covered Petry dish for 24 hours at 4 °C. Thereafter, the leaves were oven dried at 70 °C to a constant weight and their dry weights (DW) were recorded. The equation $RWC (\%) = (FW - DW) / (TW - DW)$ was used for RWC calculation. The midday leaf water potential (Ψ) measurements were made one day before irrigation was given. The leaves were selected from the middle part of shoots; they were detached and their Ψ was measured immediately by a pressure chamber (Model PMS 1505D-EXP USA).

Statistical analysis

The data obtained throughout the course of this study were statistically analyzed according to the analysis of variance (ANOVA) as the method described by Snedecor and Cochran (1980). The average for each treatment was the mean of five replicates. Differences among treatments were determined using Least Significant Differences (LSD) at probability level of 0.05. Data for the percentage of mycorrhizal colonization were analyzed after angular transformation (Steel and Torrie, 1980).

RESULTS AND DISCUSSIONS

1-Mycorrhizal root colonization and survival seedlings percentage

The percentage of root colonization was determined at the end of the experimental seasons 2014 and 2015. The percentage of root colonization was significantly increased in case of inoculated mycorrhizal plants compared to un-inoculated ones. Table 2 and 3 shows the percentage of mycorrhizal root colonization, arbuscules % and vesicles%. The mycorrhizal colonization was observed at all developed drought levels. With increasing drought stress levels, the root colonization was decreased significantly, in 2014 season, from 89.25 to 38.97%. The corresponding values for 2015 season were from 80.73 to 31.77%. There was a gradual decline in the mycorrhizal root length

percentage, with increasing drought stress. In 2014 season, the percentages of root colonization values were 89.25, 59.35 and 38.97% for pomegranate seedlings irrigated with 100, 50 and 25% of the soil field capacity, respectively. The corresponding values for arbuscules % were 70.98, 42.12 and 40.86% and the corresponding values for vesicles% were 58.26, 38.16 and 18.98%. Similar trend was noticed in 2015. The decline in colonization under stress could be caused by adverse conditions for sporulation and development of spores under unfavorable rizosphere conditions (Murkute *et al.* 2006). Inoculated plants showed a higher survival rate under conditions of drought stress as has been observed previously (Wu *et al.* 2008). There was no interaction between inoculation and the level of drought on seedlings survival.

Table 2. Mycorrhizal root colonization%, arbuscules (A) %, vesicles (V) %, and survival percentages in pomegranate seedlings during 2014 season

	Irrigation levels		
	100% FC	50% FC	25% FC
Root colonization %	89.25 ±2.16 a	59.35 ±5.02b	38.97 ±2.36c
A%	70.98 ±3.11a	42.12 ±3.31b	40.86 ±5.03b
V%	58.26 ±5.30a	38.16 ±5.12b	18.98 ±3.16c
Seedling survival %	+AM	99.86 ±0.001a	95.93 ±0.05a
	-AM	98.99 ±0.04a	94.36 ±0.02a
F-test			
	AM	FC	AM x F.C
	*	*	NS

FC= Field capacity

AM= Arbuscular mycorrhizae

Table 3. Mycorrhizal root colonization%, arbuscules (A) %, vesicles (V) %, and survival percentages in pomegranate seedlings during 2015 season.

	Irrigation levels		
	100% FC	50% FC	25% FC
Root colonization %	80.73 ±3.12 a	52.11 ±7.15b	31.77 ±3.22c
A%	66.30 ±3.50a	40.17 ±2.25b	43.23 ±5.03b
V%	50.25 ±4.20a	35.16 ±4.55b	17.32 ±4.66c
Seedling survival %	+AM	99.30 ±0.002a	95.89 ±0.05a
	-AM	98.72 ±0.02a	91.55 ±0.02a
F-test			
	AM	FC	AM x FC
	*	*	NS

FC= Field capacity

AM= Arbuscular mycorrhizae

2. Growth indices

The effect of drought stress and mycorrhizae as well as the interaction between these two variables on the growth indices of pomegranate seedlings is shown in Table 4 and Figure 1. As an overall average, the data of the present investigation generally indicated that increasing the level of drought was accompanied by a gradual decline in growth rate, leaf area, trunk cross-sectional area and total dry weight. This reduction in plant growth indices was more evident at higher (25% FC) rather than at lower drought stress level

(50%). This result is in conformity with similar results reported by numerous investigators such as: Lange and Lenz (1999), Symeonidou and Buckley (1999) and Arzani and Arji (2000). The reduction in the growth of pomegranate seedlings, observed herein, could be attributed to a noticeable decrease in the water absorbing power of the seedlings under the drought conditions.

Table 4. Results of the analysis of variance with mean square testing the effects of irrigation levels (IL), mycorrhizae (AM) and their interactions on growth rate (GR), leaf area (LA), trunk cross-sectional area (TCA), total dry weight (TDW), chlorophyll content (Chl), leaf proline content (LPC), leaf total carbohydrates (LTC), catalase activity (CAT), Leaf water potential (Ψ) and relative water content (RWC) during 2014 and 2015 seasons.

season	GR	LA (cm ²)	TCA (cm ²)	TDW (gm)	Chl SPAD	LPC mg/100 dw	LTC mg/100 dw	CAT (μ mole H ₂ O ₂ / g fw / min)	Ψ (MPa)	RWC (%)
2014										
Irrigation levels (IL)	0.27***	1088***	16.9***	273***	128***	95***	21***	44.09***	1.99***	2606***
Mycorrhizae (AM)	0.10***	435***	6.61*	207***	84***	18***	9**	7.72*	0.35***	207***
IL X AM	0.01	23.3	2.24 NS	8.60	13.57	3.91	0.23 NS	2.49 NS	0.03 NS	1.52 NS
2015										
Irrigation levels (IL)	0.14***	550***	0.187***	446***	334***	172.0***	20.604***	37.55***	3.44***	3733***
Mycorrhizae (AM)	0.05***	375***	0.028***	428***	156***	75.15***	11.408**	9.54*	0.46***	339***
IL X AM	0.002	17.5	0.004**	27.3	13.38	11.48	0.5 NS	3.03 NS	0.06 NS	61.8 NS

*Significant at P = 0.05, **Significant at P = 0.01 and ***Significant at P = 0.001

Concerning the overall effect of mycorrhizal inoculation, the data of the present study indicated that mycorrhizae seemed to affect positively most, if not all, the criteria describing the seedlings growth (Table 4 and Figure 1). Mycorrhizal seedlings, in general, had higher growth rate, leaf area, trunk cross-sectional area and total dry weight than non mycorrhizal seedlings. These positive mycorrhizal responses were supported by numerous investigators such as: Krishna *et al.* (2005), Wang *et al.* (2008), Khalil *et al.* (2011) and Khalil (2013) working on different fruit species. These superior effects of mycorrhizae on seedlings growth could be attributed to the influence of mycorrhizae in improving plant nutrition (Ruiz-Lozano *et al.*, 2012). Other researchers, however, attributed these responses to the influence of mycorrhizae in maintaining adequate plant water relationships (Evelin *et al.*, 2009). Still, a third group of scientists pointed out to the role played by mycorrhizae in alleviating oxidative stress of inoculated plants, and consequently its growth (Bressano *et al.* 2010).

As for the interactional effect of drought stress and mycorrhizal inoculation on plant growth indices, it might be concluded that although mycorrhizal seedlings often showed higher growth values than non-mycorrhizal ones, these growth responses were usually more pronounced at higher (25% FC) drought stress level. For example, in 2014 season, the 25% FC treatment reduced the total dry weight of mycorrhizal seedlings by 39.70%, whereas it reduced that of non-mycorrhizal ones by as much as 42.45%.

3. Organic components

The results of the present investigation clearly revealed that, seedlings irrigated with 50 and 25% drought stress levels showed significant decrease in leaf chlorophyll content, leaf total carbohydrates and leaf catalase activity, whereas, that of leaf free proline content tended to increase during 2014 and 2015 growing seasons (Table 4 and Figure 2). In 2014 season, seedlings grown under 25% FC drought stress level showed increase in leaf free proline content reached as much as 73.65% in comparison with those grown under control. This result is consistent with Murkute *et al.*, (2006) who reported that proline and various betaines can function as osmoprotectants and cryoprotectants, when accumulated in cell. In 2014 season, the reduction in leaf total chlorophyll, total carbohydrates and catalase in seedlings grown under 25% FC reached as much as 32.8, 24.32 and 39.2%, respectively as compared with control. The corresponding values for 2015 season were 41.4, 27.8% and 35%. Similar increase in proline content and decrease in chlorophyll and carbohydrates content and catalase activity by drought stress were recorded (Khalil, 2013). Jie *et al.* (2010) reported that apple seedlings subjected to water stress often showed decrease in total carbohydrates. They attributed the low carbohydrates content of drought plants to the decrease in photosynthetic rates in the drought-stressed leaves when photosynthetic production was insufficient to meet demand, breakdown of soluble carbohydrates could sustain metabolism. Moreover, they added that the accumulation of osmolytes, as the soluble sugar fractions, could be considered as an adaptive response to drought stress condition. The decline in CAT activity is regarded as a general response to many stresses (Gunes *et al.*, 2008 and Liu *et al.*, 2008). The reduction of CAT activity is supposedly due to its role in overcoming stress damage, particularly when CAT activity becomes a limiting factor in the scavenging of active oxygen species produced in the oxidative stresses (Shim *et al.* 1999). The adverse effects of drought stress on total chlorophyll content in the leaves could be due to the suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments (Murkute *et al.*, 2006).

Regarding the effect of mycorrhizal inoculation on organic component, the total chlorophyll content, total carbohydrates, leaf free proline content and catalase activity, the results indicated that it was significantly higher than that of the un-inoculated ones. This trend was observed during both experimental seasons. For example, in 2015 season, seedlings inoculated with AM had the highest total carbohydrates content (15.66%), while the seedlings without mycorrhizal inoculation had the lowest value (13.12 %). It has been reported that mycorrhizal fungi markedly enhanced the photosynthesis of inoculated plants and increased its stomatal conductance where might provide a reasonable explanation for the general increase in the carbohydrates content of mycorrhizal plants observed herein (Nylund and Wallander, 1989 and Khalil, 2013). Preinoculated seedlings had greater leaf chlorophyll content compared with non mycorrhizal ones. The enhanced chlorophyll level might be responsible for increasing photosynthesis in inoculated plant. This can further be attributed to increased Mg uptake which is essential for chlorophyll bio-synthesis (Krishna *et al.*, 2005 and Khalil *et al.*, 2011). Several reports have paid special attention to the importance of AM in alleviating oxidative stress; they found that the protection against oxidative damage was due to an increase in enzymatic antioxidant levels (Bressano *et al.* 2010).

4. Leaf water potential (Ψ) and relative water content (RWC)

The effect of drought stress level in particular, 100, 50 and 25% of the soil field capacity as well as the effect of mycorrhizal inoculation on leaf water potential (Ψ) and relative water content of pomegranate seedlings are presented in Table 4 and Figures 3&4. The results indicated that leaf water potential (Ψ) and relative water content respond negatively to increasing drought stress conditions. A gradual decrease in leaf water potential (Ψ) and relative water content was evident with increasing drought stress levels. These results are in general agreement with those reported by Tezara *et al.* (2002), Liu *et al.* (2004) and Ozkur *et al.* (2009). They all agreed that with increasing drought stress levels of growing media, a noticeable decrease in leaf water potential (Ψ) and relative water content (RWC) of the leaves was noticed.

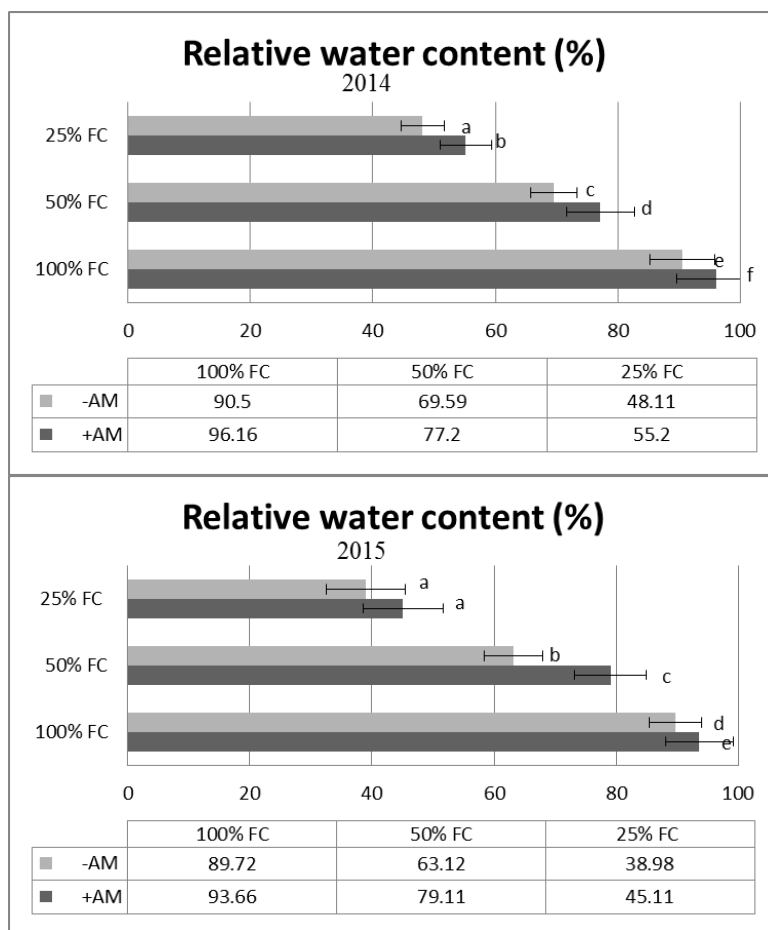


Fig.4. Effect of drought stress and mycorrhizal inoculation on relative water content (%) of pomegranate seedlings during 2014 and 2015 season. + AM with mycorrhizae, – AM without mycorrhizae and FC (field capacity).

As for the specific effect of mycorrhizal inoculation, the data generally indicated that there were statistical differences between AM inoculated and un-inoculated seedlings in their leaf water potential (Ψ) and relative water content. Significant higher leaf water potential (Ψ) and relative water content values were observed in inoculated seedlings, in comparison with un-inoculated ones. Drought stress decreased leaf water potential (-53MPa) but the decrease was larger in un-inoculated plants (- 0.90). These results are in line with those of Porcel *et al.* (2004), Colla *et al.* (2008) and Jahromi *et al.* (2008). They reported that the leaf water potential and relative water content were higher in stressed AM plants than in non-AM plants. This could be attributed to the effect of AM in improving hydraulic conductivity of the root at low water potential (Kapoor *et al.*, 2008). The improved root conductance is

associated with a longer root and an altered root system morphology induced by AM (Kothari *et al.*, 1990). Besides, Evelin *et al.* (2009) reported that mycorrhizal plants are also shown to possess a lower osmotic potential which is maintained by fungal accumulation solutes, consequently resulting in improved plant osmotic adjustment.

CONCLUSION

It can be concluded that AM symbiosis enhances osmotic adjustment in roots, which could contribute to maintaining leaf gradient favorable to the water passing from soil into the roots. This enables higher leaf water potential Ψ in AM plants during drought and keeps the plants protected against drought, as shown by their significantly higher shoot biomass production, and these cumulative effects increase the plant drought tolerance.

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REFERENCES

- Aboul-Nasr, A. (1993). Identification of VA-mycorrhizal fungi in soil of Alexandria Governorate. *Alex. J. Agric. Res.*, 38(2): 371-376.
- Aboul-Nasr, A. (2004). Method of producing inoculums of endomycorrhizal fungi. *Academy Sci. Res. and Tech. Egypt. Patent No.* 23234.
- Arzani, K. and I. Arji (2000). The effect of water stress and deficit irrigation on young potted olive cv., 'local Roghani Roodbar' *Acta Hort.*, (537): 879-885.
- Augé, R. M. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, 11: 3-42. 11: 3-42.
- Bates, B.C.; Z. W. Kundzewicz; S. Wu and J. P. Palutikof (2008). *Climate Change and Water. Technical Paper of the Intergovernmental Panel on Climate Change. IPCC Secretariat, Geneva, Switzerland*, p.p 210.
- Bates, L. S.; R. P. Waldren and I. D. Teare (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, 39: 205-207.
- Bressano, M.; M. Curetti, L. Giachero; S. Vargas Gil; M. Cabello; G. March; D. A. Ducasse and C. M. Luna (2010). Mycorrhizal fungi symbiosis as a strategy against oxidative stress in soybean plants. *J. Plant Physiol.*, 167: 1622-1626.

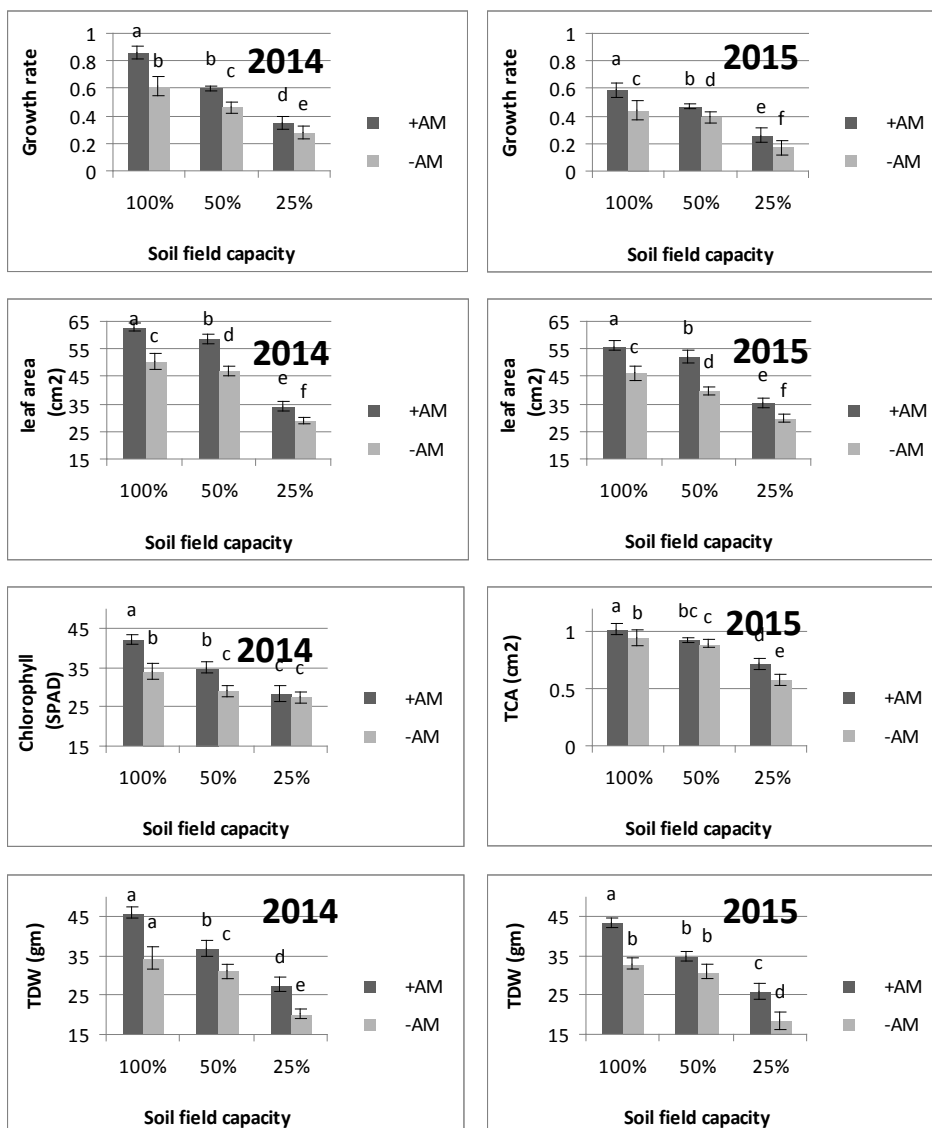
- Colla, G.; Y. Roupael; M. Cardarelli; M.Tullio; C.M. Rivera and E. Rea (2008). Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. *Biology and Fertility of Soils.*, 44: 501–509.
- Davies, F.T.; J.R. Potter and P. G. Linderman (1992). Mycorrhiza and repeated drought exposure affect drought resistance and extraradical hyphae development of pepper plants independent of plant size and nutrient content. *Journal of Plant Physiology*, 139, 289–294.
- El-Tohamy, W.; W. H. Schnitzler; U. El-Beairy and M.S. El-Betagy (1999). Effect of VA mycorrhiza on improving drought and chilling tolerance of bean plants. *Journal of Applied Botany* 73, 178–183.
- European Commission (2009). *Agricultural Statistics. Main Results 2007–2008.* Euro-stat, Luxembourg.
- Evelin, H.; R. Kapoor and B. Giri (2009). Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. *Ann Bot.* 2009 Dec; 104(7): 1263–1280.
- FAO (2015). Food and Agriculture Organization of the United Nations: Water Uses. [http://www.fao.org/nr/water/aquastat/water use/index.stm](http://www.fao.org/nr/water/aquastat/water%20use/index.stm)
- Gianinazzi, S. (2004). Genomyca Workshop on “Technological transfer in arbuscular mycorrhiza research” Mach 1-4, 2004, in INRA at Dijon, France.
- Gunes, A.; D.J. Pilbeam; A. Inal and S. Coban (2008). Influence of silicon on sunflower cultivars under drought stress, I: Growth, antioxidant mechanisms, and lipid peroxidation. *Communications in Soil Science and Plant Analysis*, 39: 1885-1903.
- Israelsen, O. W. and V. E. Hansen (1962). *Irrigation, Principles and Practices.* John Wiley and Sons, Inc. New York.
- Jahromi, F.; R. Aroca; R. Porcel and J. M. Ruiz-Lozano (2008). Influence of salinity on the *in vitro* development of *Glomus intraradices* and on the *in vivo* physiological and molecular responses of mycorrhizal lettuce plants. *Microbial Ecology*, 2008: 55:45–53.
- Jie, Z.; Y. Yuncong; J. G. Street and D. C. Ferree (2010). Influence of soil drought stress on photosynthesis, carbohydrates and the nitrogen and phosphorus absorption in different section of leaves and stem of Fuji/M.9EML, a young apple seedlings. *African Journal of Biotechnology*, 9 (33): 5320-5325.
- Kapoor, R.; D. Sharma and A.K. Bhatnagar (2008). Arbuscular mycorrhizae in micropropagation systems and their potential applications. *Scientia Horticulturae*, 116: 227–239.
- Kar, M. and D. Mishra (1976). Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant Physiol.*, 57: 315-319.
- Khalil, H.A. (2013). Influence of vesicular-arbuscular mycorrhizal fungi (*Glomus* spp.) on the response of grapevines rootstocks to salt stress. *Asian Journal of Crop Science*, 5(4): 393-404.
- Khalil, H.A.; A.M. Eissa; S. M. El-Shazly and A. M. Aboul-Nasr (2011). Improved growth of salinity-stressed citrus after inoculation with mycorrhizal fungi. *Scientia Horticulturae*, 130: 624-632.

- Khattab, M.M.; A. E. Shaban; A. H. El-Shrief and A. S. El-Deen Mohamed (2011). Growth and productivity of pomegranate trees under different irrigation levels. I: Vegetative growth and fruiting. *J. Hortic. Sci. Ornam. Plants*, **3**(2):194–198.
- Kothari, S.K.; H. Marschner and E. George (1990). Effect of VA mycorrhizal fungi and rhizosphere microorganism on root and shoot morphology, growth and water relations of maize. *New Phytologist*, 1990: 116: 303–311.
- Krishna, H.; S.K. Singh and R.R. Sharma (2005). Biochemical changes in micropropagated grape (*Vitis vinifera* L.) plantlets due to arbuscular-mycorrhizal fungi (AMF) inoculation during *ex vitro* acclimatization. *Scientia Horticulturae*, 106: 554-567.
- Lange, K. and F. Lenz (1999). Nitrogen intake and distribution in apple trees in relation to water supply, pruning methods and fruit load. *Hort. Abst.*, 69 (3): 1858.
- Lambers, H.; F.S. Chapin and T.L. Pons (2008). *Plant Physiological Ecology*, 2nd edn. Springer, New York.
- Lehto, T and J. Zwiazek (2011). Ectomycorrhizas and water relations of trees: a review. *Mycorrhiza*, 21: 71-90.
- Li, Y.P.; W. Ye; M. Wang and X.D. Yan (2009). Climate change and drought: a risk assessment of crop yield impacts. *Climate Res* 39:31–46.
- Liu, F.; C. R. Jensen and M. N. Andersen (2004). Drought stress effect on carbohydrate concentration in soybean leaves and pods during early reproductive development: its implication in altering pod set. *Field Crops Res.*, 86:1–13.
- Liu, X.; X. Hua; J. Guo; D. Qi; L. Wang; Z. Liu; Z. Jin; S. Chen and G. Liu (2008). Enhanced tolerance to drought stress in transgenic tobacco plants overexpressing VTE1 for increased tocopherol production from *Arabidopsis thaliana*. *Biotechnol. Lett.*, 30:1275–1280.
- Maclean, D.; K. Martino and H. Scherm (2014). Pomegranate production. <http://extension.uga.edu/publications/detail.cfm>.
- Miller, G.; N. Suzuki; S. Ciftci-Yilmaz and R. Mittler (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ.*, 33: 453–467.
- Murkute, A.A.; S. Sharma and S.K. Singh (2006). Studies on salt stress tolerance of citrus rootstock genotypes with arbuscular mycorrhizal fungi. *HortScience*, (PRAGUE), 33 (2): 70-76.
- Nylund, J.E. and H. Wallander (1989). Effect of ectomycorrhiza on host growth and carbon balance in a semi-hydroponic cultivation system. *New Phytol.*, 112(3): 389-398. (*Soils & Fert.*, 35: 5380).
- Nelsen, C. E. and G. R. Safir (1982). Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. *Planta*, 154: 407–413.
- Ozkur, O.; F. Ozdemir; M. Bor and I. Turkan (2009). Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* Desf. to drought. *Environ. Exp. Bot.*, 66:487–492.

- Porcel, R and J. M. Ruiz-Lozano (2004). Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. *Journal of Experimental Botany*, 55 (403): 1743–1750.
- Ruiz-Lozano, J. M. (2003). Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress: new perspectives for molecular studies. *Mycorrhiza*, 13: 309–317.
- Ruiz-Lozano, J. M.; R. Porcel; G. Barzana; R. Azcon and R. Aroca (2012). Contribution of Arbuscular Mycorrhizal Symbiosis to Plant Drought Tolerance: State of the Art, Plant responses to drought stress from morphological to molecular features. Book chapter, Springer-Verlag Berlin Heidelberg. PP. 335.
- Shim I.S., Naruse Y., Kim Y.H., Kobayashi K. and Usui K. 1999. Scavenging activity of NaCl-induced activated oxygen in two rice (*Oryza sativa* L.) cultivars differing in salt tolerance. *Jpa.J. Trop. Agr.* 43: 32–41.
- Smart, R. E. and G. E. Bingham (1974). Rapid estimates of relative water content. *Plant Physiol.*, 53: 258-260.
- Snedecor, G.W. and W.G. Cochran (1980). *Statistical Methods*, 7th Edn. Ames: Iowa State University press. 507pp.
- Subramanian, K. S.; C. Charest; L. M. Dwyer and R. I. Hamilton (1997). Effects of arbuscular mycorrhizae on leaf water potential, sugar content and P content during drought and recovery of maize. *Canadian Journal of Botany*, 75: 1582–1591.
- Steel, R.G.D. and Torrie, J.H.; 1980. *Principal and procedures of statistics*. 2nd ed. Mc Graw-Hill, New York, USA.
- Symeonidou, M.V. and G. P. Buckley (1999). The effect of pre-planting desiccation stress and root pruning on the physiological condition and subsequent field performance of one year old *Prunus avium* and *P. cerasifera* seedlings. *Hort. Abst.*, 69(8):7224.
- Tezara, W.; V.J. Mitchell; S.D. Driscoll and D.W. Lawlor (2002). Effects of water deficit and its interaction with CO₂ supply on the biochemistry and physiology of photosynthesis in sunflower. *J. Exp. Bot.*, 53:1781–1791
- Thimmaiah, S.R. (2004). *Standard Methods of Biochemical Analysis*. New Delhi, Kalyani Publishers: 267.
- USDA (2009). *Census of Agriculture 2007 United States. Summary and StateData*, vol., 1. National Agricultural Statistics Service, Washington, DC, Part 51.AC-07-A-51.
- Wang, M.; P. Christie and Z. Xiao (2008). Arbuscular mycorrhizal enhancement of iron concentration by *Poncirus trifoliata* L. Raf and *Citrus reticulata* Blanco grown on sand medium under different pH. *Biol. Fertil. Soils*, 45: 65-72.
- Wu, Q. S.; R.X. Xia and Y. N. Zou (2008). Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. *Euro. J. Soil Biol.* 44:122–128.
- Yadava, U.L. (1986). A rapid and non-destructive method to determine chlorophyll in intact leaves. *HortScience*, 21: 1449-1450.

الاستجابة المورفولوجية والفسيزيولوجية ومقاومة الإجهاد المائي لشتلات الرمان
عن طريق التلقيح بالميكوريزا
هدى علي عبد المحسن خليل
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تم اجراء هذه الدراسة خلال موسمي نمو ٢٠١٤ و ٢٠١٥ وذلك لدراسة تأثير الإجهاد المائي باستخدام ثلاث مستويات ري مختلفة وهي ١٠٠٪ ، ٥٠٪ و ٢٥٪ من السعة الحقلية وكذلك دراسة تأثير التلقيح بلفاح من فطر الميكوريزا علي الاستجابة المورفولوجية والفسيزيولوجية و مقاومة الإجهاد المائي لشتلات الرمان النامية تحت هذه الظروف من الإجهاد المائي. أوضحت نتائج الدراسة ان الإجهاد المائي أدى الي انخفاض نسبة عدوي الجذور بلفاح الميكوريزا . وقد وجد ان زيادة مستوي الإجهاد المائي أدى الي انخفاض معدل نمو الشتلات ، ومساحة سطح اوراقها ومساحة مقطع ساقها ووزنها الكلي الجاف. وأدى التلقيح بلفاح الميكوريزا الي زيادة معنوية في معدل نمو الشتلات و مساحة سطح اوراقها و مساحة مقطع ساقها ووزنها الكلي الجاف وذلك بغض النظر عن مستوي الإجهاد المائي. كما أدى الإجهاد المائي الي زيادة محتوى الأوراق من الحمض الأميني البرولين وانخفاض محتوى الأوراق من الكلوروفيل والكربوهيدرات ونشاط إنزيم الكتاليز بينما أدى التلقيح بلفاح الميكوريزا الي زيادة محتوى الأوراق من الكلوروفيل و البرولين والكربوهيدرات الكلية وزيادة مستوي نشاط إنزيم الكتاليز. وقد ادت زيادة مستوي الإجهاد المائي الي انخفاض الجهد المائي للأوراق وكذلك انخفاض المحتوى المائي النسبي للأوراق بينما أدت الميكوريزا الي زيادة الجهد المائي للأوراق وكذلك محتواه المائي النسبي.



[Fig. 1. Effect of drought stress and mycorrhizal inoculation on the growth rate, leaf area, trunk cross-sectional area (TCA) and total dry weight (TDW) of pomegranate seedlings during 2014 and 2015 seasons. + AM with mycorrhizae and – AM without mycorrhizae

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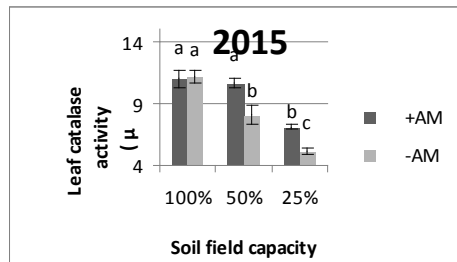
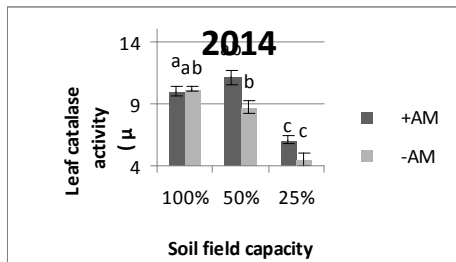
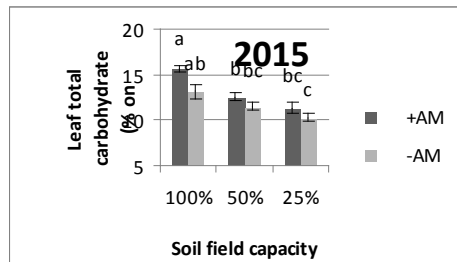
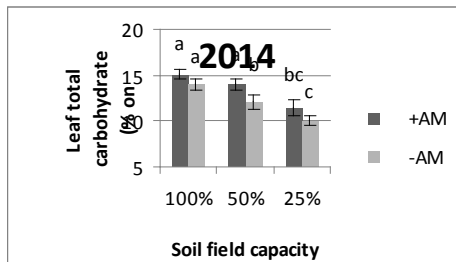
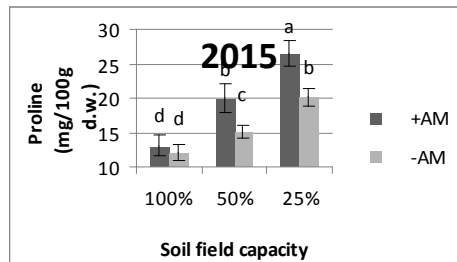
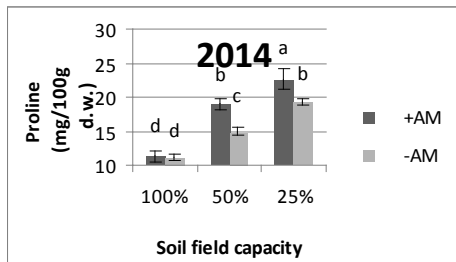
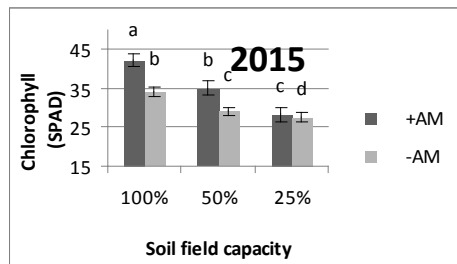
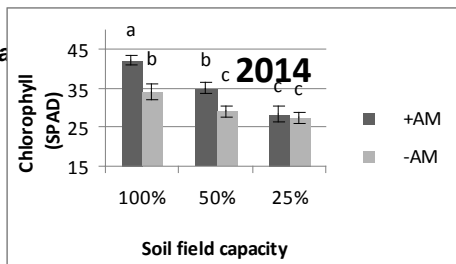


Fig. 2. Effect of drought stress and mycorrhizal inoculation on the chlorophyll content, proline content, leaf total carbohydrates and leaf catalase activity of pomegranate seedlings during 2014 and 2015 seasons . + AM with mycorrhizae and – AM without mycorrhizae

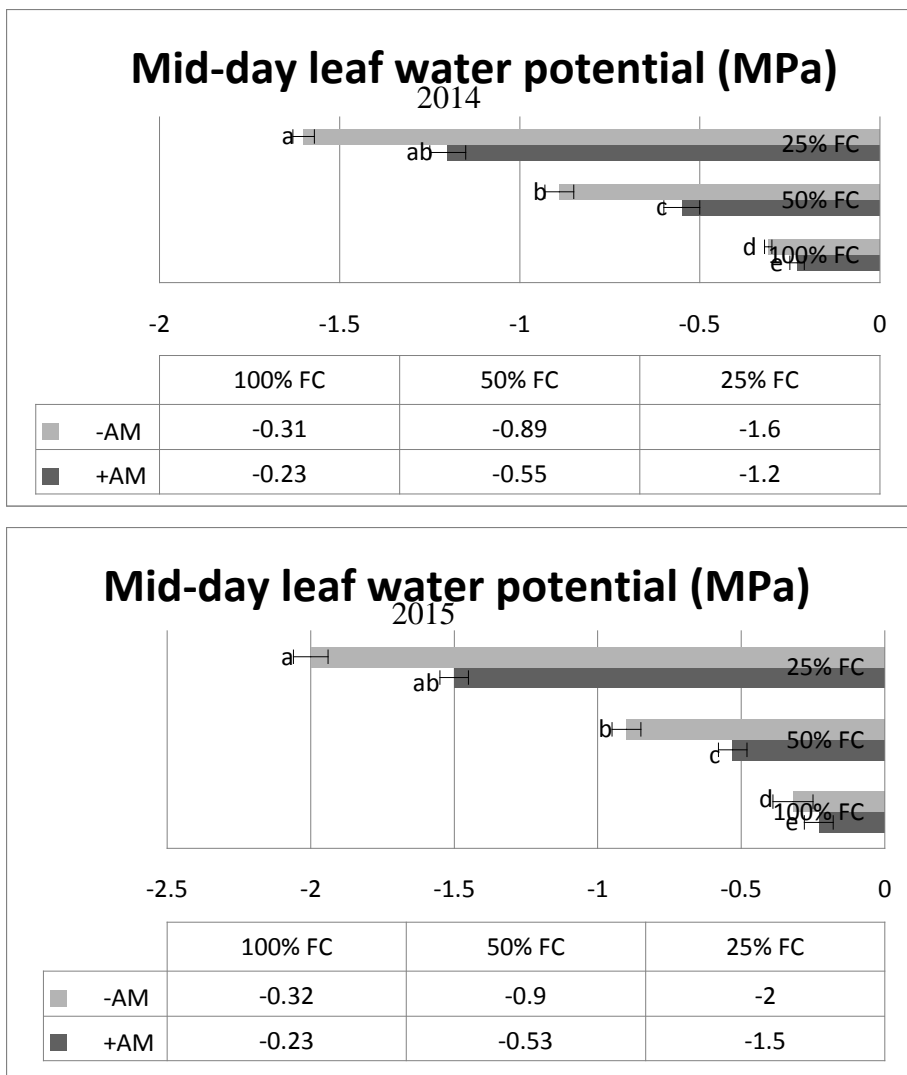


Fig. 3. Effect of drought stress and mycorrhizal inoculation on leaf water potential (Ψ) of pomegranate seedlings during 2014 and 2015 season. + AM with mycorrhizae, – AM without mycorrhizae and F.C (field capacity).