Increasing Plant Tolerance to Drought Stress by Inoculation with Arbuscular Mycorrhizal Fungi

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Abstract: The present study was aimed to evaluate the effects of *Glomus mosseae* in three levels of soil infestation (300, 600 and 900 spores pot^{-1}) to improve tolerance of maize plants (*Zea mays* L.) for drought stress conditions with bearing in mind determine some plant growth parameters (PGP) and biochemical [plant height, stem length, root length, plant fresh wt., shoot dry wt., root dry wt., root/shoot ratio, plant chlorophyll content, soluble protein, proline in leaves and Phosphorus (P) uptake] in the presence or absence of *G. mosseae*. The result shown that the drought treatment causing decrease in values of almost PGP, except plant root dry weight, which was increased when comparing with well irrigation treatment. The plants treated by *G. mossea* were recorded a significant (P<0.05) increase in all PGP comparing with untreated plants in both normal irrigation and drought stress. The highest PGP values were recorded when plant inoculated by 900 spores pot^{-1}. The water deficit treatment was caused a significant decrease in plant soluble protein by rate 29.34% comparing with plants that well irrigate by normal way. While the *G. mossea* treatments were caused increase in plant soluble protein by rate 13.33, 22.18 and 29.27% in the normal irrigation treatment, and by rate 24.89, 36.25 and45.17% in the drought treatment comparing with plant in soil free from mycorrhizae. On contrast the proline content in plant leaves was increased in drought treatment by rate 22% comparing with plant in well irrigation. The treatments with *G. mossea* causing decreased in plant proline by rate 28.88, 38.05 and 43.19% in the drought treatment respectively with three levels of soil infestation. The drought treatment caused decrease in plant P uptake by rate 72.09% comparing with well irrigation treatment. The inculcation by *G. mosseae* caused increased in plant P uptake by rate 42.66, 76.11 and 79.32% in normal irrigation treatments and 88.34, 93.58 and 94.91% in drought stress comparing with plant free mycorrhizal in both water treatments.

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1. Introduction:

Mycorrhiza is a symbiotic association between a group of soil fungi called arbuscular mycorrhizal fungi (AMF) and plants. The successful association between plants and AMF constitutes a strategy to improve the nutritional status of both associates, which reduces the use of fertilizers specially phosphorus nutrition (Almagrabi and Abdelmoneim, 2012). The AMF take carbohydrates compounds from their plant host, while the plants benefit from the association by the increased nutrients uptake, which improve tolerance to abiotic stress (drought or salinity), as well as enhanced plant disease control (Linderman, 1994; Song *et al.*, 2011). The water stress is considered the main factor that causing limitations to plant growth. The effects of drought on plant growth depend on several factors such as plant genetic resistance, stage of growth and duration of plant expose to drought (Panozzo and Eagles, 1999; Echave *et al.*, 2005; Song *et al.*, 2011). The AMF are playing a vital role in sustainable agriculture because they enhance plant water relations, which improve the drought resistance of host plants (Allen and Allen, 1986; Nelsen, 1987). The abilities of specific associations between plants and AMF to tolerate drought are of a great interest. The results in several studies on drought stress conditions indicated that the plant biomass, chlorophyll contents and rate of transpiration were greater in plants inoculated with AMF compared with plants without AMF infection (Ruz-Lozano *et al.*, 1995; Augé, 2001; Beltrano *et al.*, 2003; Asensio *et al.*, 2012). Also AMF have been observed effects on stomatal conductance with similar frequency under amply watered and drought stress (Bethlenfalvay *et al.*, 1988; Henderson and Davies, 1990; Ibrahim *et al.*, 1990; Augé *et al.*, 1992; Awotoye *et al.*, 1992; Davies *et al.*, 1993). AMF symbiosis has also affected stomatal sensitivity to atmospheric water status (Huang *et al.*, 1985).

AMF effects on plant water relations and metabolism during drought have been associated with morphological and phenological effects. AMF Acacia (Osonubi *et al.*, 1992) and rose (Henderson and Davies, 1990) showed more leaf abscission during drought than plants untreated with AMF, while wheat treated with AMF showed less leaf drop (Ellis *et al.*, 1985) and less leaf necrosis (Bryla and Duniway, 1997). AMF maize had relatively more green leaf area than non-mycorrhizal maize after drought (Subramanian *et al.*, 1995) and AMF symbiosis delayed leaf senescence of Alfalfa in drought conditions (Goicoechea *et al.*, 1997). Soybeans plant treated with AMF had less drought-induced pod abortion than untreated plants (Busse and Ellis, 1985). Leaf movements were greater in AMF than in Leucaena free AMF inoculation (Huang *et al.*, 1985). AMF plant leaves had lower cuticle weight and less epicuticular wax than plant leaves free from AMF infection (Henderson and Davies, 1990). The present study was mainly aimed to evaluate the effects of one species of AMF (*Glomus mosseae*) on the growth of maize plants (*Zea mays* L.) under drought stress conditions comparing with plants free from mycorrhiza by estimated proline accumulation under normal irrigation and drought stress. Also determined the effect of drought on some plant growth parameters (plant height, stem length, root length, plant fresh wt., shoot dry wt., root dry wt., root/shoot ratio and chlorophyll content) in the presence or absence of AM fungus inoculum to assess the role of mycorrhizal fungi on improve tolerance of maize plants to drought.

2. Material and Methods

2.1. Preparation of biological materials

One species of AMF was isolated from the rhizosphere of green grass growing in soil at North Campus of King Abdulaziz University in Jeddah city, western of Saudi Arabia. Approximately 400 intact spores in similar form of AMF were extracted by using the wet sieving and decanting according to Schenck, (1982) and identified morphologically according to Schenck and Perez, (1990), then propagated on *Cynodon dactylon* in sterilized soil for three months in greenhouse conditions. Colonized root fragments containing spores were used as AMF inocula. When AMF colonization on *C. dactylon* roots reach to 80%, the AMF spore density was estimated (300±20 spores$10g^{-1}$ of air dried roots). In the present study, AM fungal inoculum consisted of monoxenic culture of *Glomus mosseae* (Nicolson and Gerdeman) Gerdeman and Trappe, which we use in three inoculums rate 300, 600 and 900±20 spores pot^{-1} and mix with soil preparation for sowing seeds.

Zea mays (L.) seedlings were grown from commercial seeds (Hybrid T-313). Seeds were surface disinfected by shaking them in a 1% Sodium Hypochlorite (NaClO) solution for 10 min and rinsed successively 10 times for 5 min in sterile water. The seeds were germinated in plastic pots (25 cm diameter and 30cm depth vol. 2.5Kg soil). Four seeds were sown per pot, which filling by autoclaved soil and place in a greenhouse (Temperature 28±2°C and 60% relative humidity). After one week all the seedlings were showing above the soil with germination rate over ninety five percent. Plants in each pot were supplied by 1.75g of NPK (12% N_2: 12% P_2O_5: 17% K_2O) per pot twice for 7 weeks.

2.2. Experimental design

The experiment was performed by using eight treatments: Six treatments with AMF (*Glomus mosseae)* divided in two groups one of them treated by three rates of infection (300, 600 and 900±20 spores pot^{-1}) with normal irrigation and another at drought conditions. Two treatments free from AMF infection one of them in normal irrigation and other in drought conditions as a check. Three replications were used for each treatment. Plants in all treatments were left to grow for 7 weeks in a greenhouse at temperature 28±2°C and 60% relative humidity. plants in each pot were irrigated twice weekly with 600 ml pot^{-1} (soil moisture level close to field capacity) in the normal irrigation treatments and 200 ml pot^{-1} in the drought treatment.

2.3. Analytical methods

2.3.1. Plant growth parameters and biochemical

The harvested plant (shoots and roots) after 7 weeks were rinsed with tap water and then with distilled water. The plant height; shoot and root weight; root length and root/shoot ratio were estimated for all treatments. The chlorophyll concentration was measured on the second fully expanded leaf using CL-01chlorophyll content meter (Hansatech Instruments, USA).

Soluble protein content was determined by extraction method according to Zhang, (1990). The free proline content was estimated using the acid ninhydrin method as described by Bates *et al.*, (1973). Plant leaves were grounded in a mortar and pestle with % 3 (w/v) sulfosalicylic acid aqueous solutions and the homogenate was filtered through Whatman No. 1 filter paper, then 2 ml of filtered extract was taken for the analysis to which 2 ml acid ninhydrin and 2 ml glacial acetic acid were added. The reaction mixture was incubated in a boiling water bath for 1 h and the reaction was finished in an ice bath. Four milliliter of toluene was added to the reaction mixture and the organic phase was extracted, in which was read at 520 nm using toluene as blank by UV-visible spectrophotometer (Thermo Electron,

Model Bio Mate 3, Massachusetts, USA). Proline concentration was determined using calibration curve and expressed as μg proline g^{-1}fw (fresh weight).

2.3.2. Phosphorus (P) determination in plant tissue

The phosphorus concentration in plant shoot was determined by the molybdate blue ascorbic acid method according to Murphy and Riley, (1962) after the plant material was air dried and digested by nitric acid and perchloric acid for expressed as P uptake (mg g^{-1}).

2.3.3. Arbuscular mycorrhizal fungi (AMF) root colonization% and spores density

The root system of each plant was separated from the shoot, and dry weights were determined after the preparations were dried for 36 hrs at 70°C. The presence of an AMF 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 Phillips and Hayman, (1970). The stained roots placed on the glass slides for microscopic observations under 200×magnifications (***Leica*** DM550Q, USA). The calculation of AMF colonization was estimated for each sample by examination about one hundred pieces of roots (1cm long), and the AMF spores densities were calculated according to Schenck, (1982).

2.4. Data analysis

Data were analyzed using ANOVA by using SAS statistical software (SAS Institute, Cary, NC, USA). When the main effect was significant (P <0.05), differences between means were evaluated for significance by using Duncan's multiple-range test (Duncan, 1955).

3. Results

3.1. Plant growth parameters and biochemical

Data presented in Table (1) shown that the plants of *Zea mays* L., which inoculated with *Glomus mosseae* in three levels of infection 300, 600 and 900 spores pot^{-1} were recorded a significant (P<0.05) increase in all plant growth parameters (plant height, stem length, root length, plant fresh wt., shoot dry wt., root dry wt., root/shoot ratio and chlorophyll content) comparing with untreated plant in normal irrigation or drought treatment. The highest values in almost of plant growth parameter were observed when *G. mossea* used at 900 spores pot^{-1} followed by 600 spores pot^{-1} then 300 spores pot^{-1}. In general, the drought treatment causing decrease in values of almost plant growth parameters, when comparing with well irrigation treatment except plant root dry weight (g), which was increased in drought treatment with a weak significant comparing between other treatment.

The result in Table (2) shown that the water deficit (drought treatment) was caused a significant decrease in plant soluble protein by rate 29.34% comparing with soluble protein content in plants that well irrigate by normal way. While in the presence of *G. mossea* in the three levels of infection, were caused increase in soluble protein by rate 13.33, 22.18 and 29.27% in the normal irrigation treatment, and by rate 24.89, 36.25 and 45.17% in the drought treatment comparing with untreated plant with *G. mossea*. On contrast the proline content in plant leaves was increased in drought treatment by rate 22% comparing with proline value in plant leaves at normal irrigation. The treatments with *G. mossea* with different levels of soil infestation (300, 600 and 900 spores pot^{-1}) causing decreased in plant proline by rate 12.07, 38.09 and 32.98% in normal irrigation treatments and 28.88, 38.05 and 43.19% in the drought treatments comparing with plant free from AMF inoculation in both normal irrigation and drought treatments .

2.3.2. Phosphorus (P) determination in plant tissue

Plant phosphorus uptake was strongly influenced by drought treatment and inoculation with mycorrhizal fungus *Glomus mosseae* at different three levels of infection. Drought treatment was caused decrease in plant P uptake value by rate 72.09% comparing with recorded value in normal irrigation treatment (from 0.43 to 0.12 $mg^{-1}$of plant shoot wt.). On contrast the effect of *G. mosseae* caused increase in plant P uptake values by rate 42.66, 76.11 and 79.32% at 300, 600 and 900 spores pot^{-1} respectively in normal irrigation treatments. In drought stress *G. mosseae* had a great effect than its record in well irrigation treatment. The P uptake in drought treatment in the presence of *G. mosseae* was recorded increase by rate 88.34, 93.58 and 94.91% at 300, 600 and 900 spores pot^{-1} respectively comparing with plant free mycorrhizal inoculums (Figure 1).

3.3. Arbuscular mycorrhizal fungi (*Glomus mosseae*) root colonization% and spores density

Data illustrated in Figure (2) shown that the effect of drought treatment on activity of *Glomus mosseae* by determined two fungus growth parameters that fungus root colonization% and spores density 100 g^{-1} of soil. In drought treatment (200ml pot^{-1}) the fungus root colonization% on *Zea mays* plants was increased by increasing fungus infection level 300, 600 and 900 spores pot^{-1} by rate 31.45, 18.81 and 9.43% respectively comparing with same treatment in well irrigation (600ml pot^{-1}). The highest value of *G. mosseae* root colonization% was recorded at inoculum 900 spores pot^{-1} by value 90.6% causing hyper colonized Figure (3). As well as fungus spore density was increased in drought condition comparing with well irrigation treatment by rate

33.68, 12.28 and 21.46 spores100g^{-1} soil at the three levels of fungus infection respectively. Also the highest value of *G. mosseae* spores density was found when plant inculcated with 900 spores pot^{-1} (2688 spores 100g^{-1} soil).

Table 1. Influence of inoculation with *Glomus mosseae* at three levels in normal irrigation and drought treatment on plant (*Zea mays* L.) growth parameters and chlorophyll level

Treatment	Plant growth parameters							
Spores pot^{-1}	Plant height (cm)	Stem length (cm)	Root length (cm)	Plant fresh wt. (g)	Shoot dry wt. (g)	Root dry wt. (g)	Root/Shoot ratio	Chlorophyll (Unit)
Normal irrigation (600ml water pot^{-1} twice weekly for 7 weeks)								
Untreated	40.85^{a}	29.80^{b}	11.00^{a}	11.50^{b}	3.43^{a}	0.66^{a}	0.19^{a}	14.8^{a}
300	60.00^{c}	39.20^{d}	20.80^{cd}	18.50^{e}	5.32^{c}	1.00^{a}	0.18^{a}	15.9^{a}
600	60.25^{c}	40.00^{d}	20.25^{c}	22.00^{f}	7.16^{e}	1.40^{b}	0.19^{a}	15.8^{a}
900	61.75^{cd}	40.70^{d}	21.00^{d}	22.50^{f}	7.75^{de}	1.60^{b}	0.23^{a}	16.2^{b}
Drought treatment (200ml water pot^{-1} twice weekly for 7 weeks)								
Untreated	38.55^{a}	23.50^{a}	15.00^{b}	09.50^{a}	2.86^{a}	1.00^{a}	0.35^{ab}	11.8^{a}
300	42.55^{a}	30.40^{bc}	12.00^{ab}	11.00^{b}	3.00^{a}	1.30^{b}	0.43^{b}	14.3^{b}
600	49.50^{b}	32.50^{c}	17.00^{c}	13.55^{c}	4.47^{b}	1.70^{b}	0.38^{b}	15.4^{b}
900	55.25^{c}	33.00^{c}	22.25^{d}	16.25^{d}	5.36^{d}	2.20^{bc}	0.41^{b}	15.6^{b}

- Means in each column followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiplerange test. - Values are the means of three replications.

Table 2. Effect of inoculation by *Glomus mosseae* in three infection levels at normal irrigation and drought treatment on soluble protein (mg g^{-1}) and leaf proline contents (µg g^{-1}fw) of *Zea mays*

Spores pot^{-1}	Soluble protein (mg g^{-1})		Proline content (µg g^{-1}fw)	
	Normal irrigation (600 ml pot^{-1})	Drought condtion (200ml pot^{-1})	Normal irrigation (600 ml pot^{-1})	Drought condtion (200ml pot^{-1})
untreated	20.41^{a}	14.42^{a}	55.73^{c}	72.00^{c}
300	23.55^{b}	19.20^{b}	49.00^{b}	51.20^{b}
600	26.23^{b}	22.62^{c}	34.50^{a}	44.60^{a}
900	28.86^{b}	26.30^{d}	37.35^{a}	40.90^{a}

- Means in each column followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiplerange test. - Values are the means of three replications.

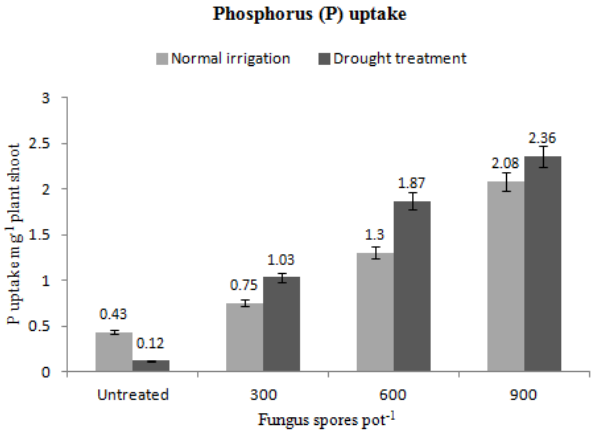

Figure 1. The effect of three different inoculations by *Glomus mosseae* on *Zea mays* L. plant phosphorus (P) uptake in normal irrigation and drought stress treatment after 7 weeks from inoculation

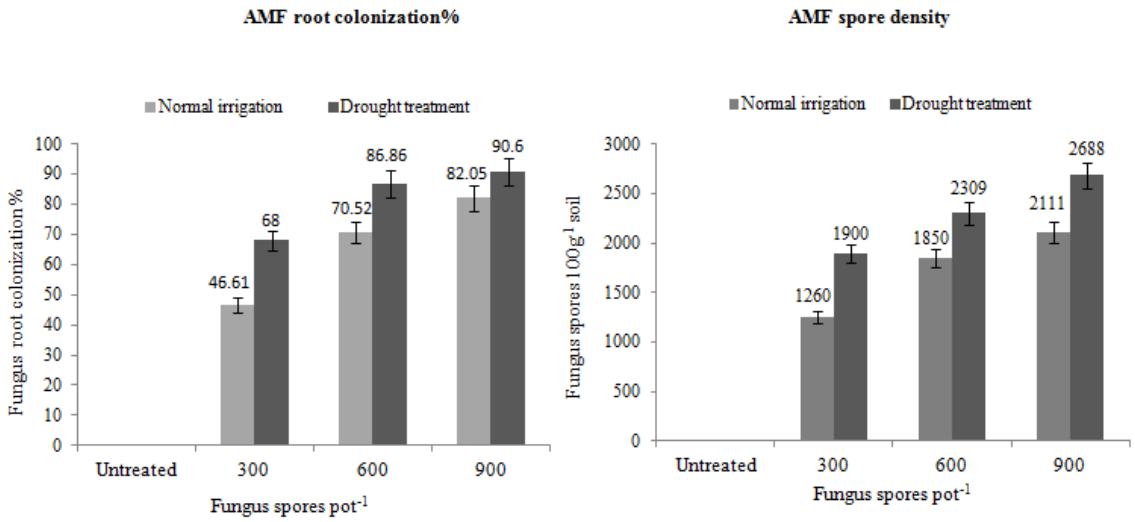

Figure 2. Influence of normal irrigation and drought treatment on *Glomus mosseae* root colonization% and spores density on *Zea mays* L. after 7 weeks from inoculation

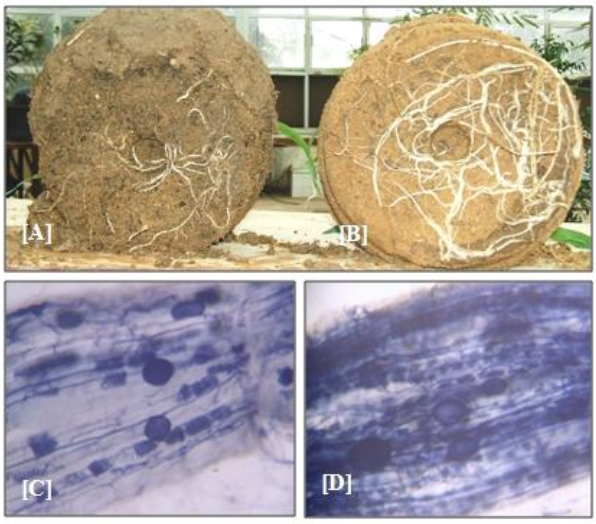

Figure 3. The effect of drought and well irrigation on root colonization% of *Glomus mosseae* on maize plant (*Zea mays* L.) [A]- Plant roots growth in well irrigation treatment (600ml pot^{-1}), [B]-Plant roots growth under drought stress treatment (200ml pot^{-1}) [C, D]- Photomicrographs for *G. mosseae* structures in plant roots after clearing and staining (200×) to comparing between fungus colonized in well irrigation treatment [C], and hyper colonized in plant roots as the effect of drought treatment [D]

4. Discussion

In this study we determined the effect of arbuscular mycorrhizal fungi (AMF) to improve plant tolerance for drought stress. The mycorrhizal fungus, which we use is *Glomus mosseae* that a widespread genus in neutral to alkaline soils. In the drought condition, almost plant growth parameters decreased comparing with well irrigated treatment this result may be due to soil moisture, which affects the movement of nutrient in the soil. On the other hand all plant treatment in the presence of *G. mosseae* causing increase in all plant growth parameter that due to extraradical fungus mycelia, which extend the root surface area and improve the uptake of water and nutrients by the roots (Bethlenfalvay *et al.*, 1988). The effects of *G. mosseae* on plant water status have been associated by enhanced host nutrition, especially phosphorus (P) nutrition (Giovannetti and Mosse, 1980, Graham and Syvertson, 1984, Almagrabi and Abdelmoneim, 2012). However, it has

also been reported that the effect of AMF on drought stress may be independent of P uptake (Sweatt and Davies, 1984, Augé *et al.*, 1986, Bethlenfalvay *et al.*, 1988, Almagrabi and Abdelmoneim, 2012, Karimi *et al.*, 2012).

The drought stress had a undesirable effect on plant soluble protein in the presence or absence infection by *G. mossease* that seems due to a sharp decline in plant photosynthesis. The plant leaves chlorophyll content values were decreased in drought stress comparing with same treatment in well irrigation condition, that indicate to plant photosynthesis decreased in drought, which lead to inhibit some essential material for protein synthesis, therefore the protein synthesis dramatically reduced or even stopped (Mohammadkhani and Heidari 2008, Karimi *et al.*, 2012). The gradual decrease in plant total soluble proteins during water deficiency was induced by proteolysis or decline in some essential mineral for protein synthesis which uptake with water as nitrogen compounds (Lqbal and Bano, 2009, Bayramov *et al.*, 2010, Costa and LoBato, 2011). Accumulation proline is the basic response to water stress in plants is the accumulation of osmo protectants, (Moradshahi *et al.*, 2004).

Proline accumulation is responsible for the utilizable energy source and serving as a nitrogen source compound during periods of inhibited growth (Kala and Godara, 2011). The plants in drought treatment, which were inculcated with *G. mossease* in different levels of infection they record decrease in proline content with different values according to level of infection. The increase of proline in plant leaves give a good indication about plant exposed high drought stress. Also proline accumulation is believed to play adaptive roles in plant stress tolerance (Ashraf and Iram, 2005, Mafakheri *et al.*, 2010, Din *et al.*, 2011, Karimi *et al.*, 2012).

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