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Alleviation drought stress of mungbean (*Vigna radiata* L.) plants by using arbuscular mycorrhizal fungi

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Abstract
In order to evaluate drought stress and arbuscular mycorrhiza on related root traits and grain yield of mungbean, a factorial experiment was carried out based on a randomized completely design in pot culture. Irrigation after 25, 50, 75, and 100 mm of evaporation from a Class A pan possessed irrigation's levels as the first factor. At the second factor arranged *Glomus mosseae*, *G. intraradices* of mycorrhiza species and non-inoculum as a control with three replications were conducted in Urmia University in 2009. Results showed that grain yield of inoculated mungbean at both species with 4.29 and 4.31g/plant had the highest values. Both species had more root dry weight, root length and root to shoot ratio of dry weight than control. Root volume of inoculated mungbean with *G. mosseae* was not significant differences with control. Colonization of *G. intraradices* and *G. mosseae*, with 53.37 and 46.29% had the most values from plants irrigated after 25 mm of evaporation. At both species had the most leaf phosphorus with 260.2 and 258.3 mg/g of leaf dry weight. Mycorrhizae Colonization (r=0.73**), leaf phosphorus (r = 0.45**), root dry weight (r = 0.71**) and root volume (r = 0.68**) had positive correlation coefficients with grain yield. Variations of leaf proline accumulations from plants irrigated after 75 and 100 mm of evaporation were between 4.48 and 5.17 µmol/g leaf fresh weights. Although drought stress reduced grain yield, but inoculated it reduced the severity of stresses. Inoculated plants increased 65% of potential yield than control.

1. Introduction

In many arid and semiarid regions of the world, drought stress limits crop production. Inoculated plants with Arbuscular mycorrhizal (AM) fungi can improve crop production under drought stress conditions (Al-Karaki and Clark 1998; Al-Karaki and Al-Raddad 1997). Mycorrhiza is a symbiotic fungus that caused beneficial relationship between soil and plant. Increasing of water absorption and nutrient uptake by mycorrhizal hyphae can be due to growth hyphae to 20mm root surface than 1.5mm hairy roots (Sylvia and Williams 1992). Also, low root penetration compared to high hyphae penetration into cracks and pores of soil. Hyphae of fungus infiltrate into the soil where the roots are unable to penetrate. Speed of inorganic phosphate into the hyphae was 2cm/h which is several times higher than diffusion in the soil (Pelletier and Dionne 2004; Robert 2001; Ruiz-Lozano 2003). Mycorrhizal plants can be affected water balance under irrigated and drought stress conditions (Robert 2001). Symbiotic plants with *G. intraradices* increase biotic and a-biotic stresses. There were several mechanisms for expression increasing root hydraulic conductivity, osmotic adjustment and improvement root contact with soil particles through connecting mycorrhizal hyphae (Auge 2004). At mycorrhizal faba-bean enhanced nodule number, nodule dry weight, days to flowering, number of pods and grain yield compared to non-mycorrhizal plants under different irrigation regimes (Faisal et al. 2000). Mycorrhizal rosemary plants with affecting water relations, gas exchanges and root growth have more root and shoot biomasses and reduced leaf water potential (Sanches-blanco et al. 2004). AM fungi by increasing mineral nutrients especially phosphorus tolerate to biotic and a-biotic stresses (Sawers et al. 2008; Smith and Read 2008; Abd-Alla et al. 2000). Mycorrhiza increases proline accumulation in plants subjected to drought stress (Ruiz-Lozano et al. 1995; Azcon et al. 1996; Goicoechea et al. 1998). Rate of photosynthesis improved due to more phosphorous absorption and chlorophyll content in mycorrhizal pepper plants (Demir 2004). In mycorrhizal mungbean plants grain yield, biological yield, leaf P, leaf N, protein percentage, protein yield, harvest index of protein, and ecosystem water use efficiency were improved compared with the non-mycorrhizal plants. Two species of mycorrhiza, *G. mosseae* and *G. intraradices* significantly improved the yield (grain, protein) and reduced the water-deficit stress in the field (Habibzadeh et al. 2013). AM symbiosis of corn plants by improving hydraulic conductivity, water absorbing, changing water relations, expanding root system, improving plant nutrition and increasing plant metabolism tolerated to water deficit (Boomsma and Vyn 2008). AM symbiosis in sugarcane, mungbean, wheat and tomato improved absorption capacity and vegetative growth (Wu and Xia 2004). At mycorrhizal sesame enhanced roots through increasing volume and dry weight of root (Boureima et al. 2007). In another experiment, lavender plants inoculated with *G. mosseae* and *G. intraradices* expanded roots 35% and 100%, respectively (Marulanda et al. 2007). The objectives of this experiment were evaluation of root traits and osmotic adjustment mungbean inoculated with *G. mosseae* and *G. intraradices* under different levels of irrigation.

2. Materials and Methods

2.1. Experimental Location

A trial was conducted in agricultural faculty of west Azerbaijan province in Iran. The experiment located in longitude 37°, 39' north, latitude 44°, 58′ east and 1365m altitude. Environmental conditions of the experimental site, including the highest and lowest temperatures and humidity, sum of sunny hours, daily and monthly solar radiation and potential evapo-transpiration of the study are shown in Table 1.

Some physicochemical properties of soil which is used in 360 pots were determined (Table 2).

Table 1. *Environmental conditions at the experimental site during summer 2009.*

Parameter	June	July	August	September
Highest temperature (°C)	29.1	35.2	32.5	28.6
Lowest temperature (°C)	5.0	10.7	11.1	7.9
Highest relative humidity (%)	79	72	73	83
Lowest relative humidity (%)	33	32	27	38
Sum of sunny hours (no.)	309	357	365	290
Solar radiation (MJ m^{-2} d^{-1})	25.2	26.5	25.4	19.9
Solar radiation (MJ $m^{-2}mo^{-1}$)	756	821.5	787.4	597.0
Potential evapo-transpiration (mm mo^{-1})	199	235	225	185

Table 2. *Some of soil physico-Chemical Characteristics.*

Saturation (%)	Electrical Conductivity (dS m^{-1})	pH	Organic carbon (%)	Phosphorus (mg kg^{-1})	Potassium (mg kg^{-1})	Soil texture
48	0.41	7.15	0.74	9.8	324	Silty clay

2.2. Experimental Design

A factorial experiment based on a completely randomized design carried out with three replications. Irrigation regimes with four levels of 25, 50, 75, and 100 mm of evaporation from a Class A pan and inoculation mycorrizal mungbean (*Vigna radiata* L.) cultivar NM92 with three levels including *Glomus mosseae*, *G. intraradices* and non-inoculation as control arranged as the first and second factors, respectively. Irrigation water needed before irrigation (V_N) is the amount of water needed during irrigation to replenish the soil moisture deficit, thereby restoring the soil to field capacity. The value of V_N was calculated according to Benami and Ofen (1984):

$$VN = \frac{(FC - WP)BD \times D(1 - ASM)A}{100}$$

where V_N is the irrigation water needed before irrigation (m^3), FC is field capacity (%), WP is the wilting point (%), BD is bulk density (g cm^{-3}), D is the root zone depth (m), ASM is the available soil moisture before irrigation (a fraction), and A is the area of the soil pot(m^2). Each plot consist 10 pots which all was 360 pots. Depth and diameter of pots was 22 cm which filled with 7kg of soil. Seeds of the mungbean cultivar NM92 were provided by the Agricultural Research Station of Dezfol. The two species of AM fungi used in this study were G. mosseae and G. intraradices, which were produced on maize (Zea mays L.) host plants by Dr. E.M. Goltapeh at Tarbiat Modarres University, Tehran, Iran. The mycorrhizal inoculum was a mixture of sterile sand, mycorrhizal hyphae, spores (20 spores g^{-1} inoculum), and colonized root fragments. Ten grams of the appropriate inoculum was placed into the hole below two seeds, and then covered with soil. For non-mycorrhizal control plants were sown with no inoculation. Seeds were sown on 29 June 2009 into a silty clay soil (a fine-loamy, mixed, superactive, mesic Typic Calcixerept) with pH 7.15 and 9.8 mg kg^{-1} of P. At three primary leaf stages were applied irrigation regimes. Total water consumption during growing season was 84, 51, 39 and 27 liters at per pot for irrigations of 25, 50, 75, and 100 mm of evaporation from a Class A pan, respectively. Root dry weights were determined after dried samples at oven 72°C. Length and volume of roots measured from 10 randomly selected plants at the end of the growing season. Grain yield and total dry matter recorded from all pots. At maturity time, the percentage of colonization of mungbean roots by AM fungi was determined on 15 plants per experimental unit. Root colonization was measured in fresh roots cleared in 10% KOH for 10 min at 90°C and stained in 0.05% lactic acid–glycerol–Trypan Blue (Phillips and Hayman 1970). The percentage of root colonization by arbuscular mycorrhizal fungi was microscopically determined using the gridline intersection method (Giovannetti and Mosse 1980). To measure leaf P, dried leaves were milled, digested, and analyzed as described by Watanabe and Olsen (1965) and Ohnishi et al. (1975). The method described for P involves drying, homogenization, and combustion (4 h at 500°C) of the leaf sample. The plant ashes (5 mg) are digested in 1 ml of concentrated HCl. The samples are then filtered, and total P is quantified as PO_4^- using the ascorbic acid method (Watanabe and Olsen, 1965). The amount of PO_4^- in solution was determined colorimetrically at 882 nm (Graca et al., 2005). Leaf proline content was measured according to Bates et al. (1973). Analysis of variance of data was performed using MSTATC software. The effects of irrigations, application of mycorrhizae, and the interactions of these two factors were analyzed by ANOVA and the means compared by the Student Neuman Keul test ($P \leq 0.05$).

3. Results and Discussion

Different levels of irrigations and mycorrhizae for traits of colonization percentage, leaf phosphorous and proline accumulations, root dry weight, root volume, root to shoot ratio and grain yield and interaction between them for leaf proline accumulation and Mycorrhizae colonization were significant differences (Table 3).

Table 3. *Mean squares traits of mungbean affected by mycorrhizal infection under different irrigation regimes*

SOV	d.f	Mean squares							
		Mycorrhizae colonization	Grain yield	Leaf phosphorus	Leaf Proline	Root dry weight	Root length	Root volume	Root/ shoot
Irrigation (I)	3	409.76**	15.57**	4072.31**	14.09**	0.09**	14.61	0.55**	18.06**
Mycorrhizae (M)	2	5237.52**	11.02**	12876.50**	6.63**	0.16**	124.22**	0.92**	37.32**
M × I	6	88.10**	0.35	686.99	0.67*	0.001	17.65	0.04	0.81
Error	24	2.31	0.35	738.35	0.21	0.01	19.41	0.07	2.69
CV (%)	-	5.87	15.78	11.30	12.08	19.43	15.17	18.85	21.10

* Significant at the 5% probability level; ns, not significant.
** Significant at the 1% probability level.

3.1. Related Root Traits

Colonization percentage of *G. intraradices* was more than *G. mosseae* and less reduced with increasing water stress. Variations of this trait were for *G. intraradices* between 29.11 to 53.27 and *G. mosseae* 25.83 to 46.29. Colonization mycorrhiza reduced due to water stress (Table 4). Volume and dry weight of roots decreased with severity stress. Irrigation after 25 and 100 mm of evaporation were 0.65g, 1.62cm^3 and 0.42, 1.03cm^3 values of them, respectively. *G. intraradices* had the most root dry weight, root length and root volume with 0.66g, 31.96cm and 1.63cm^3, respectively (Table 4). Root to shoot ratio in 25, 50 and 75 mm of evaporation were the same group but at 100 mm of evaporation was reduced. *G. mosseae* with 8.78 root to shoot ratio had the most value (Table 5). Expanded roots of mychorrhizal plants enhanced root area (Allen et al. 1981). Therefore, water uptake in mycorrhizal plants was due to more root expansion than control (Huang et al. 1985). Mycorrhizal corn plants through expanding root system, improving hydraulic conductivity and water uptake increase drought tolerance (Boomsma and Vyn 2008). In lavender inoculated plants with *G. mosseae* and *G. intraradices* improved root growth, 35% and 100%, respectively (Marulanda et al. 2007).

Table 4. *comparison of colonization percentage and proline accumulation of mungbean affected by irrigation regimes and mycorrhiza species.*

Irrigation regimes+	Mycorrhizal symbiosis	Mycorrhizae Colonization (%)	Leaf Proline (µmol/g)
25	Non-mycorrhizal	2.71h++	1.37e
	Glomus mosseae	46.29b	1.56e
	G. intraradices	53.37a	1.82de
50	Non-mycorrhizal	2h	2.12cde
	G. mosseae	39.46d	2.71bcd
	G. intraradices	43.49c	3.24b
75	Non-mycorrhizal	1.64h	2.73bcd
	G. mosseae	29.74f	4.48a
	G. intraradices	35.69e	4.63a
100	Non-mycorrhizal	1.36h	2.98bc
	G. mosseae	25.83f	4.84a
	G. intraradices	29.11g	5.17a

+ Irrigation after evaporation from a Class A pan

++Means followed by the same letter(s) in each column are not significant differences

Table 5. *means comparison of mungbean traits by mycorrhizae species.*

Mycorrhizal symbiosis	Grain yield (g/plant)	Leaf phosphorus (mg/100g dry leaf)	Root dry weight (g/plant)	Root length (cm)	Root volume (cm^3)	Root/ shoot (%)
Non-mycorrhizal	2.64b+	202.57b	0.43b	25.59b	1.08b	5.88b
Glomus mosseae	4.29a	260.20a	0.60a	29.58a	1.39b	8.78a
G. intraradices	4.31a	258.36a	0.66a	31.96a	1.63a	8.66a

+Means followed by the same letter(s) in each column are not significant differences

3.2. Osmotic Components

Leaf proline accumulation arised under drought stress and amount of it under 60% and 40% field capacity were the same group. Meanwhile, in 25 and 50 mm of evaporation had in *G. intraradices* with 1.82 and 2.71µmol/g and *G. mosseae* with 1.56, 2.12 µmol/g, respectively.

Ion phosphorus accumulation in leaves of inoculated plants at both species was higher than control. The highest and lowest accumulations were allocated to 25 and 100 mm of evaporation with 262.22 and 216.98mg/100g dry leaf, respectively (Tables 5 and 6). Nutrients uptake under drought stress decreased through reducing transpiration, disruption of active transport systems and membrane permeability and reducing root absorption. mycorrhizal hyphae uptake fixed phosphorous where plant roots couldn't absorb (Lu et al., 2007). Influence of AM plants on leaf phosphorus in this experiment coordinated by other researchers (AL-Karaki et al. 2004; Ruiz-lozano et al. 1995; Sawers et al. 2008; Smith and Read 2008; Abd-Alla et al. 2000; Wu and Xia 2004).

3.3. Grain Yield

Between different levels of irrigation, 25 mm of evaporation had the most grain yield with 5.14g/plant. Under lower water consumption reduced grain yield. Both species with 4.31 and 4.29g/plant grain yield had the most values than control (Tables 5 and 6). Grain yield differences in mycorrhizal treatments are related to water absorption and mineral nutrients (AL-Karaki, et al. 2004; Demir 2004; Faisal et al. 2000; Kaya et al. 2003; Pelletier and Dione 2004; Robert 2001; Sanches-blanco et al. 2004).

Table 6. *means comparison of mungbean traits by irrigation regimes.*

Irrigation regimes+	Grain yield (g/plant)	Leaf phosphorus (mg/100g dry leaf)	Root dry weight (g/plant)	Root length (cm)	Root volume (cm^3)	Root/shoot (%)
25	5.14a++	262.22a	0.65a	-	1.62a	6.65a
50	4.02b	254.03ab	0.61a	-	1.40b	6.52a
75	3.84b	228.27bc	0.51a	-	1.42ab	8.54a
100	1.97c	216.98c	0.42b	-	1.03c	9.38b

+Irrigation after evaporation from a Class A pan

++Means followed by the same letter(s) in each column are not significant differences

3.4. Relationships of Traits

Correlation coefficients of traits showed that mycorrhizae colonization with grain yield (r=0.73**), leaf phosphorus (r=0.69**), root dry weight (r=0.70**), root Length (r=0.43**), root volume (r=0.65**) and root/shoot (r= 0.34*) were significant differences (Table 7). In addition, leaf phosphorus (r = 0.45**), root dry weight (r = 0.71**) and root volume (r = 0.68**) had significant differences with grain yield. Therefore, they could be indirect criteria in selecting superior genotypes in breeding programs.

Table 7. *Correlation coefficients between mungbean traits.*

Treatment	Mycorrhizae colonization	Grain yield	Leaf phosphorus	Leaf proline	Root dry weight	Root length	Root volume
Grain yield	0.73**						
Leaf phosphorus	0.69**	0.45**					
Leaf proline	0.11	-0.33	0.14				
Root dry weight	0.70**	0.71**	0.49**	-0.06			
Root length	0.43**	0.21	0.43**	0.44**	0.44**		
Root volume	0.65**	0.68**	0.49**	-0.07	0.89**	0.44**	
Root/shoot	0.34*	-0.08	0.52**	0.68**	0.11	0.29	0.11

* and ** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

4. Conclusions

Inoculated plants with *G. intraradices* and *G. mosseae* showed more root dry weight, root length and volume than control. Root related traits such as root dry weight, root volume and root to shoot weight ratio increased in more water consumption and consequently will lead to increase grain yield. *G. intraradices* is recommended due to produce further colonization percentage in mungbean inoculated plants. Relationships between traits showed that with increasing leaf phosphorus, root dry weight and root volume in inoculated mycorrhizal mungbean plants enhanced grain yield

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