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Arbuscular Mycorrhizal Fungi in Alleviation of Drought Stress on Grain Yield and Yield Components of Mungbean (Vigna Radiata L.) Plants

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Abstract: To investigate the effect of mycorrhizal fungi on reduction of drought stress on related grain yield and yield components of mungbean plants, a pot culture was conducted based on Randomized Completely Design with three replications in Urmia University in 2009. The experiment with four irrigation regimes (25, 50, 75 and 100 mm of evaporation from a class A pan) were assigned at the first factor and two mycorrhiza species; *Glomus mosseae*, *Glomus intraradices* and a non-inoculated treatment at the second factor. Results showed that in both mycorrhizae species significantly (P<0.05) increased the grain yield, so *Glomus intraradices* (4.29 g/plant) and *Glomus mosseae* (4.31 g/plant) had the highest grain yield. Non inoculated treatment had the lowest (2.64 g/plant) grain yield. The maximum (5.14 g/plant) and minimum (1.97 g/plant) grain yield achieved in irrigation after 25 and 100 mm evaporation from pan, respectively. With increasing water deficit stress decreased relative water content, pod length, seeds/pod, pods/plant and seeds/plant. Mycorrhizae colonization (r=0.72**), relative water content (r = 0.76**), pod length (r = 0.90**), seeds/pod (r = 0.74**), pods/plant (r = 0.71**) and Seeds/plant (r = 0.86**) had the positive correlation coefficients with grain yield. Also, results showed that mycorrhizae species affected grain yield of mungbean plants through their effect on pod length, seeds/pod, pods/plant and seeds/plant under well-watered and drought stress conditions.

Keywords: Colonization, Drought stress, Grain yield, Mungbean, Mycorrhiza, Yield components

Introduction

Arbuscular mycorrhizal (AM) symbiosis is formed by 70–90% of land plant species (Smith and Read, 2008). AM fungi that all members belong to a monophyletic phylum, the Glomeromycota (Schussler *et al*., 2001) occur in almost all terrestrial ecosystems (Heinemeyer and Fitter, 2004; Martin, 2008). Inoculation of plant roots with (AM) fungi may be effective in improving crop production under drought conditions. Colonization of roots by AM fungi has been shown to improve productivity of numerous crop plants in soils under drought stress (Al-Karaki and Al-Raddad, 1997; Al-Karaki and Clark, 1998; Faber *et al*., 1990; Sylvia *et al*., 1993).

Arbuscular mycorrhizal (AM) symbiosis has been shown upon hundreds of occasions to change the water relations of host plants (Auge, 2001). Earlier, Al-Karaki *et al*., (2004) showed that plant recovery after water-deficit stress occurred faster in mycorrhizal plants than nonmycorrhizal ones. Stomatal conductance, transpiration rate, and leaf water potential are generally enhanced in mycorrhizal plants under water-limited conditions (Duan *et al*., 1996; Caravaca *et al*., 2003). In legume crops, mycorrhizal fungi were found to increase the vegetative growth and seed yield (Lambert and Weidensaul, 1991; Mathur and Vyas, 2000). 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).

Arbuscular mycorrhizal (AM) fungi have been reported to improve nutrient uptake, particularly phosphorus (Abd-Alla *et al*., 2000). Arbuscular mycorrhizal colonization was found to increase soluble carbohydrates and chlorophyll in host plants which often causes to change the level of host amino acids (Auge, 2001), reducing osmotic pressure, or

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maintaining protein structures in their cells (Bray, 1997) and enhancement of root hydraulic conductivity (Auge, 2004) under drought stress conditions.

The main objective of this research was to evaluate the effects of mycorrhizal fungi species (*Glomus mosseae* and *G. intraradices* compared with uninoculated plants) on grain and yield components of pot-grown mungbean that has been subjected to various levels of water-deficit conditions (irrigation after 25, 50, 75, and 100 mm of evaporation from a Class A pan).

Materials and Methods

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 temperature and humidity, sum of sunny hours, daily and monthly solar radiation and potential evapotranspiration of the study are shown in Table 1. Some physicochemical properties of soil which is used in 360 pots were determined (Table 2).

A factorial experiment based on completely randomized design carried out with three replications. Four irrigation regimes 25, 50, 75 and 100 mm of evaporation from a Class A pan and inoculation mycorrizal mungbean (*Vigna radiata* L.) 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 consisted of 10 pots which all was 360 pots. Depth and diameter of pots was 22 cm with containing 7kg of soil. Seeds of the mungbean cultivar NM92 were provided by the Agricultural Research Station of Dezfol. The two species of mycorrhizal 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 each seed, and then covered with soil. For non-mycorrhizal control plants were sown with no inoculation. At first 4 seeds were planted in each pot and reduced two plants in the third weeks. At three primary leaf stages were applied irrigation regimes. Total water consumption during growing season per pot was 84, 51, 39 and 27 liters for irrigations of 25, 50, 75 and 100 mm of evaporation from a Class A pan, respectively. Pod length, Seeds/pod, Pods/plant and Seeds/plant measured from 10 randomly selected plants at the end of the growing season. Grain yield recorded from all pots.

At plant maturity, 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 AM fungi was microscopically determined using the gridline intersection method (Giovannetti and Mosse, 1980).

The leaf relative water content (RWC) was ascertained by measuring the fresh weight, rehydrated weight on distilled water, and dry weight (DW, 70°C for 48 hours) using the following formula (Turner, 1986):

RWC = (FW – DW)/ (SW- DW) × 100

In which the fresh weight (FW) of leaves was determined by immediately weighting one fully expanded young leaf, which was allowed to rehydrate for 4 h by floating 1 cm from the cutting part into a covered beaker with distilled water. The rehydrated leaves were weighted (SW) to determine saturate mass, and then the leaf was dried at 70 °C for 24 h to determine dry weight.

Total soluble carbohydrates were determined based on phenol sulfuric acid method (Dubois et al., 1956). In this method, 0.5 g of fresh weight of leaves was homogenized with ethanol. The extract was filtered and then treated with 5% phenol and 98% sulfuric acid. This mixture remained for 1 hour and then its absorption at 485 nm was measured by spectrophotometer. Soluble carbohydrate contents were shown as mg g^{-1} of fresh weight.

Analysis of variance of data was performed using MSTATC software.The effects of irrigation, 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

≤ 0.05). Also, correlation coefficients were calculated.

Results and Discussion

Different levels of irrigations and mycorrhizae for traits of colonization percentage, relative water content, pod length, seeds/pod, pods/plant, seeds/plant, grain yield and interaction between them for Mycorrhizae colonization were significant differences (Table 3).

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 53.37 and *G. mosseae* 25.83 to 46.29. Colonization mycorrhiza reduced due to water stress (Table 4). Pod length, seeds/pod, pods/plant and seeds/plant decreased with severity stress. Irrigation levels of 25 and 100 mm of evaporation from a Class A pan were 8.56cm, 9.08, 8.31, 76.16 and 7.53cm, 7.24, 4.14, 30.14 values of them, respectively. *G. intraradices* had the seeds/pod and seeds/plant with 9.05 and 75.03, respectively (Table 5 and 6). It has been suggested that number of seed pod^{-1} was reduced when water stress occurred during reproductive stage. More number of seed in a pod results in more fertilization and optimum seed development. Water stress at flowering induce flower abortion and drying of stigma which reduce the germination of pollen grain in stigma and reduce fertilization capability and ultimately causes less number of seeds in a pod and poor seed development (Liu *et al*., 2003). Other report also show that generally, severity stress at the flowering stage caused increase flower abortion (Kokubun *et al*., 2001). Auge (2001) reported that the mycorrhizal soybean plants produced less drought-induced pod abortion than non-mycorrhizal plants, because of increasing photosynthesis, photosynthetic storage and export at the same time.

Relative water content was higher in both species *G. mosseae* and *G. intraradices* (88.08%) than non-mycorrhizal (80.82%) plants (Table 5). The highest (92.25%) and lowest (76.06%) relative water content was obtained from plants irrigated after 25 and 100 mm of evaporation, respectively (Table 6). Hardie, (1985) reported that AM fungal hyphae with a diameter of 2–5 μm can penetrate soil pores inaccessible to root hairs (10–20 μm diameter) and so absorb water that is not available to non-mycorrhizal plants. Ruiz-Lozano and Azcon, (1995) in their experiment with lettuce plants designed an AM hyphal compartment in the root zone using a 50-μm nylon screen that allowed penetration by AM hyphae but not by roots, water was applied by injection to this compartment. They found an increase in plant fresh weight of nearly 150% in mycorrhizal as compared to the non-mycorrhizal plants. Similarly, leaf water content increased in mycorrhizal plants with water applied to the hyphal compartment.

The minimum total soluble carbohydrates (52.82 mg/g FW) was obtained from non-mycorrhizal mung bean plants and in both species *G. mosseae* (72.53 mg/g FW) and *G. intraradices* (70.42 mg/g FW) the maximum level of total soluble carbohydrates was obtained (Table 5). AM symbiosis can increase the drought tolerance of plants if the commonly observed higher rates of photosynthesis lead to an increased accumulation of nonstructural carbohydrates that, acting as osmoprotectants, can lower the osmotic potential (Auge, 2001; Porcel and Ruiz-Lozano, 2004; Khalvati *et al*., 2005). Several studies have reported the accumulation of carbohydrates when plants are subjected to water stress in both woody species such as Citrus (Wu and Xia, 2006) and Macadamia cultivars (Yooyongwech *et al*., 2013) and in herbaceous species such as lettuce cultivars (Baslam and Goicoechea, 2012) and pistachio (Abbaspour *et al*., 2012).

Both species *G. mosseae* (4.29g/plant) and *G. intraradices* (4.31g/plant) significantly improved the grain yield compared with the non-mycorrhizal (2.64g/plant) plants and reduced the water-deficit stress in mungbean plants. The highest (5.14g/plant) and lowest (5.14g/plant) grain yield were obtained from plants irrigated after 25 and 100 mm of evaporation, respectively. Grain yield differences in mycorrhizal plants with control 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).

Correlation coefficients of traits showed that mycorrhizae colonization with grain yield (r=0.72**), relative water content (r=0.55**), total soluble carbohydrate (r=0.58**), pod length (r=0.67**), seeds/pod (r=0.60**), pods/plant (r=0.70**) and seeds/plant (r= 0.70*) were significant differences (Table 7). In addition, relative water content (r = 0.76**), pod length (r = 0.90**), seeds/pod (r = 0.74**), pods/plant (r = 0.71**) and seeds/plant (r = 0.86**) had significant differences with grain yield. These observations indicate that plants having a higher pod length and seeds/plant produce higher grain yield.

Conclusion

Inoculated plants with *G. intraradices* and *G. mosseae* showed more relative water content, total soluble carbohydrate, pod length, seeds/pod, pods/plant and seeds/plant than control. Moreover, the extent of growth of *G. intraradices* and *G. mosseae* mycorrhizal plants positively correlated with the rate of AM root colonization and Mycorrhizal symbiosis clearly increased the grain yield. Yield components such as pod length, seeds/pod, pods/plant and seeds/plant increased in higher irrigation levels and consequently will lead to increase grain yield.

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

Parameter	2009 June	July	Aug.	Sept.
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 evapotranspiration, mm mo^{-1}	199	235	225	185

Table 2. Some soil physico-Chemical Characteristics.

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

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

S.O.V.	df	Mycorrhizae colonization	Relative water content	Total soluble carbohydrate	Pod length	Seed/pod	Pod/plant	Seed/plant	Grain yield
Irrigation (I)	3	409.76**	420.63**	89.73	3.42**	5.21**	29.93**	4446.50**	15.57**
Mycorrhizae	2	5237.52**	21.66**	1404.75**	2.64**	11.78**	24.50**	3607.20**	11.02**
M × I	6	88.10**	13.94	68.06	0.07	0.19	0.90	287.05	0.35
Error	24	2.31	26.97	62.16	0.21	0.93	1.59	170.16	0.35
CV (%)	-	5.87	6.06	7.90	5.48	11.69	18.54	20.62	15.78

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

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

Irrigation Regimes+	Mycorrhizal symbiosis	Mycorrhizae Colonization (%)
25	Non-mycorrhizal	2.71h
	Glomus mosseae	46.29b
	G. intraradices	53.37a
50	Non-mycorrhizal	2h
	G. mosseae	39.46d
	G. intraradices	43.49c
75	Non-mycorrhizal	1.64h
	G. mosseae	29.74f
	G. intraradices	35.69e
100	Non-mycorrhizal	1.36h
	G. mosseae	25.83f
	G. intraradices	29.11g

+ 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	Relative water content (%)	Total soluble carbohydrate (mg/g FW)	Pod length (cm)	Seeds/pod	Pods/plant	Seeds/plant	Grain yield
Non-mycorrhizal	80.82b	52.82b	7.91b	7.15b	5.14b	43.35b	2.64b
Glomus mosseae	88.08a	72.53a	8.61a	8.56a	7.69a	71.40a	4.29a
G. intraradices	88.08a	70.42a	8.80a	9.05a	7.54a	75.03a	4.31a

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

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

Irrigation Regimes+	Relative water content (%)	Total soluble carbohydrate (mg/g FW)	Pod length (cm)	Seeds/pod	Pods/plant	Seeds/plant	Grain yield
25	92.25a	-	8.56a	9.08a	8.31a	76.16a	5.14a
50	86.96a	-	8.82a	8.36a	7.43a	76.04a	4.02b
75	87.37a	-	8.84a	8.33a	7.28a	70.71a	3.84b
100	76.06b	-	7.53b	7.24b	4.14b	30.14b	1.97c

+ Irrigation after evaporation from a Class A pan
Means followed by the same letter in each column are not significant differences

Table 7. Correlation coefficients between mungbean traits.

Treatment	Mycorrhizae colonization	Relative water content	Total soluble carbohydrate	Pod length	Seeds/pod	Pods/plant	Seeds/plant
Relative water content	0.55**						
Total soluble carbohydrate	0.58**	0.27					
Pod length	0.67**	0.77**	0.27				
Seeds/pod	0.60**	0.69**	0.25	0.78**			
Pods/plant	0.70**	0.55**	0.36*	0.55**	0.54**		
Seeds/plant	0.70**	0.71**	0.26	0.91**	0.84**	0.55**	
Grain yield	0.72**	0.76**	0.20	0.90**	0.74**	0.71**	0.86**

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