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Effects of Water Deficit Stress and Two Mycorrhiza Species on Some Physiological Attributes and Tuber Fresh Weight of Potato cv. Agria Under Field Condition

Saman Khosravifar¹, Farhad Farahvash^{1*}, Nasser Aliasgharzad², Mehrdad Yarnia¹ and Farrokh Rahimzadeh Khoie¹

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Abstract

In order to evaluate the tuber fresh weight and some physiological attributes of Agria potato cultivar in response to water deficit stress and mycorrhiza inoculation, a field trial was conducted as a split plot scheme based on randomized complete block design with three replications at the research site of Islamic Azad University, Tabriz Branch in 2012. Four water levels including: irrigation after 60 (S₀) mm evaporation from class A pan as the control and irrigation after 90 (S₁), 120 (S₂) and 150 (S₃) mm evaporation from the pan as water deficit stress conditions were arranged in the main plots and four levels of arbuscular mycorrhizal fungi (AMF) including: no AMF (M₀), inoculation of *Glomus etunicatum* (G_e) (Becker and Gerdemann), *Glomus intraradices* (G_i) (Schenck and Smith) and dual inoculation of the two species (G_{ei}) were arranged in the sub-plots. Water application and AMF inoculation had significant effect on all of studied attributes. Increasing of water deficit stress intensity increased stomatal resistance and decreased leaf relative water content and tuber fresh weight. On other hand, positive effect of AMF inoculation was observed on stomatal resistance, leaf relative water content, leaf chlorophyll content and tuber fresh weight. Also, significant interaction between water application and AMF inoculation was observed for stomatal resistance, leaf relative water content, leaf chlorophyll content and tuber fresh weight in non-mycorrhizal potatoes. In all of water levels, maximum tuber fresh weight was observed by inoculation with *G. intraradices*.

Keywords: Arbuscular mycorrhiza; Potato; Stomatal resistance; Tuber fresh weight; Water deficit

Introduction

Drought stress has an adverse effect on crop plants (Xoconostle-Cazares *et al.* 2010) and limits productivity of agricultural systems and food production (Boyer 1982). Half of the human death by natural disasters is caused by drought (Hoerling and Kumar 2003).

Arbuscular mycorrhiza (AM) is a widespread mutualistic symbiosis (non-pathogenic association) between soil-borne fungi and plant roots (Marschner and Dell 1994; Quilambo 2003) in majority of crop and horticultural plants (Smith and Read 2008). Studying the arbuscular

mycorrhizal fungi (AMF) is difficult because of their multiple ecological functions in the soil system (Hamel and Strullu 2006). Mycorrhiza with important role in plant production can improve nutrient uptake in crops and increase their yield. Also, it plays a key role in sustainable agriculture (Moawad 1979; Sharif and Moawad 2006). AMF can protect host plant (Ellis *et al.* 1985; Huang *et al.* 1985; Puppi and Bras 1990; Sanchez-Diaz and Honrubia 1994; Ruiz-Lozano *et al.* 1999; Quilambo 2003), increase its drought tolerance by changing soil-plant water relations and enhancement of water absorption capacity

¹Department of Agronomy and Plant Breeding, Tabriz Branch, Islamic Azad University, Tabriz, Iran

²Department of Soil Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

^{*}Corresponding author; Email: farahvash@iaut.ac.ir

(Allen 1982; Fitter 1985; Hardie 1985; Koide 1985; Kothari *et al.* 1990; Faber *et al.* 1991; Sylvia *et al.* 1993; Vonreichenbach and Schonbeck 1995; Dell'Amico *et al.* 2002; Echave *et al.* 2005). Also, AM plants recover faster than non-mycorrhizal (NM) plants in the water stress conditions (Hardie and Leyton 1981; Al-Karaki and Clark 1998).

Potato (*Solanum* spp.) with extraordinary adaptation range, easy cultivation and high nutritional content has helped sustain humanity for centuries (DeFauw *et al.* 2012). Potato can't be matched with other crops in view of its production of food energy and food value per unit area (Guenthner 2010). As a staple food, potato is the fourth most important crop after rice, wheat and maize (Gaurav *et al.* 2010) and is very

drought sensitive because it consumes great amount of water during growth period (Lahlou *et al.* 2003).

The aim of this study was to evaluate the effect of water deficit stress on some physiological attributes and tuber fresh weight of potato in the presence of two mycorrhiza species under field condition.

Materials and Methods

The field experiment was conducted at the Agricultural Research Station of Islamic Azad University, Tabriz Branch (38°5′ N, 46°17′ E, 1360 m) in 2012. Metherological data of the station during the growing season are shown in Table 1.

Table 1. Average monthly temperature and precipitation of the research site for 2012

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	May	June	July	August	September	October
Temprature	19.05	23.88	26.30	31.99	36.30	16.40
Percipitation	0.87	0.59	0.53	0.53	0.17	0.19

The experiment was conducted as the split plot scheme based on randomized complete block design with three replications. Four water levels, irrigation after $60~(S_0)$ mm evaporation from class A pan (located in the experimental field) as the control and irrigation after $90~(S_1)$, $120~(S_2)$ and $150~(S_3)$ mm evaporation from the pan as water deficit stress conditions were arranged in the main plots and four types of arbuscular mycorrhizal fungi (AMF) including no application of AMF (M₀), inoculation with *Glomus etunicatum* (G_e) (Becker and Gerdemann), *Glomus intraradices* (G_i) (Schenck and Smith) and dual inoculation of the two species (G_{ei}) were arranged in the subplots. In order to supply dual inoculum of the two

species, their inoculum was mixed with the 1:1 ratio (w/w). Sixty g of inocula were placed directly under potato tubers. Water levels were exerted from the flowering time.

Certified potato seeds (*Solanum tuberosum* cv. Agria, a mid to late-maturing cultivar) were supplied from the Center of Agricultural Services, Khodabande city, Zanjan Province, Iran. Average weight of seeds was 70 g. No fungicide or any other kind of pesticides were used before planting the seeds in the experimental site. The two AMF inoculation species, *G. etunicatum* and *G. intraradices* were provided from the Department of Soil Science, Faculty of Agriculture, University of Tabriz, Iran. The inoculums were the mixture

of 2/3 sterile sand, 1/3 sterile soil, spores (60 spores per ml of inoculum), hyphae and infected fragment of sorghum roots.

Plots were prepared after plowing and disk harrowing. Soil sampling at depth of 30 cm showed that its texture was sandy loam having some chemical attributes as follow: pH of 8.2, 550 mg kg⁻¹ available K, 28.4 mg kg⁻¹ available P and organic carbon of 0.98%. The field was kept in the fallow condition during the previous year in order to decrease endogenous mycorrhizal fungi, remove the fungi propagules and decompose the root debris of the previous crop. Potato seeds were planted on May 9 with between-row space of 75 cm and within-row space of 25 cm in plots of 4.5m×5.25m size, with 6 rows. Planting depth was 7 cm. Weeding was done by hand, without applying any kind of herbicide.

All physiological attributes were measured from the third leaf situated on the top of the canopy one day before the final irrigation. Stomatal resistance was measured on the basis of sec cm⁻¹ through a steady state with a poremeter model AP4 (Delta-T Devices, Ltd., Cambridge, UK). For the measurement of leaf relative water content sampled leave were weighted by a digital scale with 0.01 g accuracy to determine the fresh weight (W_f). The leaves were subsequently saturated in the distilled water for 12 h, reweighed to determine saturated weight (W_s), and weighted again to determine dried weight (W_d) after drying at 80°C. Following formula was used to determine leaf relative water content (Cvikrová et al. 2013).

RWC (%) = $(W_f-W_d)/(W_s-W_d) \times 100$.

Leaf chlorophyll content was measured by the chlorophyll meter (CCM-200, Opti-Science, USA). The chlorophyll meter calculates a unit-less chlorophyll content index (CCI) on the basis of absorbance measurement at 655 and 940 nm. At harvesting time (October 7) tubers of three plants in the center rows of each plot were weighed and their mean was recorded.

Statistical analysis of the collected data was carried out using MSTATC software and the means were compared by the Least Significant Difference (LSD) test at the 5% and 1% probability levels.

Results

Stomatal resistance

Water level, mycorrhiza inoculation and their interaction had significant effects on stomatal resistance (Table 2). Water deficit stress increased stomatal resistance (r_s), as there were significant differences between stomatal resistance of S₀ and all water deficit stress levels. The maximum and the minimum values of r_s were observed for S_3 and S_0 , respectively. S_3 was 524.84% more than S_0 (Table 3). It is noticeable that r_s value did not change significantly after S_1 . Table 4 shows that AMF species and their dual inoculation had different effects on stomatal resistance. Gei had the maximum stomatal resistance which was 45.38% more than M₀ as the minimum. Stomatal resistance of M₀, G_e and G_i were not significantly different, but their r_s value were significantly different from Gei. Table 5 shows that the highest and the lowest r_s values were observed in S₁G_{ei} and S₀G_e, respectively. In both NM and AM plants stomatal resistance of water deficit stress

levels were significantly more than that of S_0 as the control. Stomatal resistance of S_3 decreased in AM plants as compared to NM. This decrease was significant for G_i and G_{ei} . It is noticeable that stomatal behavior at S_3 was nearly similar for M_0 and G_e .

Relative water content

Based on Table 2, water level, mycorrhiza inoculation and their interaction had significant effects on leaf RWC. The maximum and the minimum RWC was observed for S_0 and S_1 , respectively. At S_1 , RWC decreased 14.32% as compared to S_0 . There were significant differences between RWC of water deficit stress levels and S_0 as the control, but RWC values of S_1 , S_2 and S_3 did not differ significantly (Table 3). In AM plants, RWC was more than NM, as the highest and the lowest RWC were recorded in G_{ei} and M_0 , respectively. RWC of dual inoculated plants was 8.99% more than M_0 . There was not

significant difference between RWC of Ge and M₀, but both G_i and G_{ei} were significantly different from M₀. Also, there was not significant difference between RWC values of Gi and Gei (Table 4). Table 5 shows that among the combinations of water and AMF inoculation levels, the maximum and the minimum RWC were obtained from S_0M_0 and S_3M_0 , respectively. It is obvious that gradient of RWC decrease in M₀ plants was more pronounced than AM as water stress intensity increased from S₀ to S₁. RWC values of M₀ plants did not significantly change as the water level changed from S_1 to S_3 . This condition was also observed for Gi and Gei. In Ge plants the differences of S_0 level with S_1 and S_3 were significant. However, S₁ and S₃ did not differ significantly. It is noticeable that the differences of RWC value of S_2 plants with S_1 and significant non-significant, S_3 were and respectively.

Table 2. Analysis variance of the effects of water stress level and mycorrhiza inoculation on the studied attributes of potato

			Mean square	es	
Source of variation	df	Stomatal resistance	Leaf relative water content	Chlorophyll content	Tuber fresh weight
Replication	2	7.30	5.94	4.36	3574.87
Water level (S)	3	3161.48**	248.68**	316.43**	210262.44**
Main plot error	6	16.46	4.52	23.83	1860.80
Mycorrhiza inoculation (M)	3	390.88**	61.01**	240.52**	82320.28**
S×M	9	350.18**	45.92**	160.68**	8542.24*
Sub- plot error	24	32.83	4.85	13.61	3425.38
CV (%)		18.76	3.66	6.95	16.16

^{*:} P\u20.05, **: P\u20.01, CV: Coefficient of Variation, df: degrees of freedom

Chlorophyll content

Water level, mycorrhiza inoculation and their interaction had significant effects on chlorophyll

content (Table 2). Table 3 shows that the differences of chlorophyll content of S_0 with other water application levels were not significant, but a

significant difference was observed between S_1 and S_2 . On the basis of Table 4, maximum and minimum chlorophyll content were observed in G_e and M_0 plants, respectively. In G_e plants it was 21.20% more than M_0 . Chlorophyll content of

AM plants was significantly higher than NM plants. However, there were not significant differences among chlorophyll content of AM plants.

Table 3. Means of potato traits under study at different water levels

Water level	Stomatal resistance	Leaf relative water	Chlorophyll	Tuber fresh weight
water lever	(sec cm ⁻¹)	content (%)	content (CCI)	(g)
S_0	6.48	66.89	52.85	551.12
S_1	40.17	5731	59.67	349.16
S_2	34.99	59.04	47.13	304.04
S_3	40.49	57.41	52.59	244.31
LSD (1%)	6.14	3.22	7.38	65.29

 S_0 represents water application after 60 mm evaporation from class A pan as control. S_1 , S_2 and S_3 show water stress levels after 90, 120 and 150 mm evaporation from the pan, respectively. LSD: Least significant difference.

On the basis of Table 5, chlorophyll content of S₁G_e, having the maximum value, and that of S₂G_{ei}, having the minimum value, were 62.88% higher and 5.89% lower than S₀M₀ condition, were significant respectively. There not differences among chlorophyll content of all water levels in M₀ plants. In G_e and G_{ei} plants, the chlorophyll content was increased significantly as the water level changed from S_0 to S_1 . The chlorophyll content of S₀ was not significantly different from those of S2 and S3 as inoculated with G. etunicatum. This status was also observed between So and S2 levels in the condition of dual inoculation of AMF. Chlorophyll content was decreased with the increase of water deficit from S_1 to S_2 for both G_e and G_{ei} . In G_i plants, chlorophyll content of all water deficit stress levels were less than S_0 as the control. Moreover, the difference between S₀ and S₂ was significant in G_i plants.

Tuber fresh weight

Effects of water levels, mycorrhiza inoculation and their interaction were significant on tuber fresh weight (Table 1). Tuber fresh weight was decreased with the increase in water stress intensity as the maximum and minimum values were observed at S_0 and S_3 , respectively. At S_3 it was 55.67% less than S₀. No significant differences were observed between S1 and S2 and between S₂ and S₃ conditions. But, the difference between S_1 and S_3 was significant (Table 3). Inoculation with AMF increased tuber fresh weight significantly as compared to M₀ plants. The lowest and the highest values of tuber fresh weight were recorded for M₀ and G_i, respectively. Inoculation with G. intraradices showed 77.80% more tuber fresh weight than M₀. There was no significant difference between tuber fresh weight of Ge and Gei. However, the differences of Gi with G_e and G_{ei} were significant (Table 4). Tuber fresh weight of NM and AM plants decreased significantly at S₁, S₂ and S₃ as compared to S₀

condition. This happened when the plants were inoculated with G. etunicatum until the S_2 level, but it stayed nearly constant at S_3 . In all water levels the maximum tuber fresh weight was recorded when inoculated with G. intraradices. The highest and the lowest values of tuber fresh

weight were observed for S_0G_i and S_3G_{ei} , respectively (Table 5). Tuber fresh weight for S_0G_i was 84.26% more than S_0M_0 . Also, at S_3G_{ei} condition it was 4.29% higher and 54.05% lower than S_3M_0 and S_0M_0 , respectively.

Table 4. Means of potato traits under study at different arbuscular mycorrhizal fungi types

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Arbuscular	Stomatal resistance	Leaf relative water	Chlorophyll	Tuber fresh weight
mycorrhizal fungi	(sec cm ⁻¹)	content (%)	content (CCI)	(g)
M_0	26.46	57.28	46.50	260.83
Ge	26.29	59.57	56.36	356.07
G_{i}	30.92	61.36	55.38	463.76
Gei	38.47	62.43	54.00	367.96
LSD (1%)	6.54	2.51	4.21	66.83

M₀, G_e, G_i and G_{ei} represent no application of AMF, inoculation with G. etunicatum and G. intraradices and their dual inoculation, respectively. LSD: Least significant difference

Table 5. Means of studied attributes as affected by water levels and arbuscular mycorrhizal fungi

Water level	Arbuscular mycorrhizal fungi level	Stomatal resistance (sec cm ⁻¹)	Leaf relative water content (%)	Chlorophyll content (CCI)	Tuber fresh weight (g)
S_0	M_0	0.87	71.52	45.80	362.38
	G_{e}	0.81	63.91	53.00	580.38
	G_{i}	4.84	64.53	62.01	667.75
	G_{ei}	19.4	67.59	50.62	593.97
S_1	M_0	30.5	53.53	49.88	272.89
-	G_{e}	31.67	54.43	74.60	320.45
	G_{i}	41.67	59.03	54.27	432.24
	G_{ei}	56.83	62.24	59.92	371.06
S_2	M_0	23.47	52.07	45.94	234.11
	G_{e}	25.67	62.27	50.49	261.50
	G_{i}	42.67	60.55	48.99	380.24
	G_{ei}	48.17	61.26	43.10	340.32
S_3	M_0	51.00	52.00	44.41	173.97
	G_{e}	47.00	57.68	47.35	261.95
	G_{i}	34.50	61.34	56.24	374.84
	G_{ei}	29.47	58.64	62.37	166.49
LSD		13.08	5.03	8.42	98.63
LSD		P≤0.01	P≤0.01	P≤0.01	P≤0.05

 S_0 , S_1 , S_2 and S_3 represent water level after 60, 90, 120 and 150 mm evaporation from class A pan, respectively. M_0 , G_e , G_i and G_{ei} show no application of AMF, inoculation with G. etunicatum and G. intraradices and their dual inoculation, respectively. LSD: Least significant difference

Discussion

Water deficit increased stomatal resistance as compared to the normal condition (S_0). It has been reported that stomatal resistance of potato plants (*Solanum tuberosum* cv. Agria) was increased with the enhancement of water deficit stress (Khosravifar *et al.* 2008). Also, Kawakami *et al.*

(2006) showed that in dry plots, stomatal conductance of two potato cultivars (Norin 1 and Konafubuki) derived from both microtubes and conventional seeds, were less than wet plots. It has been suggested that stomatal resistance increase is one of the drought avoidance mechanisms that is used by plants as a strategy to

resist against drought stress (Levitt 1980; Kozlowski and Pallardy 2002; Poorter and Markesteijn 2008). It seems that the potato plant uses this mechanism to withstand drought via avoidance of water waste by stomata.

This study showed that AMF inoculation increased stomatal resistance as compared with M₀. Ruiz-Lozano et al. (1995) suggested that AMF may affect the stomatal sensitivity via increasing abscisic acid production. With the exception to S₀G_e, stomatal resistance of AM plants was more than NM plants as water level changed from S_0 to S_2 . At S_3 , the stomatal resistance of AM plants was less than M₀. At all water levels, the stomatal behavior was similar in M₀ and G_e. There are evidences that AM plants can overcome to water deficit stress through stomatal regulation (Levy and Krikun 1980; Stevens and Peterson 1996). However, it was shown that stomatal conductance of lettuce plants (Lactuca sativa L. cv. Romana) colonized with deserticola, Glomus fasciculatum, Glomus Glomus mosseae, Glomus etunicatum, Glomus intraradices, Glomus caledonium and Glomus occultum were more than NM plants in the wellwatered condition. This status was also observed in the drought stress condition except for G. occultum (Ruiz-Lozano et al. 1995). Our results showed that AM potato plants may not overcome the water deficit stress at S₃ through increase of the stomatal resistance.

Levels of water deficit stress significantly decreased RWC, as compared to S₀. Results of Khosravifar *et al.* (2008) on potato (*Solanum tuberosum* cv. Agira) showed that RWC decreased with the enhancement of water deficit

stress. Our study showed that RWC of AM plants was more than NM plants. Gholamhoseini et al. (2013) also, reported that RWC of AM leaves was significantly more than NM leaves in sunflower (Helianthus annuus cv. Alestar) in 2006 but not significant in 2007. In our study application of AMF at S₀ condition could not increase the RWC as compared with M_0 . Hernandez-Sebastia et al. (1999) showed that RWC of leaf discs of in-vitro strawberry plantlets was not affected by AMF under high humidity levels. Furthermore, Abo-Ghalia and Khalafallah (2008) reported that the shoot water content of mycorrhizal wheat plants (inoculated by mixture of Glomus spp.) was increased as compared to NM plants in the well water condition but the difference was not significant. Our results showed that AMF application may not be effective in the improvement of RWC at S₀ condition, but, in all water stress levels, the RWC of AM plants was more than NM plants. Increasing of leaf RWC in AM potato plants under water deficit condition indicates that these plants have high root hydraulic conductivity at this condition. The root conductance improvement can be related to the longer root and an alteration in the root system induced by mycorrhizae (Kapoor et al. 2008). Aliasgharzad et al. (2006) reported that in all soil moisture levels with the field capacity (FC) of 0.85, 0.7 and 0.6, leaf RWC of the inoculated soybean plants with Glomus mosseae and Glomus etunicatum was more than NM plants at both flowering and maturation stages.

The chlorophyll content of water stress treatments was not significantly different from the control. There are different reports about the

effect of water deficit on chlorophyll content in different plants. Khosravifar *et al.* (2008) showed that chlorophyll content of potato plants increased with the enhancement of water deficit stress. Also, Heidari and Golpayegani (2012) reported that chlorophyll content of basil (*Osimum basilicum* L.) increased with changing of soil humidity from 80% field capacity to 40%. However, on the base of other studies, chlorophyll content of *Erythrina variegate* (Manoharan *et al.* 2010) and marigold (Asrar *et al.* 2011) decreased with increasing of water deficit.

Our study showed that chlorophyll content was obviously increased in the AM potato plants as compared to NM plants. This increase was also reported by Mathur and Vyas (2000) in AM of Ziziphus mauritiana as compared to NM. Moreover, results of Meenakshisundaram and Santhaguru (2010) revealed that chlorophyll content in the leaves of sorghum (Sorghum bicolor L.) plants colonized with Glomus fasciculatum, Glomus **Glomus** mosseae, dimorphicum and Glomus tubaeformis was significantly more than NM plants. Also, there are other reports about the increase of chlorophyll a and b content in the leaves of AM Rosa hybrida L. (Auge et al. 1987), pepper (Demir 2004) and wheat (Abo-Ghalia and Khalafallah 2008). The carbon derived from photosynthesis to maintain the performance of AM symbioses is often referred to the "cost", and the nutrients obtained through the symbiosis are often referred to the "benefit" of the symbiosis (Koide and Elliot 1989). The increase in chlorophyll content by AMF inoculation may improve the carbon requirement as the cost of AM symbiosis and

consequently improves the symbiosis especially in the drought condition. Furthermore, it has been suggested that high chlorophyll content of AM plants as compared to NM plants, is sometimes related to high photosynthesis rate enhancement in nitrogen and magnesium content (as major components of chlorophyll molecules) in the AM plants (Mathur and Vyas 1995). With the exception to S₂G_{ei}, chlorophyll content of AM plants was more than M₀ in all water levels. It has been reported that in both water deficit and wellwatered conditions, chlorophyll a and b content of AM (inoculated with Glomus constricum) was more than AM plants in marigold (Asrar et al. 2011). It has been suggested that AM fungi can help plants to confront photoinhibition and photodestruction of pigments under stress condition through the increase of carotenoids. Caratenoids protect photosynthetic apparatus of plants in the drought condition dealing with oxygen radical as agent of photoinhibitory damaging (Foyer and Harbinson 1994).

Water deficit had adverse effect on tuber fresh weight. Khosravifar *et al.* (2008) reported that water deficit stress decreased tuber fresh weight of potato plants (*Solanum tubersum* L. cv Agria). Lahlou *et al.* (2003) showed that final tuber fresh yield of different potato cultivars decreased by drought stress in the field. Potato yield decrease in the drought condition can be mediated by the decrease in plant leaf size as the first morphological change (Jefferies and MacKerron 1987) and consequently decrease dry matter accumulation in tubers as a result of decrease in radiation interception (Jefferies 1993).

Our study revealed that AMF inoculation increased tuber fresh weight. However, this increase was different at AMF levels. Yao et al. (2002) also reported the increase of tuber fresh weight in micropropagated potato plantletss through incolation with Glomus intraradices and Glomus etunicatum, but the increase was more pronounced in G. etunicatum. Duffy and Cassells (2000) suggested that mycorrhizal fungi can increase or decrease crop yield depending on the mycorrhizal isolate and host genotype. Our results showed that tuber fresh weight of all NM and AM plants decreased with the enhancement of water deficit intensity as compared to S₀. Also, in all water levels tuber fresh weight of AMF potato plants was more than NM plants, except for the dual inoculation of AMF at the S₃ level. Guarav et al. (2010) showed that potato tuber yield of Kufri Badshah cultivar decreased in both AM and NM plants under water deficit stress, but the yield was higher in the stressed and non-stressed AM plants as compared to their related NM controls. Some researchers suggested that the increase of crop yield by AMF colonization under drought stress may occur through high root surface absorption area and densely proliferate root growth (Kothari *et al.* 1990) or differences in the root systems hydraulic attribute (Auge *et al.* 1994).

Conclusion

The results indicated that the enhancement of water deficit stress increased stomatal resistance and decreased relative water content and tuber fresh weight. For both stomatal resistance and relative water content, the higher values were observed with the dual inoculation of Glomus etunicatum and Glomus intraradices. Also, the maximum chlorophyll content and tuber fresh weight were recorded when inoculated with Glomus etunicatum and Glomus intraradices, respectively. In both control and water deficit conditions, the inoculation with Glomus intraradices had the best effect on potato tuber fresh weight.

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