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Full Length Research Paper

Effects of arbuscular mycorrhizal fungi, different levels of phosphorus and drought stress on water use efficiency, relative water content and proline accumulation rate of Coriander (*Coriandrum sativum* L.).

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An experiment was carried out using a split factorial based randomized complete block design with 4 replications to study the effects of AMF, phosphorus and drought stress on some characteristics of Coriander. The factors studied included application and non-application of mycorrhiza (*Glomus hoi*) 0.35 and 70 kg ha^{-1} phosphorus applications, and two levels of drought stress irrigation. The results showed that drought stress significant effect on water use efficiency, relative water content and proline accumulation rate (α = 1%). Highest water use efficiency and proline accumulation rate were achieved under stress conditions and highest relative water content was achieved under without stress conditions. Also, mycorrhiza and phosphorus significant effects on water use efficiency (α = 5%). Highest water use efficiency was achieved under application of mycorrhiza and application of 70 kg ha^{-1} phosphorus respectively. Relative water content and proline accumulation rate were not significantly affected due to phosphorus and mycorrhiza. The results this experiment showed that water use efficiency was increased under application of mycorrhiza that can increase absorb of phosphorus and water in drought conditions.

Key words: Arbuscular mycorrhizal fungi, phosphorus, drought stress, water use efficiency, coriander.

INTRODUCTION

Coriander (*Coriandrum sativum* L.) is an important cash crop of India. It is also extensively grown in Russia, Central Europe, Asia and Morocco. The stem leaves and fruits have a pleasant aroma and the young plant (green coriander) is used in preparing sauces and for flavouring of curries and soups. The fruits are extensively employed as a condiment. In the USA and Europe, coriander is also used for flavouring liquors (Kapoor et al., 2001). Coriander seed oil is an aromatic stimulant, a carminative (remedial in flatulence), an appetizer and a digestant stimulating the stomach and intestines. It is generally beneficial to the nervous system. Its main use is in masking foul medicines, especially purgatives, where it has anti-griping qualities. Coriander cakes were once taken against 'St. Anthony's fire', or 'Rose' a severe streptococcal skin infection called "erysipelas", which caused many deaths before the advent of antibiotics. In Asia the herb is used against piles, headache and swellings; the fruit in colic, piles and conjunctivitis; the essential oil in colic, rheumatism and neuralgia; the seeds as a paste for mouth ulceration and a poultice for other ulcers (Arganosa et al., 1998).

Water deficit occurs when water potentials in the rhizosphere are sufficiently negative to reduce water availability to sub-optimal levels for plant growth and development. On a global basis, it is a major cause limiting productivity of agricultural systems and food production (Boyer, 1982). In cereal crops which provide the major carbohydrate staples for humans, even intermittent water stress at critical stages may result in considerable yield

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Table 1. The results of soil analysis.

Soil texture	Sand (%)	Silt (%)	Clay (%)	K mg/kg	P mg/kg	N mg/kg	Na Ds/m	EC 1: 2.5	pH	Depth of sampling
Sa	49	30	21	147.2	6.2	34.7	0.04	0.19	8.1	0 – 15 cm
Sa.c.L	56	25	19	124.3	3.7	28.2	0.03	0.16	7.9	15 – 30 cm

reduction (Ludlow and Muchow, 1990) and crop failure. However, the wild progenitors of crop species are often found to be relatively drought-resistant since they grow in environments that are far more adverse than crop environments (Richards, 1993). Moreover, wild cereals have been shown to be a repository of characteristics important for drought resistance since they possess vast genetic diversity which may be missing in crop species (Nevo, 1992).

Mycorrhizal fungi live in a ‘symbiotic’ relationship with plants. They grow in close association with the roots and play an important role in the concentration and transfer of soil nutrients to the plant. In exchange, the plant supplies the fungus with sugars. In some cases of poor establishment of young plants, especially from seed, this can be associated with failure to establish a mycorrhizal relationship with suitable fungi. Mycorrhizal fungi have been suggested as having a role in mediating the uptake of water at times of drought stress, and of heavy metals on contaminated ground (Courtecuisse, 1999). Root systems of crop and native plants are commonly colonized by one or more mycorrhizal fungi, naturally occurring soil fungi that increase nutrient absorption and improve soil structure. The hyphae of arbuscular mycorrhizal fungi penetrate roots and grow extensively between and within living cortical cells, forming a very large and dynamic interface between symbionts. The hyphae also extend from root surfaces into the surrounding soil, binding particles and increasing micro- and macro-aggregation (Auge, 2001). Although specific fungus-plant associations with respect to drought tolerance are of great interest (Ruiz-Lozano et al., 1995), the exact role of arbuscular mycorrhizal fungi (AFM) in drought resistance remains unclear (Auge et al., 1992a). More studies are therefore needed to determine the direct or indirect mechanisms which control plant-water relations in AMF-plant symbiosis. Although the effects of AMF on plant water status have been ascribed to the improved host nutrition (Graham and Syverten, 1987; Fitter, 1985), there are reports that drought resistance of AMF plants is somewhat independent of plant P nutrition status of plants (Bethenfalvay et al., 1988; Khalvati et al., 2005). Although improved host nutrition has been ascribed to AMF effects on plant water status, there are reports that the drought resistance of AMF plants is somewhat independent of phosphorous levels. Therefore mycorrhiza fungi can increase absorb of phosphorus by symbiosis with plant of root. This symbiosis can decrease application of phosphorus fertilizers in fields, without decrease quantity and quality yield of plant.

MATERIAL AND METHODS

This study was carried out in the Iran Research Institute of Forest and Rangelands. The field experiment was carried out in a split factorial based randomized complete block design with 4 replications. The factors which studied were application and non-application of mycorrhiza (*Glomus hoi*), 0.35 and 70 kg ha^{-1} phosphorus fertilizer (triple super phosphate) applications, and two levels of drought stress comprised of irrigation after 30 mm water evaporation from evaporation pan (without stress conditions) and irrigation after 60 mm water evaporation (drought stress conditions). The soil consisted of 25% clay, 30% silt and 45% sand (Table 1). The soil bulk density was 1.4 g cm^{-3} and further the field was prepared in a 15 m^2 area (5 m × 3 m) totally 48 plots. The irrigation system was a piping system. Water usage was determined water by meter for each plot. *Glomus hoi* was provided from the Department of Biosafety and Microorganisms, Agricultural Biotechnology Research Institute of Iran (ABRII) which was consisting of root fragments and adhering spores mixed with soil (90 – 110 propagules per 10 g soil) and the seeds of coriander were obtained from the Research Institute of Forest and Rangelands, Iran. At the growth period and between two according irrigation, we collected 20 young leafs from each plot for determined relative water content (RWC) by under formula.

$$\mathrm{RWC} = \frac{\text{Leafs fresh weight} - \text{Leafs dry weight}}{\text{Leafs turgid weight} - \text{Leafs dry weight}}$$

Also in this period we collected 0.5 g young leafs from each plot for determined proline accumulation rate. At the end of growth period determined dry matter yield and water used by evapotranspiration. Finally, for determined water use efficiency (WUE) use from under formula.

$$\mathrm{WUE} = \frac{\text{Dry matter yield (kg)}}{\text{Water used by evapotranspiration } (\mathrm{m}^3)}$$

Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System and followed by Duncan's multiple range tests and terms were considered significant at $P < 0.05$ by MSTAT-C software.

RESULTS

The results showed that drought stress significantly effects WUE, RWC and proline accumulation rate in $P<0.01$ (Table 2). Highest WUE (0.45 kgm^{-3}) and proline accumulation rate (6.77 mmol mm^{-1}) were appeared under stress conditions and highest RWC (90.60%) was appeared under without stress conditions (Figure 1, 2 and 3). Also, the results showed that mycorrhiza and phosphorus significant effects on water use efficiency in $P<0.05$ (Table 2). Highest water use efficiency (0.40 kg

Table 2. Variance analysis of WUE, RWC and proline accumulation rate.

		Mean Squares		
Value Sources	**df**	**WUE**	**RWC**	**Proline**
Replication	3	0.013*	0.137	0.049
Mycorrhiza	1	0.007*	0.032	0.004
Error a	3	0.001	0.097	0.064
Phosphorus	2	0.004*	0.048	0.071
Mycorrhiza × phosphorus	2	0.001	0.188	0.001
Drought stress	1	0.195**	7585.745**	486.795**
Mycorrhiza × drought stress	1	0.001	0.015	0.002
Phosphorus × drought stress	2	0.002	0.024	0.058
Mycorrhiza × phosphorus × drought stress	2	0.001	0.054	0.001
Error bc	30	0.001	0.09	0.047
CV (%)		8.14	0.39	6.07

* and ** : Significant at 5% and 1%% levels respectively.

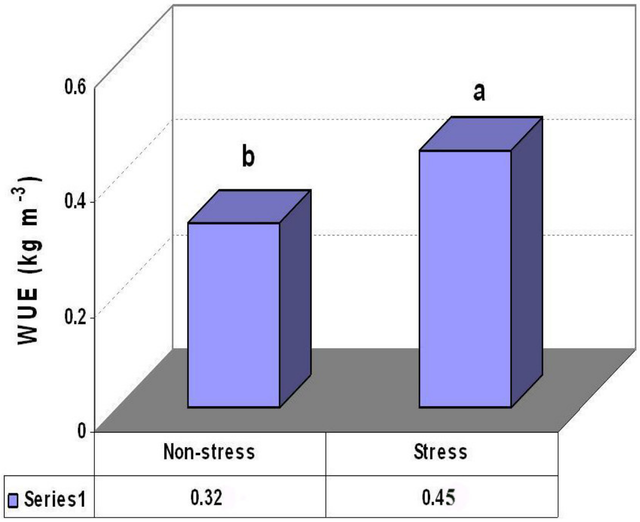

Figure 1. Effect of drought stress on WUE.

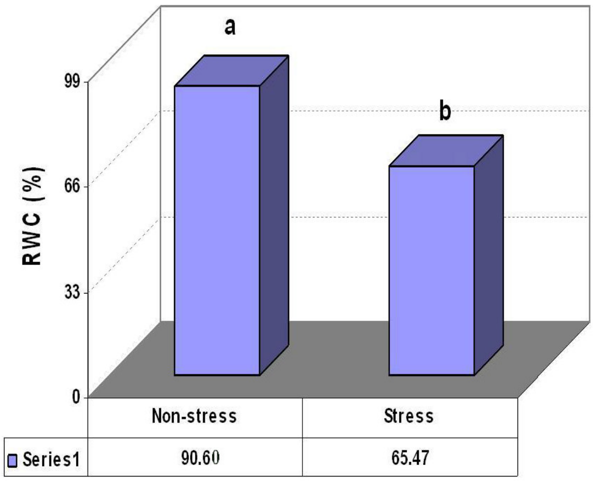

Figure 3. Effect of drought stress on RWC.

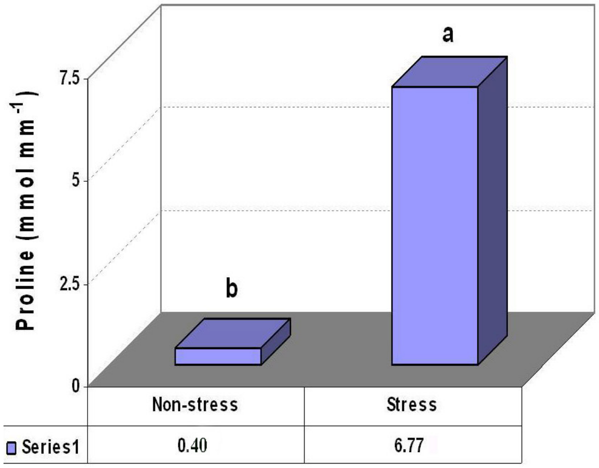

Figure 2. Effect of drought stress on proline.

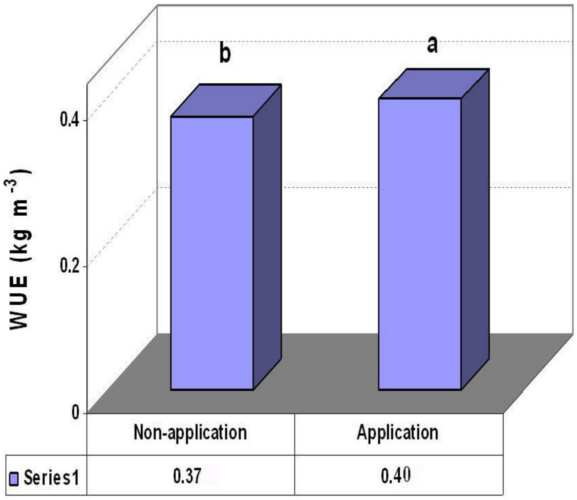

Figure 4. Effect of mycorrhiza on WUE.

Table 3. Means comparison of WUE, RWC and proline accumulation rate.

	Survey instance	Qualifications		WUE (kg m^{-3})	RWC (%)	Poroline (mmol mm^{-1})
		Non application of phosphorus		0. 36 c	87.70 a	2.05 a
	Non application	35 (kg ha^{-1}) phosphorus		0. 37 bc	87.93 a	2.14 a
		70 (kg ha^{-1}) phosphorus		0. 39 abc	88.10 a	2. 30 a
Mycorrhiza						
		Non application of phosphorus		0. 37 bc	87.90 a	2. 31 a
	Application	35 (kg ha^{-1}) phosphorus		0. 40 ab	88.20 a	2. 53 a
		70 (kg ha^{-1}) phosphorus		0. 41 a	88.50 a	2.63 a
		Non stress		0. 31 c	90.66 a	0.41 b
	Non application					
		Stress		0. 44 a	65.48 b	6.75 a
Mycorrhiza						
		Non stress		0. 34 b	90.00 a	0.41 b
	Application					
		Stress		0. 46 a	65.59 b	6.78 a
		Non application of phosphorus		0. 30 c	90.79 a	0.42 b
	Non stress	35 (kg ha^{-1}) phosphorus		0. 31 c	90.11 a	0.41 b
		70 (kg ha^{-1}) phosphorus		0. 35 b	90.39 a	0.41 b
Drought stress						
		Non application of phosphorus		0. 43 a	65.61 b	6.42 a
	Stress	35 (kg ha^{-1}) phosphorus		0. 46 a	65.82 b	6.75 a
		70 (kg ha^{-1}) phosphorus		0. 45 a	65.02 b	6.70 a
			Non application of phosphorus	0. 29 d	90.70 a	0.48 b
		Non stress	35 (kg ha^{-1}) phosphorus	0. 31 d	90.65 a	0. 39 b
			70 (kg ha^{-1}) phosphorus	0. 32 d	90.45 a	0.41 b
	Non application					
			Non application of phosphorus	0. 43 b	65.55 b	6. 90 a
		Stress	35 (kg ha^{-1}) phosphorus	0. 44 ab	65.53 b	6.66 a
			70 (kg ha^{-1}) phosphorus	0. 45 ab	65.20 b	6.82 a
Mycorrhiza						
			Non application of phosphorus	0. 32 d	90.52 a	0.42 b
		Non stress	35 (kg ha^{-1}) phosphorus	0. 32 d	90.69 a	0. 39 b
			70 (kg ha^{-1}) phosphorus	0. 37 c	90.63 a	0. 39 b

Table 3. Contd.

	Application					
			Non application of phosphorus	0. 43 b	65.53 b	6. 93 a
		Stress	35 (kg ha^{-1}) phosphorus	0. 48 a	65.37 b	6.70 a
			70 (kg ha^{-1}) phosphorus	0. 46 ab	65.41 b	6.72 a

Means within the same column and factors, followed by the same letter are not significantly difference (P<0.05) using Duncan's multiple range test

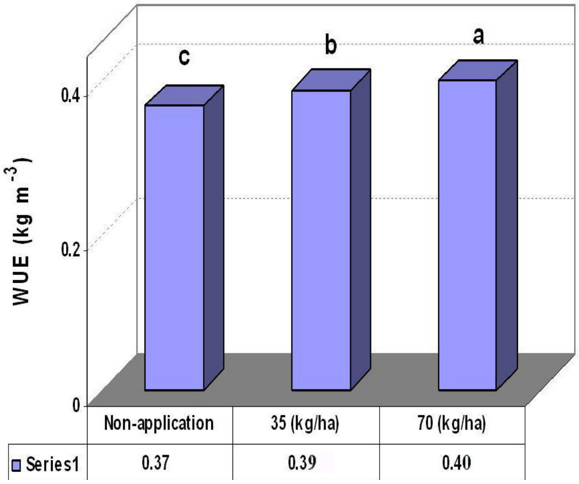

Figure 5. Effect of phosphorus on WUE.

m^{-3}) and (0.40 kg m^{-3}) was appeared under application of mycorrhiza and application of 70 kg ha^{-1} phosphorus respectively (Figures 4 and 5). Relative water content and proline accumulation rate were not significantly affected due to phosphorus and mycorrhiza. Interaction of mycorrhiza and phosphorus wasn't significant effect on WUE, proline accumulation rate and RWC (Table 2). A means comparison showed that highest WUE (0.41 kg m^{-3}) was appeared under application of mycorrhiza and 70 kg ha^{-1} phosphorus (Table 3). Also, the interaction of mycorrhiza and drought stress had no significant effect on upon plant characteristics (Table 2). The highest WUE and proline accumulation rate (0.46 kg m^{-3}) and (6.78 mmol mm^{-1}) respectively appeared under application of mycorrhiza in drought stress conditions. The highest RWC (90.60%) appeared under mycorrhiza application without drought stress conditions (Table 3). Interaction of phosphorus and drought stress had no significant effect on WUE, proline accumulation rate and RWC (Table 2). Highest WUE (0.46 kg m^{-3}) appeared under the application of 35 kg ha^{-1} phosphorus in drought stress conditions and the highest proline accumulation rate (6.91 mmol mm^{-1}) appeared under non-application of phosphorus in drought stress conditions and the highest RWC (90.61%) appeared under non-application of phosphorus and without stress conditions (Table 3). The interaction of mycorrhiza, phosphorus and drought stress showed no signifycant effect on the plant characteristics (Table 2). The highest WUE (0.48 kg m^{-3}) appeared under mycorrhiza application and 35 kg ha^{-1} phosphorus in drought stress conditions and the highest proline accumulation rate (6.90 mmol mm^{-1}) appeared under non-application of mycorrhiza and phosphorus in drought stress conditions. Also, highest RWC with was appeared under non-application of mycorrhiza and phosphorus and without stress conditions (Table 3).

DISCUSSION

Plants colonized by mycorrhizal fungi have been shown to deplete soil water more thoroughly than non-mycorrhizal plants (Auge, 2001). One reason for this is the fact that the shoots of plants with AMF usually have a larger biomass (more evaporative leaf surface area) than non-AMF plants (Fitter, 1985; Nelsen, 1987). Also the root systems of plants with AMF are often more finely divided and thus have more absorptive surface area (Allen et al., 1981; Busse and Ellis, 1985; Ellis et al., 1985; Huang et al., 1985; Sharma et al., 1991; Osonubi et al., 1992; Osonubi et al, 1994; Okon et al., 1996). Furthermore, the roots of plants with AMF dry the soil more quickly than non-AMF plants of similar size (Bryla and Duniway, 1997). In our experiment, mycorrhizal coriander signifycantly WUE throughout the improvement plant water relations under drought conditions corresponding of mycorrhiza's contribution in P uptake to AMF-plants and act to synthesis of certain phytohormones as like as ABA and cytokinin. In the present study, mycorrhizal (*Glomus hoi*) treatment of coriander significantly improved WUE through improvement of plant water relations under drought conditions. These improvements were likely achieved via the mycorrhizal contribution to phosphorous uptake and the ability of AMF to stimulate plant synthesis of certain phytohormones such as ABA and cytokinins. Consequently, plants with AMF had higher phosphorous content in shoots than non-AMF plants, in agreement with the observation of Labour et al. (2003) and Dhanda et al. (2004) our data also revealed that roots of AMF-inoculated coriander were longer with increasing fungal hyphae growth, similar to the findings of Ruiz-Lozano et al. (1995). In addition, we found highest WUE among AMF-inoculated plants under drought conditions compared to well-watered plants. Absorb of phosphorus is by plants in forms $H_2SO_4^-$ and HSO_4^{-2} that for absorb of this anion, pH of soil must be acidic. Hyphaes of mycorrhiza splash solvent acid of phosphorus (For example: Malic acid) that cause increasing absorb of phosphorus by plan in non-acid soils. WUE increased by mycorrhiza because application of mycorrhiza increased absorb of phosphorus by plan and also, phosphorus increased biological yield. Therefore each factor that increase of biological yield cause increasing of WUE, Finally mycorrhiza and phosphorus increased WUE in Coriander. Also, increaseed WUE under drought stress conditions because in these conditions, plant deleted surplus leafs and decreased leafs area and also closed or semi closed it stomatals because least of water wasted by evapotranspiration. Therefore Coriander optimum used from water for product dry matter and caused that increased WUE in these conditions. Proline is an important amino acid in plant under drought stress that prevent from inside of cells oxidation. Also it regularize osmotic pressure of plant under drought stress for absorb of water, Therefore proline accumulation rate increased in Coriander under drought stress. Water deficits induce dramatic increases in the proline concentration of phloem sap in alfalfa (Girousse et al., 1996), suggesting that increased deposition of proline at the root apex in water stressed plants (Voetberg and Sharp, 1991) could in part occur via phloem transport of proline (Girousse et al., 1996). A proline transporter gene, *ProT2*, is strongly induced by water and salt stress in *Arabidopsis thaliana* (Rentsch et al., 1996). Homologous proline transporter genes have been identified in tomato; *LeProT1* is strongly expressed in mature and germinating pollen, and may encode a general transporter for compatible solutes. LeProT1 transports proline and GABA with low affinity and glycinebetaine with high affinity (Schwacke et al., 1999). Relative water content under without stress conditions was more from drought stress conditions. In stress conditions decrease water of soil and plant for absorb of water decrease inside of osmotic pressure until water enter from soil to plant with more pressure. Therefore RWC decreased by drought stress in Coriander. Our findings indicate that AMF-inoculation improves WUE and decreases the phosphorous requirement for coriander plants subjected to water stress.

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