

Biochemical Response and Nutrient Uptake of Two Arbuscular Mycorrhiza-Inoculated Chamomile Varieties under Different Water Potentials

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Abstract

Background

Water-deficit stress is one of the most important sources of damage to crop production worldwide. Adopting appropriate varieties using soil microorganisms such as arbuscular mycorrhiza (AM) fungi can significantly reduce the adverse *effects of* water deficiency. This study is aimed to evaluate the role of *Funneliformis mosseae* on nutrients uptake and some physiological traits of two chamomile varieties namely Bodgold (Bod) and Soroksári (Sor) under water-deficit stress. The pot experiment was performed in a completely randomized design with three factors: water-deficit stress (PEG 6000) was applied along with Hoagland solution at three levels (0, -0.4 and -0.8 MPa), two German chamomile varieties (Bodgold (Bod) and Soroksári (Sor)) and AM inoculation (*Funneliformis mosseae* species (fungal and non-fungal)) at four replications in perlite substrate.

Results

Water-deficit stress significantly reduced the uptake of macro-nutrients (N, P, and K) and micro-nutrients (Fe, Cu, Mn, and Zn) in the shoots and roots. Moreover, the level of osmolytes (total soluble sugars and proline) and the activity of antioxidant enzymes in the shoots of both varieties increased under water-deficit stress. In the case of Sor variety, the level of these compounds was more satisfactory. AM improved plant nutrition uptake and osmolyte contents while enhancing antioxidant enzymes and reducing the adverse *effects of* water-deficit stress. Under water-deficit stress, the growth and total dry weight improved upon AM inoculation.

Conclusions

In general, inoculation of chamomile with AM balanced the uptake of nutrients increased the level of osmolytes, antioxidant enzymes, and hence improved plant characteristics under water-deficit stress in both varieties, however, it was more effective in reducing stress damages in Sor variety.

1. Introduction

Water-deficit stress (due to global warming and climate change) is the main cause of the decremented annual plant performance. In arid and semi-arid regions, plants are exposed to water-deficit stress due to the simultaneous increase in the rate of transpiration and temperature and the reduction of the root access to water (Halo et al. 2020). Water-deficit stress affects plant life in many ways; for example, shortage of water to roots reduces rate of transpiration as well as induces oxidative stress (Hasanuzzaman et al. 2013). Water-deficit stress imparts deleterious effects on plant growth by affecting enzyme activity, nutrients uptake, and nutrient assimilation (Ahanger et al. 2017).

The use of herbal medicinal products and supplements has tremendously increased over the past three decades. More than 80% of the world's population rely on these products as a part of primary healthcare (Sharma 2004; Ekor et al. 2014). German chamomile (*Matricaria chamomilla* L.) belongs to the family of Asteraceae, one of the most common medicinal plants (Wichtl 2004). Chamomile is a prominent medicinal plant whose compounds are considered safe (Sharifi et al. 2014). German chamomile flower and its extracts have antimicrobial and antioxidant activity and have been used as a painkiller, anti-anxiety, antispasmodic, anti-inflammatory, and anti-gastrointestinal agent (Rehmat et al. 2020). With the increase in the global demand for medicinal plants, there is an urgent need to increase their cultivation and production. The increasing demand for medicinal plants, especially chamomile, necessitates deeper knowledge to adopt drought-resistant varieties. In addition to water management, the selection of the right genotype can also contribute to preventing water-deficit stress damages and promoting sustainable use of water resources. Concerning medicinal plants, although water-deficit stress increases the synthesis of secondary metabolites, it can also decline the growth of the plant especially its vegetative and reproductive organs (biomass) which generally contain medicinal compounds (Selmar et al. 2017). It also reduces the nutrient uptake of chamomile (Salehi et al. 2018), which plays a vital role in its total dry weight and essential oil content (Andrzejewska et al. 2014). Under water-deficit stress, lipids, proteins, and nucleic acids are damaged due to the rise in the content of reactive oxygen species (ROS) (Uzilday et al. 2012). Plants exploit effective systems such as antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and osmolytes such as soluble sugars to combat toxic ROSs and reduce their consequent damages (Al-Arjani et al. 2020).

As one of the commercial varieties of chamomile tetraploid, Bodgold (Bod) has shown favorable performance in terms of total dry weight and essential oil among other types of diploid and tetraploid chamomile (Banatska (2x), Lutea (4x), Zloty Lan (4x), and Goral (4x)) (Tsivelika et al. 2018). Soroksári (Sor) is another important and diploid variety of chamomile with a desirable essential oil content compared to Lutea, Goral (tetraploid), and Bona (diploid) varieties according to Gosztola et al. (2010). Heretofore, no comparative study has been carried out to explore the activity of antioxidant enzymes, absorption of nutrients, and dry weight of these varieties under stress conditions. Nonetheless, a study reported an increase in proline and antioxidant activity of the Bod variety under water-deficit stress (Benabdellah et al. 2011). Arbuscular mycorrhizal (AM) fungi coexist with the root of most plants and have exhibited great potential for counteracting environmental stresses, as they can increase the availability of plants to a larger volume of the rhizosphere and also improve water and nutrients uptake (Zhang et al. 2018) via morphological change of root volume and through their hyphae (Hameed et al. 2014). On the other hand, these fungi enhance the nutrient uptake by increasing the synthesis of compounds and enzymes involved in the absorption process, such as phosphatase (Hu et al. 2013). However, there is a strong evidence of drought stress alleviation by AMF in different crops (Begum et al. 2019).

Improving the nutrients and water absorption by AM promotes plant growth and reduces the adverse effects of water-deficit stress caused by PEG (Benabdellah et al. 2011; Wu et al. 2013). The beneficial effects of AM have been reported in many Asteraceae families under water-deficit stress. For instance, under water-deficit stress condition, AM improves the absorption of macro and micro-nutrients and increments the total dry weight of plants from the Asteraceae family, including *Echinacea angustifolia* (Attarzadeh et al. 2019), German chamomile (Benabdellah et al. 2011), *Helianthus annuus* (Gholamhoseini et al., 2013), Marigold (Asrar and Elhindi 2011), Safflower (Abbaspour et al. 2010) and Scabious (*Knautia arvensis*) (Doubková et al. 2013). AM also improves

growth and yield in *Echinacea angustifolia* by increasing the defensive level of antioxidant (catalase and peroxidase) and osmolyte (proline) enzymes (Attarzadeh et al. 2019).

Therefore, the goal of the present study was to evaluate the effect of *Funneliformis mosseae* on reducing the impact of water-deficit stress in two most important German chamomile varieties to determine a more resistant one, according to the physiological traits and nutrients uptake. Deciphering the AM-mediated mechanisms in the plant protection responses and metabolic pathways under unfavorable conditions is required to gain insight into their potential, and it will open up new approaches to exploit AM as a bioprotective tool against drought in sustainability and food security.

2. Materials And Methods

2.1 Experimental Design

A pot experiment was performed in the factorial arrangement within a completely randomized design with four replications in the research greenhouse of the Faculty of Agriculture, Yasouj University. The greenhouse temperature was $25 \pm 2^\circ\text{C}$. The PEG treatment (Water-deficit stress) was applied along with Hoagland solution at three water potentials (control, -0.4, and -0.8 MPa). Tap water ($\text{EC } 450 \mu\text{S cm}^{-1}$) was used as control treatment with Hoagland solution. Different levels of water-deficit stress were prepared using polyethylene glycol 6000 (PEG) via the formula proposed by Michel and Kaufman (1973) and applied in nutrient solutions. The second studied factor was the use of arbuscular mycorrhiza (AM) fertilizer (*Funneliformis mosseae* species (Zist Fanavar Sabz Company, Iran) (fungal and non-fungal)) which was initially inoculated in the culture medium (Chitarra et al. 2016). In mycorrhizal plants, each pot of mycorrhizal treatment received 40 g of AM inoculant (containing spore numbers of 120 g^{-1} substrate) at a depth of 5 cm and incorporated well with the soil below. Two German chamomile varieties (Bodgold (Bod) and Soroksari (Sor)) were considered. The Bod variety seeds were purchased from Isfahan Agricultural and Natural Resources Research Center while the Sor variety was supplied from Yasouj Zardband Company. The seeds of both varieties were sterilized with sodium hypochlorite solution (1%) for 3 min and then washed several times by distilled water. To produce seedlings, the seeds were first transplanted in a bed of peat moss and cocopeat (1: 2) in a 72-cell (30 cc) seedling tray. At the 4-6-leaf stage, they were transferred to plastic pots with a height of 25 cm and a diameter of 18 cm (6 seedlings per pot), filled with sterilized perlite (sterilized with autoclave (105°C for 30 minutes)). After transferring the plants to the pot, the plants were pre-cultured with 1/4 Hoagland's nutrient solution for 4 weeks to adaptation and establishment of seedlings to the new condition. During the transplanting phase, the modified Hoagland's nutrient solution was used in this protocol, which contained 5 mM KNO_3 , 5 mM $\text{Ca}(\text{NO}_3)_2$, 1 mM $\text{NH}_4\text{H}_2\text{PO}_4$, 2 mM MgSO_4 , 0.4 mM H_3BO_3 , 0.08 mM MnCl_2 , 1.8 μM ZnSO_4 , 0.1 μM $(\text{NH}_4)_6\text{Na}_2\text{MoO}_4$, 3 μM CuSO_4 and 0.1 mM FeSO_4 (Hoagland and Arnon, 1950).

The water-stress treatments were started 4 weeks after transplanting and drought stress was gradually applied and maintained for 4 weeks. The pH of the solutions was adjusted to 5.8 ± 0.1 before each irrigation. It should be noted that all the seed-starting containers, pots, and seedling beds were decontaminated with a greenhouse autoclave, and distilled water was used to make all the nutrient solutions.

2.2 Sampling to measure dry weight, physiological, and nutrients traits

21 days after water-deficit stress, two plants were selected from each pot, and young upper leaves were sampled to measure physiological traits. The samples were transferred to the laboratory after being placed in a liquid nitrogen container where they were stored at -40°C . To measure the traits associated with the dried sample, four plants were harvested from each pot and dried at 70°C for 48 hours.

2.3 Mycorrhizal Determinations

The percentage of mycorrhizal root colonization was estimated by visual observation of fungal colonization after clearing washed roots with tap water and cut into segments of 1 cm length. Approximately 100 root segments were randomly chose and cleared in 10% KOH after which they were placed in a water bath at 90°C for 30 min and stained with 0.05% Trypan blue in acetic acid (v/v), according to Cao et al. 2013. The rate of mycorrhizal colonization was estimated by Linderman and Biermann (1981) method.

2.4 Enzyme Activity

To prepare the enzymatic extract, 3 ml of extraction buffer (100 mM potassium phosphate at pH = 7.8, 0.1 M EDTA, and 0.1 M PVP) was homogenized with 0.1 g of leaf sample using a mortar in an ice bath. The obtained homogeneous samples were centrifuged for 30 minutes (14000 rpm at 4°C) and the supernatant was used to measure the enzymes activity. Catalase (CAT) activity was evaluated by monitoring the reduction of the absorption of hydrogen peroxide in the reaction mixture at 240 nm using a spectrophotometer (Aebi 1984). Peroxidase (POD) activity was also assessed according to the absorption of the reaction mixture (enzymatic extract, potassium phosphate buffer, and guaiacol along with 30% H_2O_2) at 470 nm (Zhou and Leu 1999). Polyphenol oxidase (PPO) activity was measured based on the intensity of the orange color of methyl catechol at a wavelength of 420 nm produced in the reaction mixture (Kahn 1975). The CAT, POD, and PPO activity of the extract was expressed as enzyme unit mg^{-1} protein min^{-1} . One unit of enzyme activity is defined as the amount required to decompose μl mol of the substrate within one min.

2.5 Determination Of Proline Content

To determine the amount of proline in the shoot, 0.1 g of fresh tissues were homogenized with 10 ml of 3 % aqueous sulfosalicylic acid followed by centrifugation. Two milliliters of the supernatant were blended with acid ninhydrin and glacial acetic acid (two milliliters of each). The mixture was kept in a water bath for 1 h at 100°C. The reaction mixture was then extracted with toluene (four milliliters) whose absorbance was determined at 520 nm after cooling down to room temperature (Lechasseur and Paquine 1979).

2.6 Determination Of Total Soluble Sugar

Total soluble sugar was determined based on the method specified by Irigoyen et al. 1992. Fresh leaves (0.1g) were added to 5 ml of 80 % ethanol in a water bath and heated for 1 hour at 80 °C. Then, 1 ml of the sample extract was taken to another set of test tubes and mixed with 1 ml each of 18 % phenol and distilled water. They were then allowed to stand at room temperature for an hour. Finally, 5 ml of sulfuric acid was added and the whole mixture was vortexed. The absorbance was read at 490 nm using a UV spectrophotometer. Ethanol 80 % was used as a blank sample. Absorbance was recorded at 625 nm using a spectrophotometer.

2.7 Measurement Of Nutrients

The extract for measuring nutrients was prepared based on digestion by the H₂SO₄-salicylic acid-H₂O method. This extract was employed to measure nitrogen(N) (Novozamsky et al. 1974), manganese Mn (Atomic Absorption), potassium (K) (Film Photometer) (Knudsen et al., 1982), and phosphorus (P) (Røtset 1984) contents. To measure iron (Fe), zinc (Zn) and copper (Cu), the samples were placed at 500 °C for 4 h, 5 ml of 2 normal hydrochloric acids (2n) was added to the samples and placed on the heater. The concentration of Fe, Zn, and Cu in the shoot and root of the plant was determined by the atomic absorption (Chapman and Pratt 1961).

2.8 Statistical Analysis

Main and interaction effects of experimental factors were determined from analysis of

variance (ANOVA) using a general linear model and means were separated by using a least significant difference (LSD) test with $p < 0.05$ using the SAS software 9.1(SAS Institute, Cary, NC, United States) and the graphs were drawn by Excel 2013 software. Comparison of means was performed using the LSD at a P-value of 5%. In the case of significant interaction, LS means procedure was used to compare significant interactions. When an *F*-test indicated statistical significance at $P < 0.05$, the protected least significant difference was used to separate the means of main effect and the significant interactions were separated by slicing method. When the interactions were not significant, we only discussed the main effects. When the main effects, two-way and three-way interactions effects traits were significant we only discussed on three-way interactions effect or when the main effects, two-way interactions traits were significant we only discussed on two-way interactions effect. Pearson correlation analysis was carried out by the XLSTAT (Addinsoft, Paris, France) program.

3. Results

3.1 Root Colonization

No AM colonization was found in the roots of non-inoculated chamomile varieties seedlings. The effect of drought stress on the percentage of root colonization was significant (Fig. 1). When the root segments were stained the root mycorrhizas clearly visible intraradical a: arbuscules, b: vesicles, c: internal hyphae were found (Fig. 1). Drought stress reduced the mycorrhizal colonization in both varieties. The rate of root colonization was similar between the two varieties. Also, colonization in the control treatment was about 9% higher than the - 0.4 MPa, while its rate did not significantly between the - 0.4 and - 0.8 MPa treatments (Fig. 1).

3.2 Content of macro-nutrients (N, P, and K) in roots and shoots

The three-way interaction between different levels of drought stress, mycorrhiza, and variety was significant on the nitrogen (N) and phosphorus (P) contents of the shoot (Table 1). However, the P and N contents of roots and potassium (K) level of the shoot and root were evaluated based on their significance at the 5% level as interaction and main effects (Table 1). Water-deficit stress reduced N accumulation in the shoot. High levels of water stress (-0.8 MPa) significantly declined these nutrients uptake in Bodgold (Bod) variety. Regardless of AM application, the N content of the shoot of the Soroksári (Sor) variety was much higher than the Bod variety; nonetheless, the difference got more evident under water stress; so that the N content of the shoot of Bod was 4% lower than Sor variety for + AM treatment under normal condition. Under stress at an osmotic potential of -0.4 and - 0.8 MPa and AM-inoculated roots this value reached 13 and 35%, respectively (Fig. 2). The water stress also reduced the N content of the root, but AM partially increased the content of this nutrient uptake in the roots under stress and control conditions. Also, the N content of the root of both varieties was almost the same. However, the effect of AM on increasing the content of this nutrient was higher in Sor as compared with the Bod variety (Table 2). AM reduced the adverse effects on the P content of shoot. At the water potential of -0.4 and - 0.8 MPa, the impact of AM on the amount of P in shoot was higher in the Bod variety. Regarding the + AM treatment, the P content of shoot of the Bod variety was 20 and 27.5% at the stress level of -0.4 and - 0.8 MPa, respectively. In the case of Sor variety, this parameter was 12 and 10% higher during the -AM treatment. In general, the P level of the shoot of the Bod was lower than the Sor variety, so that the highest P content of the shoot of the Sor variety was observed upon AM inoculation (Fig. 2). The P content of the root also decreased with a reduced amount of osmotic potential. The P amount of root was higher in Sor variety. With increasing stress, the mentioned difference declined as the P content of Sor root was 25% under control condition, which decremented to 11 and 3% at osmotic potentials of -0.4 and - 0.8 MPa, respectively (Table 2). According to Table 2, AM caused a 12%

enhancement in the P content of the root. Water-deficit stress increased the K level of shoots and roots. At non-stress conditions, the K content of the shoot of the Sor was 10% higher than the Bod variety. By enhancing the stress rate, the difference narrowed so that the K content of the shoot did not significantly differ between the two varieties at a water potential of -0.8 MPa. According to the mean comparison of the main effects of the treatments (Table 2), AM generally increased the K content of shoot in the chamomile by 9%. Both water stress and AM treatments enhanced the K content of the root; where the Sor variety exhibited higher root K content. As already stated, AM increased the root K content. Under stress conditions, this increase was more profound, so that the potassium content of the AM-inoculated roots was respectively 17%, 25%, and 19% higher than the non-AM treatment under normal (Control) and water potential of -0.4 and -0.8 MPa (Table 2).

Table 1
Analysis of variance of osmotic potential, arbuscular mycorrhiza and variety and their interactions effects on nutrients uptake in

Source of variation	df	Shoot concentration						Root concentration				
		N	P	K	Zn	Fe	Cu	Mn	N	P	K	Zn
O	2	**47.2114	**81.29	**17.1565	**23.65040	**14.13444	**89.2636	**09.3939	**26.164	**61.131	**65.2966	**02.519
AM	1	**96.181	**66.25	**29.208	**68.4504	**38.1906	**66.2813	**61.3236	**25.41	**8.26	**32.506	02 ^{ns} .36
Var	1	**80.201	**32.5	**60.55	**52.500	**13.550	**66.338	*24.413	**28.1	**1.26	**16.606	**65.151
O×AM	2	**38.13	**65.0	041 ^{ns} .0	**45.289	**88.43	66 ^{ns} .9	66 ^{ns} .26	**32.3	66 ^{ns} .0	**50.31	52 ^{ns} .25
O×Var	2	**56.54	**69.2	**20.22	*84.111	**06.129	60 ^{ns} .60	*69.196	21 ^{ns} .0	**69.3	**66.18	**00.465
AM×Var	1	**58.1	**96.0	98 ^{ns} .2	33 ^{ns} .133	16 ^{ns} .1	*69.141	*56.269	*62.0	30 ^{ns} .0	**65.19	68 ^{ns} .4
O×AM×Var	2	**46.5	**06.1	08 ^{ns} .5	28 ^{ns} .28	**s35.68	39 ^{ns} .50	29 ^{ns} .80	16 ^{ns} .0	35 ^{ns} .0	69 ^{ns} .0	68 ^{ns} .4
Error	36	223.0	019.0	46.2	11.34	16.1	68.28	64.45	146.0	30.0	62.2	40.14
CV%		05.2	20.1	15.3	69.3	34.1	34.8	23.10	63.4	08.5	64.4	12.3

O: Osmotic potential, Var: Varieties, AM: Arbuscular mycorrhizal inoculation, ns non-significance at $P \leq 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$, statistical significance

Table 2

Interaction effect of osmotic potential with variety, osmotic potential with arbuscular mycorrhiza, arbuscular mycorrhiza with variety and main effect of arbuscular mycorrhiza on macro nutrients uptake of shoot and root of chamomile.

		Treatments		Shoot concentration		Root concentration	
				K (mg g ⁻¹)	N (mg g ⁻¹)	P (mg g ⁻¹)	K (mg g ⁻¹)
Two-way interactions	O×Var	Control	Bod	36.5e	10.7b	9.7e	18.6f
		PEG-0.4	Bod	51.8c	8.6c	13.2c	30.1d
		PEG-0.8	Bod	58a	4.5e	16.5a	43.7b
		Control	Sor	41d	11.3a	12.2d	24.33e
		PEG-0.4	Sor	54b	8.7c	14.7b	38.4c
		PEG-0.8	Sor	57.8a	4.9d	17a	53.7a
	O×AM	Control	Am+	40.8d	11.9a	11.5e	23.1e
		PEG-0.4	Am+	55.0b	10.1b	14.9c	38.1c
		PEG-0.8	Am+	60.0a	5.2d	17.6a	53.0a
		Control	Am-	36.7e	10.2b	10.5f	19.8f
		PEG-0.4	Am-	50.8c	7.2c	13.1d	30.5d
		PEG-0.8	Am-	55.7b	4.1e	15.8b	44.4b
	AM×Var	Am+	Bod	51.1b	8.8b	13.8b	33.4c
		Am-	Bod	46.4d	7.1c	12.5c	28.2d
		Am+	Sor	52.7a	9.3a	15.5a	42.7a
		Am-	Sor	49.1c	7.2c	13.8b	34.9b
Main effects	AM	Am+		51.9a	9.0a	14.7a	38.1a
		Am-		47.8b	7.2b	13.1b	31.6b

Water-deficit stress at three osmotic potentials (control, -0.4, and -0.8 MPa). Plants inoculated (+ AM) or not inoculated (-AM) with the arbuscular mycorrhiza fungus *F. mosseae*, two German chamomile varieties (Bodgold (Bod) and Soroksari (Sor)). Means followed by common letter are not significantly different at the level of 5% (LSD test)

3.3 Content of micronutrients (Fe, Zn, Mn, and Cu) in roots and shoot

The three-way interaction between different water-deficit stress levels, AM, and the variety was significant only on the iron (Fe) content of the shoot (Table 1). However, comparisons between Fe, zinc (Zn), and copper (Cu), and manganese (Mn) contents of the roots and other micro-elements in the shoot were evaluated based on the significance level of 5% for the main effects and the two-way interaction effects (Table 1). Water-deficit stress reduced the Fe content of the shoot in both varieties; however, AM significantly increased the amount of this nutrient at all levels. In comparison with -AM, + AM enhanced the Fe content of the shoot of the Bod variety by 12%, 12.5%, and 44% under water-deficit water potentials of 0, -0.4, and -0.8 MPa, respectively. Moreover, the Fe content of Sor was correspondingly increased by 5%, 22%, and 29 % (Fig. 2). AM also incremented the accumulation of Fe in the roots of these plants under stress conditions. On the other hand, the Fe content of the roots of the two studied varieties was not significantly different under normal conditions. A reduction in the osmotic potential declined the Fe content of root in both varieties, nonetheless, its effect was more significant on the Bod variety (Table 3).

Table 3

Interaction effect of osmotic potential with variety, osmotic potential with arbuscular mycorrhiza, arbuscular mycorrhiza with variety, and main effect of osmotic potential, arbuscular mycorrhiza on micro nutrients uptake of shoot and root of chamomile.

		Shoot concentration			Root concentration						
		Treatments	Zn (mg.kg ⁻¹)	Mn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Zn (mg.kg ⁻¹)	Mn (mg kg ⁻¹)	Cu (mg kg ⁻¹)		
Two-way interactions	O×Var	Control	Bod	94.7e	74.6a	74.7	53.4a	182.2b	64.1b	182.2b	
		PEG-0.4	Bod	227.5b	66.9b	60.3	36.6c	73.4e	53.4d	73.4e	
		PEG-0.8	Bod	142.5c	47.8c	49.7	19.7e	92.5d	40.0e	92.5d	
		Control	Sor	102.5d	78.1a	78.8	53.4a	188.4a	68.1a	188.4a	
		PEG-0.4	Sor	238.4a	80.6a	70.3	40.9b	77.2e	55.6c	77.2e	
		PEG-0.8	Sor	143.1c	48.1c	51.6	21.9d	116.3c	39.4e	116.3c	
	O×AM	Control	Am+	110.0e	83.6a	84.4a	55.9a	192.8a	74.1a	71.6a	
		PEG-0.4	Am+	237.8a	83.4a	73.8b	43.4c	84.4e	57.5b	63.4b	
		PEG-0.8	Am+	155.6c	55.6c	57.5c	22.8e	114.4c	46.9d	40.0e	
		Control	Am-	87.2f	69.1b	69.1b	50.9b	177.8b	58.1b	60.3c	
		PEG-0.4	Am-	228.1b	64.1b	56.9c	34.1d	66.3f	51.6c	54.1d	
		PEG-0.8	Am-	130.0d	40.3d	43.8d	18.8f	94.4d	32.5e	29.1f	
	AM×Var	Am+	Bod	162.9b	68.9b	67.5b	39.6b	125.2b	57.7b	56.7b	
		Am-	Bod	146.9c	57.3c	55.6c	33.5d	106.9d	47.3c	46.5d	
		Am+	Sor	172.7a	79.6a	76.3a	41.9a	135.8a	61.3a	60.0a	
		Am-	Sor	150.0c	58.3c	57.5c	35.6c	118.8c	47.5c	49.2c	
	Main effects	O	Control		98.6c	76.4a	76.7a	53.4a	185.3a	66.1a	65.9a
			PEG-0.4		233.0a	73.8a	65.3b	38.8b	75.3c	54.5b	58.8b
PEG-0.8				142.8b	48.0b	50.6c	20.8c	104.4b	39.7c	34.5c	
AM		AM+		167.8a	74.2a	71.9a	40.7a	130.5a	59.5a	58.3a	
		AM-		148.4b	57.8b	56.6b	34.6b	112.8b	47.4b	47.8b	

Water-deficit stress at three osmotic potentials (control, -0.4, and -0.8 MPa). Plants inoculated (+ AM) or not inoculated (-AM) with the arbuscular mycorrhiza fungus *F. mosseae*, two German chamomile varieties (Bodgold (Bod) and Soroksari (Sor)). Means followed by common letter are not significantly different at the level of 5% (LSD test)

Water-deficit stress reduced the Cu content of the shoots (Table 3). At normal conditions, there was no significant difference between the Mn content of the shoots of the two varieties. A decline in Mn of the shoot of the Bod variety was observed at all levels. The reduction of this nutrient was observed in the shoot of the Sor variety only at the level of -0.8 MPa. The Mn and Cu levels of the shoots in both varieties did not significantly differ, but AM increased the content of both nutrients, with a higher rate of increase in the shoot of the Sor variety. Water-deficit stress reduced Mn and Cu contents of the root. Under both stress and normal conditions, the level of these nutrients was higher in Sor root (Table 3). AM also increased the Zn level of the shoot under osmotic stress conditions. The Zn content of the shoots was higher at a water potential of -0.4 and -0.8 MPa as compared with the control condition. Regarding the root, the trend was the opposite, and under water-deficit stress, the root content of these nutrients was less than the controls. Also, the Zn level of shoot was higher in the Sor variety under all conditions, nonetheless, this was significant in normal and water potential of -0.4 MPa (Table 3). AM inoculation increased the Zn content of chamomile by 16% in (Table 3). There was no significant difference between the Mn contents of the shoots of the two varieties under normal conditions. A decrease was, however, observed in the Mn level of the Bod at all stress levels, nonetheless, the decrease in Mn content of the shoot of Sor was observed only at the stress level of -0.8 MPa. AM enhanced the Mn and Cu levels of the shoots of the Sor and Bod varieties although their difference was not significant. Water-deficit stress reduced Mn and Cu contents of the root, the level of these nutrients was higher in Sor roots than Bod under stress and control conditions. AM also increased the Zn content of the shoot under osmotic stress conditions. Result showed, the Zn amount of the shoot was higher than that of controls at a water potential of -0.4 and -0.8 MPa. Concerning the root, the trend was the opposite, as the amount of these nutrients in the root was less than the controls. Also, the Zn content of Sor shoot was higher than that of Bod in all conditions, which was significant under normal and water potential of -0.4 MPa.

3.4 Osmolytes

According to the result (Table 4), the three-way interaction of variety, water-deficit stress and AM significantly affected the proline level, whereas, the amount of total soluble sugar was affected by the interaction of water-deficit stress × variety and water-deficit stress × AM. The water-deficit stress generated by PEG increased the levels of proline and total soluble sugar in both varieties. The highest level of these osmolytes was observed in the osmotic potential of -0.8 MPa. The proline level of Sor was higher than Bod variety in all treatments. Also, in stress and non-stress conditions, the total soluble sugar content of Sor was more than that of Bod. Under stress conditions, AM increased the amount of proline in both varieties, however, its effect on increasing the osmolyte was more evident in the case of Bod variety. An increase was also observed in the amount of total soluble sugar of the AM-inoculated samples at different osmotic potentials (Fig. 3).

Table 4
Analysis of variance of osmotic potential, arbuscular mycorrhiza and variety and their interactions effects on osmolytes, activity of antioxidant enzymes (catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO)), and shoot and root dry weights.

Source of variation	df	Proline	Total soluble sugar	CAT	POD	PPO	Shoot dry weight	Root dry weight
O	2	**50.136	**390.36248	**92.3864	**62.2	**80.217	**09.1	**002.0
AM	1	**54.16	**25.2693	**51.281	**13.0	**63.36	**083.0	**008.0
Var	1	**56.30	**58.753	**45.210	**34.0	**87.33	**015.0	**00086.0
O×AM	2	**12.1	**88.419	**07.26	**009.0	**40.4	**017.0	**00083.0
O×Var	2	**04.0	**14.79	**14.43	**09.0	**19.6	**148.0	**0001.0
AM×Var	1	**44.0	87 ^{ns} .6	59 ^{ns} .2	**01.0	**35.1	**0068.0	**0003.0
O×AM×Var	2	**43.0	17 ^{ns} .17	**18.8	**013.0	**9.0	**0058.0	00009 ^{ns} .0
Error	36	006.0	40.7	832.0	0003.0	126.0	0001.0	00003.0
CV%		32.1	92.2	41.4	46.3	08.5	25.2	88.5

O: Osmotic potential, Var: Varieties, AM: Arbuscular mycorrhizal inoculation, ns non-significance at $P \leq 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$, statistical significance

3.5 The Activity Of Antioxidant Enzymes

The three-way interaction of water-deficit stress, AM, and variety was significant on the levels of POD, PPO, and CAT enzymes (Table 4). Water-deficit stress increased the activity of all three enzymes relative to the non-stress conditions. Under stress conditions, the activities of CAT and PPO were higher in Sor as compared with Bod variety. Under stress conditions, the activity of the POD enzyme in Sor variety was higher. AM also enhanced CAT and POD at control conditions as well as water potential levels of -0.4 and -0.8 MPa. The uppermost activity of these enzymes was observed in both varieties upon AM inoculation under the stress potential of -0.8 MPa. For both varieties, the uppermost activity of these enzymes was observed in AM inoculation and the stress potential of -0.8 MPa. Although the level of PPO enzyme in the stress condition was higher than the control, the highest activity was observed under water-deficit conditions (-0.4 MPa) in AM-inoculated plants (Fig. 4).

3.6 Dry Weight Of Root And Shoot

A significant three-way interaction was observed regarding water-deficit stress, AM, and variety on the dry weight of root and the shoot (Table 4). The water-deficit stress reduced the dry weight of the root and shoot (Fig. 5). On the other hand, AM + decremented the adverse effect of osmotic potential and increased root dry weight. AM + treatment, at all levels, led to higher shoot and root dry weight as compared with AM-treatment. In general, AM improved the dry weight of chamomile at different water potential levels. With decreasing the osmotic potential, shoot dry weight was declined so that the highest shoot dry weight was obtained in PEG 0, AM+, and Sor variety (Fig. 5).

The correlation analysis (Fig. 6) showed a strong correlation between different nutrients uptake and the dry weight of chamomile. The data under normal and water-deficit stress conditions with and without AMF colonization used for correlation analysis. For example, P and N contents of the shoot and Mg, Fe, and Cu levels of root showed a slightly positive and significant correlation with shoot dry weight. In chamomile, the content of each nutrient showed some relations with the uptake of other nutrients and most of these correlations were synergistic (positive). For example, a slightly positive and significant correlation was observed between the Mg, P, N, Cu, and Fe nutrients of the shoot. Moreover, the Mg and P contents of the root, in addition to being highly correlated with each other, showed a positive correlation with other nutrients uptake such as N, Fe, and Cu of shoots and roots. Although the effect of the nutrients on each other was more synergistic, but a significant negative correlation was also observed between the shoot and root as well as between K and N contents of the shoot.

4. Discussion

The colonization rate of mycorrhizal fungi reflects the degree of infection and affinity between AM and the host plant. Under all water potentials, the AM colonization rate was over 57%, indicating a relatively high affinity between the selected AM and chamomile varieties. Under water stress, however, mycorrhizal colonization rate decreased significantly, which likely had an impact on the microbial activities, and affected the function of AM symbionts to a certain extent. This decrease in colonization was due to water shortage in the studied pots because environmental factors strongly affect colonization, also, this decrease may be due to the low carbon availability in the host plants under drought stress, or because drought stress could have inhibited spore germination and hyphal growth in the rhizosphere soil (Chen et al. 2020). Wu and Xia (2006) found that drought stress significantly decreased the mycorrhiza colonization of *Glomus versiforme*. They suggested that arid and semiarid environments had adverse effects on mycorrhiza fungi developments in host plants.

Under water-deficit stress, the uptake of many nutrients declines due to reduced nutrient mass flow and diffusion (Zhao et al. 2020). Our results and other studies showed that osmotic stress caused by PEG impairs the uptake of micro and macronutrients (Mouradi et al. 2016). Although AM improved nutrient levels and reduced the stress damage by expanding root depth and more soil access through their hyphae, the variation trends of the uptake, accumulation, and transfer of nutrients vary in different species of plants and AM under water-deficit stress condition. In many studies, AM has reported to increase the uptake of nutrients such as N (Hashem et al. 2019), P (Zardak et al. 2018), K (Zhao et al. 2015), Cu, Zn, Fe (Abbaspour et al. 2012), and Mn (Wu and Zou 2009) under the water-deficit condition in different plants. Moreover, AM can affect the uptake of nutrients by producing different compounds. For instance, AM increased the amount of P absorbed by plants via increasing the activity and production of enzymes such as phosphatase (Hu et al. 2013). It has been shown that AM not only improved the P uptake but also increased the uptake of N and nutrition in the plant through enhancing the hydraulic conductivity of the root under water-deficit stress (Gholamhoseini et al. 2013; Kong et al. 2014).

Another compound produced by AM is chelating agents such as siderophores, which can ameliorate the uptake of micro-nutrients such as Zn and Fe in the plants (Dehghanian et al. 2018). Although some studies have shown that increasing the concentration of P by mycorrhiza has a positive effect on the Zn content, but another reason for the increase in the amount of Zn in the roots and shoots of mycorrhizae-inoculated plants is the rise in the diffusion-limited process of Zn (Lehmann et al. 2014). By increasing the osmotic potential, the pores will be closed which will reduce transpiration and imbalance the active transport, thereby reducing the transfer of nutrients from the root to the shoot (Silva et al. 2009) while increasing the K content of the shoot by mycorrhizae, increasing stomatal conductance and improved the transport of nutrients from the roots to the shoots (Ruiz-Lozano and Azcón 1995).

The increased activity of POD, CAT (Uzilday et al. 2012) and PPO (Thipyapong et al. 2004) enzymes under stress condition indicated their crucial role in enduring water-deficit stress. CAT has been considered as the most indispensable enzyme for counteracting the hydrogen peroxide produced under stress conditions (Khanna-chopra and Selote 2007). POD is among the major H_2O_2 -binding enzymes in cytosol and chloroplasts whose level also rapidly increases rose under water-deficit stress. Under water-deficit stress, an increment was observed in the CAT and POD activities in diverse members of the Asteraceae family, such as *Silybum marianum* (Nouraei et al. 2018), *Carthamus tinctorius* L (Chavoushi et al. 2019), *Helianthus annuus* L (Ghobadi et al., 2013). In line with our results, other studies have also reported that AM has increased the levels of POD and PPO (Meddich et al. 2015; Tyagi et al. 2017) in various plants under water-deficit stress. One of the reasons for the increase in POD enzyme by AM could be the expression of its encoding genes in inoculation with AM (Mustafa et al. 2017). Although CAT is a metalloenzyme and thus its activity depends on the availability of metal nutrients (Armada et al. 2016), in the present study, AM improved the uptake of metal nutrients; however, the effect of mycorrhizal inoculation on CAT enzyme levels under stress conditions was very different and depended on the plant species and even the species of mycorrhizal fungi (Wu and Zu 2009). The increase in CAT activity by *F. mosseae* (Amiri et al. 2015) and other species has been reported in many plants under water-deficit stress (Aalipour et al. 2020; Al-Al-Arjani et al. 2020). Osmolytes such as total soluble sugars and proline increased under water-deficit stress; playing a significant role in regulating the osmotic potential of the plant (Khan et al. 2015). Proline is an amino acid and can be stored in the cytoplasm, which in addition to osmotic regulation of the cell, detoxifies ROS and protects membrane integrity and stabilizes proteins/enzymes, and serve as one of the plant's solutions to reduce stress damage (Ashraf et al. 2007). In the current study, the increased leaf proline level was observed by *Funneliformis mosseae* under water-deficit stress.

The increase in proline content can be assigned to the effect of AM on increasing the N content of the plants under water-deficit stress (Augé 2001). High N levels in the plant under water stress can significantly influence the genes involved in proline biosynthesis which finally increase proline (Monreal et al. 2007; Wang et al. 2011). In another study, an increase was reported in total soluble sugar under drought stress conditions, which is consistent with our results (Al-Arjani et al. 2020). AM increased the level of total soluble sugar in plants as it increased the activity of sucrose-metabolized enzymes which had a positive and significant relationship with glucose, fructose, and sucrose contents (total soluble sugars) (Wu et al. 2017). As observed, under water-deficit stress, plant growth decreased due to reduced osmotic regulation ability, disruption of the solute uptake system, disturbance of osmotic balance, and excessive energy requirements to produce osmolytes (Munns et al. 1993). Based on the findings of this study, a loss was observed in the dry weight of shoots, roots, and flowers of chamomile under water-deficit stress (Baghalian et al. 2011). One of the causes of reduced chamomile growth under stress may be the osmoregulation imbalance and the disruption in the salt absorption system or the high level of energy required for counteracting the stress (Salehi et al. 2018). An increment was also detected in the dry weight of shoots, roots, and flowers of chamomile (Bączek et al. 2019) due to the improved absorption, distribution

of nutrients, the increment of proline, total soluble sugars, and antioxidant enzymes by AM, which improved the growth performance, lowered the stress damage, enhanced the plant growth and elevated the dry weight (Al-Arjani et al. 2020).

According to the results, the dry weight of chamomile shoots, roots, and flowers reduced under drought stress (Baghalian et al. 2011). Under drought stress, plant growth was reduced due to the reduction of osmotic regulation, osmotic imbalance, and the requirement of excessive energy needs to cope with stress (i.e. the production of osmolites and disruption of the nutrient uptake) (Munns et al. 1993). All nutrients play a vital role in plant growth; the nutrients (macro and micro) were positively correlated with the plant growth (Daur et al. 2011). The effects of each nutrient on the uptake of other nutrients are very complex. According to the correlation shown in Fig. 6, the synergistic effect between many nutrients in chamomile reflects the diverse roles of these nutrients in the growth, yield, and uptake of other nutrients by chamomile. For example, sufficient Mg causes a proportional distribution of carbohydrates in the root and shoot; promoting the chamomile root growth (He et al., 2020). On the other hand, Mg affects biomass production and plant growth by proper distribution of carbohydrates and the appropriate allocation of hydrocarbons to different parts of plants (Verbruggen and Hermans 2013) or improving the plant access to N (Haberman et al. 2019) and iron (due to its vital role in photosynthesis) (Dong et al. 2019) with an effective role in vegetative growth and ultimately the accumulation of plant dry weight. This is consistent with a positive and high correlation of the dry weight of chamomile with the mentioned elements. According to the results, one of the most important causes of reduced growth of chamomile under stress conditions is the disturbed nutrients uptake (Salehi et al. 2018). The level of nutrients in the plant and the ability to uptake these nutrients are important factors in selecting the best cultivar under stress. In this regard, the Sor cultivar was almost superior to Bod in terms of both factors. On the other hand, improved absorption and distribution of elements as well as increased proline, total soluble sugar and antioxidant enzymes by mycorrhiza inoculation enhanced the growth while reducing stress-induced damages (Al-Arjani et al. 2020). In line with previous reports (Bączek et al. 2019), mycorrhiza increased the dry weight of shoots, roots, and flowers of chamomile.

5. Conclusions

Water-deficit stress increased the levels of the total soluble sugar, proline, and the activity of antioxidant enzymes (CAT, POD, and PPO) in both chamomile varieties. The amount of these enzymes and osmolytes was higher in the Soroksári variety as compared with the Bodgold variety. Water-deficit stress also reduced the uptake and transport of many nutrients from the roots to the shoots, which resulted in decreased content of nutrients such as N, P, Fe, and Mn in the shoots of both varieties. Such a reduction of nutrients in the plant declined the plant dry weight under water-deficit stress, however, the dry weight of the shoot was higher in Soroksári variety under the control treatment and water potential of -0.4 MPa as compared with the Bod variety. AM + reduced the negative effects of drought stress on the plant through increasing the nutrients uptake, osmolytes contents and the activity of antioxidant enzymes.

6. Abbreviations

AM

Arbuscular mycorrhiza

Bod

Bodgold

Sor

Soroksári

PEG

polyethylene glycol

N

Nitrogen

P

Phosphorus

K

Potassium

Fe

Iron

Zn

Zinc

Cu

Copper

Mn

Manganese

CAT

Catalase

SOD

Superoxide dismutase

APX

Ascorbate peroxidase

ROS

Reactive oxygen species

+AM

Plants inoculated with the arbuscular mycorrhiza fungus

-AM

Not inoculated with the arbuscular mycorrhiza fungus

DW

Dry weight

O

Osmotic potential

Var

Varieties

PPO

Polyphenol oxidase

7. Declarations

Authors' contributions

FE and AS designed the study, AS, MMD and FE performed experiments; FE, SH, MH and AM wrote the manuscript; all authors commented on the manuscript; All authors read and approved the final manuscript.

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Conflict of interest

The authors acknowledge that there was no conflict of interest in performing this study or analyzing its results.

Availability of data and materials

Not applicable.

Declarations Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

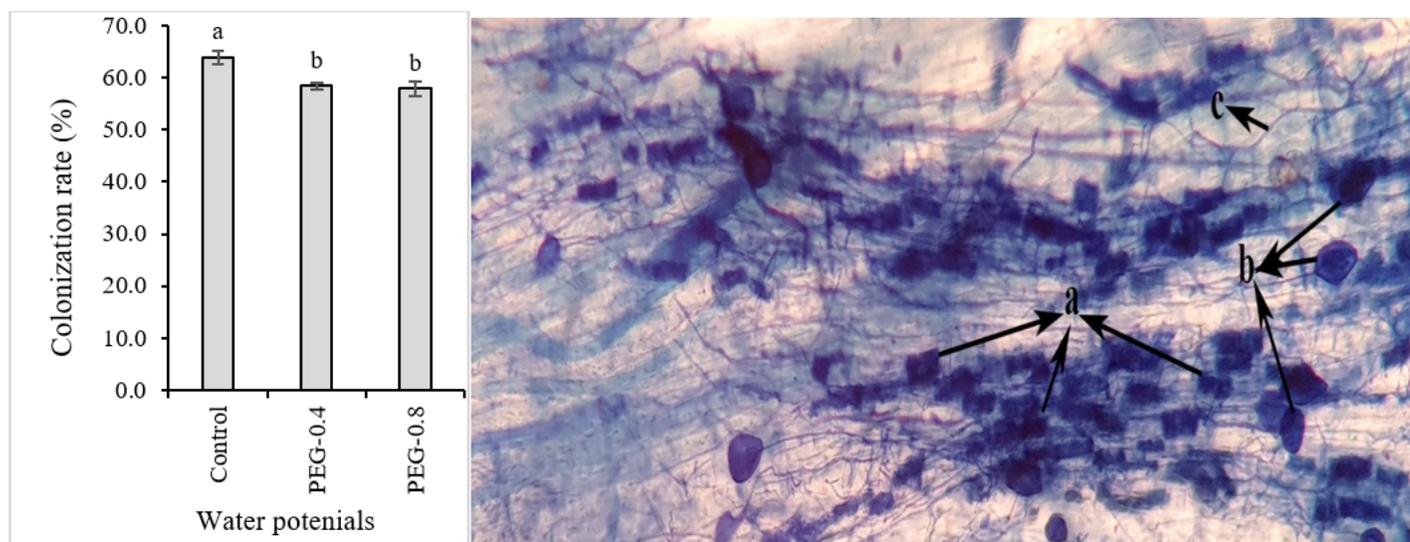


Figure 1

Structures of Arbuscular Mycorrhizal Fungi (AM) in Chamomile root, the stained in figure includes the components of mycorrhizae a: Arbuscules, b: Vesicles, c: internal hyphae (A). Colonization rate of mycorrhizal *F. mosseae* under osmotic potential (B). O** Var ns O×Var ns

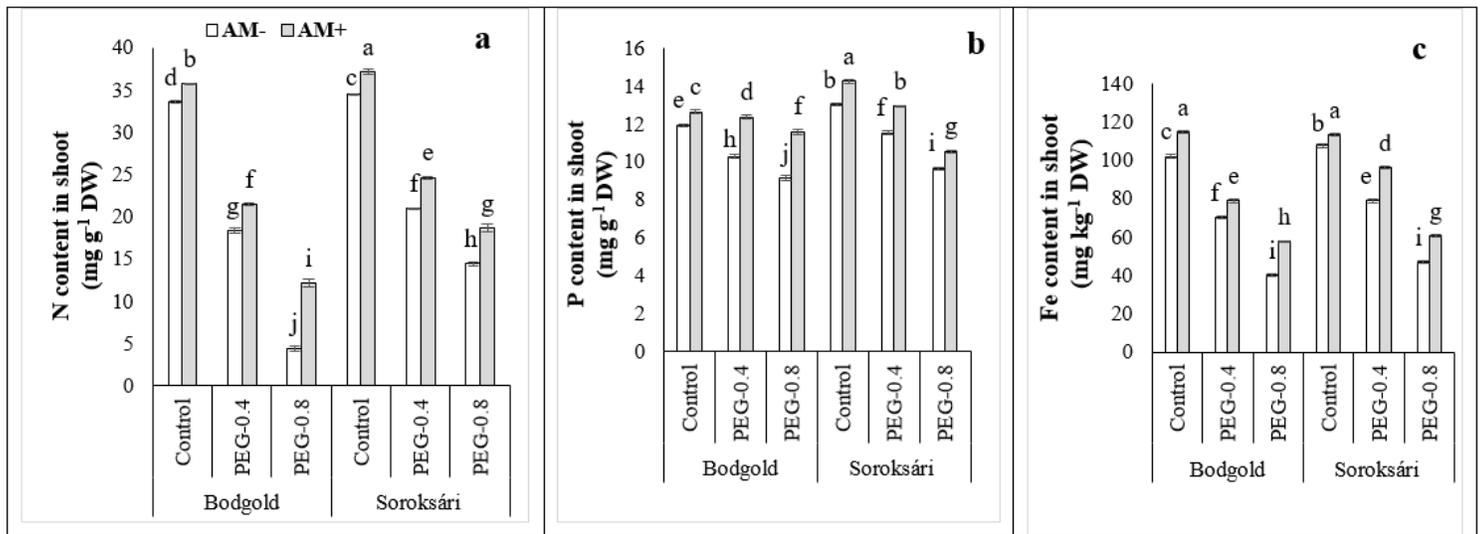


Figure 2
Interaction effect of osmotic potential (control, -0.4, and -0.8 MPa) × arbuscular mycorrhiza (AM) × variety on N content in shoot (a), P content in shoot (b) and Fe content in shoot (c) of chamomile. Means within a column followed by the different letter are significantly different at $P \leq 0.05$. Standard error of the mean (n = 3)

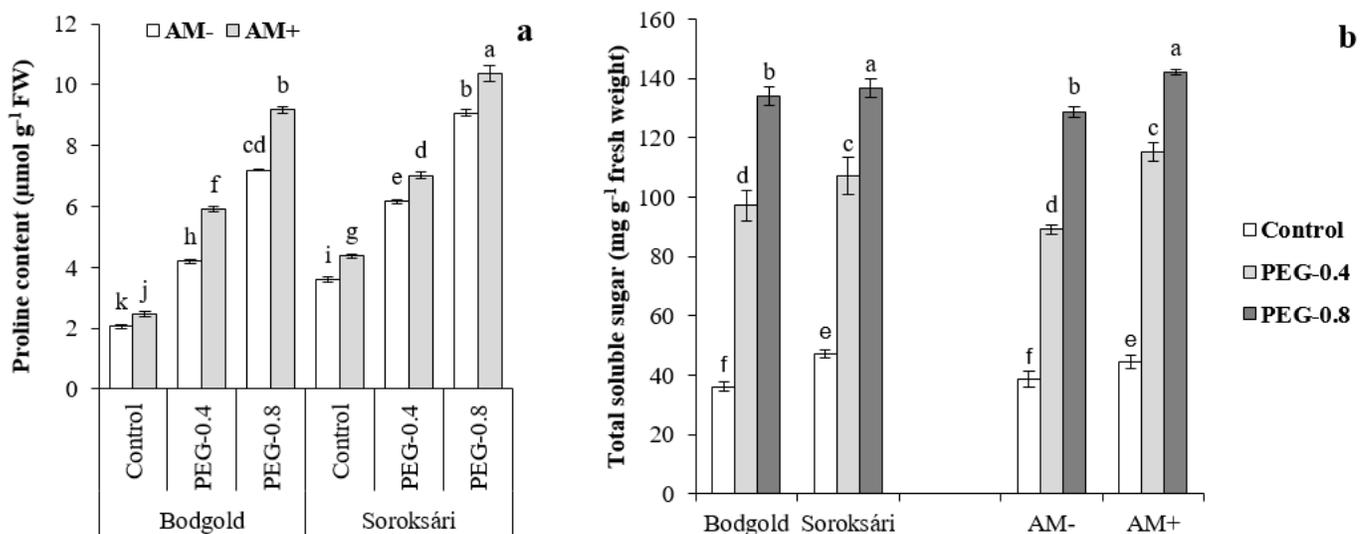


Figure 3
Interaction effect of osmotic potential (control, -0.4, and -0.8 MPa) × arbuscular mycorrhiza (AM) × variety and on proline (a) and total soluble sugar (b) content of chamomile. Means within a column followed by the different letter are significantly different at $P \leq 0.05$. Standard error of the mean of total soluble sugar (n = 6) and proline (n = 3)

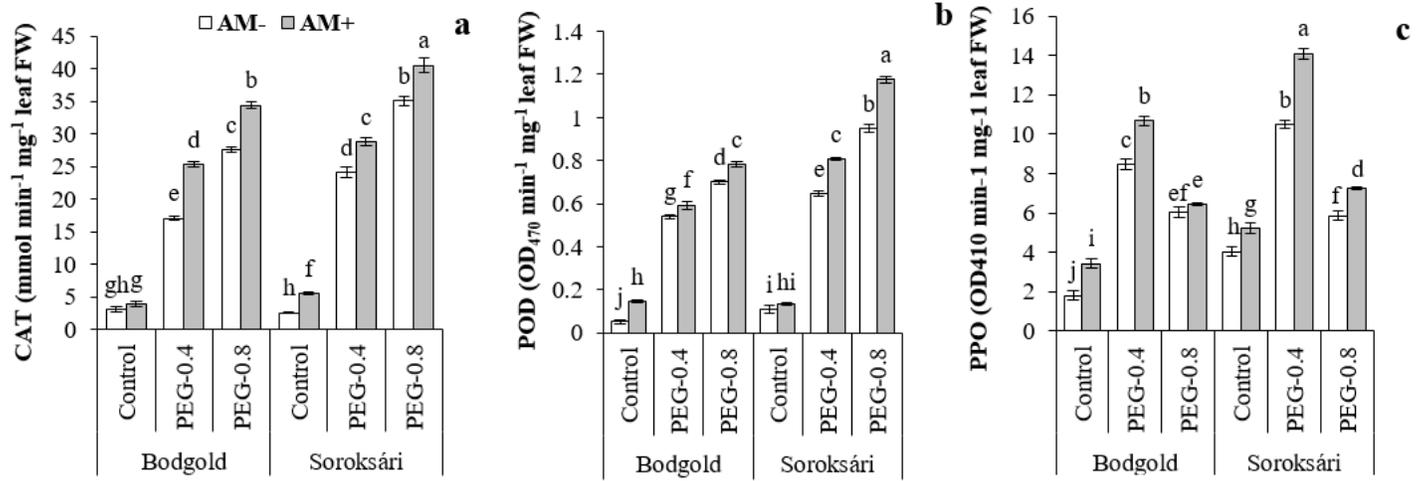


Figure 4 Interaction effect of osmotic potential (control, -0.4, and -0.8 MPa) × arbuscular mycorrhiza (AM) × variety and on catalase (CAT) (a), peroxidase (POD) (b) and polyphenol oxidase (PPO) (c) activities of chamomile. Means within a column followed by the different letter are significantly different at $P \leq 0.05$. Standard error of the mean ($n = 3$)

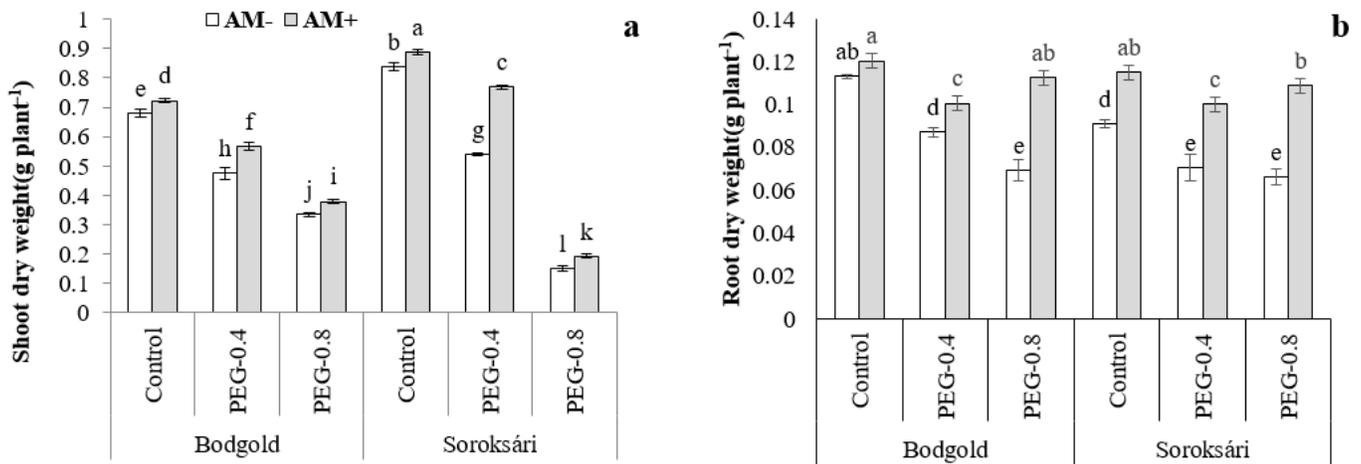


Figure 5 Interaction effect of osmotic potential (control, -0.4, and -0.8 MPa) × arbuscular mycorrhiza (AM) × variety and on dry weight of shoot (a), root (b). Means within a column followed by the different letter are significantly different at $P \leq 0.05$ (LSD). Standard error of the mean ($n = 3$)

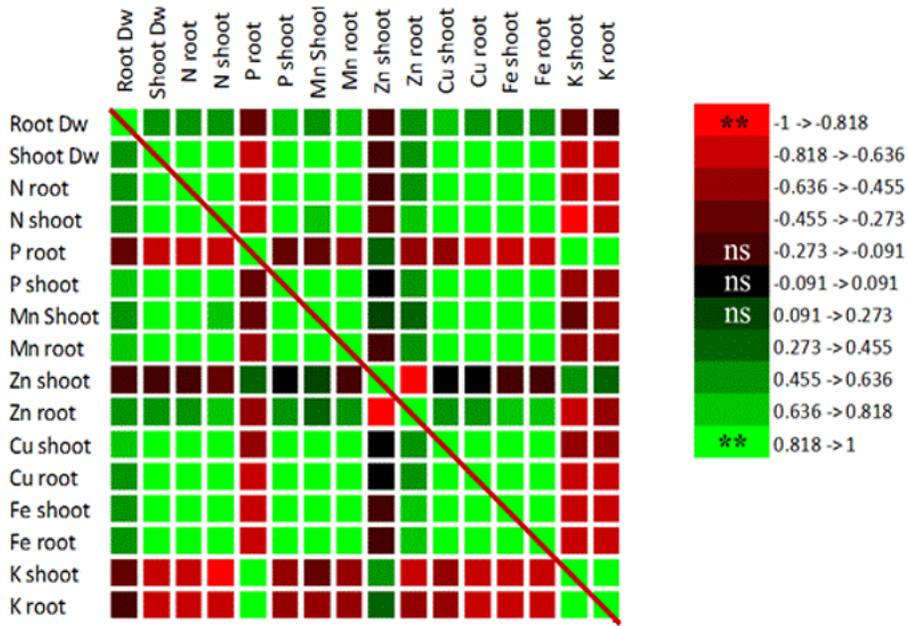


Figure 6

Correlation coefficients among nutrients uptake and root and shoot dry weight (DW). ns non-significance; ** Significant at 0.01 probability levels.

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