The combined use of silicon and arbuscular mycorrhizal fungus mitigate the adverse effects of drought stress on *Matthiola incana*

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Abstract

Effects of the application of potassium silicate (PS) and an arbuscular mycorrhiza fungus (AMF) (*Glomus mosseae*) were investigated on alleviating the impacts of drought stress on *Matthiola incana* in a split-split-plot experiment in two consecutive years. The main plot was assigned to drought stress at three levels of irrigation (25%, 50%, 70%, of field capacity) comparing with control (100% of field capacity) and the sub-plots were assigned to AMF at two levels (0 and 200 g of fungi in pots) and sub-sub plots were assigned to potassium silicate (PS) at three levels (0, 200, and 400 ppm). The application of PS with fungus increased colonization percentage regardless of the drought stress level. Regarding the interaction of drought stress and PS, it was revealed that the application of 400 and 200 ppm of PS elevated the contents of chlorophylls *a*, *b*, and total at all drought stress levels. Also, deficit irrigation versus the normal irrigation increased malondialdehyde (MDA) content both in non-AMF and AMF-containing treatments. The lowest catalase (CAT) and peroxidase (POX) contents were obtained from the treatment of 400 ppm of PS, while the highest were related to the treatment of 0 ppm of PS. Proline content was reduced by the application of 200 g of AMF and 400 ppm of PS at moderate and severe (25% FC) drought stress levels. In general, at moderate and severe drought stress levels, some physiological traits were improved by the foliar application of 400 ppm PS and *Glomus mosseae* inoculation.

Keywords: antioxidant; membrane stability; oxidative stress; relative water content; silica.
Abbreviations: Field Capacity: FC; potassium silicate: PS; Reactive Oxygen Species: ROS; peroxidase: POX; catalase: CAT; Malondialdehyde: MDA; Membrane stability: MSI; Relative Water Content: RWC; Leaf proline content: LPC; Arbuscular mycorrhiza fungus: AMF

Introduction

Matthiola incana, known as night-scented stock, is one of the most prominent ornamental species of flowers in Iran, the demand for which has significantly increased in recent years due to their colour diversity and pleasant aromas (Elmi, 2009). *Matthiola incana* plant belongs to the Brassicaseae (Cruciferae) family. The flowers are in various colours and because of the beauty of the flowers and their bright colors and fragrant aromas are used in garden design (Al-Khafaji, 2020). Global climate change, rising temperatures, and soil moisture fluctuations, including drought and flood, have reduced crop growth and yields over the past 50 years. Drought has a profound effect on agriculture as it disrupts crop production programs, reduces stocks, and decreases farmer incomes so that drought is considered the most important abiotic factor limiting plant growth and yields (Fazeli Kakhaki and Moayedi, 2018; Saleh *et al.*, 2018). lack of water in the plant adversely affects the growth, development and yield of plants and a prolonged Drought severely diminishes plant productivity (Basu *et al.*, 2016; Asefpour, 2020). Plants have developed complex physiological and biochemical adjustments to tolerate Drought, including the activation of antioxidative enzymes and water status through the accumulation of organic osmolytes such as soluble carbohydrates and free amino acids, particularly proline (Singh *et al.*, 2017). The association of roots with arbuscular mycorrhizal fungi (AMF) is the most abundant symbiosis in the plant kingdom (Willis *et al.*, 2013). The colonization of roots by mycorrhizal fungi increasing nutrient uptake and plant tolerance to stress (Abdel *et al.*, 2016). The hypha of AMF can serve as an important channel absorbing nutrients from the soil by plants, thereby promoting nutrient uptake by host plants (Karagiannidis *et al.*, 2002), enhancing plant growth, yield, and quality (Bowles *et al.*, 2016; Rozpądek *et al.*, 2016), improving plant photosynthetic capacity (Mathur *et al.*, 2018), accelerating plant growth, and enhancing plant stress resistance through impacting root exudation (Gong *et al.*, 2003). Previous studies have demonstrated that drought stress can be mitigated by using AMF (Wu and Zou, 2017) and potassium silicate (PS) (Liang *et al.*, 2003).

According to Miransari (2010) and Das *et al.* (2021), AMF can alleviate the adverse effects of drought stress by increasing the plant's ability to take up water and nutrients. These fungi increase the absorption surface area for nutrient and water absorption by spreading their hyphae into the soil. AMF significantly increases water, nitrogen, and phosphorus absorption. Arbuscular mycorrhizae fungi (AMF) have been shown to increase biochemical access to phosphorus sources so that increasing phosphatase activity of root may lead to the acidification of the rhizosphere (Ruiz-Llozno, 2003). The increased exploitation of the soil by the hyphae (mycelium growth beyond the P depletion zone) and the competitive ability of the hyphae to absorb localized sources of orthophosphate result in higher efficiency in P acquisition and often in a growth promotion of mycorrhized plants (Bucher, 2007). It has been revealed that in inoculated plants, mycorrhizal a increases water efficiency by increasing photosynthesis and production of more photosynthetic assimilates per unit of water consumed (Fitzsimons and Miller, 2010). In a study on the coexistence of onion with mycorrhizal a, an increase in water use efficiency was reported (Bolandnazar *et al.*, 2007). Moreover, mycorrhizal al fungi had a significant direct contribution to the uptake of phosphorus, zinc, and copper under water stress (Smith *et al.*, 2011).

Silicon is present by about 2% of shoot dry weight (Hodson *et al.*, 2005) in most plant tissues and organs such as cell walls, intercellular space, epidermis, roots, leaves, and reproductive organs (Voleti *et al.*, 2008). Although Si is not considered an essential element for higher plants, numerous studies have demonstrated that (Si) is a beneficial element that alleviates abiotic and biotic stresses in plants (Etesami and Jeong, 2018). Si supplementation alleviates drought stress of plants. Several mechanisms including the activation of photosynthetic enzymes (Yin *et al.*, 2014). the activation of enzymatic antioxidant defense systems, increased water use efficiency (Hajiboland *et al.*, 2017). nutrient uptake (Chen *et al.*, 2011) root growth and hydraulic conductance (Lux *et al.*, 2002) and the accumulation of organic osmolytes (Ming *et al.*, 2012) are involved in (Si)-mediated growth improvement under Drought stress (Rizwan *et al.*, 2015). This element increases the

strength of epidermal cell walls and protects plants against water loss by cuticular transpiration, thereby preventing water evaporation from the leaves (Mehrgan *et al.*, 2018). Optimal nutrition of silica (about 2% of shoot dry weight) improves the growth and volume/weight development of roots, which ultimately increases the total area for element adsorption (Liang *et al.*, 2005). Gunes *et al.* (2008) observed that Si applications provided higher Si and proline concentrations in sunflower plants exposed to drought stress. Probably a more efficient osmotic adjustment as a function of higher proline concentrations is part of the tolerance mechanism to water deficit.

Drought stress has diverse destructive effects on plant morphology and physiology, and it can damage the cell components through enhanced production of reactive oxygen species (ROS) (Ebrahimi *et al.*, 2021; Amiri Forotaghe *et al.*, 2022). The presence of ROS in the cellular environment destroys major cellular macromolecules, such as DNA, RNA, and vital enzymes, which is called oxidative damage (Ashraf and Ali, 2008). Also, one of the conspicuous effects of oxygen free radicals on cell health is the destruction of cell membranes (Bhattacharjee and Mukherjee, 2002). Plants can eliminate oxygen free radicals and their toxic effects by synthesizing various antioxidant enzymatic compounds, such as peroxidase and catalase (Meloni *et al.*, 2003). Therefore, given the potential of PS and AMF for mitigation of drought stress effects, the objectives of the present study are to elucidate the influence of PS and AMF on water status, and activity of antioxidative defense system in *Matthiola incana* plants under drought conditions. Also, this study was conducted to investigate the alleviation of drought stress effects by using the symbiosis effects of AMF and PS in *M. incana*.

Materials and Methods

To study the effects of potassium silicate (PS) foliar application and arbuscular mycorrhiza fungi (AMF) application on the alleviation of drought stress effects in *M. incana*, an experiment was carried in completely randomized design with a split-split plot design, with three replications at the Parandis Greenhouse Complex located in Khorasan Razavi province, Mashhad, Iran (36°42′N, 59°65′E) during 2018-2019. The experiment was conducted in a heated polyethylene greenhouse. The relative humidity of the greenhouse was 50±5% and the minimum and maximum temperatures were 12 °C and 25 °C, respectively. The main plot was assigned to drought stress at four levels (25%, 50%, 70% of FC) comparing with control (100% of field capacity), the sub-plot was assigned to AMF (*Glomus mosseae*) at two levels (0 and 200 g in pot), which was added to the substrate in each pot (as recommended by Turan Biotechnology Company), and the sub-sub-plot was assigned to PS at three levels (0, 200, and 400 ppm). In this experiment, we used 10 kg pots, which contained a mixed substrate of soil, sand, and manure (rotten cow dung) at an equal ratio (Table 1). Soil, sand and manure mixture Sterilized by steaming at 100 °C for 1 h. The F1 hybrid seeds of *M. incana* cv. 'Regal White' were sown in plastic pots (5 seeds/pot). Before planting, seeds were surface sterilized in sodium hypochlorite for 20 min, washed five times in sterile water. After germination, only one plant was kept in each pot. The AMF was first added to the substrate in each pot. Drought stress was applied based on the weight method by the use of a tensiometer (Farzi and Golami, 2018). The irrigation was withdrawn and the necessary measurements were made at the 50% flowering stage. PS (with the trade name of SILICOCARB) was prepared at predetermined concentrations (0.4 and 0.2 g L^{-1}), and the plants were sprayed weekly with the prepared solutions two weeks after establishment in the pots until the end of the experimental period. Eight-week-old plants (five weeks after starting PS treatments) were harvested and samples were taken for biochemical analyses immediately. At the flowering stage, the physiological traits were measured. At this stage, leaf samples prepared from each treatment were placed in plastic and immediately stored in the freezer (-80 °C) until measurement and 5 sample were prepared from each replication. Calculation amount of each trait was performed according to the given references.

Table 1. Some chemical and physical properties of the substrate

Soil texture	Total N (%)	P (ppm)	K (ppm)	EC (ds.m^{-1})	pH
Loam	0.06	8	270	2.8	7.4
Rotten cow dung	0.58	4800	1500	3.1	7.2

Biochemical analysis and antioxidant enzyme activities

Proline content

Free proline content was determined according to the method described by Bates *et al*. (1973) with slight modifications. A 2% homogenate of the fresh leaf was prepared with 3% sulfosalicylic acid and centrifuged at 11500 rpm at 4 °C for 15 min. Two milliliter of supernatant was taken and 2 mL of glacial acetic acid and acid ninhydrin reagent was added. The reaction mixture was boiled in water bath for 60 min and then cooled on ice. Then 4 mL of toluene was added and incubated at room temperature for 30 min. Tubes were then shaken for 15 sec and allowed to stand for 10 min for phase separation. The upper phase was separated and absorbance was measured at a wavelength of 520 nm by using spectrophotometer (UV-1800, Shimadzu, Japan). The proline concentration was determined based on comparison with 0-160 µM proline standard curve and expressed on a (µmol/g leaf FW) basis.

Measuring plant pigments

For extraction and measurement of plant pigments, one gram of fresh leaves was ground, with 5 mL of acetone 80% and centrifuged at 8000 rpm for 10 min. The rate of light absorbance by extract was measured at 645, 663, 470, 480, and 510 nm wavelengths using a spectrophotometer (UV-1800, Shimadzu, Japan). The concentrations of leaf pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) were calculated using leaf tissue and methanol equations (Dere *et al*., 1998).

Equation (1): $C_a = 15.65A_{666} - 7.340A_{653}$

Equation (2): $C_b = 27.05A_{653} - 11.21A_{666}$

Equation (3): $C_{x+c} = (1000 A_{470} - 2.860 C_a - 129.2 C_b)/245$

in which C_a and C_b are the chlorophyll *a* and *b* contents, respectively, and C_{x+c} is the total carotenoid content.

Determination of the malonyldialdehyde (MDA) content

For the measurement of lipid peroxidation in leaves, the thiobarbituric acid (TBA) test, which determines MDA as an end product of lipid peroxidation was used, according to the method of Velikova *et al*. (2000). The amount of MDA–TBA complex (red pigment) was calculated from the extinction coefficient 155 mM^{-1} cm^{-1}.

Membrane stability index measurement

The membrane stability Index (MSI) was assessed by measuring leaf electrolyte leakage (Sairam and Saxena, 2000). For this purpose, fully developed leaves of each plant were utilized for RWC measurement and discs of leaves were prepared. Discs were washed with distilled water and placed in a tube. 20 mL of distilled water was added to them. They were placed on a rotary shaker at 25 °C for 24 h. Electrical conductivity (EC) was measured using an EC-meter (EC$_1$). In order to measure the total electrolyte leakage, the discs were placed in an autoclave at 120 °C for 20 min. Their EC was measured again (EC$_2$). The membrane stability index (MSI) was calculated as:

Equation (4): MSI= [1-(EC1/EC2)]× 100

Relative water content measurement

To determine leaf relative water content (RWC), fully-developed young leaves were first weighed, soaked in distilled water for 24 h, thereafter, they were blotted dry gently on a paper towel, and the turgid weight was determined. After drying in an oven at 70 °C for 24 h, RWC was calculated using the following equation (Smart and Bingham, 1974).

Equation (5): RWC = [(Fresh weight − dry weight) /(turgid weight − fresh weight)] × 100%

Determination of leaf catalase and peroxidase content

The catalase activity was measured using the method described by Srinivas *et al.* (1999) at 25 °C. To prepare enzyme extract, 150 mg of ground fresh leaf samples were mixed by 1.5 mL of extract buffer containing 2 mL of 0.1 M phosphate buffer with pH = 6.8 and centrifuged at 13000 rpm for 20 min. 3 mL of reaction mixture containing 2800 µl of 50 mM phosphate buffer with pH = 6.8, 100 microliters of 25 mM H_2O_2 and 100 microliters of enzymatic extract were then added to the solution. Samples were read at time phases of 0, 20, 40, and 60 seconds with a spectrophotometer (UV-1800, Shimadzu, Japan) at a wavelength of 240 nm. The catalase (CAT, EC 1.11.1.6) activity was assayed by monitoring the decrease in absorbance of H_2O_2 at 240 nm; unit activity was taken as the amount of enzyme which decomposes 1 µmol of H_2O_2 in one min. An extinction coefficient of 40 $mM^{-1}cm^{-1}$ was used to calculate the enzyme unit.

For the measurement of peroxidase activities, the following reaction mixture (3 mL) was used: 10 mM potassium phosphate buffer, pH 7.0, 0.02–0.06 mL enzymatic extract and 0.6 mL guaiacol 1% (w/v) aqueous solution. The reaction was started by adding 0.15 mL of 100 mM H_2O_2 and the optical density at 470 nm was recorded in a spectrophotometer (UV-1800, Shimadzu, Japan) against an identical mixture to which no H_2O_2 was added. The linear initial reaction rate was used to estimate the activity, which was expressed in mmol of guaiacol dehydrogenation product (GDHP) formed per milligram protein per minute, using the extinction coefficient of 26.6 mM^{-1} cm^{-1}(Velikova *et al.*, 2000).

Root colonization measurement

For evaluation of the AMF colonization, the fine roots (1 g FW) were cleared in 10% (v/v) KOH and stained with 0.05% (v/v) trypan blue in lacto–glycerin. The colonization rate of the roots (%) was estimated by counting the proportion of root length containing fungal structures (arbuscules, vesicles and hyphae) using the gridline intersect method (Mc Gonigle *et al.*, 1990). To measure the percentage of root colonization, 100-cm pieces of stained roots were put on girded Petri dishes in a 1 cm dimension. Then, the percentage of colonization was determined using the intersection of root segments with the grid lines, and the average percentage of colonization was calculated for each treatment (Rejaea *et al.*, 2016).

Data analysis

At the flowering stage, 5 sample were prepared from each replication. Data were analysed in the Minitab-18 and MSTATC software packages, and the means were compared using Duncan's test at the 5% probability level. Graphs were drawn in MS-Excel2010 software.

Results

Leaf proline content (LPC)

The application of AMF at all stress levels decreased LPC compared to non-fungal treatments. The plants exposed to 25% of FC but no fungus and those exposed to 75% of FC and AMF exhibited the highest and the lowest LPC (9.58 and 1.31 µmol/g leaf FW), respectively (Figure 1A). PS and drought stress had significant effects on LPC so that increasing drought stress resulted in elevated LPC. However, the application of PS to plants exposed to drought stress decreased LPC (Figure 1B). The application of 400 ppm PS in all

drought stress levels, except normal irrigation conditions, reduced LPC versus the control. The interaction of AMF and PS was significant (P < 0.01) so that increasing PS concentrations and the presence of fungus reduced LPC (Table 4). The comparison of the interactions among drought, mycorrhizal fungus, and PS revealed that LPC was increased at more severe stress levels. Moreover, the application of fungus and PS at a rate of 400 ppm at all of stress levels had a reducing effect on LPC, and these treatments contained lower concentrations of LPC than those not treated with the fungus and PS (Table 6).
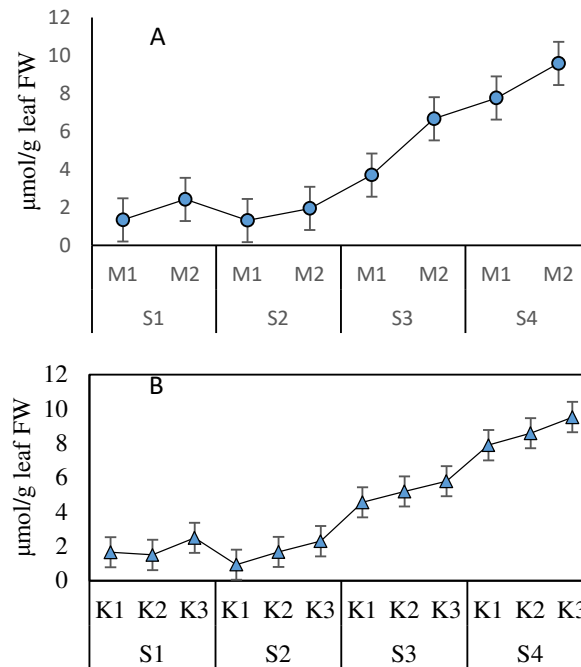

Figure 1. Interactive effect of A) drought stress and AMF and B) drought stress and potassium silicate on leaf proline content (LPC)
Drought stress: S1, S2, S3, and S4 represent 100%, 75%, 50%, and 25% of FC, respectively. AMF: M1 and M2 indicate the application and non-application of the fungus and Potassium silicate: K1, K2, and K3 denote 400, 200, and 0 ppm, respectively. Vertical bars indicate ±SE.

Carotenoid and chlorophyll a, b and total contents
At the severe stress level of 25% of FC, the carotenoid content was significantly increased (by about 31%) versus the control (Figure 2A). The interaction of drought stress and AMF boosted the carotenoid content by increasing the stress level, but the carotenoid content was decreased with the application of the AMF at all drought levels (Figure 2A). The interaction of drought stress and PS revealed that PS at the rates of 400 and 200 ppm elevated the chlorophyll *a*, *b*, and total contents at different levels of drought stress compared to no PS application (Figure 2B). As shown in Table 4, the PS × fungus interaction led to an elevated level of total chlorophyll. Concerning the interaction of drought stress, AMF, and PS (Table 5), the lowest carotenoid content (1.43 mg/g FW) was measured in the fungal treatment with 100% of FC and 400 ppm of PS. This reduction was observed at each level of stress in the treatment with AMF and PS. The highest levels of chlorophylls *a*, *b*, and total (26.75, 30.58, and 76.46 mg/g, respectively) belonged to the -AMF + 100% of FC + 400 ppm of PS treatment. All the chlorophyll types were the lowermost in the AMF-free treatment + 25% of FC + - PS (Table 5).

MDA levels

MDA levels rose with increasing drought stress under the interaction of drought stress and AMF, but the AMF application at all stress levels reduce MDA concentrations, thereby improving the plant status (Table 2). The drought and PS interaction (Table 3) demonstrated that the MDA level was maximal at 25% stress level when no PS was applied and the minimal (3.26 μmol/g leaf FW) was obtained at 100% stress level applied with 400 ppm of PS. The antioxidant defense system is one of the strategies plants use to cope with oxidative stress. In the interaction of drought stress, AMF and PS (Table 5), it was observed that - AMF + 100% FC + - PS treatments resulted in the lowest level of MDA (3.03 μmol/g leaf FW).
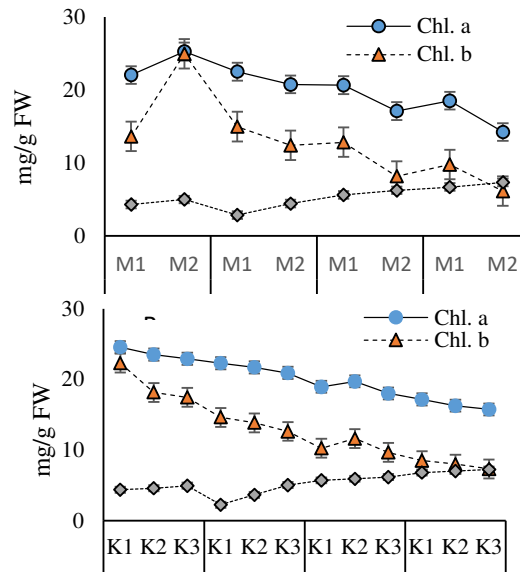

Figure 2. Interactive effect of A) drought stress and AMF and B) drought stress and potassium silicate on chlorophyll *a* and *b* and carotenoid

Drought stress: S1, S2, S3, and S4 represent 100%, 75%, 50%, and 25% of FC, respectively. AMF: M1 and M2 indicate the application and non-application of the AMF and Potassium silicate: K1, K2, and K3 denote 400, 200, and 0 ppm, respectively. Vertical bars indicate ±SE.

Table 2. The comparison of means for the interactive effect of the AMF and drought stress on physiological traits of *Matthiola incana*

Drought	Fun.	Fun. Colonize. (%)	Total Chl. (mg/g)	CAT (μmol H$_2$O$_2$/g leaf FW)	MDA (μmol/g leaf FW)	POX (μmol H$_2$O$_2$/g leaf FW)	RWC (%)	MSI
S1	M1	71.8[a]	61.66[c]	0.51[g]	3.61[g]	4.69[g]	53.42[b]	25.79[b]
	M2	54.3[b]	71.07[a]	0.46[g]	3.19[h]	1.96[h]	75.61[a]	33.71[a]
S2	M1	50.6[b]	63.54[b]	0.75[f]	4.30[f]	8.01[f]	48.25[c]	23.02[c]
	M2	41.0b[c]	58.97[d]	0.97[e]	5.0[e]	10.50[e]	41.88[de]	18.26[d]
S3	M1	34.57[c]	59.76[d]	1.26[d]	5.66[d]	12.00[d]	45.07[cd]	20.38[d]
	M2	21.2[d]	53.88[f]	2.29[b]	6.25[a]	13.70[c]	39.14[e]	15.20[e]
S4	M1	4.6[e]	56.16[e]	1.90[c]	6.91[b]	16.71[b]	34.52[f]	10.99[f]
	M2	0[f]	48.42[g]	2.65[a]	8.73[c]	21.25[a]	33.55[g]	6.38[g]

Drought: S1, S2, S3, and S4 represent 100%, 75%, 50%, and 25% of FC, respectively. AMF: M1 and M2 indicate the application and non-application of the AMF, respectively. Means with a similar letter are not significantly different at a level of 0.05 based on the Duncan's test.

Table 3. The comparison of means for the interactive effect of PS and drought stress on physiological traits of *Matthiola incana*

Drought	Fun.	Fun. Colonize (%)	Total Chl. (mg/g)	CAT (µmol H_2O_2/g leaf FW)	MDA (µmol/g leaf FW)	POX (µmol H_2O_2/g leaf FW)	RWC (%)	MSI
S1	K1	57.6[c]	69.50[a]	0.40[g]	3.26[g]	2.00[f]	63.22[a]	33.16[a]
	K2	67.1[b]	65.19[b]	0.49[fg]	3.39[g]	3.38[ef]	58.99[ab]	29.45[b]
	K3	75.1[a]	64.41[b]	0.57[f]	3.56[g]	4.61[e]	56.33[b]	26.64[b]
S2	K1	31.5[e]	62.61[c]	0.78[e]	4.66[f]	9.48[d]	46.14[c]	21.29[c]
	K2	35.6[de]	61.85[c]	0.88[e]	5.03[ef]	9.88[cd]	45.31[c]	20.62[c]
	K3	40.4[d]	59.31[d]	0.92[e]	5.25[de]	10.67[cd]	43.75[c]	20.01[cd]
S3	K1	11.5[f]	56.26[e]	1.86[d]	5.44[cd]	11.24[cd]	42.93[c]	19.03[cde]
	K2	15.0[f]	58.89[d]	1.71[d]	5.60[cd]	11.82[bc]	42.16[c]	17.49[de]
	K3	15.0[f]	55.30[ef]	1.76[d]	4.83[e]	13.25[b]	41.23[c]	16.86[e]
S4	K1	0[g]	54.66[f]	2.55[a]	7.66[b]	19.26[a]	33.53[d]	10.34[f]
	K2	0[g]	53.39[g]	2.02[c]	7.73[b]	18.45[a]	31.12[d]	8.79[fg]
	K3	0[g]	48.83[h]	2.26[b]	8.15[a]	19.24[a]	28.45[d]	6.93[g]

Drought: S1, S2, S3, and S4 represent 100%, 75%, 50%, and 25% of FC, respectively. Potassium silicate: K1, K2, and K3 denote 400 200, and 0 ppm, respectively. Means with a similar letter are not significantly different at a level of 0.05 based on the Duncan's test.

Leaf relative water content (RWC) and membrane stability index (MSI)

Leaf RWC was used as an important indicator of plant water status in the assessment of cell damage. The interaction of drought stress and AMF treatment showed that the highest and lowest leaf RWC contents (75.61% and 33.55%) were at 100% and 25% of FC both in the AMF and non-fungi treatments, respectively (Table 2). Except for the normal irrigation, however, the presence of AMF at stress levels increased leaf RWC from 3% to 8% compared to the fungus-free treatment at the same drought level. The interaction of all three treatments including stress, AMF, and PS revealed that the highest leaf RWC (68.62%) belonged to -AMF + 100% of FC + 400 ppm of PS. Except for the 100% of FC treatment, the presence of AMF had an increasing effect on leaf RWC, but different levels of PS did not affect this trait significantly (Table 6). The results showed that except for the normal irrigation, the highest MSI (33.71) was obtained from the fungus treatments. With increasing stress intensity, the amount of MSI was decreased, but the amount of the decrease in MSI in non-fungal treatment was more than that in the AMF treatments (Table 2). MSI was used as a membrane permeability index for the assessment of cell damage. Due to the interaction between the drought stress and the AMF, the highest MSI (33.71) and the lowest MSI (6.38) were measured in the fungus-free treatments at 100% and 25% of FC, respectively (Table 2). The result of drought stress × PS concentrations (Table 3) showed that with increasing the stress levels, MSI decreased significantly. In all irrigation treatments, the highest amount of MSI was in 400 ppm PS treatment. The results showed that the application of AMF and 400 ppm PS caused the highest MSI. The interaction of drought stress, AMF, and PS showed that MSI was maximized (38.44%) in the -AMF +100% of FC+ 400 ppm of PS treatment. Except for the treatment at 100% of FC, the other AMF containing, stress treatments had an increasing effect on MSI, but different PS levels had no significant effects on the MSI (Table 6).

Catalase (CAT) and Peroxidase (POX) contents

The interaction between stress levels and AMF showed that CAT levels rose with increasing stress levels, but the AMF treatment could reduce the level of this enzyme at all stress levels compared to the fungus-free treatment (Table 2). In the interaction of drought stress and PS (Table 3), the highest CAT level (2.55 µmol

H_2O_2/g leaf FW) was obtained from the plants treated with 25% of FC and 400 ppm of PS. At the other stress levels, different levels of PS had no significant effects on CAT reduction. In the interaction of AMF and PS (Figure 3), the presence of fungus and PS had a reducing effect on CAT levels, and various levels of PS were significantly different. The interaction of the three treatments showed, the lowest CAT level (0.39 μmol H_2O_2/g leaf FW) was found in the 100% of FC + AMF + 400 ppm of PS treatment. The highest CAT level was in drought stress of 25% of FC + AMF + PS free treatment (Table 5). The results for the interaction of stress levels and AMF showed that the POX levels were increased with rising stress levels, but the AMF could reduce this enzyme at all stress levels compared to the AMF -free treatment (Table2). In the interaction of drought stress and PS (Table3), the highest POX level (19.26 μmol H_2O_2/g leaf FW) was obtained from the plants exposed to 25% of FC and treated with 400 ppm of PS. The interaction of the three treatments (drought stress, AMF, and PS), showed the lowest POX level (1.29 μmol H_2O_2/g leaf FW) belonged to normal irrigation of 100% of FC+ AMF + - PS treatment (Table 6).
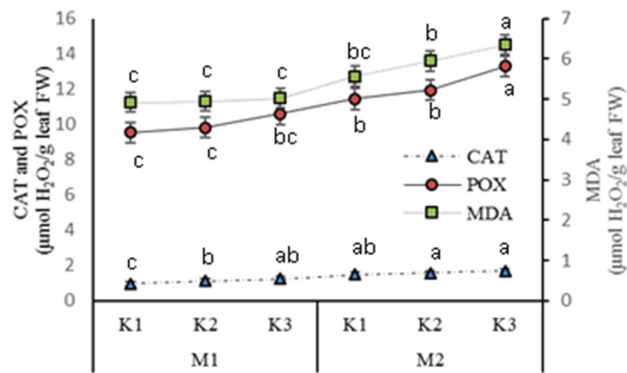

Figure 3. The interactive effect of AMF and potassium silicate on catalase (CAT), peroxidase (POX), and MDA of *Matthiola incana*

AMF: M1 and M2 indicate the application and non-application of the AMF, respectively; Potassium silicate: K1, K2, and K3 denote 400, 200, and 0 ppm, respectively. Vertical bars indicate ±SE.

Table 4. The comparison of means for the interactive effect of AMF and potassium silicate on physiological traits of *Matthiola incana*

Fun.	PS	Chl. a (mg/g)	Chl. b (mg/g)	Caroten. (mg/g FW)	Fun. Coloniz. (%)	Total Chl. (mg/g)	RWC (%)	MSI	LPC (μmol/g leaf FW)
M1	K1	21.07[ab]	14.73[a]	4.33[c]	53.2b	60.80[b]	47.27[a]	21.40[a]	3.19[d]
	K2	21.48[a]	13.38[ab]	4.75[d]	55.9ab	61.58[a]	45.64[ab]	20.52[ab]	3.45[d]
	K3	20.29[c]	13.13[bc]	5.56[bc]	64.5a	58.46[c]	45.48[ab]	18.82[bc]	3.93[c]
M2	K1	20.42[bc]	11.93[cd]	5.26[c]	25.4d	60.72[b]	43.30[bc]	19.92[abc]	4.24[c]
	K2	19.13[d]	12.46[bcd]	5.89[ab]	33.1cd	58.07[c]	43.19[bc]	18.25[c]	5.11[b]
	K3	18.50[d]	11.63[d]	6.16[a]	37.9c	55.46[d]	41.69[c]	16.40[d]	6.12[a]

AMF: M1 and M2 indicate the application and non-application of the AMF, respectively; Potassium silicate: K1, K2, and K3 denote 400 200, and 0 ppm, respectively. Means with a similar letter are not significantly different at a level of 0.05 based on the Duncan's test.

Table 5. The comparison of means for the interaction of the drought stress, AMF and PS on morphological traits of *Matthiola incana*

Drought	Fun	PS	Chl. a (mg/g)	Chl. b (mg/g)	Carotenoid (mg/g FW)	Total Chl. (mg/g)	CAT (µmol H$_2$O$_2$/g leaf FW)	MDA (µmol/g leaf FW)	Fun. Coloniz. (%)
S1	M1	K1	22.38[c-g]	14.02[cde]	1.43[p]	62.55[de]	0.39[l]	3.50[nop]	51.5b
		K2	22.05[d-g]	13.52[cde]	2.24[op]	61.44[ef]	0.41[l]	3.59[nop]	59.7b
		K3	21.76[d-h]	13.38[c-f]	3.08[no]	61.00[cfg]	0.48[l]	3.76[mno]	70.1a
	M2	K1	26.75[a]	30.58[a]	4.71[j-m]	76.46[a]	0.49[l]	3.35[op]	35.6cd
		K2	24.99[ab]	22.81[b]	4.87[j-m]	68.93[b]	0.52[kl]	3.20[op]	42.5c
		K3	24.07[bc]	21.54[b]	5.43[f-k]	67.83[b]	0.63[jkl]	3.03[p]	45.6bc
S2	M1	K1	23.35[bcd]	16.48[c]	4.06[mn]	65.76[c]	0.74[jk]	4.02[lmn]	41.8c
		K2	22.82[cde]	15.30[cd]	4.37[lm]	64.74[c]	0.76[jk]	4.31[klm]	45.0bc
		K3	21.39[e-i]	13.18[c-g]	4.92[j-m]	60.13[fgh]	0.76[j]	4.58[g-l]	46.6bc
	M2	K1	21.25[e-i]	12.79[c-g]	4.49[klm]	59.46[f-i]	0.82[ij]	5.30[ghi]	24.0
		K2	20.61[f-j]	12.43[d-h]	5.11[i-l]	58.96[g-j]	1.01[hi]	5.75[fgh]	26.1
		K3	20.45[g-k]	12.09[d-h]	5.18[h-l]	58.50[h-k]	1.09[h]	5.92[cfg]	32.7d
S3	M1	K1	19.83[h-l]	11.80[d-i]	5.30[g-l]	58.06[i-l]	1.18[gh]	4.77[ijk]	23.4e
		K2	22.48[c-f]	15.07[cd]	5.65[e-j]	63.86[cd]	1.39[fg]	5.00[ij]	27.1de
		K3	19.70[i-l]	11.69[d-i]	5.97[d-i]	57.36[j-m]	1.23[gh]	5.23[hij]	27.8de
	M2	K1	18.08[lmn]	8.74[h-l]	6.15[c-h]	54.46[nop]	2.29[cd]	6.11[ef]	5.6f
		K2	16.96[mno]	8.20[i-l]	6.24[c-g]	53.93[op]	2.25[d]	6.21[def]	7.6f
		K3	16.30[no]	7.66[jkl]	6.40[b-f]	53.24[p]	2.33[bcd]	6.43[de]	7.1f
S4	M1	K1	18.74[j-m]	10.22[c-j]	6.57[b-c]	56.83[klm]	1.55[f]	7.50[c]	0g
		K2	18.59[klm]	9.64[f-k]	6.73[a-d]	56.30[lmn]	2.18[de]	6.80[d]	0g
		K3	18.33[lm]	9.46[g-k]	6.86[a-d]	55.36[mno]	1.98[e]	6.57[de]	0g
	M2	K1	15.59[op]	6.81[jkl]	7.08[abc]	52.50[pq]	2.49[bc]	7.83[c]	0g
		K2	13.98[pq]	6.39[kl]	7.37[ab]	50.48[q]	2.54[b]	8.65[b]	0g
		K3	13.17[q]	5.22[l]	7.61[a]	42.30[r]	2.91[a]	9.72[a]	0g

Drought stress: S1, S2, S3, and S4 represent 100%, 75%, 50%, and 25% of FC, respectively; AMF: M1 and M2 indicate with and without the AMF, respectively; Potassium silicate: K1, K2, and K3 denote 400, 200, and 0 ppm, respectively. Means with a similar letter are not significantly different at a level of 0.05 based on the Duncan's test.

Table 6 (continued). The comparison of means for the interactions of drought stress, AMF, and PS on morphological traits of *Matthiola incana*

Drought	Fun	PS	POX (μmol H$_2$O$_2$/g leaf FW)	RWC (%)	MSI	LPC (μmol/g leaf FW)
S1	M1	K1	2.70^{lm}	57.83^{bc}	27.88^{cd}	1.17^{klm}
		K2	4.53^{kl}	52.48^{cd}	25.46^{cde}	1.31^{jklm}
		K3	6.85^{jk}	49.95^{cde}	24.03^{def}	1.57^{i-m}
	M2	K1	2.37^{lm}	68.62^{a}	38.44^{a}	1.10^{klm}
		K2	2.23^{lm}	65.49^{ab}	33.45^{b}	1.04^{lm}
		K3	1.29^{m}	62.71^{ab}	29.24^{bc}	0.76^{m}
S2	M1	K1	7.41^{j}	49.07^{c-f}	23.72^{d-g}	1.83^{i-l}
		K2	7.71^{ij}	48.87^{c-f}	22.88^{e-h}	2.00^{i-l}
		K3	8.92^{g-j}	46.80^{d-g}	22.46^{e-h}	3.41^{fg}
	M2	K1	11.54^{fgh}	43.21^{d-i}	18.86^{h-l}	2.13^{h-k}
		K2	12.06^{fg}	41.74^{e-i}	18.36^{h-l}	2.300^{hij}
		K3	12.41^{efg}	40.70^{e-j}	17.56^{ijkl}	2.47^{ghi}
S3	M1	K1	9.57^{g-j}	45.62^{d-h}	21.71^{e-i}	3.19^{fgh}
		K2	10.53^{f-i}	45.32^{d-h}	20.05^{f-j}	3.77^{f}
		K3	11.40^{fgh}	44.28^{d-i}	19.40^{g-k}	4.13^{f}
	M2	K1	12.91^{ef}	40.23^{f-j}	16.37^{j-m}	5.93^{e}
		K2	13.11^{ef}	39.00^{g-k}	14.93^{k-n}	6.64^{de}
		K3	15.10^{de}	38.18^{g-k}	14.32^{lmn}	7.45^{cd}
S4	M1	K1	18.45^{bc}	36.57^{h-k}	12.28^{mno}	7.66^{cd}
		K2	16.49^{cd}	35.27^{i-l}	11.30^{no}	7.70^{c}
		K3	15.21^{de}	31.72^{j-m}	9.38^{op}	7.91^{c}
	M2	K1	20.03^{b}	30.49^{j-m}	8.40^{opq}	8.12^{c}
		K2	20.41^{b}	26.98^{lm}	6.27^{pq}	9.49^{b}
		K3	23.31^{a}	25.19^{m}	4.48^{q}	11.15^{a}

Drought stress: S1, S2, S3, and S4 represent 100%, 75%, 50%, and 25% of FC, respectively; AMF: M1 and M2 indicate with and without the AMF, respectively; Potassium silicate: K1, K2, and K3 denote 400, 200, and 0 ppm, respectively. Means with a similar letter are not significantly different at a level of 0.05 based on the Duncan's test.

Colonization percentage

In all inoculated samples, the percentage of colonization was increased compared to the control, and this fungal behavior was well visible in the interactions. With increasing drought stress levels, the percentage of colonization was significantly reduced in both AMF and non- AMF samples. Lower available water in 50% FC resulted in a decline in colonization percentage (by about 50%) compared with the control (Table 2). The application of PS at stress levels of 100%, 75%, and 50% of FC improved the percentage of colonization, except in 25% FC treatment in which the use of PS did not effect on colonization percentage (Table 3). Abbasdokht and Safdari (2016) showed that the colonization percentage in soybean plants was increased by the increment of potassium fertilizer under natural irrigation conditions. In our study, it was observed that the highest colonization was recorded by increasing PS level to 400 ppm in 100% FC treatment (Table 3). The amount of this trait was decreased with the increase in the drought stress level. However, the effect of PS in improving colonization percentage was observed at lower levels of drought stress. It seems that the presence of fungi in the root environment can improve water uptake and nutritional elements by increasing the length and development of their hyphae in the root environment. The maximum colonization was obtained by applying 400 ppm potassium silicate with using of AMF under natural irrigation conditions. The amount of this trait

was decreased with incremental the drought stress level. However, the effect of potassium silicate in improving of colonization percentage was observed at low levels of drought stress (Table 5).

Discussion

Our results showed that the application of Si and (AMF) in *Matthiola incana* might alleviate the adverse effects of drought stress in a synergistic manner. Drought is a major abiotic stress, that interrupts the normal physiological and biochemical processes in plants and causes significant yield losses in almost all major field crops (Ullah *et al.*, 2019). Water-deficit stress reduces tissue water content and cell turgidity followed by a decrease in transpiration rate due to a partial stomatal closure that restricts CO_2 entry into the leaf for photosynthesis (Yin *et al.*, 2010). It has been found that water stress causes certain disorders at different structural and functional levels (Zhang *et al.*, 2015; Vaghar and Ehsanzadeh, 2018).

Increasing the soil water tension induced stress in the stock plants and consequently stimulated the production of proline. PS application could reduce the proline level. The accumulation of organic osmolytes such as proline, as a common response in plants under water stress that leads to an osmotic gradient with the environment (Ashraf *et al.*, 2011), which was observed in the stock plants in this work. The alleviating effect of AMF and PS, however, was not mediated by osmotic-adjustment, and the concentration of proline rather decreased in the leaves of +AMF and +PS plants. Leaf RWC was used as an important indicator of plant water status in the assessment of cell damage. The results showed that the presence of AMF and PS at stress levels increased leaf RWC. Thus, the method of stress alleviation of AMF and PS for an increase in water uptake capacity may be less expensive than the strategy of osmotic-adjustment (Nadian, 2011; Munns, 2002).

At all drought levels, proline accumulated in the leaves while the application of AMF and PS reduced leaf proline concentrations. The accumulation of proline in the leaves under water stress is a well-documented phenomenon, but the role of proline in osmo-tolerance remains controversial. In some studies, the accumulation of proline has been correlated with stress tolerance (Zou *et al.*, 2013), but other researchers suggest that proline accumulation is a symptom of stress impairment rather than stress tolerance (Crusciol *et al.*, 2009). Our results support the view that proline accumulation under stress is a symptom of stress, so the Si-mediated reduction of proline concentrations is a sign of stress alleviation. Similarly, the AMF-mediated reduction of the proline concentration suggests that the AMF colonization of plants, partially mitigated the effects of drought stress and reduced proline concentrations in leaves. These results are in agreement with a previous report (Porcel *et al.*, 2012).

The results showed that with increasing stress intensity, the amount of MSI was decreased and the application of AMF and PS have positive effect on this index. MSI was used as a membrane permeability index for the assessment of cell damage. Decreased MSI under drought stress has also been reported in other studies (Turkan *et al.*, 2005; Salehpour *et al.*, 2009). Increased ROS compounds under stress can induce lipid peroxidation and the oxidation of fatty acids in membrane, and increasing electrolytes leakage (Eraslan *et al.*, 2007). *Arbuscular mycorrhiza* inoculation improves polyunsaturated fatty acid concentrations in cellular membranes for maintaining their structural and functional integrity (Alqarawi *et al.*, 2014; Bearden, 2001; Begum *et al.*, 2019).

In this study, Si application improved membrane stability index under drought stress. It is generally accepted that the maintenance of integrity and stability of membranes under water stress is a major component of drought tolerance in plants (Bajji *et al.*, 2002). However, numerous studies have demonstrated that Si is one of the important elements in plants and plays an important role in their drought tolerance. Inside the plant, the silicate is a non-mobile element, which, after being deposited inside the cell, becomes a polymer gel and reduces ion leakage from the bio-membranes (Liang *et al.*, 2007). The results of this study are consistent with the results of Zhu *et al.* (2004).

The concentration of photosynthesis pigments in leaves was significantly affected by PS, AMF and drought stress. At the severe stress level, the carotenoid content was significantly increased versus the control. The PS and AMF interaction led to an elevated level of chlorophyll *a*, *b*, and total. In the present study, the decrease in the pigment content may have been resulted from decreased RWC (Matthews et al., 1990). The chlorophyll content is an important factor in plant productivity since it is directly proportional to the photosynthesis rate of plant for biomass production (Wang *et al.*, 2002). The chlorophyll *a:b* ratio significantly was increased with water deficit. Therefore, chlorophyll *b* was more sensitive than chlorophyll *a* (Sultana *et al.*, 1999). A decrease in chlorophyll concentration in stress conditions could be attributed to the increased activity of the chlorophyll-degrading enzyme chlorophyllase (Epstein, 1999). Furthermore, PS and AMF application increased chlorophyll *a* and *b* under drought stress treatment (Figure 2). However, in this research, with increasing the intensity of drought stress, the amount of chlorophyll *a* and *b* decreased and the amount of carotenoids increased. The carotenoid content, which protects chlorophyll against light oxidation, was increased at higher levels of stress to hinder further chlorophyll degradation, which is consistent with the results reported by Abdalla *et al.* (2007), Mohammadkhani and Heidari (2007) and Mittler (2002).

In this study drought stress caused the activation of antioxidative defense enzymes (CAT and particularly of POX). The Application of AMF in drought stress treatments increased the amount of CAT, POX and MDA. Also, the amount of CAT and MDA increased with the application of PS in severe drought stress treatments. The elevated activity of CAT in plants is an adaptive mechanism protecting cells against oxidative damage by reducing hydrogen peroxide concentration produced from cellular metabolism (Gill and Tuteja, 2010). By stimulating the activity of CAT through the detoxification of hydrogen peroxide, Si prevents oxidative stress and prevents hydroxyl radical production (Rastgoo and Alemzadeh, 2011). Results showed, PS application increased the antioxidant activity enzymes and lead to enhanced the protected plant against the stress condition. The findings of the present study are consistent with the results of Balakhnina and Borkowska (2013) and Gong *et al.* (2003).

The results showed an increase in MDA content under severe drought stress and the application of AMF. In all drought stress levels, PS application decreased MDA. MDA is a peroxidation product of unsaturated fatty acids in phospholipids. Therefore, its synthesis under stress conditions is used as a marker to indicate lipid peroxidation (Katsuhara *et al.*, 2005). Si which removes reactive oxygen species, decreases the permeability of cell membranes and increases the activity of catalase and peroxidase and superoxide dismutase enzymes, thereby indirectly reducing the peroxidation of cell membrane lipids and the amount of MDA (Liang *et al.*, 2007). According to Ma and Yamaji (2006), Lata *et al.* (2011) and Ouzounidou *et al.* (2016) the Si-mediated increase in antioxidant defense abilities is a beneficial result of Si rather than a direct effect.

Leaf RWC was used as an important indicator of plant water status in the assessment of cell damage. The presence of AMF at stress levels increased leaf RWC in all stress treatments. In general, the presence of an extensive external hyphae network of AMF as an extension of the root system of the host plant can absorb and transfer water and nutrients to the plant through tiny pores that are out of the plant's access, thereby increasing the moisture of the shoot and roots of symbiotic plants compared to non-symbiotic species (Nadian, 2011). In all drought stress treatments, the application of AMF and PS increased RWC compared to their non-application. There are reports on the increased root hydraulic conductance by Si, and the increase was attributed to the Si-mediated upregulation of transcription of some aquaporin genes (Liu *et al.*, 2014).

The inoculation of plants with AMF increased the level of colonization in the roots. This increase was greater in plants grown under normal irrigation than under drought stress conditions. These data agreed with the general observation that AMF levels are lower in water stress conditions than in normal irrigation (Al-Karaki *et al.*, 2004). In severe drought stress, the percentage of root colonization was significantly reduced. Soil moisture can affect spore germination and development. However, the effect of PS in improving colonization percentage was observed at lower levels of drought stress (Wu and Xia, 2006). In similar study results demonstrated a synergistic effect of (AMF) and Si on improving the growth of strawberry not only under

Drought stress but also under control conditions (Moradtalab *et al*., 2019). In our experiment, it was observed that with increased moisture in the rhizosphere and the application of 400 ppm PS under the use of AMF, the fungus colonization was increased. It seems that, when suitable conditions are provided, the plant provides carbohydrates to the AMF and the fungi provide more water and micronutrients to the host plant. Then, the symbiotic association becomes beneficial and the AMF colonization increases.

Conclusions

The results showed that drought stress had destructive effects on plant growth by reducing the amount of plant pigments and RWC and sharply increasing electrolyte leakage, MDA, LPC, POX, and CAT contents. Based on the results, the application of AMF and PS improved some physiological traits of the stock plants under drought stress. At all levels of drought stress (75, 50, and 25% of FC), the use of AMF and high rates of PS (400 ppm) improved membrane stability index and RWC and reduced the amount of antioxidant enzymes (CAT and POX). The maximum colonization was obtained by applying 400 ppm PS with the use of AMF under non-stress conditions (100% FC treatment). The amount of this trait was decreased with an increase in the drought stress level. In this context, the protective effects of PS and AMF treatments seems to be related to an improved micronutrients status, an increased expression of the enzymatic antioxidative defense system, and an elevated water uptake capacity and water use efficiency. results indicate that PS and AMF alleviated water stress in a synergistic manner. In general, the results revealed that the application of PS (400 ppm) and AMF improved some physiological and antioxidant enzymes content at mild drought stress levels. Our results revealed that the application of PS (400 ppm) and mycorrhizal fungi improved some physiological and antioxidant enzymes content at mild drought stress levels. Our results provide a theoretical basis for the application of Si supplements and AMF in *Matthiola incana* cultivation.

Authors' Contributions

A.M. and A.S. perceived the idea. A.S. conducted the field experiments. A.S., S.F.F.K. and Sh.Kh. collected and analysed the data. A.S. wrote first draft of manuscript. A.M., S.F.F.K., and Sh.Kh. reviewed and prepared final draft of manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abbasdokht H, Safdari F (2016). Relationship between board inoculation of Rhizobium japonica and mycorrhizal fungus with root colonization and yield quantitative and qualitative of the soybean plant (*Glycine max* L) in different levels of potassium. Agricultural Science and Sustainable Production 26(3):57-70.

Abdalla MM, El-Khoshiban NH (2007). The influence of water stress on growth, relative water content, photosynthetic pigments, some metabolic and hormonal contents of two *Triticum aestivum* cultivars. Journal of Apply Science Research 3:2062-2074.

Al-Karaki GN, McMichael B, Zak J (2004). Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14:263-269. *https://doi.org/10.1007/s00572-003-0265-2*

Alqarawi AA, Abd Allah EF, Hashem A (2014). Alleviation of salt-induced adverse impact via mycorrhizal fungi in *Ephedra aphylla* Forssk. Journal of Plant Interactions 9(1):802-810. *https://doi.org/10.1080/17429145.2014.949886*

Amiri Forotaghe Z, Souri MK, Ghanbari Jahromi M, Mohammadi Torkashvand A (2021). Influence of humic acid application on onion growth characteristics under water deficit conditions. Journal of Plant Nutrition 45(7):1030-1040. *https://doi.org/10.1080/01904167.2021.1994604*

Asefpour Vakilian K (2020). Machine learning improves our knowledge about miRNA functions towards plant abiotic stresses. Scientific Reports 10:3041. *https://doi.org/10.1038/s41598-020-59981-6*

Ashraf M, Akram NA, Al-Qurainy F, Foolad MR (2011). Drought tolerance: roles of organic osmolytes, growth regulators, and mineral nutrients. Advances in Agronomy 111:249-296. *https://doi.org/10.1016/B978-0-12-387689-8.00002-3*

Ashraf M, Ali Q (2008). Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (*Brassica napus* L.). Environmental and Experimental Botany 63:266-273. *https://doi.org/10.1016/j.envexpbot.2007.11.008*

Bajji M, Kinet J, Lutts S (2002). The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. Plant Growth Regulation 36:61-70. *https://doi.org/10.1023/A:1014732714549*

Balakhnina T, Borkowska A (2013). Effects of silicon on plant resistance to environmental stresses. International Agrophysics 27(2). *https://doi.org/10.2478/v10247-012-0089-4*

Basu S, Ramegowda V, Kumar A, Pereira A (2016). Plant adaptation to drought stress. F1000Research 5. *https://doi.org/10.12688/f1000research.7678.1*

Bates LS, Walderen RD, Taere ID (1973). Rapid determination of free proline for water-stress studies. Plant and Soil 39:205-702. *https://doi.org/10.1007/BF00018060*

Bearden BN (2001). Influence of arbuscular mycorrhizal fungi on soil structure and soil water characteristics of vertisols. Plant and Soil 229:245-258. *https://doi.org/10.1023/A:1004835328943*

Begum NC, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, Ahmed N, Zhang LX (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. Frontiers in Plant Science 10:1068. *https://doi.org/10.3389/fpls.2019.01068*

Bhattacharjee S, Mukherjee AK (2002). Salt stress-induced cytosolute accumulation, antioxidant response and membrane deterioration in three rice cultivars during early germination. Seed Science and Technology 30:279-287.

Bolandnazar S, Aliasgarzad N, Neishabury MR Chaparzadeh N (2007). Mycorrhizal colonization improves onion (*Allium cepa* L.) yield and water use efficiency under water deficit condition. Scientia Horticulturae 114:11-15. *https://doi.org/10.1016/j.scienta.2007.05.012*

Bowles TM, Barrios-Masias FH, Carlisle EA, Cavagnaro TR, Jackson LE (2016). Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. Science of the Total Environment 56:1223-1234. *https://doi.org10.1016/j.scitotenv.2016.05.178*

Bucher M (2007). Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. New Phytologist 173:11-26. *https://doi.org/10.1111/j.1469-8137.2006.01935.x*

Chen W, Yao X, Cai K, Chen J (2011). Silicon alleviates drought stress of rice plants by improving plant water status, photosynthesis and mineral nutrient absorption. Biological Trace Element Research 142(1):67-76. *https://doi.org/10.1007/s12011-010-8742-x*

Crusciol CC, Pulz AL, Lemos LB, Soratto RP, Lima GPP (2009). Effects of silicon and drought stress on tuber yield and leaf biochemical characteristics in potato. Crop Science 49:949-954. *https://doi.org/10.2135/cropsci2008.04.0233*

Das D, Basar NU, Ullah H, Salin KR, Datta A (2021). Interactive effect of silicon and mycorrhizal inoculation on growth, yield and water productivity of rice under water-deficit stress. Journal of Plant Nutrition. *https://doi.org/10.1080/01904167.2021.1927087*

Dere Ş, Güneş T, Sivaci R (1998). Spectrophotometric determination of chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. Turkish Journal of Botany 22(1):13-18.

Ebrahimi M, Souri MK, Mousavi A, Sahebani N (2021). Biochar and vermicompost improve growth and physiological traits of eggplant (*Solanum melongena* L.) under deficit irrigation. Chemical and Biological Technologies in Agriculture 8(1):1-14. *https://doi.org/10.1186/s40538-021-00216-9*

Elmi M (2009). A brief overview of the global flower market. Business Think Tank, Iran Trade Development Organization.

Epstein E (1999). Silicon. Annual Review of Plant Biology 50:641. *https://doi.org/10.1146/annurev.arplant.50.1.641*

Eraslan F, Inal A, Gunes A, Apalsan M (2007). Impact of exogenous salicylic acid on the growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity. Scientia Horticulturae 113:120-128. *https://doi.org/10.1016/j.scienta.2007.03.012*

Etesami H, Jeong BR (2018). Silicon (Si): Review and future prospects on the action mechanisms in alleviating biotic and abiotic stresses in plants. Ecotoxicology and Environmental Safety 147:881-896. *https://doi.org/10.1016/j.ecoenv.2017.09.063*

Farzi R, Golami M (2018). The effect of different mulch types on some photosynthesis and water relation parameters of olive cultivar 'Manzanilla' under water stress conditions. Journal of Crop Production and Processing 8(3).

Fazeli Kakhaki SF, Moayedi AA (2018). Stress in plants: from physiology to genome. SokhanGostar Publications, pp 473.

Fitzsimons MS, Miller M (2010). The importance of soil microorganisms for maintaining diverse plant communities in tallgrass prairie. American Journal of Botany 97(12):1937-1943. *https://doi.org/10.3732/ajb.0900237*

Gill SS, Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48:909-930. *https://doi.org/10.1016/j.plaphy.2010.08.016*

Gong HJ, Chen KM, Chen GC, Wang SM, Zhang CL (2003). Effects of silicon on growth of wheat under drought. Journal of Plant Nutrition 26(5):1055-1063. *https://doi.org/10.1081/PLN-120020075*

Gunes A, Pilbeam DJ, Inal A, Coban S (2008). Influence of silicon on sunflower cultivars under drought stress, I: Growth, antioxidant mechanisms, and lipid peroxidation. Communications in Soil Science and Plant Analysis 39(13-14):1885-1903. *https://doi.org/10.1080/00103620802134651*

Hajiboland R, Cheraghvareh L, Poschenrieder C (2017). Improvement of drought tolerance in tobacco (*Nicotiana rustica* L.) plants by silicon. Journal of Plant Nutrition 40:1661-1676. *https://doi.org/10.1080/01904167.2017.1310887*

Hodson MJ, White PJ, Mead A, Broadley MR (2005). Phylogenetic variation in the silicon composition of plants. Annals of Botany 96:1027-1046. *https://doi.org/10.1093/aob/mci255*

Karagiannidis N, Bletsos F, Stavropoulos N (2002). Effect of Verticillium, wilt (*Verticillium dahliae*, Kleb.), and mycorrhiza (*Glomus mosseae*) on root colonization, growth, and nutrient uptake in tomato and eggplant seedlings. Scientia Horticulturae 94:145-156. *https://doi.org/10.1016/S0304-4238(01)00336-3*

Katsuhara M, Otsuka T, Ezaki B (2005). Salt stress-induced lipid peroxidation is reduced by glutathione S-transferase, but this reduction of lipid peroxides is not enough for a recovery of root growth in *Arabidogsis*. Plant Sciences 169(2):369-373. *https://doi.org/10.1016/j.plantsci.2005.03.030*

Lata C, Jha S, Sreenivasulu N, Prasad M (2011). Differential antioxidative responses to dehydration-induced oxidative stress in the core set of foxtail millet cultivars. Protoplasma 248:817-828. *https://doi.org/10.1007/s00709-010-0257-y*

Latef AAHA, Hashem A, Rasool S, Abd_Allah EF, Alqarawi AA, Egamberdieva D, ... Ahmad P (2016). Arbuscular mycorrhizal symbiosis and abiotic stress in plants: a review. Journal of Plant Biology 59(5):407-426.

Liang Y, Sun W, Zhu Y, Christie P (2007). Mechanisms of Silicon-mediated alleviation of abiotic stresses in higher Plants-a review. Environmental Pollution 147:422-428. *https://doi.org/10.1016/j.envpol.2006.06.008*

Liang YC, Chen Q, Liu WHZ, Ding RX (2003). Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgar* L.). Journal of Plant Physiology 160:1157-1164. *https://doi.org/10.1078/0176-1617-01065*

Liang YC, Sun CWYC, Romheld V (2005). Effects of foliar- and root applied silicon on the enhancement of induced resistance to powdery mildew in *cucumis sativus*. Journal of Plant Pathology 54:678-685. *https://doi.org/10.1111/j.1365-3059.2005.01246.x*

Liu P, Yin L N, Deng X P, Wang S W, Tanaka K, Zhang SQ (2014). Aquaporin-mediated increase in root hydraulic conductance is involved in silicon-induced improved root water uptake under osmotic stress in *Sorghum bicolor* L. Journal of Experimental Botany 65:4747-4756. *https://doi.org/10.1093/jxb/eru220*

Lux A, Luxová M, Hattori T, Inanaga S, Sugimoto Y (2002). Silicification in sorghum (*Sorghum bicolor*) cultivars with different drought tolerance. Physiologia Plantarum 115:87-92. *https://doi.org/10.1034/j.1399-3054.2002.1150110.x*

Ma JF, Yamaji N (2006). Silicon uptake and accumulation in higher plants. Trends in Plant Science 11:392-397. *https://doi.org/10.1016/j.tplants.2006.06.007*

Mathur S, Sharma MP, Jajoo A (2018). Improved photosynthetic efficacy of maize (*Zea mays*) plants with arbuscular mycorrhizal fungi (AMF) under high temperature stress. Journal of Photochemistry and Photobiology B: Biology 180:149-154. *https://doi.org/10.1016/j.jphotobiol.2018.02.002*

Matthews R, Azam-Alisn B, Peacock JM (1990). Response of four sorghum lines to midseason drought: II. Leaf characteristics. Field Crops Research 25:297-308. *https://doi.org/10.1016/0378-4290(90)90011-Y*

Mc Gonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JAA (1990). New method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal Fungi. New Phytologist 115:495-501. *https://doi.org/10.1111/j.1469-8137.1990.tb00476.x*

Mehrgan B, Mousavifard P, Rezainejad A (2018). The effect of potassium silicate foliar application on some morphological, physiological, and biochemical traits of *Alternanthera repens* L. under drought stress. Journal of Crop Improvement 20(1):299-314.

Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environmental and Experimental Botany 49(1):69-76. *https://doi.org/10.1016/S0098-8472(02)00058-8*

Ming, DF, Pei ZF, Naeem MS, Gong HJ, Zhou WJ (2012). Silicon alleviates PEG-induced water-deficit stress in upland rice seedlings by enhancing osmotic adjustment. Journal of Agronomy and Crop Science 198(1):14-26. *https://doi.org/10.1111/j.1439-037X.2011.00486.x*

Miransari M (2010). Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stresses. Review article. Plant Biology 12:563-569. *https://doi.org/10.1111/j.1438-8677.2009.00308.x*

Mohammadkhani N, Heidari R (2007). Effects of water stress on respiration, photosynthetic pigments and water content in tow Maize cultivar. Pakistan Journal Biological Science 10:4022-4028.

Moradtalab N, Hajiboland R, Aliasgharzad N, Hartmann TE, Neumann G (2019). Silicon and the association with an arbuscular-mycorrhizal fungus (*Rhizophagus clarus*) mitigate the adverse effects of drought stress on strawberry. Agronomy 9:41. *https://doi.org/10.3390/agronomy9010041*

Munns R (2002). Comparative physiology of salt and water stress. Plant Cell and Environment 25:239-250. *https://doi.org/10.1046/j.0016-8025.2001.00808.x*

Nadian H (2011). Effect of drought stress and mycorrhizal coexistence on growth and phosphorus uptake by two different sorghum cultivars in root morphology. Journal of Agricultural Science and Technology and Natural Resources 15:127-140.

Ouzounidou G, Giannakoula A, Ilias I, Zamanidis P (2016). Alleviation of drought and salinity stresses on growth, physiology, biochemistry, and quality of two *Cucumis sativus* L. cultivars by Si application. Brazilian Journal of Botany 39(2):531-539. *https://doi.org/10.1007/s40415-016-0274-y*

Porcel R, Aroca R, Ruiz-Lozano JM (2012). Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. Agronomy for Sustainable Development 32(1):181-200. *https://doi.org/10.1007/s13593-011-0029-x*

Raghda'a Ali Al-Khafajy D, AL-Taey KA, AL-Mohammed MH (2020). The impact of water quality, bio fertilizers and selenium spraying on some vegetative and flowering growth parameters of *Calendula officinalis* L. under salinity stress. International Journal of Agricultural and Statistical Sciences 16:1175-1180.

Raghda'a Ali Al-Khafajy D, AL-Taey KA, AL-Mohammed MH (2020). The impact of water quality, bio fertilizers and selenium spraying on some vegetative and flowering growth parameters of *Calendula officinalis* L. under salinity stress. International Journal of Agricultural and Statistical Sciences 16:1175-1180.

Rastgoo L, Alemzadeh A (2011). Biochemical responses of gouan *(Aeluropus littoralis)* to heavy metals stress. Australian Journal of Crop Science 5(4):375-383.

Rejaea F, Esmaeilzadeh A, Shamshiripoor M, Saberi M (2016). Investigation of different species of mycorrhizal fungi on increasing the herbal efficiency of heavy metal contaminated soils using two plants, corn and sunflower. Iranian Journal of Soil and Water Research 47(3):475-483.

Rizwan M, Ali S, Ibrahim M, Farid M, Adrees M, Bharwana SA, ... Abbas F (2015). Mechanisms of silicon-mediated alleviation of drought and salt stress in plants: a review. Environmental Science and Pollution Research 22(20):15416-15431. *https://doi.org/10.1007/s11356-015-5305-x*

Rozpądek P, Rąpałakozik M, Wêżowicz K, Grandin A, Karlsson S, Wazny R (2016). Arbuscular mycorrhiza improves the yield and nutritional properties of onion (*Allium cepa*). Plant Physiology and Biochemistry 107:264-272. *https://doi.org/10.1016/j.plaphy.2016.06.006*

Ruiz-Llozno JM (2003). Arbuscular mycorrhiza symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13:309-317. *https://doi.org/10.1007/s00572-003-0237-6*

Sairam RK, Saxena DC (2000). Oxidative stress and antioxidant in wheat genotypes: possible mechanism of water stress tolerance. Journal of Agronomy and Crop Science 184:55-61. *https://doi.org/10.1046/j.1439-037x.2000.00358.x*

Saleh S, Liu G, Liu M, Ji Y, He H, Gruda N (2018). Effect of irrigation on growth, yield, and chemical composition of two green bean cultivars. Horticulturae 4(1):3. *https://doi.org/10.3390/horticulturae4010003*

Salehpour M, Ebadi A, Izadi M, Jamaati-e-Somarin S (2009). Evaluation of water stress and nitrogen fertilizer effects on relative water content, membrane stability index, chlorophyll and some other traits of lentils (*Lens culinaris* L.) under hydroponics conditions. Research Journal of Environmental Sciences 3:103-109. *https://doi.org/10.3923/rjes.2009.103.109*

Singh R, Parihar P, Singh S, Mishra RK, Singh VP, Prasad SM (2017). Reactive oxygen species signaling and stomatal movement: Current updates and future perspectives. Redox Biology 11:213-218. *https://doi.org/10.1016/j.redox.2016.11.006*

Smart RE, Bingham E (1974). Rapid estimates of relative water content. Plant Physiology 53:258-260. *https://doi.org/10.1104/pp.53.2.258*

Smith FA, Smith SE (2011). What is the significance of the arbuscular mycorrhizal colonization of many economically important crop plants? Plant Soil 348:63-79. *https://doi.org/10.1007/s11104-011-0865-0*

Srinivas ND, Rashmi K, Raghavarao K (1999). Extraction and purification of a plant peroxidase by aqueous two-phase extraction coupled with gel filtration. Process Biochemistry 35:43-48. *https://doi.org/10.1016/S0032-9592(99)00030-8*

Sultana N, Ikeda T, Itoh R (1999). Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. Environmental and Experimental Botany 42:211-220. *https://doi.org/10.1016/S0098-8472(99)00035-0*

Turkan I, Melike B, Ozdemir F, Koca H (2005). Differential response of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Science 168:223-231. *https://doi.org/10.1016/j.plantsci.2004.07.032*

Ullah H, Santiago-Arenas R, Ferdous Z, Attia A, Datta A (2019). Improving water use efficiency, nitrogen use efficiency, and radiation use efficiency in field crops under drought stress: A review. Advances in Agronomy 156:109-157. *https://doi.org/10.1016/bs.agron.2019.02.002*

Vaghar M, Ehsanzadeh P (2018). Comparative photosynthetic attributes of emmer and modern wheats in response to water and nitrogen supply. Photosynthetica 56(4):1224-1234. *https://doi.org/10.1007/s11099-018-0825-5*

Velikova V, Yordanov I, Edreva A (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamins. Plant Science 151:59-66. *https://doi.org/10.1016/S0168-9452(99)00197-1*

Voleti SR, Padmakumari AP, Raju VS, Mallikarjuna Babu S, Ranganathan S (2008) Effect of silicon solubilizers on silica transportation, induced pest and disease resistance in rice (*Oryza sativa* L.). Crop Protection 27:1398-1402. *https://doi.org/10.1016/j.cropro.2008.05.009*

Wang J, Possw A, Donovanw TJ, Shannonz MC, Leschw SM (2002). Biophysical properties and biomass production of elephant grass under saline conditions. Journal of Arid Environments 52:447-456. *https://doi.org/10.1006/jare.2002.1016*

Willis A, Rodrigues BF, Harris PJ (2013). The ecology of arbuscular mycorrhizal fungi. Critical Reviews in Plant Sciences 32(1):1-20. *https://doi.org/10.1080/07352689.2012.683375*

Wu QS, Xia RX (2006). Arbuscular mycorrhiza fungi influence growth, osmotic adjustment, and photosynthesis of citrus under well-watered and water stress conditions. Journal of Plant Physiology 163(4):417-425. *https://doi.org/10.1016/j.jplph.2005.04.024*

Wu QS, Zou YN (2017). Arbuscular mycorrhizal fungi and tolerance of drought stress in plants. In: Arbuscular mycorrhizas and stress tolerance of plants (pp. 25-41). Springer, Singapore. *https://doi.org/10.1007/978-981-10-4115-0-2*

Yin L, Wang S, Liu P, Wang W, Cao D, Deng X, Zhang S (2014). Silicon-mediated changes in polyamine and 1-aminocyclopropane-1-carboxylic acid are involved in silicon-induced drought resistance in *Sorghum bicolor* L. Plant Physiology and Biochemistry 80:268-277. *https://doi.org/10.1016/j.plaphy.2014.04.014*

Yin Y, Li S, Liao W, Lu Q, Wen X, Lu C (2010). Photosystem II photochemistry, photoinhibition, and xanthophyll cycle in heat stressed rice leaves. Journal of Plant Physiology 167:959-966. *https://doi.org/10.1016/j.jplph.2009.12.021*

Zhang J, Lin Y, Zhu L, Yu S, Kundu SK, Jin Q (2015). Effects of 1-methylcyclopropene on function of flag leaf and development of superior and inferior spikelets in rice cultivars differing in panicle types. Field Crops Research 177:64-74. *https://doi.org/10.1016/j.fcr.2015.03.003*

Zhu Z, Wei G, Li J, Qian Q, Yu J (2004). Si alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). Plant Science 167:527-533. *https://doi.org/10.1016/j.plantsci.2004.04.020*

Zou Y N, Wu Q S, Huang Y M, Ni Q D He X H (2013). Mycorrhizal mediated lower proline accumulation in *Poncirus trifoliata* under drought derives from the integration of inhibition of proline synthesis with increase of proline degradation. PLoS ONE 8:e80568. *https://doi.org/10.1371/journal.pone.0080568*

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