Interactive effects of silicon and arbuscular mycorrhiza on root growth, uptake of several soil elements, antioxidant potential, and secondary metabolites of Licorice (*Glycyrrhiza glabra* L.) under water-deficit condition

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Abstract

Aims

Water deficit affect agricultural systems negatively globally. This research objective was to mitigate drought detrimental effects on Licorice growth, utilizing biofertilizer and mineral nutrition. Exogenous application of silicon (Si) and arbuscular mycorrhizal fungi (AMF) may help licorice plant cope with drought stress.

Methods

An experiment was designed with two Si solute levels in irrigation water (i.e. 0 (Si₀) and 300 mg/L (Si₁)), two levels of AMF inoculation (i.e. inoculation with *Claroiedoglomus etunicatum* (F₁) and without inoculation (F₀)), and five soil moisture regimes (i.e. 100, 80, 60, 40 and 20% of field capacity (FC)). The impacts of Si and AMF were assessed on licorice yield, mineral uptake, antioxidant potential, and secondary metabolites under water-deficit stress. After two months, the plants were harvested and their morphological traits were measured. Root extracts were utilized for secondary metabolites and nutrient determination.

Results

Under water deficit conditions, there were significant decreases in root length, diameter, and dry weight (DW) ($p < 0.05$), compared to the control. Si and AMF could significantly ($p < 0.05$) enhance root area (47.75-150.64 cm²) under all irrigation levels. Also, Si significantly ($p < 0.05$) increased the level of root colonization in licorice. The maximum glycyrrhizic acid (GA) (26.62 mg/g_{DW}) was achieved by the interaction between Si and AMF in response to 40% FC. A reduction in total flavonoids and phenolics of licorice was observed under severe drought levels; however, the Si and AMF acted to increase sinapic acid (16.46 mg/g_{DW}), and trans ferulic acid (1.11 mg/g_{DW}) in roots. The results indicated that the interaction between Si and AMF positively affected nitrogen (N) and phosphorous (P) concentrations in roots (53.51 and 74.07 %, respectively). At low irrigation levels, the concentrations of P and Si elements increased significantly in the roots. Also, there was an increase in potassium concentration (K) in response to 100, 80, and 40% FC among Si-treated plants (38.5-44.7%).

Conclusions

The exogenous application of Si and AMF showed a synergistic impact on the ability to mitigate the adverse effects of drought stress by improving plant growth and enhancing metabolite accumulation in licorice. These findings bring prospective insight into world water deficit crisis conquering.

Introduction

Licorice (*Glycyrrhiza glabra* L.) is a medicinal plant from the Fabaceae family. It is a well-known industrial herb, widely utilized in medicine for its therapeutic characteristics because of its broad range of pharmacological actions (Han et al., 2022). Licorice is a key plant resource in arid and semiarid environments for wind protection and sand fixation. Rhizomes of licorice have a large quantity of glycyrrhizic acid, a key bioactive triterpenoid saponin (Heidari et al., 2021; Karkanis et al., 2018). However, careless overexploitation of wild ecotypes of *G. glabra* has recently resulted in a rapid reduction of its populations, if not extinction. Because of the plant's high economic value and the risk of extinction owing to overharvesting, it appears vital to grow and domesticate this valuable species (Zhang et al., 2017). Wild licorice plants are well-suited to growing in challenging situations, including drought, and on lands with nutrient deficiency. These are the two main causes that usually limit licorice production. *Glycyrrhiza* plants are grown to help restore ecosystems that have been degraded, especially in arid and semi-arid areas (Han et al., 2022). In this regard, plants have evolved a variety of stress-resistance strategies. Plant-microbe mutualism may influence plant development, nutrient uptake, and resistance to water-deficit stress (Zou et al., 2021). Arbuscular mycorrhizal (AM) fungi can also create a symbiotic relationship with legumes, improving plant mineral nutrition, particularly N and P (Wang et al., 2021). Inoculating plants with *C. etunicatum* (previously *Glomus etunicatum*) has reportedly induced drought tolerance in various crops, including *Vigna radiata* (Musyoka et al., 2020), *Cinnamomum migao* (Liao et al., 2021), and *Glycine max* Merr. (Iggehon and Babalola, 2021). Mycorrhizal fungi are heterotrophic, meaning that they obtain their carbon content from the plants they live on (Sharma et al., 2020). Mycorrhizal associations are known to promote growth via contribution to intensive P uptake, induction of drought-responsive genes, and activation of different metabolic pathways (Wen-Ya et al., 2021). AMF-colonization can improve the establishment of extensive hyphal networks which assist in water absorption and nutrient uptake, leading to better soil structures (Jajoo and Mathur, 2021). Moreover, among mineral nutrients, Si is widely regarded as a favorable element for plant development because its uptake by plants can promote growth in the face of abiotic stresses (Majumdar and Prakash, 2020). Silicon was reportedly capable of mitigating the negative effects of drought stress on *Brassica juncea* (Alamri et al., 2020), *G. max* (Koentjoro et al., 2020), *Triticum aestivum* L. (Bukhari et al., 2020), and *Solanum lycopersicum* L. (Chakma et al., 2021). The key mechanisms that support the ability of Si to mitigate the effects of drought stress include ways of enhancing water uptake and transport, regulating stomatal behavior and water loss, as well as accumulating solutes and osmoregulatory substances. The mechanisms also promote the root osmotic driving force, improve root/shoot ratios, regulate aquaporins, increase root hydraulic conductance, enhance mineral nutrient uptake, maintain nutrient balance, and induce plant defense associated with signaling events. These are collectively known to assist plants in maintaining water balance (Rehman et al., 2021; Thakral et al., 2021; Thorne et al., 2021; Wang et al., 2021). In the light of the said cases of research, the goal of the present work was to evaluate how licorice responds to Si and AMF, especially under water-deficit stress. To the best of our knowledge, such information has not been published before. To achieve this aim, licorice plants were treated by five levels of drought stress (i.e. 100, 80, 60, 40, and 20% of FC) in the presence or absence of Si (300 mg/L) and *C. etunicatum* symbiosis.

Materials And Methods

Soil characteristics Soil samples were taken from the upper (0–30 cm) horizon of the soil profile before being air-dried. They were sieved (via a 2-mm sieve) and blended in appropriate ways. The soil characteristics including texture (Beretta et al., 2014), pH (Bargrizan et al., 2017), electrical conductivity (EC) (Aboukila and Norton, 2017), organic matter (OM) (Miyazawa et al., 2000), cation exchange capacity (CEC) (Meimaroglou and Mouzakis, 2019), the available amount of phosphorus (Shen et al., 2019), total nitrogen quantity (Guebel et al., 1991), potassium content (Kumar and Gill, 2018), and micronutrients' concentrations such as Zn, Fe, Cu, and Mn (Lindsay and Norvell, 1978) were determined via referenced methods. The physicochemical characteristics of the soil are presented in Table S1.

Plant material

This experiment was carried out in the College of Agriculture, Shiraz University. The seeds of *G. glabra* were collected from Eghlid (Aspas village, 52° 23' 58" E and 30° 38' 31" N), a region in the north of Fars province, Iran. The seeds were scarified by soaking in concentrated H₂SO₄ (97%- Merck) for 10 min, washed with running water several times, and immediately sown in transplant trays (Peat moss and perlite mix, 2:1). In each cell of the trays, two seeds were sown. They were allowed to grow in the greenhouse after sowing (Day: 27±1°C, night: 23±1°C, humidity: 70±3%, light: 40000 Lux). A month after germination, the plantlets were transferred into small plastic pots containing 250 mL of field soil and sand mixture (2:1) (Lang et al., 2019). Bigger pots (50 cm height × 29 cm diameter) were used for transplanting 6-month-old seedlings in a sandy medium (soil and sand mixture (2:1)) (Lang et al., 2019). Each pot had two seedlings.

Preparation of microbial inoculation

C. etunicatum was supplied by the soil biology lab at Shiraz University, department of soil science, and was previously separated by Dr. Mehdi Zarei. This fungus was propagated in a sterilized mixture of soil and sand (1:1) for five months using *Sorghum bicolor* as the host plant. The inoculum potential for infectivity as root colonization was estimated at 85%. A number of 10 spores existed per gram of substrate. The inoculum (250 g) was added to the root zone before doing the final transplantation. Each mycorrhizal pot was filled with soil containing fungal spores, mycorrhizal roots, and mycelia of *C. etunicatum*. Meanwhile, non-mycorrhizal pots were supplied with an equal amount of washed sand (Zarei et al., 2020).

Experimental design and growth conditions

A pot experiment was performed in a factorial experiment that was arranged in a completely randomized design with three factors, including five levels of drought stress (i.e. 100 (control), 80, 60, 40 and 20% of field capacity, represented by W (i.e. W₁₀₀ (control), W₈₀, W₆₀, W₄₀, and W₂₀), two levels of AMF, denoted by F (i.e. F₀: without inoculation, F₁: inoculated), and two levels of Si solute in irrigation water (i.e. Si₀: without Si application, Si₁: 300 mg/ L of SiO₂). Four replications were considered. After two weeks of

adaptation and plant establishment in pots, irrigation treatments were carried out for two months. Irrigation was performed differently to make the soil reach the five different levels of FC. The weighting approach was used for adjusting the stress treatments (Abbaszadeh et al., 2020). As the plants consumed water and as evaporation occurred, the weight of pots decreased gradually through the course of observations. For each treatment group, the five drought levels were applied. In the case of Si nutrition, SiO_2 was dissolved in the irrigation water. Every other day, water was added to the pots after weighing the pots so that the amount of soil moisture could be measured indirectly and the specifications of each treatment could be upheld. After eight weeks, and under the respective conditions of growth, the plants were harvested for analysis.

Parametric measurements

Morphological parameters

The plants were entirely removed from the pots at the end of the experiment. After cleaning the roots, various parameters were examined and measured. Root length was measured by a scientific ruler and root diameter was measured by a caliper (0.01 mm accuracy) (Shan et al., 2018). The root fresh weight (FW) was determined promptly after harvesting the plants. Then, the roots were oven-dried at 80 °C for 24 h before measuring their dry biomass. Dry and fresh weights of the roots were measured with a four-digit scale (Lamacque et al., 2020). Root volume was calculated according to the volume displacement method (Harrington et al., 1994). Root area was measured by MATLAB software (Jadhav, 2021).

Assessment of AMF colonization

Fresh root segments (2 cm long) were dyed as described by Rahimi et al., 2021 with minor modifications so that root colonization by AMF could become measurable. Stained root segments were selected randomly for evaluation under the microscope and according to a relevant approach in the literature (Trouvelot et al., 1986). A Nikon Eclipse E200 microscope was used for examining the segments (at 40× magnification). The mycorrhizal inoculation parameters and confirmation were calculated utilizing Mycocalc (<http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/MYCOCALC.EXE>) (Etemadi et al., 2014).

***Glycyrrhiza glabra* root extraction and quantitative analysis by HPLC**

The well-ground powder was prepared from the dried roots of each sample (500 mg) and its extract was taken by ethanol: water (70:30). After adding the solvent to the powder and after sonicating them for half an hour in an ultrasonic bath, the extracts were centrifuged and injected into High-Performance Liquid Chromatography (HPLC). Extraction and injection were done in three replicates (Esmaeili et al., 2019).

The glycyrrhizic acid content in the licorice roots was evaluated by Knauer liquid chromatography device consisting of a 2695 Separations Module (Germany), equipped with a 100 µl loop and Photodiode Array Detectors (PDA) using a C18 column (Knauer, 25 cm × 4.6mm Eurospher 100-5), with water that contained 0.3% H_3PO_4 (solvent A) and acetonitrile (solvent B) as the mobile phase. The flow rate was 1

ml/min. The samples were monitored at a wavelength of 250 nm in the case of glycyrrhizic acid (Esmaeili et al., 2019).

Antioxidant activity and 50% inhibition (IC-50) determination

Total antioxidant activity was calculated according to the protocol of Taban et al., 2021. The absorbance was read at 517 nm (Taban et al., 2021). According to the DPPH free-radical scavenging method, the sample concentration was assessed in relation to IC-50 (Half maximal Inhibitory Concentration). The inhibition (%) was plotted against sample concentrations and each test was performed three times (Taban et al., 2018).

Determination of total phenols and flavonoids

The total phenolic quantity of the ethanolic extracts was assessed by the method described by Haghighi and Saharkhiz, 2021. The absorbance read by a spectrophotometer at 750 nm (Epoch Biotek, Winooski, VT, USA). The absorbance of ethanolic extracts to estimate the concentration of total flavonoids done by the method described by Taban et al., 2020. The absorbance was measured at 510 nm by a microplate reader in the spectrophotometer (Taban et al., 2020).

Extraction and profile assay of polyphenolic compounds by HPLC

The extraction was carried out using a solvent (85% methanol/15% acetic acid) with a concentration with the method described by Gholami et al., 2018 with little modifications. The polyphenols were collected using a syringe that had a filter on its head (0.45 µm). Then, they were transferred to glass containers and remained frozen until HPLC analysis (Gholami et al., 2018).

Polyphenolic components in *G. glabra* extracts were separated, identified, and quantified by HPLC analysis on an Agilent 1200 series (USA), equipped with a reverse-phase Zorbax Eclipse (XDB)-C18 column (10 cm × 5 mm i.d.; 150 mm film thickness) and with the assistance of a photodiode array detector (PAD). The method completely described by (Mousavi et al., 2021).

Mineralogical analysis by X-ray Diffraction

The root powder ash (1g) of each sample was prepared at 500°C and then became subjected to X-ray powder diffraction (XRD) analysis. The quantity of N, K, P, and Si in the samples was measured by X-ray diffraction (XRD) (Bruker's X-ray Diffraction Model: D8 Advance; Germany) while Cu Kα radiation had a wavelength of 1.54 Å in the three replicates. The relative abundance of minerals was measured using peak height intensity as an indication. Scanning was carried out from 15 to 75° 2θ, with a step interval of 0.05° and with a counting time of 1 s per step. Quantitative analysis of mineral abundance was carried out using the X'Pert HighScore software (Oliveira et al., 2012).

Statistical Analysis

This research mostly involved a pot experiment in a factorial arrangement with four replications in a completely randomized design. It had three factors, i.e. drought, AMF, and Si. A general linear model was employed for statistical analysis and for dealing with the entire experimental data using Minitab software (version 17). Statistical significance was defined at $p < 0.05$ (Tukey's test). In the case of significant interactions, the slice method was applied to obtain mean comparisons. Principal component analysis was also used according to a correlation matrix by Minitab software. Corplot was drawn by R software version 4.1.1 (corrplot package) and path analysis was performed by IBM SPSS (version 27).

Results

Plant Biomass

In the present study, the fresh and dry weights of roots were evaluated under experimental treatments. The results showed that lower irrigation levels led to a significant decrease in both types of root weight ($p < 0.05$). It is noteworthy that the effects observed from the three irrigation levels (W_{60} , W_{80} , and W_{100}) were not significantly different from each other ($p < 0.05$). The lowest fresh and dry weights were observed in response to severe drought treatment (W_{20}) which brought about a decrease of 67% compared with the control. While different irrigation levels had a significant ($p < 0.05$) impact on the fresh and dry biomass of licorice rhizomes (Figs. 1A and 1B), Si and fungi did not have significant effects on the root weight.

Root characteristics (length, volume, area, and diameter)

An analysis of licorice root length showed that the longest roots occurred as a result of the well-irrigated treatment (W_{100}), while other treatments showed a significant ($p < 0.05$) reduction in root length, compared to the control ($p < 0.05$). It is noteworthy that no significant difference was observed among other irrigation levels (i.e. W_{20} , W_{40} , W_{60} , and W_{80}) (Fig. 2A).

In the case of root volume, a decrease in irrigation levels caused the root volume to decrease significantly ($p < 0.05$). In this respect, however, the three irrigation levels (W_{60} , W_{80} , and W_{100}) were not significantly different from each other. The smallest root volume was observed in response to the severe drought treatment (W_{20}) which showed a decrease of 64% compared with the W_{100} . While different irrigation levels had a significant ($p < 0.05$) effect on licorice root volume (Fig. 2B), Si and fungi did not have significant effects on this parameter.

According to an analysis of root area (Table S2), maximum root area in well-irrigated treatments was observed as a matter of interaction between Si and F (150.64 cm^2). In plants of severe drought stress (W_{40}), however, Si application per se was the cause of maximum root area (113.36 cm^2) (Table S2).

In general, root diameter decreased by a lowered level of irrigation (i.e. severe drought stress). F and Si each, per se, maintained the value of root diameter almost akin to the control group under severe drought stress (W_{20} and W_{40}). However, the interaction between Si and F in W_{20} caused a significant decrease in

root diameter, compared with the control ($p<0.05$). The highest values were observed by the effect of W_{80} when using either mycorrhiza (19.45 mm) or Si (18.77 mm) (Table S2).

Mycorrhizal colonization

Microscopic assessments revealed that non-inoculated plants acquired no or low levels of AMF colonization. *C. etunicatum* successfully colonized the roots of licorice under various treatments. Plants that were inoculated with *C. etunicatum*, and were integrated with Si, showed a significant ($p<0.05$) increase in root colonization. Specifically, these rates of increase were 3321% and 1685% in response to drought stress and well-watered conditions, respectively, compared with the control. Evaluations showed that W_{40} led to a minimum of root colonization. Meanwhile, Si application increased the amount of this variable in all irrigated levels, except in the case of W_{20} . Evaluations among inoculated plants showed that Si_0 treatments exhibited fewer percentages of root colonization, compared with the Si_1 treatment. Observations also revealed that the Si improved root colonization by 11.51% under severe drought stress, i.e. at W_{40} . In general, Si had a positive effect on root colonization in all irrigation levels, except at W_{20} which meant an unrelenting effect of severe drought stress (Fig. 3).

Glycyrrhizic acid

An analysis of GA in licorice roots showed that the highest amount of this metabolite (26.62 mg/g DW) was obtained at W_{40} by applying integrated Si and F (Fig. 4). In well-irrigated treatments, the Si application alone was able to enhance GA content (18.39 mg/g DW). Regardless of Si usage, the severe drought level (W_{40}) caused mycorrhizal-inoculated plants to show a notable increase in the amount of GA (16.43 mg/g DW), compared with the same irrigation level in non-mycorrhizal plants (Fig. 4).

Total flavonoid, antioxidant activity, and IC-50

Total flavonoids in licorice roots were increased significantly by Si and F ($p<0.05$). They caused 7.85 and 9.28% enhancement in the total flavonoid content, respectively (Figs. 5A and 5B). *G. glabra* root extracts showed an ability to scavenge DPPH free radicals which have been often seen as a measure of total antioxidant activity (Fig. 6). The total antioxidant activity in licorice roots at different levels of irrigation and mycorrhizal inoculation showed that the well-irrigated treatment (W_{100}) led to the highest antioxidant activity (60.86%). In severe drought stress (W_{20}), the presence of mycorrhiza ultimately maintained the level of antioxidant activity at 59.65%, although this had no significant difference with the control. Regardless of Si and F, root extracts of plants in the 80% FC irrigation demonstrated a higher ability to scavenge free radicals (IC-50 = 0.73 mg/ml) as compared to other irrigation levels. As a matter of Si and mycorrhizal interaction, severe drought stress (W_{40}) induced a higher capacity in plants to scavenge free radicals (IC-50 = 1.63 mg/ml). Si alone was able to keep the IC-50 at a low quantity (0.95 mg/ml) in response to W_{80} , hence its great capacity to scavenge free radicals (Table S3).

Total phenol and polyphenol profile

The results of total phenol determination showed that Si and F, separately, did not have a significant effect on the amount of total phenol at different drought stress levels. But interactions between mycorrhiza and Si significantly ($p < 0.05$) increased the amount of total phenol in the W_{40} group (1.74 mg/g DW) compared with other irrigation levels. In plants without Si and mycorrhizal inoculation, no significant difference was observed between the effects of the well-irrigated treatment (W_{100}) and those of the severe drought stress (W_{20} and W_{40}) on total phenol content (Table S3).

The HPLC examination of polyphenolic compounds in licorice roots and their profile showed that the highest amount of sinapic acid (10.11 mg/g DW) occurred in well-irrigated treatments when plants were inoculated with mycorrhiza. In severe drought stress levels (W_{40}), however, the combined application of Si and F could significantly ($p < 0.05$) increase the amount of sinapic acid (16.46 mg/g DW). As the irrigation level decreased, the quercetin content reduced significantly (44.1%) compared with the well-irrigated treatment (W_{100}) ($p < 0.05$). Irrespective of Si and F, more severe drought stress levels caused a significant increase ($p < 0.05$) in the amount of trans-ferulic acid (0.74-0.91 mg/g DW). Nonetheless, Si application on plants of the W_{100} treatment maximized the amount of trans-ferulic acid (1.11 mg/g DW) ($p < 0.05$) (Table S4).

Concentration of plant nutrients

Concentrations of select nutrients were evaluated in licorice roots (Table S5). Regardless of Si and mycorrhiza treatments, the concentration of K reached a maximum amount in the well-irrigated treatment (W_{100}). In plants of the Si or F treatments, there was no significant variation in K amount at various drought stress levels. The amount of N in inoculated plants, at all irrigation levels, showed a significant increase ($p < 0.05$), compared to non-inoculated plants. Si and F interactions were able to maximize the N concentration in plants of the severe drought stress treatment (W_{20}). However, Si alone could not significantly increase the N concentration in response to the severe drought treatment (W_{20}). Regardless of Si, the concentrations of P in inoculated plants were significantly ($p < 0.05$) higher than in non-inoculated plants in response to well-irrigated and moderated drought levels (W_{100} , W_{80} , and W_{60}). In the case of Si and F interaction, W_{80} and W_{20} treatments led to the maximum amount of P. Drought stress had no significant effect on P concentration at 40, 60, 80, and 100% FC, irrespective of AMF and Si treatments (Table S5).

Principal component analysis of the measurable traits of *G. glabra* in a combination of effects caused by mycorrhiza, silicon, and drought

In the biplot of PC analysis, the first two PCs represented 46% of the variation in the measured traits among the treatment groups (Fig. 7). The first PC explained 24% of the variation and comprised root diameter, root fresh and dry weights, root volume, root area, root length, quercetin, and Si. In contrast, the second PC accounted for 21.9% of trait-related variations, comprising root colonization, total phenol and flavonoid contents, and nitrogen content. In the bi-plot and PC analyses, the cosine of the angles between

vectors showed the extent of correlation between traits. In the present study, the projection of treatment groups on the two PCs in the bi-plot showed that the studied treatments were divided into two distinct groups. The first group comprised 12 treatments, i.e. T1, T2, ... and T12 in association with the first PC-linked measurements of parameters. Meanwhile, the treatments T13, T14, ... and T20 were categorized into the second group for having a higher association with the second PC-linked traits (Fig. 7).

Correlation and path analysis of measured parameters in *G. glabra* under a combination of mycorrhiza, silicon, and drought

Using correlation and path coefficient analysis, there were successful evaluations of the relations among licorice root traits, including root colonization, elemental analysis, and secondary metabolite production (Figs. S1 A and S1 B). Stronger positive correlations were found between root traits. These were, namely, root fresh weight, dry weight, volume, and diameter. Among the secondary metabolites, total phenol and total flavonoids showed a good positive correlation between each other (Fig. S1 A). In comparison with the simple correlation analysis, path analysis was aimed at the dependent and independent traits of licorice. The results demonstrated that sinapic acid and GA maintained the highest direct influence as expressed in $\beta=0.719$ ($p<0.001$). Also, total phenol and total flavonoids showed a significant direct effect ($p<0.01$) as expressed in $\beta=0.707$ (Fig. S1 B). In sum, these traits can be improved in their performance through path analysis.

Discussion

Drought is the most important abiotic stress that limits crop productivity worldwide (Tahmasebi et al., 2019). In the present study, the interaction between Si and AMF inoculation was seen as a mitigation factor against stress, thereby assisting in the maintenance of plant yield, antioxidant potential, and secondary metabolites of *G. glabra* under water-deficit conditions. According to biomass analysis in the present study, lower amounts of dry and fresh biomass were achieved under reduced irrigation levels. Similarly, previous research indicated how water deficit caused variations in the decrease of fresh and dry biomass in *T. aestivum* L. (Pour-Aboughadareh et al., 2019) and *Tetraena mandavillei* L. (Alam et al., 2021). The interaction between Si and mycorrhiza did not cause a substantial difference in licorice biomass production but, contrary to the present study, several studies have shown that Si and mycorrhiza can increase plant biomass in *Glycyrrhiza uralensis* (Zhang et al., 2020), *Arachis hypogaea* L. (Patel et al., 2021), and *Nicotiana tabacum* L. (Begum et al., 2021). This increase in plant biomass supposedly occurs by a higher rate of carbon assimilation and photosynthesis. Previous studies have reported that drought stress can reduce root diameter and root length in *Gossypium hirsutum* L. (Xiao et al., 2020) and *Brassica napus* L. (Arifuzzaman and Rahman, 2020), thereby confirming the results of the current work. In legumes and gramineae, mycorrhiza is known to support the growth of root diameter (Liu et al., 2020) which is in agreement with current observations.

Si has reportedly assisted plant growth in *G. max* Merr. (Hussain et al., 2021) and *Phaseolus vulgaris* L. (Boshkovski et al., 2020). Specifically, it increased root length and biomass, nodule growth, and improved

nodule function when supplied into the root medium under drought conditions (Tripathi et al., 2021). According to the current results, root volume was affected and notably reduced in response to severe drought stress only. Using mycorrhiza at moderate drought stress and/or using Si at severe drought levels led to the maximum root area. These variations in root system morphology influenced the function of roots. The characteristics of roots improved in response to Si nutrition and assisted plants in better absorption of water and nutrients. Similarly, applying Si to plants growing in Si-deficient soils may help the absorption of water and nutrients by plants. AM-induced phosphate assimilation usually improves in mycorrhizal plants and contributes to plant development (Liu et al., 2019). AMF helps host-plants partly maintain their rate of development, despite the effects of stress, by regulating a series of signaling pathways between the plant and the fungus, thereby facilitating the photosynthetic rate and other gas exchange-related features (Huang et al., 2020). Inside plant roots and in the soil, AMF can substantially increase the surface area available for nutrient absorption by plants (Gavito et al., 2019; Ma et al., 2021). Therefore, in the case of mycorrhiza and Si, root traits in many cases are frequently bound to improve. Using mycorrhiza and Si, either alone or in combination, can result in a better performance of roots while improving their features despite drought levels. Similar to the current study, root traits and total biomass production of *Zea mays* L. seedlings were enhanced by Si supplementation in the soil (Ali et al., 2021; Rivero et al., 2018). In the face of drought stress, root function can be largely affected, although specific mechanisms can help regulate the growth and architecture of the root system, while also modifying cell water permeability (Maurel and Nacry, 2020).

In the current study, Si and AMF each had a considerable contribution to the content of licorice roots, especially the flavonoids. Meanwhile, the interaction effect of Si and AMF led to a significant increase in total phenol and sinapic acid content. By modifying secondary metabolism in plants, AMF has the major role of strengthening plant resistance against abiotic stress. As a result, AMF may increase the biosynthesis of polyphenols and flavonoids (Liang et al., 2021). In the scientific literature, researchers indicated that AMF can help produce more amounts of phenols in the shoots when colonized by *Ocimum basilicum* L., compared to non-colonized plants (Hazzoumi et al., 2015). Similarly, the present study showed that the positive effect of AMF was observed more noticeably in response to W₄₀ which enhanced the total phenol content. The formation of mycorrhizal symbioses between plant roots and commercially-important groups of useful fungi can be seen as one of the most important mechanisms influencing phytochemical biosynthesis (da Cruz et al., 2020). Research on AMF revealed its effectiveness by altering terpenoid metabolism and the shikimate pathway, thereby boosting the biosynthesis of isoprenoids, polyketides, and polyphenols by regulating shikimate pathways (Tahat and Al-Momany, 2021; Verma et al., 2021). Such compounds with high antioxidant potential are known by their mechanisms to enhance plant tolerance against unfavorable drought conditions. This may be one of the causes that led to a lower level of IC-50 because of fungal inoculation in the present study and also in a previous work (Haghighi et al., 2022). Flavonoids from legumes have reportedly helped the growth of vesicular-arbuscular mycorrhizal fungi and promoted the transcription of nodulation genes in symbiotic relationships (Agnolucci et al., 2020; Ramesha et al., 2020). Other studies also found that colonized root cells underwent a variety of cytological and metabolic changes, including changes in the regulation of

plastid biosynthetic pathways and the Krebs cycle, as well as increases in the production of flavonoids, quercetin, isoprenoids, polyketides, and polyphenols (Mousavi and Karami, 2022). As stated in the current study, trans ferulic acid increased under drought stress, whereas quercetin decreased. It was discovered that water deficit caused an increase in the amount of plant phenolics, primarily ferulic acid. Since drought stress makes the photosynthetic apparatus more sensitive to the radiation it receives, a lack of water in the leaf tissue might trigger defensive mechanisms such as the creation of phenolic compounds (Riaz et al., 2019). These increases in phenolics could suggest that the system which stands against drought tolerance is triggered for the plant to better receive radiation and to restrict the negative impacts on the photosynthetic apparatus at times of stress (Kosobryukhov et al., 2020; Zahedi et al., 2019). During water deficit, phenolics may be important in scavenging reactive oxygen species (ROS) and in relieving oxidative stress during recovery. A reduction in the water potential in leaves causes a rise in total phenolic content, including ferulic acid, which could emanate from protective mechanisms being triggered and could denote a sign of drought resistance (Dias et al., 2021; Sharma et al., 2019).

The findings of the present study showed that maximum GA was achieved by the interaction between Si and AMF under severe drought stress. Partially, similar to the present findings, a study on *G. uralensis* also showed that drought stress was able to increase root GA concentration, regardless of the mycorrhizal status. In particular, this increase was significant in the case of non-AMF plants under extreme drought stress (Awan et al., 2021; Zhang et al., 2017). While the major triterpenoid saponin in licorice is GA, its antioxidant activity is expected to play a key role in ROS scavenging, thereby reducing oxidative damage considerably (Harikrishnan et al., 2021). Furthermore, an increase in GA concentrations during drought stress could be associated with a decrease in root biomass. More biosynthesis gene expressions occurred in AMF plants under mild drought stress, implying that the up-regulation of genes for the GA biosynthesis ultimately caused the increase in GA concentrations in drought-stressed AMF plants (Hu et al., 2020; Shao et al., 2019).

In agreement with the current study, which observed that Si application improved several parameters of colonization, the exogenous Si increased the Si content in *Cicer arietinum* plants and improved the plant potential to colonize with mycorrhiza in the roots (Garg and Cheema, 2021). Plant aquaporins may be affected directly by mycorrhiza, which could stimulate active Si uptake. AM fungi can selectively absorb various elements, including Si, to improve their colonization (Cibils-Stewart et al., 2020). Also, previous research indicated that stressed plants are at a better advantage of becoming hosts for fungal colonization (Zou et al., 2021). Drought acclimation improved external hyphal growth and soil aggregation of mycorrhizal plants (Cheng et al., 2021). As observed in the current study, AMF inoculation enhanced N, P, K, and Si concentration in licorice roots under various water-deficit levels. The ability of mycorrhizal inoculation to alleviate the impacts of drought stress in semiarid areas of the world is demonstrated when plants are observed to have better growth, yield, and nutrient uptake (Bahraminia et al., 2020; Pereira et al., 2021). In the present study, the concentration of Si in licorice roots increased by more than 50% under inoculated mycorrhizal treatments. This high concentration of Si plays a useful and effective role in drought tolerance. The uptake of P by plants can be increased by the effect of exogenous Si, according to previous studies on crops (Bhalla and Garg, 2021; Etesami and Jeong, 2021; Vega et al.,

2021). The available literature suggests that having a combination of Si and microbe treatments can effectively increase plant growth and nutrition. Regardless of the stress conditions, AMF and Si cooperate in key mechanisms to stimulate plant growth. Si synergistically helps plants absorb P efficiently. Si would allow farmers to use organic processes such as mycorrhiza to maintain fertility and improve plant growth instead of artificial P fertilizers (Wang et al., 2022). To enhance plant growth, AMF can be used with the intention of improving plant tolerance to abiotic stresses, while enhancing mineral uptake for establishing optimal concentrations of minerals in plant tissues (especially of P). Also, AMF can improve water relations between the plant and the soil, in addition to the provision of plant protection against soil-borne diseases. AMF can aid plants to obtain macronutrients and micronutrients such as Cu, K, Mg, N, and Zn, especially when they are present in less soluble forms in soils (Vishwakarma et al., 2021). AMF is known to promote nutrient absorption by increasing the absorption area of the roots, as well as by releasing substances like glomalin which is a glycoprotein that exudes from AMF hyphae and spores (Etesami and Jeong, 2021; Rezakhani et al., 2019). Glomalin in the soil facilitates the uptake of difficult-to-dissolve nutrients like Fe and P. There is a high efficiency with which hyphal surfaces absorb P from the soil. By comparison, it functions more productively than cylindrical root surfaces and accounts for the higher P uptake rate where AMF exists (Das et al., 2019).

Conclusion

The findings of the present study showed that Si application and mycorrhizal inoculation can positively affect morphological and biochemical parameters in licorice, despite various drought stress levels. The protective effects of Si and AMF treatments appear to be connected with the accumulation of secondary metabolites and mineral absorption, thereby improving morphological growth so that licorice production could remain partly unaffected despite water-deficit situations. The present observations showed that Si and AMF synergistically relieved the effects of drought stress on many features of the plants. Si promoted AMF colonization and the development of fungal structures which, in turn, enhanced Si absorption during mycorrhization. The current findings provide a practical foundation for the use of Si fertilizers and AMF to better enable licorice production where irrigation systems lean toward a policy of water conservation.

Declarations

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Author contribution

Tahereh Movahhed Haghighi: Investigation, Methodology, Writing- Original draft preparation, Data analysis; **Mohammad Jamal Saharkhiz:** Supervision, Validation, Funding acquisition, Reviewing, and

Editing; **Mehdi Zarei**: Methodology, Reviewing and Editing.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

Figure 1

Root biomass variation. (a) Root fresh weight variation and (b) root dry weight variation among various drought stress levels in examined Licorice plants. According to the analysis of variance that only the main effect of drought levels showed a significant difference, just its mean comparison is shown. Mean values with the same letters are not significantly different ($p<0.05$), Tukey test.

Abbreviations: W stands for water deficit treatment (5 levels including 100% (W₁₀₀), 80% (W₈₀), 60% (W₆₀), 40% (W₄₀) and 20% (W₂₀) of field capacity).

Figure 2

Root traits variation. (a) Root length variation and (b) root volume variation among various drought stress levels in examined Licorice plants. According to the analysis of variance that only the main effect of

drought levels showed a significant difference, just its mean comparison is shown. Mean values with the same letters are not significantly different ($p < 0.05$), Tukey test.

Abbreviations: W stands for water deficit treatment (5 levels including 100% (W_{100}), 80% (W_{80}), 60% (W_{60}), 40% (W_{40}) and 20% (W_{20}) of field capacity).

Figure 3

Root colonization variation among drought stress, Si application, and mycorrhiza inoculation interactions in examined Licorice plants. According to the analysis of variance that triple effects of fungi, drought levels, and Si showed a significant difference, the slice method was used for mean comparisons. Mean values with the same letters are not significantly different ($p < 0.05$), Tukey test.

Abbreviations: W stands for water deficit treatment (5 levels including 100% (W_{100}), 80% (W_{80}), 60% (W_{60}), 40% (W_{40}), and 20% (W_{20}) of field capacity), Si stands for silicon application (two levels including Si_0 (no Si application) and Si_1 (Si application)) and F stands for mycorrhiza inoculation (two levels including F_0 (no inoculation) and F_1 (inoculated)).

Figure 4

Glycyrrhizic acid content variation among drought stress, Si application, and mycorrhiza inoculation interactions in examined Licorice plants. According to the analysis of variance that triple effects of fungi, drought levels, and Si showed a significant difference, the slice method was used for mean comparisons. Mean values with the same letters are not significantly different in each treatment ($p < 0.05$), Tukey test.

Abbreviations: W stands for water deficit treatment (5 levels including 100% (W_{100}), 80% (W_{80}), 60% (W_{60}), 40% (W_{40}), and 20% (W_{20}) of field capacity), Si stands for silicon application (two levels including Si_0 (no Si application) and Si_1 (Si application)) and F stands for mycorrhiza inoculation (two levels including F_0 (no inoculation) and F_1 (inoculated)).

Figure 5

(a) Total flavonoid content variation among two Si levels in examined Licorice plants. (b) Total flavonoid content variation among two mycorrhiza inoculation levels in examined Licorice plants. According to the analysis of variance, only the main effects of fungi and Si showed a significant difference, just those mean comparisons are shown. Mean values with the same letters are not significantly different ($p < 0.05$), Tukey test.

Abbreviations: Si stands for silicon application (two levels including Si₀ (no Si application) and Si₁ (Si application)) and F stands for mycorrhiza inoculation (two levels including F₀ (no inoculation) and F₁ (inoculated)).

Figure 6

Antioxidant activity variation among various drought stress and mycorrhiza inoculation levels in examined Licorice plants. According to the analysis of variance that only the multiple effects of fungi and drought levels showed a significant difference, just its mean comparison is shown. Mean values with the same letters are not significantly different ($p < 0.05$), Tukey test.

Abbreviations: W stands for water deficit treatment (5 levels including 100% (W_{100}), 80% (W_{80}), 60% (W_{60}), 40% (W_{40}) and 20% (W_{20}) of field capacity) and F stands for mycorrhiza inoculation (two levels including F_0 (no inoculation) and F_1 (inoculated)).

Figure 7

Principal component analysis of studied treatments on various measured parameters

of licorice. **Abbreviations:** Treatments (T) are shown with numbers; 1: $W_{100} Si_0 F_0$; 2: $W_{100} Si_1 F_0$; 3: $W_{100} Si_0 F_1$; 4: $W_{100} Si_1 F_1$; 5: $W_{80} Si_0 F_0$; 6: $W_{80} Si_1 F_0$; 7: $W_{80} Si_0 F_1$; 8: $W_{80} Si_1 F_1$; 9: $W_{60} Si_0 F_0$; 10: $W_{60} Si_1 F_0$; 11: $W_{60} Si_0 F_1$; 12: $W_{60} Si_1 F_1$; 13: $W_{40} Si_0 F_0$; 14: $W_{40} Si_1 F_0$; 15: $W_{40} Si_0 F_1$; 16: $W_{40} Si_1 F_1$; 17: $W_{20} Si_0 F_0$; 18: $W_{20} Si_1 F_0$; 19: $W_{20} Si_0 F_1$; 20: $W_{20} Si_1 F_1$.

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