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Research Article

Effects of arbuscular mycorrhizal fungi inoculation and salinity stress on phenolic compounds, antioxidant capacity, and fatty acid profile of *Portulaca oleracea* L.

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Abstract

***Portulaca oleraceae* L. (purslane) is one of the most nutritious leafy vegetables that contain high fatty acids and antioxidants. Salinity was the main environmental stress limiting plant growth and development. Arbuscular mycorrhizal fungi (AMF) can colonize a wide range of host plants and facilitate the absorption of soil resources. The purpose of the study was to survey the impacts of NaCl treatments (0, 40, 80, 120, and 160 mM) and symbiosis of AMF (*Glomus intraradices* and *Glomus mosseae*) on growth parameters, phytochemical compounds, and fatty acid profile on purslane. The seeds of purslane were treated with a mycorrhizal fungus and salt stress. Root colonization percentage, growth parameters, flavonoid content, phenolic compounds, antioxidant capacity, and fatty acid profile on purslane were evaluated. The results indicated that the highest colonization rate was observed in roots inoculated with *G. intraradices*. Salinity progressively reduced the growth, but AMF symbiosis did not have a significant effect. The highest phenolic compound and flavonoid content belonged to the plants subjected to 120 mM NaCl. The lowest DPPH (IC_{50}) was observed in plants inoculated with *G. mosseae*. The phenolic compounds and flavonoid content in the leaves colonized with *G. mosseae* are significantly higher than in *G. intraradices*. Mycorrhizal symbiosis and increasing salinity negatively affected the fatty acid profile. The highest α-Linolenic content was observed with 40 mM NaCl treatment. Purslane can tolerate a moderate level of salt stress and has a high amount of antioxidants and fatty acids, which makes it possible to use it in saline localities.**

Keywords*:* Antioxidant capacity, Fatty acid, Mycorrhiza, Purslane, Salt stress

Introduction

Portulaca oleracea L. (purslane) is a dicotyledonous plant with succulent leaves belonging to the family Portulacaceae (Hosseinzadeh *et al.*, 2020). Purslane is a major cultivated vegetable due to its rich fatty acids, vitamins, and mineral elements, and it is a good source of omega-3 and omega-6 (Yazici *et al.*, 2007; Chowdhary *et al*., 2013; Uddin *et al*., 2014). There are 300-400 mg of alpha-linolenic acid (omega-3) in 100 grams of purslane (Oliveira *et al.*, 2009). Fatty acid exists in three forms: Saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) (Saffaryazdi *et al*., 2022). Omega-3 and omega-6 fatty acids as PUFA have fatty acid chains with 18 to 24 carbon atoms and three or more double bonds (Uddin *et al*., 2014). *Portulaca oleracea* is a good vegetable for a healthy diet that can neutralize free radicals, prevent cancers, asthma, cardiovascular diseases, type-I diabetes, and strengthen the immune system (Hosseinzadeh *et al.*, 2020; Saffaryazdi *et al*., 2022).

Soil salinity is the major abiotic stress that severely affects plant growth and production (Behdad *et al.*, 2020). Salt stress can lead to ionic stress through the uptake of excessive ions and nutritional imbalances, and decreasing the soil-water potential causes osmotic stress (Yazici *et al.*, 2007; Behdad *et al.*, 2021). Different studies have reported that purslane can tolerate and grow under unfavorable abiotic stress (Sdouga *et al.*, 2019). Purslane can withstand the ionic and osmotic effects of salinity due to reallocating resources and energy (Yazici *et al.*, 2007). Kafi and Rahimi (2011) confirmed that purslane is moderately salt-tolerant. An increase in phenolic compounds and antioxidant capacity in salt-treated purslane has been stated by Alam *et al.* (2015). Carvalho *et al.* (2009) reported that the salinity treatment at a moderate level led to an enhanced lipid content of plant tissue, but the lipid synthesis was disturbed at higher levels of salinity. In some purslane varieties, the levels of C18:3 fatty acids (Yeilaghi *et al.*, 2012) and total lipids (Khalid, 2017) reduce when exposed to NaCl treatment.

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AM fungus is an obligate biotrophic fungus that obtains the carbon required for its growth and development from the lipids and sugars of host plants (Feng *et al.*, 2020). Mycorrhizal fungi, as bio-fertilizers, play very important functions in plant resistance and growth to stress by elevating the nutrient status and water absorption (Yousefi Rad, 2016). Safari Sinegani and Elyasi Yeganeh (2017) reported that the inoculation of plants with mycorrhiza leads to maintaining the environment healthy, and enhances the quality and quantity of the yield and phytochemical compounds. Hristozkova *et al.* (2017) stated that AM fungus led to the elevated antioxidant capacity of *Ocimum basilicum*. Luginbuehl *et al.* (2017) showed that AM fungi receive essential fatty acids from their symbiotic host, and the content of host fatty acids may be reduced. A significant increase in oleic acid and linoleic acid levels of the inoculated sunflowers with *Glomus* species was observed (Sharma *et al*., 2021).

Several studies reported that purslane could grow readily in arid and saline soils (Kafi and Rahimi, 2011; Hnilickova *et al*., 2021). Moreover, a good solution for reinforcing secondary metabolites in medicinal herbs is their growth under stress (Baghbani-Arani *et al*., 2017). Considering that purslane has nutritional compounds and unique secondary metabolites, elicitors such as salt stress and mycorrhizal fungi can be used to increase these compounds. However, so far no studies are available on its symbiotic associations with *G. intraradices*, *G. mosseae*, and purslane. Hence, the present study aimed to investigate the effect of five levels of salt stress and the effects of the symbiotic relationship between host and AMF on growth, phenolic compounds, flavonoids, antioxidant capacity, and fatty acid profile of *P. oleracea*.

Materials and methods

Plant materials and cultivation: Seeds of *P. oleracea* were obtained from Pakan-Bazr Company (Isfahan, Iran). Voucher specimens (no 21808) were identified by the herbarium of Ferdowsi University of Mashhad (Mashhad, Iran).

NaCl treatments: Ten purslane seeds were cultured in plastic pots (23 cm in diameter and 21.5 cm in height) filled with loamy sand soil. The pots were kept in the 14 h light/10 h dark photoperiod and 25/20˚C day and night temperatures, respectively, in a greenhouse at the Faculty of Agriculture, Ferdowsi University of Mashhad (geographical coordinates: 34° 55′ 17.94″ N, 59° 41′ 2.11″ E). During the growth period, the soil moisture of pots was constant near 80% field capacity (FC). Two-week-old seedlings were subjected to 0, 40, 80, 120, and 160 mM NaCl. The treatments of salinity were kept constant throughout the growth period and were regularly adjusted by measuring the electrical conductivity (EC) of the drainage water. The leaves of plants were collected after 42 days of salinity treatment (Borsai *et al.*, 2020). The harvested purslane was air-dried at 25 ± 3˚C and powdered in a mechanical grinder.

Fungi inoculation: To investigate the symbiosis between purslane and AM, the inoculum of mycorrhizal fungi (*G. mosseae* and *G. intraradices*), was purchased from the Organic Herbal Medicine Clinic (Isfahan, Iran). After the soil was sterilized by autoclaving, the inoculum of AM fungus (100 g inoculum per 1 kg of soil) was added to the soil along with seed cultivation. Irrigation of pots was done every week based on field capacity (FC). The pots were kept in the greenhouse conditions mentioned above. After 56 days, the roots were washed and placed in FAA fixative (Formaldehyde Alcohol Acetic Acid) (Phillips and Hayman, 1970). Purslane leaves were harvested for other assays.

Growth parameters: After harvesting an eight-week-old plant (flowering stage), shoot lengths were determined. Then, the samples were dried at a high temperature (70˚C) for two days, and dry weight (DW) was measured (Saffaryazdi *et al.*, 2020).

Determination of mycorrhizal colonization: Root segments fixed in FAA are washed three times and heated at high temperatures (90˚C for 1 hour) in 10% KOH (v/v). The fixed parts of the root were washed three times with water and acidified with 1% HCl (v/v) for 3–5 min and stained with lactophenol cotton blue (Manoharachary and Mukerji, 2006). The root colonization (%) was measured by the following formula (Phillips and Hayman, 1970).

Root colonization (%) = Number of infected segments / Total number of examined segments × 100

Preparation of purslane extracts: To prepare the alcoholic extracts, 0.25 g of the powdered leaves (shadow-dried) were macerated in 25 ml of 80% methanol (v/v) for 24 h. The mixtures were filtered with Whatman filter paper No. 1, and then the filtrate was evaporated under the CH612 fume hood (Fater Electronic, Iran). The produced extracts were weighed and stored at -20˚C for further analyses.

Determination of phenolic compounds: Phenolic compound content was measured according to the colorimetric method by the procedure of Singleton and Rossi (1965) with some modifications. The absorbance was measured at 765 nm using a spectrometer (Shimadzu, AA-670, Japan). Gallic acid (GA) was applied as standard. The phenolic compound content was reported as mg (GAE)/100 g dry weight.

Determination of flavonoids: Flavonoid content was determined by the protocol of Chang *et al*. (2002), and the absorbance of the reaction mixture was read at 415 nm by spectrophotometer (Shimadzu, AA-670, Japan). The flavonoid content was expressed as mg of QE equivalents per 100 g dry weight.

Determination of DPPH radical scavenging capacity: To determine the values of IC_{50}, first, the different concentrations (0.156–10 mg ml^{-1}) of methanolic extracts of purslane were prepared. Then, the DPPH (2, 2-diphenyl-1-picrylhydrazyl) antioxidant capacity was assayed, and absorbance was read at 515 nm by spectrophotometer (Shimadzu, AA-670, Japan)

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(Brand-Williams *et al*., 1995). IC_{50} refers to the extract concentration at which 50% of the DPPH radicals are scavenged or reduced. A control = absorbance of the control sample; A sample = absorbance of the extract.

$$Percentage\ of\ free\ radical\ scavenging = \frac{(A\ control - A\ sample)}{A\ control} \times 100$$

Determination of ferric reducing antioxidant power (FRAP): FRAP analysis was performed according to the method by Sulaiman *et al*. (2011), and the absorbance was read at 593 nm by a spectrometer (Shimadzu, AA-670, Japan). The calibration curve was drawn with different concentrations of $FeSO_4 7H_2O$, and the data were shown in µmol of Fe per g dry weight.

Fatty acid assay: For gas chromatography (GC) assay, the direct trans-esterification method (Talebi *et al.*, 2013). First, 20 mg of powdered leaf was extracted by 1.5 ml of extraction buffer containing methanol and 2% sulfuric acid (v/v) (20 mg/1.5 ml). Then, the extracts were shaken (WiseCube, WIS-20R, Ukraine), and NaCl solution (w/v) and 1.5 mL of hexane were added; the obtained supernatant was used for GC analysis. The fatty acid (FA) was measured using gas chromatography (6890, Agilent, USA) with a flame ionization detector (FID). One microliter of the sample (split ratio of 20:1) was injected into an H88 capillary column (100 m, 0.25 mm I.D., film thickness 0.2 µm). The oven temperature was maintained at 140˚C for 5 min and raised to 240˚C at a rate of 4˚C min^{-1}. Both the injector and detector temperatures were maintained at 260˚C. Helium as carrier gas was used at a constant flow rate of 1.5 mL min^{-1}. The individual fatty acids were identified by retention time comparison with a mixture of 37 fatty acid methyl ester (FAME) standards (Supelco, USA, Catalog No: 18919-1AMP).

Statistical analysis: Analysis of variance (ANOVA) was carried out using SPSS version 22 (SPSS, Inc., USA) software. Significances among means were determined using the Duncan test at a $P<0.05$ significance level. The experiment was done based on a completely randomized design (3 fungi and 4 salinity treatments, with control) with three replications for all the treatments and phytochemical analysis.

Results

Root colonization: According to the staining of purslane root (Fig. 1), mycorrhizal symbiosis formed between plant roots and AM fungus. A study of fungal structures revealed that vesicles were abundant in the roots infected with both AM species, but arbuscular was detected only in roots colonized by *G. mosseae*. The average root colonization percentage was 82.33% and 71.66% in roots infected with *G. intraradices* and *G. mosseae*, respectively (Fig. 2).

Growth parameters: Analysis of variance indicated that all the treatments had significant effects ($P \leq 0.001$) on the shoot growth (Table 2). According to Table 1, the shoot length and dry weight reduced progressively in the purslane with the elevation of the NaCl concentration. As compared to the controls, the shoot grown under the highest salt stress (160 mM NaCl) showed a significant decline in length (66%) and dry weight (67%), respectively (Table 1). But, AM fungus (*G. mosseae* and *G. intraradices*) did not significantly affect the height and dry weight of shoot purslane.

Phenolic compound and flavonoid content: Based on the results, both AM fungi and salt stress had a significant impact on the flavonoid and phenolic compounds content of leaves ($P \leq 0.001$) (Table 2). According to Figure 3. A and B, phenolic compounds and flavonoid contents elevated significantly in the purslane leaves with the elevation of the salt treatments (up to 120 mM NaCl) in comparison to the controls. In the treated leaves with 120 mM and 160 mM NaCl, phenolic compounds (1.6–1.1 fold) and flavonoids (1.8–1.6 fold) were increased compared to the controls, respectively (Figure 3). In all treatments, the highest levels of phenolic compound were observed in the leaves of purslane inoculated with *G. mosseae*. The phenolic compounds and flavonoid content in the leaves colonized with *G. mosseae* are significantly 1.2 fold higher than *G. intraradices* (Figure 3 A and B).

Antioxidant capacity by DPPH and FRAP: Analysis of variance indicated that all the treatments caused marked ($P \leq 0.01$) changes in antioxidant capacity (DPPH and FRAP) (Table 2). Based on Figure 4 (A), the DPPH radical scavenging of purslane leaves increased significantly under the NaCl treatments in comparison to the controls. The lowest level of IC_{50} was shown under the 120 mM NaCl concentration (Figure 4A). The different salinity levels had a significant effect on FRAP radical scavenging of leaves (Figure 4B). IC_{50} of DPPH scavenging was significantly reduced in purslane plants inoculated with *G. mosseae* (3.03 mg ml^{-1}) and *G. intraradices* (3.33 mg ml^{-1}), compared to the control (3.87 mg ml^{-1}) (Figure 4). On the contrary, mycorrhiza treatment had no significant effect on FRAP antioxidant capacity, compared to the control.

Fatty acid content: Based on the evaluation of fatty acid profiles of purslane, the presence of seven fatty acids, including C4:0, C6:0, C16:0, C22:0, and C24:0 as saturated fatty acids, and C18:3n3 and C18:2n6 as unsaturated fatty acids, were identified. The GC/FID chromatogram of the profile of fatty acids in purslane leaves was mentioned in supplementary fig. Analysis of variance indicated that salt stress and mycorrhizal treatments caused a significant effect ($P \leq 0.05$) on the fatty acid profile (Table 3). As compared to the controls, the content of saturated fatty acids, except C4:0 and C16:0, reduced with increasing NaCl concentration. The highest level of butyric acid was detected in purslane grown under 120 mM NaCl (Table 4). According to table 4, α-Linolenic acid and linoleic acid as unsaturated fatty acids decreased under the higher NaCl concentrations (160 mM). The highest C18:3n3 content was observed in 40 NaCl concentrations (%12-fold) when compared to the controls (Table 4). Mycorrhizal symbiosis significantly decreased the fatty acid content

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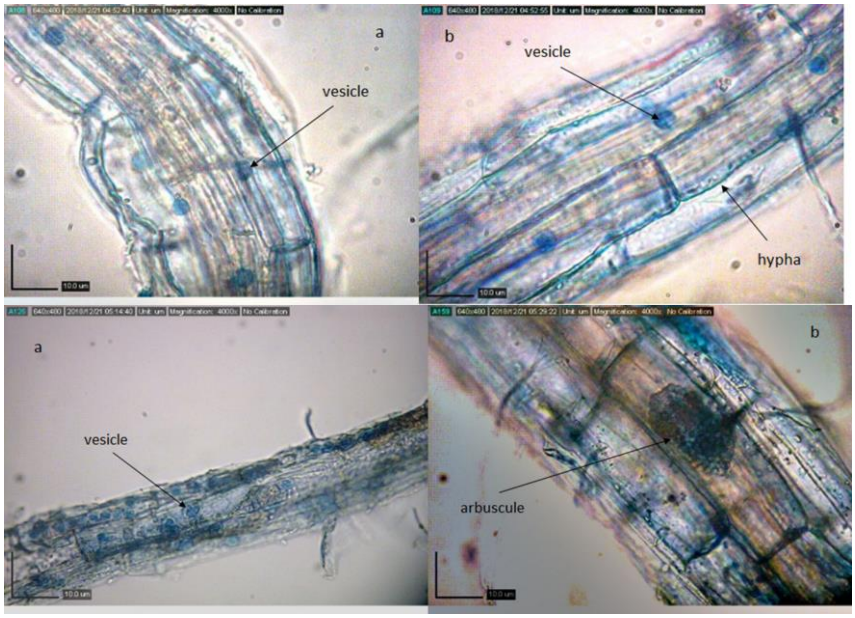

Figure 1. A fungal structure (vesicle) (a) and fungal structures (vesicle and hyphae) (b) in lactophenol cotton blue-staining roots colonized with *G. mosseae* (Up) and *G. intraradice* (down) 8 weeks after inoculation (40x magnification).

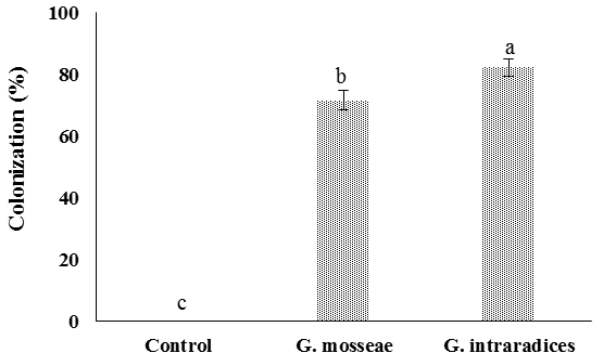

Figure 2. Effects of AM fungi (*G. mosseae* and *G. intraradices*) on root colonization percentage of purslane, compared to control.

Table 1. Effects of NaCl concentrations and AM fungi on shoot height and dry weight of purslane.

NaCl concentrations (mM)	Shoot height (cm)	Shoot dry weight (g)
0	33.33±2.41^{a}	45.63±2.31^{a}
40	20.33±0.60^{b}	27.33±0.82^{b}
80	17.86±1.12^{bc}	23.60±0.90^{bc}
120	15.36±1.02^{c}	19.70±1.31^{c}
160	11.26±0.62^{d}	15.06±1.21^{d}
G. mosseae	32.80±1.34^{a}	43.70±0.81^{a}
G. intraradices	32.30±0.81^{a}	42.93±1.62^{a}

Different small letters indicate significant difference between the mean ± SD values of three replicates based on Duncan test (P < 0.05).

Table 2. Analysis of variances (ANOVA) of shoot height, shoot dry weight, phenolic compounds, flavonoid, DPPH and FRAP in purslane leaves under salinity and mycorrhizal treatments

Parameters	DF	Shoot height	Shoot dry weight	Phenolic compounds	Flavonoids	DPPH	FRAP
Treatments	6	258.98***	483.73***	9555***	550.17***	0.25***	4862**
Error	14	3.27	6.44	657.10	8.54	0.00	1028
Coefficient of variation (CV)	-	7.71	8.12	2.83	4.46	2.62	10.66

*** (P ≤ 0.05), ** (P ≤ 0.01), *** (P ≤ 0.001), NS: Non Significant**

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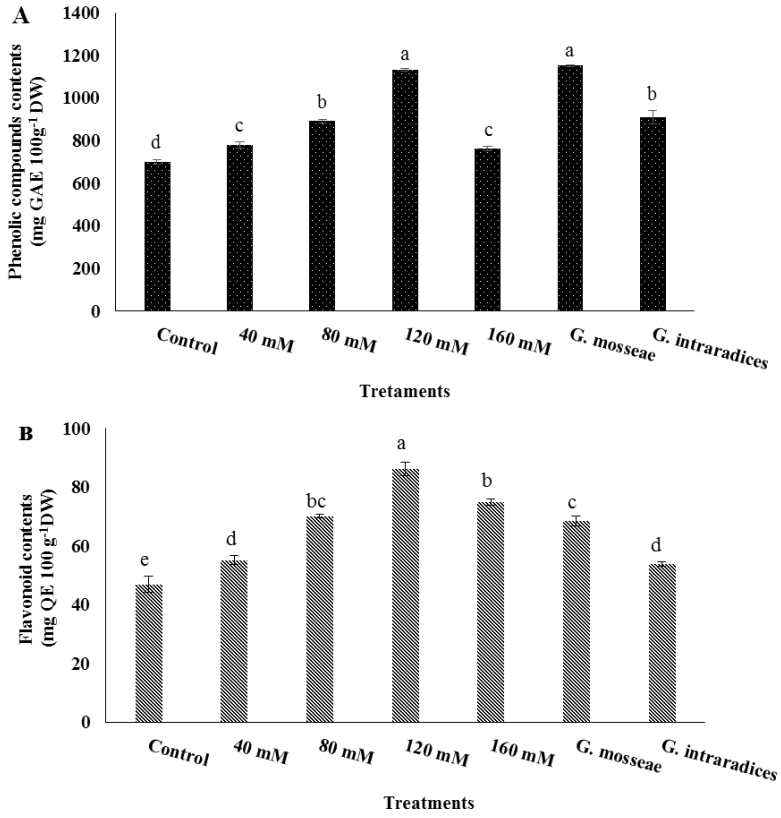

Figure 3. Effects of AM fungi (*G. mosseae* and *G. intraradices*) and salt stress on phenolic compounds contents (A) and flavonoid content (B) of purslane leaves.

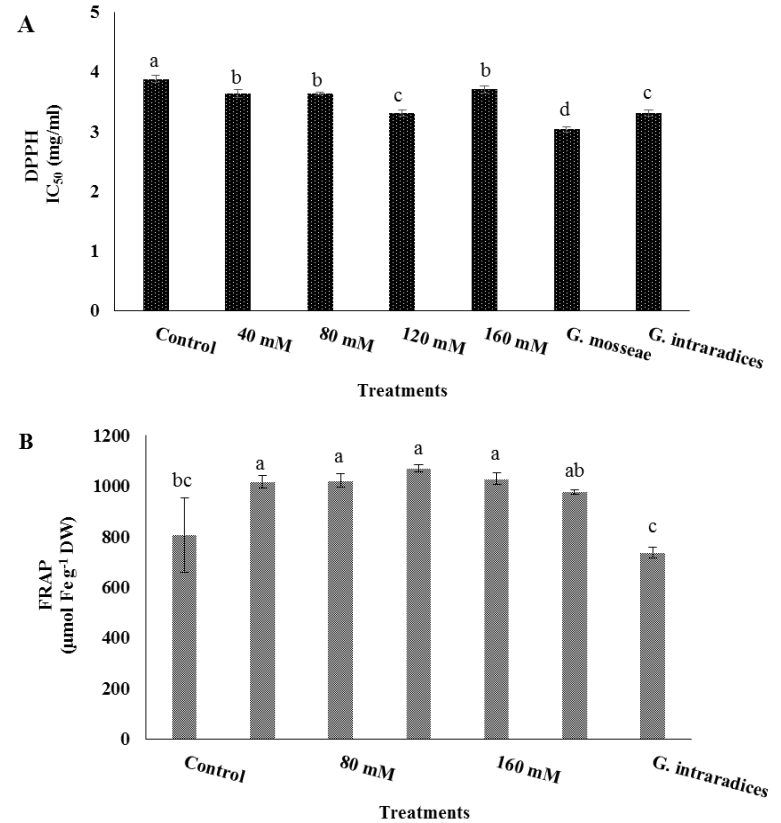

Figure 4. Effects of AM fungi (*G. mosseae* and *G. intraradices*) and salt stress on antioxidant capacity (DPPH) (A) and FRAP (B) of purslane leaves.

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Table 3. Analysis of variances (ANOVA) of fatty acids profile (C4:0, C6:0, C16:0, C22:0, C24:0, C18:2n6 and C18:3n3 in purslane leaves under salinity and mycorrhizal treatments

Parameters	DF	C4	C6	C16	C22	C24	C18:2n6	C18:3n3
Treatments	6	1490^{***}	266.97^{***}	1.31^{*}	0.30^{***}	0.48^{***}	1.81^{***}	8.33^{***}
Error	14	89.47	1.41	0.33	0.00	0.0086	0.02	0.02
Coefficient of variation (CV)		15.80	8.60	15.22	14.61	11.06	9.07	5.07

*** (P ≤ 0.05), ** (P ≤ 0.01), *** (P ≤ 0.001), NS: Non Significant**

Table 4. Effects of AM fungi and salt stress on fatty acids profile of purslane leaves

Treatments	Fatty acid content (mg g^{-1} DW)						
	C4:0	C6:0	C16:0	C22:0	C24:0	C18:2n6	C18:3n3
0 mM	52.56 ± 6.14^{c}	21.01 ± 0.51^{a}	2.42 ± 0.177^{a}	0.75 ± 0.01^{a}	1.02 ± 0.01^{a}	3.27 ± 0.11^{a}	4.19 ± 0.06^{b}
40 mM	74.64 ± 2.74^{ab}	20.01 ± 0.75^{ab}	3.12 ± 0.22^{c}	0.71 ± 0.05^{ab}	1.02 ± 0.09^{a}	2.21 ± 0.05^{b}	$4.70\pm0.1a^{a}$
80 mM	77.64 ± 4.48^{ab}	18.99 ± 0.97^{abc}	3.07 ± 1.00^{c}	0.63 ± 0.02^{b}	0.77 ± 0.01^{b}	1.93 ± 0.07^{bc}	4.09 ± 0.00^{b}
120 mM	82.42 ± 1.60^{a}	18.46 ± 0.04^{bc}	3.21 ± 0.06^{bc}	0.29 ± 0.05^{d}	0.69 ± 0.03^{bc}	1.74 ± 0.18^{c}	3.76 ± 0.13^{c}
160 mM	63.19 ± 9.81^{bc}	17.51 ± 1.22^{c}	4.18 ± 0.00^{ab}	0.43 ± 0.03^{c}	0.57 ± 0.00^{c}	0.99 ± 0.01^{d}	2.63 ± 0.08^{d}
G. mosseae	17.50 ± 3.34^{d}	0.00 ± 0.00^{d}	4.60 ± 0.69^{a}	0.00 ± 0.00^{e}	0.14 ± 0.00^{d}	1.02 ± 0.03^{d}	0.56 ± 0.06^{f}
G. intraradices	51.00 ± 5.77^{c}	0.00 ± 0.00^{d}	4.37 ± 0.46^{a}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	1.70 ± 0.09^{c}	0.88 ± 0.00^{e}

Butyric acid (C4:0); Hexanoic acid (C6:0); Palmitic acid (C16:0); Behenic acid (C22:0); Lignoceric acid (C24:0). Linoleic acid (C18:2n6c); α-Linolenic acid (C18:3n3); different small letters indicate significant differences between the mean ± SD values of three replicates based on Duncan's test (P < 0.05).

(except palmitic acid) compared to controls. Under the mycorrhizal treatments, the highest amounts of saturated and unsaturated fatty acids were observed in control plants (Table 4).

Discussion

In the study, mycorrhizal symbiosis formed between purslane roots and *G. intraradices* and *G. mosseae* with a high colonization percentage. Similarly, other findings reported the existence of symbiotic relationships between *G. intraradices* with *Solanum lycopersicum* (root colonization, 65%) (Malekzadeh *et al*., 2012), and *G. mosseae* with *Robinia pseudoacacia* L. (root colonization, 79.2%) (Gong *et al*., 2012). Up to now, little research has been carried out on symbiosis between AM fungus and species from Portulacaceae (Vatovec *et al*., 2005). For instance, Kamble and Pal (2016) indicated that *G. geosporum* and *G. macrocarpum* colonized *P. oleracea* root cortex cells and formed vesicles and extra-radical hyphae.

Similar to our results, no marked changes were observed in the biomass of *Cicer arietinum* inoculated with *G. mosseae* and *G. intraradices* (Moradi *et al*., 2018) and the height and shoot biomass of *Oryza sativa* inoculated with *G. mosseae* (Hajiboland *et al*., 2009). According to the report of Hawkins *et al*. (2000), *G. mosseae* can absorb nitrogen from organic sources and convey it to plants, which leads to better growth of the plant. In this study, the opposite result was obtained. Probably, the growth haltering of plants that are symbiotic with the fungus may be due to saving energy for the plant's defense responses. Similar to the research, Veiga *et al*. (2013) stated that the reduction of *Arabidopsis thaliana* growth incubated with AM fungal (*Rhizophagus irregularis*) is probably due to nutrient elimination to the plant side of the rhizosphere by AM hyphae that devote nutrients to the symbiont. According to Table 1, Yazici *et al*. (2007) reported that exposure of *P. oleracea* plants to 140 mM NaCl for 30 days decreased plant height and weight. Uddin *et al*. (2012) showed that purslane could tolerate mild salinity levels (66 and 132 mM). A possible reason for the decrease in shoot growth of purslane might be owing to less turgor pressure at severe salt stress, which causes cell enlargement decline (Behdad e*t al*., 2020). Also, the decrease in growth under salt treatment saves energy for the defense process and improves the risk of possible damage (Behdad *et al*., 2021). Another probable reason reported is that increasing NaCl concentration could limit the production of plant hormones such as cytokinins and increase the biosynthesis of inhibitors such as abscisic acid (Kafi and Rahimi, 2011).

Consistent with these results, several reports show that symbiotic relationships between plants and AMF enhanced phenolic compounds and flavonoid production (Hassan and Mathesius, 2012). Sarker *et al*. (2018) indicated that flavonoid compounds were elevated in the roots of *Glycyrrhiza uralensis* colonized with *G. mosseae*. Blilou *et al*. (2000) reported that accumulation of salicylic acid (SA) during the early stages of infection has been observed in rice inoculated with *G. mosseae*. Moreover, the accumulation of SA in plants inoculated with AM was correlated with an increase in the gene expression of chalcone synthase (CHS) and phenylalanine ammonia-lyase (PAL) as the key enzymes of the flavonoid biosynthesis pathway (Blilou *et al*., 2000; Bonanomi *et al*., 2001). The flavonoids affect hyphae growth, differentiation, and root colonization (Steinkellner *et al*., 2007). Similar to our results, Petridis *et al*. (2012) reported that moderate saline conditions lead to the biosynthesis of phenolic compounds in leaves of *Olea europaea* L.. Under salt stress, the accumulation of phenolic compounds that protect the cellular structures from oxidative stress is caused by elevated production of ROS and maintenance of osmotic balance (Alam *et al*., 2015). Studies showed

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that in response to salinity stress, jasmonic acid biosynthesis increased in plants, which could stimulate PAL activity (Sarker *et al*., 2018).

In the study, AMF mycorrhiza inoculation significantly had positive effects on the DPPH of purslane. Moreover, the lowest level of IC_{50} of DPPH scavenging was shown under the *G. mosseae* treatment. The salinity stress had a significantly positive effect on antioxidant capacity (DPPH and FRAP). *G. mosseae* and *Glomus caledonium* enhanced the abundance of DPPH antioxidant capacity (Harrison and Dixon, 1993). AMF symbiosis caused the increase in antioxidant capacity by using different mechanisms such as (a) elevation of nutrient uptake, (b) enhancing the efficiency of the host plants by increasing their growth, and (c) biosynthesis of phenolic compounds such as flavonoids (Mollavali *et al*., 2015). According to a report by Banuelos *et al*. (2014), mycorrhizal inoculation can promote antioxidant capacity by elevating phenolic compounds and changing the expression of genes involved in their biosynthesis, as a plant defense mechanism. Rivero *et al*. (2018) indicated that salt stress caused the accumulation of antioxidant metabolites in *Lycopersicon esculentum* inoculated with AMF compared to the controls. Hristozkova *et al*. (2017) indicated that the antioxidant properties of phenols are associated with their ability to chelate metal ions, which generate free radicals, and their capacity to scavenge ROS. Also, Alam *et al*. (2015) indicated that different levels of salinity resulted in an 18–35% increase in FRAP in *P. oleracea.*

Similar to the obtained results, the presence of C4:0, C22:0, C18:2n6, and C18:3n3 as unsaturated fatty acids (Teixeira, 2009), and C16, and C24:0 (Petropoulos *et al*., 2019) in leaves of *P. oleracea* have been reported. Montoya-Garcia *et al*. (2018) identified palmitic acid, stearic acid, linoleic acid, and, α-linolenic acid in leaves of purslane. Polyunsaturated fatty acids (PFUs), especially alpha-linolenic acid and linoleic acid, play an essential function in the growth and development of plants (Saffaryazdi *et al*., 2022). Our study showed that mycorrhizal symbiosis decreased the content of fatty acids compared to controls. *G. intraradices* are not able to synthesize some fatty acids, such as palmitic acid, and these compounds are only synthesized in the roots of mycorrhizal plants (Trepanier *et al*., 2005). Sugars and lipids, as the major sources of organic carbon, are transferred from the plant to the fungi, and the symbionts use them to synthesize mycorrhizal lipids (Luginbuehl *et al*., 2017). According to the results, the saturated fatty acid content (except C4:0, and C16) and C18:2n6 fatty acid reduced with increasing NaCl concentration. However, the highest C18:3n3 level was detected in 40 NaCl concentrations compared to the controls. The saturation degree and chain length of fatty acids also can change due to environmental conditions (Sivaprakasam *et al*., 2016). Purslane growth under salt treatment up to 40 mmol of NaCl concentration may ameliorate their fatty acid profile (Carvalho *et al*., 2009), the same as the results of the study. Under saline conditions, water scarcity probably reduces lipid accumulation and damages all enzymatic activities of fatty acids (Yeilaghi *et al*., 2012). Sui *et al*. (2018) indicated that the activity of ω-3 fatty acid desaturase and unsaturated fatty acid content in peanuts (*Arachis hypogaea* L.) decreased under salt stress.

Conclusion

The results of the study showed fungal structures (hypha and vesicle) were in purslane roots colonized with both arbuscular mycorrhizae (*G. mosseae* and *G. intraradices*). The highest colonization rate (82%) was observed in roots inoculated with *G. intraradices*. The leaves of the plants incubated with *G. mosseae* contained the maximum amount of phenolic compounds and antioxidant capacity (DPPH). The most phenolic compounds, flavonoid contents, and antioxidant capacity (FRAP) were reported in plants treated with 120 mM NaCl. The mycorrhiza and salinity treatments had negative impacts on the contents of the fatty acid profile. Based on the study, mycorrhizal (*G. mosseae*) and moderate salt treatments (up to 120 mM NaCl) can be used to increase the phenolic and antioxidant compounds of the purslane plant and thus improve its nutritional value. However, the salinity level of 40 mM NaCl was suitable for the maximum increase of fatty acids.

Author contributions

A.S. performed the experiments, did sampling and data analysis, and wrote the manuscript. A.G. supervised the whole research work and revised the manuscript. A.B. wrote and revised the manuscript.

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Conflicts of interest

We wish to confirm that there are no known conflicts of interest associated with this publication.

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