



Response of some physiological and biochemical traits of Purslane (*Portulaca oleracea*) to silica fertilizer under salinity stress

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ABSTRACT

Purpose: Purslane plant is used in the pharmaceutical and food industries. This study aimed to study some physiological and biochemical reactions of *Portulaca oleracea* to salinity stress and the effect of silica fertilizer application in reducing the harmful effects of salinity stress on climatic conditions of Behbahan city in southwestern of Iran. **Research Method:** Purslane seeds were planted in plastic pots. Salinity treatment was considered at two levels of 0 and 200 mM NaCl and silica fertilizer treatment were considered at two levels of 0 and 2g/l. **Findings:** Results revealed that with increase salinity concentration, plant height, amount of soluble sugar, amount of soluble protein and chlorophyll b significantly decreased. The use of silica fertilizer had a positive effect on the mentioned traits in comparison with its non-use. Based on the results, the use of silica fertilizer increased plant height, soluble sugar, and soluble protein by 16.19, 25.35 and 28.74%, respectively, compared to its non-use in salinity conditions on the *Portulaca oleracea* plant, which is very important due to the salinity of a large areas of agricultural lands in Iran. **Research limitations:** No limitations were founded. **Originality/Value:** This study showed that silica fertilizer, compared to its non-application, increased plant height and photosynthetic pigments and reduced the harmful effects of salinity stress on *Portulaca oleracea*. Therefore, both in the condition of lack of salinity and in the condition of salinity stress, the use of silica fertilizer is suggested in comparison with its non-use in order to improve the measured traits.

INTRODUCTION

Portulaca oleracea L. is a summer plant with broad leaves of the Portulacaceae family that grows in different parts of the world. This plant is very important in terms of nutrition and medicine. *Portulaca oleracea* has been named by the World Health Organization as the most common medicinal plant in the world due to its antioxidant properties and abundant nutrients (Hollosoy, 2002; Mpoloka, 2008). Due to the fleshy and juicy leaves of *Portulaca oleracea*, it is used to relieve heat, sunburn, and burn pain. Other therapeutic properties of *Portulaca oleracea* mentioned in some sources include the removal of corns, exfoliating properties, and elimination of contusions. These anti-inflammatory properties have been attributed to the omega-3 and omega-6 fatty acids in *Portulaca oleracea* (Xu et al., 2006). The effects of *Portulaca oleracea* on the nervous system include decreased motor activity and anticonvulsant effects (Hassan et al., 2014). In addition, other therapeutic properties of *Portulaca oleracea* include the inhibition of neuromuscular contractions following electrical stimulation and muscle relaxation activity in conscious rats (Smrkolj et al., 2006). *Portulaca oleracea* has therapeutic properties with properties such as antioxidants, blood purifier, prevention of heart attack, antiseptic, intestinal anthelmintic, muscle relaxant, and strengthening the immune system (Simioni et al., 2014).

Non-biotic stresses such as salinity are the main causes of agricultural decline worldwide, especially in arid and semi-arid regions, and reduce their fertility (Song et al., 2008). Among the severe problems in agriculture can be the limitation of crop development due to extensive saline lands (Ghanbari et al., 2006). The complexity of plants' response to salinity stress can be due to the effect of salinity through various mechanisms such as osmotic stress, nutrient imbalance, reduction of carbon dioxide concentration (closing of pores), increase in production of oxygen free radicals, and stimulate oxidative stress (AL-Taey et al., 2018). Studies have shown that soil salinity reduces growth through primary and secondary effects and thus reduces plant biomass, in this regard; various plant organs such as roots, stems, and branches react differently due to different sensitivity to salinity (Setia et al., 2013).

Silica makes up about 30% of the Earth's crust. Silica is present in the raw solution as dissolved silica mono silicic acid, which is absorbed by the plant in the same way. Silica is not an essential element of the plant, but its beneficial effects on growth, yield, and tolerance to the environment.

Stresses in many plants have been observed (Ma et al., 2006). Silica can protect plants against biological and non-biological stresses. Silica reduces abiotic stresses, including chemical stresses (salinity), physical stresses (high temperature, drought, ultraviolet, and radioactive radiation), and other stresses (Ma et al., 2006; Ahmed et al., 2011; Chen et al., 2011; Al-Taey et al., 2022). The researchers studied showed that silicon confers *Soybean* resistance to salinity stress through the regulation of reactive oxygen and reactive nitrogen species (Yong et al., 2020). Improving photosynthesis and increasing chlorophyll content under salinity conditions were positive effects of silica in this study (Lee et al., 2010). This study aimed to investigate the effect of salinity stress on some physiological and biochemical traits of *Portulaca oleracea* and the role of silica fertilizer in reducing the adverse effects of salinity stress and improving these traits in the climate of Behbahan.

MATERIALS AND METHODS

Experimental details

In order to study the physiological and biochemical traits of *Portulaca oleracea* under salinity stress and silica fertilizer, a factorial experiment was conducted in a completely randomized

design with three replications in June 2021 in the greenhouse of the Department of Biology, the Khatam Alanbia University of Technology Behbahan. Behbahan city is located between $50^{\circ} 9'$ to $50^{\circ} 25'$ east and $30^{\circ} 45'$ to $30^{\circ} 32'$ north in Khuzestan province, Iran. The mean minimum and maximum annual temperatures are 18.1 and 32.37°C, respectively, and the climate of the region is dry according to the Dumartin method. It is 313 meters above sea level and the mean rainfall in the last 30 years is 345 millimeters. During the experiment, the mean temperature of the greenhouse was 24°C and the relative humidity was about 35%. According to the molar mass of salt, the desired salinity concentration was obtained by dissolving salt in water. In this experiment, *Portulaca oleracea* seeds were obtained from a specialized store selling plant seeds and planted in plastic pots in such a way that there were five plants in each pot in June 2021. Experimental factors included salinity treatment with sodium chloride salt at two levels (0 and 200 mM) (Dayanti- Tilki et al., 2017) and silica fertilizer at two levels (0 and 2 g l⁻¹) according to the recommendation on the fertilizer bag of Ariashimi company) (Table 1). The experiment was performed in pots with a capacity of 1.2 kg using garden soil and leaf soil (ratio 1:3). To treat the plant with silica fertilizer, after seedling growth and the emergence of true leaves, the pots were treated with silica fertilizer (0 and 2 g l⁻¹) in three stages at intervals of 3 days with irrigation water (Hajihashemi et al., 2022). After that, in three weeks, irrigation of pots with 200 mM salt concentration (To determine the concentration of salt used in the experiment (200 mM), multiply the molar mass of salt by two tenths to obtain the amount of salt in grams, then weigh the calculated amount of salt with a digital scale and pour it into the graduated cylinder and calculate its volume, and we brought the volume to one liter). Moreover, at the same time, irrigation of pots with regular irrigation was done, and plant leaves were harvested to measure traits. Traits such as plant height, amount of soluble sugar, amount of soluble protein, photosynthetic pigments, chlorophyll index, and the maximum quantum efficiency of photosystem II (Fv / Fm) were evaluated.

Plant height

The height of the plant from the soil surface to the top plant was measured with a ruler.

Soluble sugar

The method of Bates was used to measure the amount of soluble sugar (DuBois et al., 1956). First, 2 ml of pure ethanol was added to 0.02 g of dried and ground leaves. Then 1 ml of 5% phenol was added to 0.5 ml of the resulting solution and mixed well. 4 ml of concentrated sulfuric acid was added to the composition and after 30 minutes, the absorbance dose of 485 nm was read using a spectrophotometer. Finally, the concentration of soluble sugars in mg g⁻¹ dry weight was determined using the standard glucose curve.

Soluble protein of leaves

Bradford method was used to measure the amount of soluble protein in leaves (Bradford, 1976). 0.1 g of fresh leaf tissue in a porcelain mortar was thoroughly ground on ice with 1 ml of 50 mM potassium phosphate buffer with an acidity of 6.8. The extracted extracts were then centrifuged at 10,000 rpm for 30 minutes at 4°C. The resulting supernatant was used to measure the solution's protein content, and the samples were adsorbed at 595 nm by a spectrophotometer.

Table 1. Different levels of salinity stress and silica fertilizer used in the experiment.

Abbreviation	Salinity level (mM NaCl)	Abbreviation	Silica fertilizer level (g/l)
Treatment level		Treatment level	
N ₁	0	S ₁	0
N ₂	200	S ₂	2

Measurement of photosynthetic pigments

The amount of chlorophyll a, b, carotenoids, and total chlorophyll were measured by taking 0.1 g of fresh leaf sample with 10 ml of 80% acetone ground in a Chinese mortar. The crushed samples were poured into a test tube and centrifuged at 3000 rpm for 10 minutes, and then the absorbance of the supernatant solution was read to determine the photosynthetic pigments by a spectrophotometer at the wavelengths of 663, 645, and 470 nm. Using equations 1, 2, 3, and 4, the amount of chlorophylls a, b, carotenoids, and total chlorophyll in µg per ml solution of the sample was calculated (Lichtenthaler & Wellburn, 1983).

$$\text{Chl.a} = (12.25A_{663} - 2.79A_{646}) \quad (1)$$

$$\text{Chl.b} = (21.21A_{646} - 5.1 A_{663}) \quad (2)$$

$$\text{Car} = (1000A_{470} - 1.82 \text{ Chl.a} - 85.02\text{Chl.b})/198 \quad (3)$$

$$\text{ChlT} = \text{Chla} + \text{Chlb} \quad (4)$$

SPAD value

Leaf chlorophyll concentration (SPAD value) was measured with a model chlorophyll meter (CCM-200 plus, Opti-Sciences Inc, NH., USA).

The maximum quantum efficiency of photosystem II

The maximum quantum efficiency of photosystem II was read with the model chlorophyll fluorometer (Pocket PEA, Hansatech, Instruments Ltd., King's Lynn, Norfolk, England) (Kalaji et al., 2011).

Data analysis

SAS statistical program version 3.9 was used for data analysis. The means were compared using Duncan's multiple range tests at a 5% probability level.

RESULTS AND DISCUSSION

Plant height

The results showed that plant height was affected by the interaction of salinity stress and silica fertilizer ($p \leq 0.01$) (Table 2). A comparison of data means showed the highest plant height with an average of 27.44 cm in conditions without salinity stress and silica fertilizer. In contrast, the lowest, with an average of 16.20 cm in salinity stress conditions, was obtained without silica fertilizer. Also, the reduction rate of *Portulaca oleracea* plant in salinity stress conditions - application of silica fertilizer compared to salinity stress conditions - without silica fertilizer was 16.19% less (Table 3), which was consistent with the research results of Arouiee et al. (2014) about the effects of salinity stress and silica application on stem height in *Trigonella foenum-graecum* L. The researchers have reported in a study that salinity stress reduces plant growth and plant productivity by affecting morphological traits (Muhammad & Hussain, 2010).

The amount of soluble sugar

The amount of soluble sugar was significantly affected by the interaction of salinity stress and silica fertilizer ($p \leq 0.05$). However, the sole application of salinity stress and silica fertilizer had no significant effect on this trait (Table 2). A comparison of the mean data showed that the highest amount of soluble sugar was related to salinity stress - application of silica fertilizer with 9.74 mg g^{-1} dry weight and the lowest amount related to salinity stress - no application of silica fertilizer with 7.27 mg/g was dry weight per gram (Fig. 1). The study of the effects of salinity and silica application on *Arabidopsis thaliana* plant showed that the application of silica increased the amount of sugar and decreased the amount of starch in comparison with the absence of its application under salinity stress conditions (Shams et al., 2019). According to Hajjhashemi et al. (2022) report, silica treatment in radish plants under salt stress increased the amount of carbohydrates consistent with the results observed in the present study. The studies of Shams et al. (2019) on *Arabidopsis* showed that salt stress increased soluble sugars and decreased starch, which probably reveals the plant's attempt to regulate insufficient osmosis. Feeding silicon, along with increasing the amount of potassium and magnesium and increasing the activity of antioxidant enzymes, caused a decrease in oxidative stress caused by salinity. In addition, increasing the amount of reducing sugars, reducing starch and increasing the amount of relative water with silicon feeding in plants under salinity shows the improvement of the water status of plants. As a result, plants fed with silicon under salinity had better growth than plants without silicon. These results showed that silicon alleviates salinity stress in *Arabidopsis* by reducing oxidative stress and improving water.

Table 2. Analysis of variance of the effect of salinity stress and silica fertilizer on morphological and physiological traits in *Portulaca oleracea*.

S.O.V	DF	Plant height	Amount of soluble sugar	Amount of soluble protein	Chlorophyll a	Chlorophyll b
Salinity	1	204.43**	0.03 ^{ns}	9.48**	0.17*	5.38*
Silica	1	0.06 ^{ns}	2.67 ^{ns}	1.63 ^{ns}	0.14*	3.20*
Salinity ×Silica	1	26.79**	6.94*	4.21*	0.008 ^{ns}	5.68*
Error	8	2.98	0.78	0.50	0.02	0.51
C.V. (%)	-	7.89	10.47	10.75	21.48	44.59

Table 2. (Continued).

S.O.V	DF	Carotenoid	Total chlorophyll	Leaf chlorophyll concentration (SPAD)	Maximum quantum efficiency of photosystem II
Salinity	1	2.68**	6.99**	17.59**	0.002**
Silica	1	0.17 ^{ns}	1.34 ^{ns}	0.92 ^{ns}	0.0000 ^{ns}
Salinity ×Silica	1	0.47 ^{ns}	4.20*	5.69*	0.00003 ^{ns}
Error	8	0.16	0.41	0.60	0.0001
C.V. (%)	-	15.63	28.1	9.17	1.79

*, $P \leq 0.05$ and **, $P \leq 0.01$

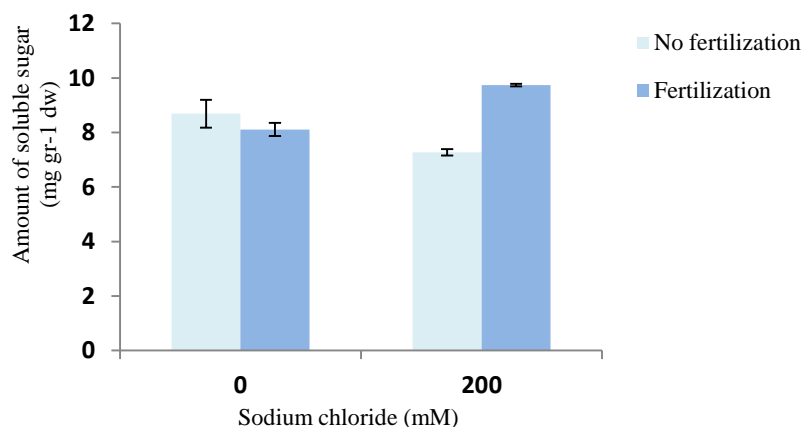


Fig. 1. The interaction of salinity and silica on the amount of soluble sugar in *Portulaca oleracea*.

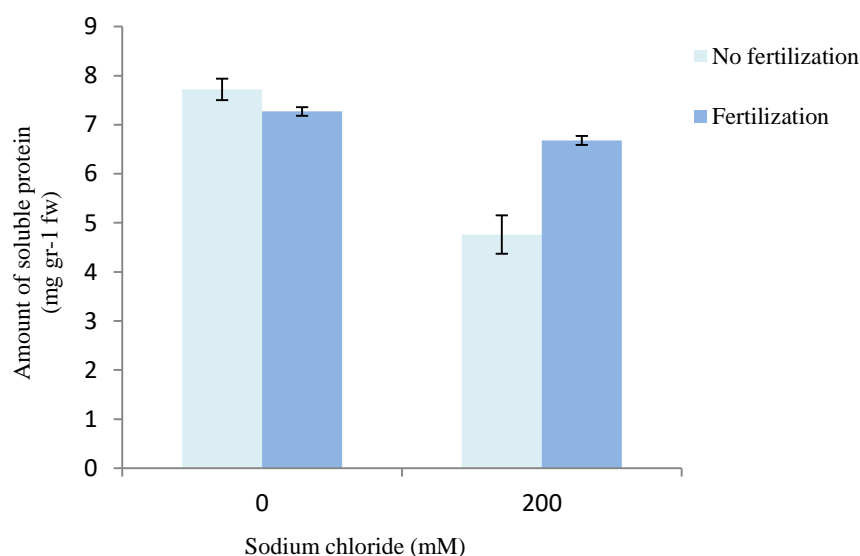


Fig. 2. The interaction of salinity and silica on the amount of soluble protein in *Portulaca oleracea*.

The amount of soluble protein

The analysis of variance (Table 2) showed that the amount of soluble protein under salinity stress ($p \leq 0.01$) was significant. The interaction effect of salinity and silica fertilizer ($p \leq 0.05$) was also significant. Salinity stress significantly reduced the amount of soluble protein in *Portulaca oleracea* compared to conditions without salinity stress. A comparison of the mean interaction of salinity stress and silica fertilizer showed that in salinity stress - application of silica fertilizer compared to salinity stress - no application of silica fertilizer, the amount of protein increased to 28.76, which indicates the positive effect of application of silica fertilizer in these conditions (Fig. 2). Plant proteins are induced by salinity. Researchers have shown that salinity stress-related proteins increase only in the salinity state and provide a form of stored nitrogen that plays an essential role in osmotic regulation (Ashraf & Harris, 2004). The studies of Hajiboland et al. (2017) showed that the application of silica in the conditions of salt stress in wheat plants increased the amount of leaf soluble protein, which is consistent with the results observed in the present study. The researchers mentioned that the amount of

soluble protein in 100 mM salinity and the application of 100 mg/ml silica increased the amount of soluble protein in peppermint compared to 100 mM salinity and the non-application of silica, probably due to the role silica compounds reduce salinity stress and increase protein content (Danaei & Abdoosi, 2021). Different concentrations of sodium chloride reduced the amount of protein in *Portulaca oleracea* L. (Rahdari et al., 2012). In cauliflower, salinity decreased protein content, but treatment with silica reduced the adverse effects of salinity stress and increased protein content (Enteshari et al., 2011).

Concentration of photosynthetic pigments

The results showed that salinity and silica fertilizer had a significant effect ($p \leq 0.05$) on chlorophyll a and b and also, the interaction of salinity and silica fertilizer had a significant effect ($p \leq 0.05$) on chlorophyll b (Table 2). The results showed that individual effects of salinity, silica, and interaction between them had a considerable impact on the carotenoids and total chlorophyll pigments (Table 2). Salinity stress reduced the concentration of chlorophyll b and total chlorophyll in *Portulaca oleracea*. A comparison of the mean interaction of salinity and silica fertilizer showed that salinity stress - no application of silica fertilizer had the lowest concentrations of chlorophyll b and total chlorophyll (Table 3). Also, the concentration of chlorophyll b and total chlorophyll in salinity stress - application of silica fertilizer compared to salinity stress - non-application of silica fertilizer increased by 30.63% and 28.97%, respectively, indicating the beneficial effects of silica fertilizer application in comparison with silica fertilizer is not used in salinity conditions (Table 3). Salinity stress due to its effect on stomatal factors and reduced entry of carbon dioxide into cells reduced nitrogen uptake as an important mineral in chlorophyll pigment synthesis, reduced phytochemical activity, and reduced leaf area. Consequently, chlorophyll contents are also reduced in the plant. The use of silica and nano-silica is induced through detoxifying free radical species. The plant defense system controls the adverse effects of salinity stress and improves chlorophyll content (Haghighi & Pessarakli, 2013; Hajiboland & Cheraghvareh, 2014). Researchers have reported a decrease in total chlorophyll concentration in salinity stress and its improvement in silica foliar application in peppermint (Danaei & Abdoosi, 2021). Studies on *Portulaca oleracea* have shown that salinity stress reduces chlorophyll a, b and total; still, at the same salinity concentrations, the amount of photosynthetic pigments increases under ascorbic acid consumption, because ascorbic acid as an antioxidant increased tolerance to salt stress and reduced the harmful effects of sodium chloride (Pazoki et al., 2012).

Table 3. Means comparison of silica levels in each salinity level for the measured traits of *Portulaca oleracea*.

Salinity (mM)	Silica (gr.l ⁻¹)	Plant height (cm)	Chlorophyll b (mg.gr ⁻¹ Fw)	Total chlorophyll (mg.gr ⁻¹ Fw)	Leaf chlorophyll concentration (SPAD)
0	0	27.44 ^a	3.48 ^a	3.96 ^a	9.27 ^a
	2	24.60 ^b	1.07 ^b	2.11 ^b	10.10 ^a
200	0	16.20 ^b	0.77 ^b	1.25 ^b	8.23 ^a
	2	19.33 ^a	1.11 ^a	1.76 ^a	6.30 ^b

Table 4. Correlation coefficients between some of the measured traits tested.

	Maximum quantum efficiency of photosystem II	Leaf chlorophyll concentration (SPAD)	Plant height	Chlorophyll a	Total chlorophyll	Amount of soluble protein
Maximum quantum efficiency of photosystem II	1					
Leaf chlorophyll concentration (SPAD)	0.63*	1				
Plant height	0.72**	0.57*	1			
Chlorophyll a	0.33	0.35	0.45	1		
Total chlorophyll	0.63*	0.47	0.72**	0.12	1	
Amount of soluble protein	0.38	0.15	0.76**	0.61*	0.55	1

*, $P \leq 0.05$ and **, $P \leq 0.01$

Leaf chlorophyll concentration (SPAD)

The results showed that leaf chlorophyll concentration (SPAD) in *Portulaca oleracea* under the influence of salinity stress ($p \leq 0.01$) and the interaction of salinity and silica fertilizer ($p \leq 0.05$) were significant (Table 2). A comparison of the mean interaction of salinity and silica fertilizer showed that in conditions without salinity stress - application of silica fertilizer compared to conditions without salinity stress - non-application of silica fertilizer, leaf chlorophyll concentration increased by 8.21% (Table 3). Also, leaf chlorophyll concentration in conditions without salinity stress - application of silica fertilizer compared to salinity stress conditions - application of silica fertilizer increased by 37.62%, indicating the positive effect of silica fertilizer on leaf chlorophyll concentration in conditions without salinity stress (Table 3). Salinity causes changes in chloroplasts. On the other hand, the decrease in leaf chlorophyll in salinity stress may be due to membrane destruction and damage to the electron transfer chain in photosystems (Mane et al., 2010). Studies have shown that the effect of salicylic acid on chlorophyll content is not the same in all plants (Memarpour & Hadi, 2012). The researchers reported that chlorophyll content in wheat was reduced by salicylic acid treatment (Moharekar et al., 2003).

Traits correlation

The correlation analysis between some measured traits (Table 4) in this study showed that there was a positive and significant relationship between the maximum quantum yield of photosystem II with leaf chlorophyll concentration and total chlorophyll ($p \leq 0.05$) and plant height ($p \leq 0.01$). Also, according to Table 4, there was a positive and significant correlation ($p \leq 0.01$) between the amount of soluble protein and plant height, and between the amount of protein and chlorophyll a ($p \leq 0.05$). The positive relationship between physiological traits indicates cooperation to reduce the adverse effects of salinity stress by activating a mechanism to respond to salinity stress in *Portulaca oleracea*. Studies on plants such as maize (Omrani & Moharramnejad, 2018) and alfalfa (Yaryura et al., 2009) showed a relationship between physiological and biochemical traits with plant biomass (Ma & Yamaji, 2008).

CONCLUSION

This study showed that silica fertilizer, compared to its non-application, increased plant height and photosynthetic pigments and reduced the harmful effects of salinity stress on *Portulaca oleracea*. Increasing the amount of soluble sugar and soluble protein and decreasing starch improved the plant's water status, which increased the growth of *Portulaca oleracea* under salinity. Therefore, silica fertilizer can reduce the harmful effects of salinity on the medicinal plant of *Portulaca oleracea*, which is very important due to the salinity of a large percentage of Iran and the abundant medicinal and food use of *Portulaca oleracea*.

Conflict of interest

The author has no conflict of interest to report.

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