



# Morphological and Physiological Response of *Conocarpus erectus* and *Salvadora persica* Species to Salinity with the Aim of Selection for Cultivation in Urban Spaces in Hot and Dry Regions

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## ABSTRACT

Due to challenges in water quality and quantity, as well as the need to select resilient species for cultivation in urban green spaces (UGS), this study aimed to evaluate the effects of six months of salinity stress (SS) on the morpho-physiological traits and mineral composition of *Conocarpus erectus* and *Salvadora persica*. Salinity stress was induced using NaCl at concentrations of 0, 250, 500, and 750 mM, applied to three-month-old seedlings. The results revealed significant changes in various traits, including plant height (PH), leaf fresh weight (LFW), leaf dry weight (LDW), root diameter (RD), root fresh weight (RFW), and root dry weight (RDW). Chlorophyll content was highest at 0 mM salinity and lowest at 750 mM, with reductions of 47.11% in *C. erectus* and 34.24% in *S. persica*. Proline (Pr) content increased with rising salinity levels, whereas total protein content (TPC) and total sugar content (TSC) decreased in both species. However, PH, TPC, and TSC showed no significant changes under salinity levels ranging from 0 to 500 mM NaCl. Leaf mineral analysis indicated that increasing salinity levels led to a decrease in calcium (Ca) content and an increase in sodium (Na) and chlorine (Cl) levels. Species-specific differences were observed under salinity stress; for instance, traits such as PH, stem diameter (SD), and leaf area index (LAI) showed no significant changes in *S. persica*. Overall, *S. persica* exhibited fewer morphological and physiological changes than *C. erectus* under salinity stress, suggesting it is better adapted to such conditions. This makes *S. persica* a promising candidate for cultivation in hot and arid urban environments.

**Abbreviation:** Leaf area (LA), Leaf area index (LAI), Leaf fresh weight (LFW=), leaf length, Leaf dry weight (LDW), Plant height (PH), Primary root diameter (PRD), Root diameter (RD), Root fresh weight (RFW), Root dry weight (RDW), Stem diameter (SD), Urban green space (UGS), Proline (Pr), Total protein content (TPC), Total sugar content (TSC), Salinity stress (SS), Electrical conductivity (EC), Chlorine (Cl), Sodium (Na), Potassium (K)

## Introduction

In recent years, climate change has significantly impacted water resources, agriculture, human health, and ecosystems worldwide (Khondoker et

al., 2023). Approximately 70% of freshwater resources are used in agriculture, and due to global warming and population growth, water

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demand is projected to rise by 55% by 2050 (Smedley and Kinniburgh, 2017). Consequently, the management and preservation of existing freshwater and unconventional water resources are of paramount importance. In the future, the use of lower-quality water sources, such as saline water, will become increasingly necessary, along with the cultivation of plant species that can tolerate such conditions for purposes such as food production, greening urban spaces, and other applications.

Global and regional climatic changes have exacerbated salinity in water resources, necessitating the use of unconventional water in agriculture (Ibrahim et al., 2023; Shabani Fard et al., 2024). Moreover, the rapid growth of the global population and increased migration to urban areas have significantly heightened the demand for urban green spaces (UGSs), a trend expected to intensify in the coming years (FAO, 2021). At present, UGSs represent a critical component of cities for environmental conservation (Nguyen et al., 2021), yet they require considerable water resources. Using unconventional water sources for irrigating UGSs presents a promising solution to water scarcity. However, given the limitations in water quantity and quality, only highly durable and salt-tolerant plant species can be cultivated in these spaces, particularly in arid regions.

In Iran, a country characterized by hot and dry climatic conditions, water salinity and scarcity are particularly severe in the southern and eastern regions. Between 2000 and 2019, the urban population in Iran increased from 64.20% to 75.94%, intensifying the demand for UGSs (Pilehvar, 2021). This issue is especially pronounced in southern Iran, where low rainfall, high temperatures, and water limitations make plant selection for UGS cultivation particularly critical. Only a limited number of native, resilient species are used in these regions, including *Conocarpus erectus* L. and *Salvadora persica* L. *Conocarpus erectus* is an evergreen tree from the Combretaceae family (Austin, 2004; Hussein and El-Dawy, 2023). Known for its high resistance to environmental stress (Afifi et al., 2021), it is widely cultivated in UGSs as a key species (Chehrazi et al., 2021). Similarly, *Salvadora persica*, a member of the Salvadoraceae family, is an evergreen species with a wide range of applications, including its traditional use as a chewing stick or "miswak" for dental care (Aumeeruddy et al., 2018). This species possesses several medicinal properties, including abrasives, antiseptics, astringents, detergents, enzyme inhibitors, and fluoride (Hussein and El-Dawy, 2023). Additionally, due to limitations in fodder

production, both species hold significant importance as sources of animal feed in hot and arid regions (Rao et al., 2017; Mohammadabadi et al., 2023).

Considering the scarcity of water resources in southern Iran and the need to employ unconventional and saline water sources for UGS irrigation, this study aimed to compare the effects of salinity stress on the morpho-physiological traits of *C. erectus* and *S. persica*. The findings of this research can guide the selection of more resistant species for UGS cultivation and other purposes, such as providing animal feed in stressful environments.

## Material and methods

### *Plant materials and growth condition*

The experiment was conducted from December 2022 to July 2023 at the Department of Horticultural Sciences, Faculty of Agriculture, Islamic Azad University, Jahrom Branch, Jahrom, Iran (latitude 28°30'N, longitude 53°33'E, altitude 1050 m above sea level). The study area experiences a semi-arid climate, with an average annual minimum and maximum temperature of 2.4 °C and 40.5 °C, respectively. The average annual rainfall is 285 mm, and the relative humidity is 45.4%. The study was performed on three-month-old *Conocarpus erectus* and *Salvadora persica* seedlings of similar height. A randomized complete block design with six replicates was used. Each pot contained one seedling, and treatments were applied to assess the target characteristics. Seedlings were planted in pots placed in an open space. Each plastic pot, measuring 29 cm in diameter and 24 cm in height, was filled with 10 kg of sandy clay loam (CL) soil. The soil composition included 2.90% organic matter and 7.5% lime content. Its pH was 7.90, with an electrical conductivity (EC) of 7.93 dS m<sup>-1</sup>. The soil also contained 1491 ppm of chlorine (Cl), 954.5 ppm of sodium (Na), and 15.40 ppm of potassium (K).

### *Treatments*

Irrigation water with an electrical conductivity (EC) of 1.5 dS m<sup>-1</sup> was sourced from the green space water distribution network at the university campus. Salinity treatments were applied using NaCl solutions at concentrations of 0 (control), 250, 500, and 750 mM. Irrigation with saline water at these specified concentrations was performed at 6-day intervals over a period of six months. The irrigation volume was determined based on the in-situ field capacity (FC) as reported by Akca and Sahin (2022). To prevent salt accumulation in the pots, additional

distilled water was applied after each irrigation session, following the method outlined by Li et al. (2011).

### ***Measurement of morphological traits***

At the end of the test period, each plant was carefully removed and washed. Samples of leaves, stems, and roots were collected from both control and NaCl-treated plants to evaluate plant parameters. Leaves from identical positions were selected for analyzing mineral content, as well as morphological and biochemical traits. Plant height (PH), primary root length (PRL), leaf length (LL), and lateral root length (LRL) were measured using a ruler. Stem diameter (SD), primary root diameter (PRD), and lateral root diameter (LRD) were determined with a digital caliper. Leaf area index (LAI) was measured using a leaf area meter (LI-3100C, LI-COR Inc., Lincoln, NE, USA). Leaf fresh weight (LFW) and root fresh weight (RFW) were measured by weighing the total leaves and roots with an accuracy of 0.001 g. Subsequently, leaf and root samples were dried in an oven at 80 °C until a constant weight was achieved, and their dry weights were recorded.

### ***Measuring biochemical traits***

#### ***Chlorophyll content***

Healthy and mature leaves were collected from each seedling during sampling. Chlorophyll a, chlorophyll b, and total chlorophyll contents were determined using the method described by Moran (1982). For this purpose, 0.1 g of fresh leaves was extracted with 80% acetone and filtered through filter paper. The absorbance of the extract was measured using a spectrophotometer (Jenway 6300) at wavelengths of 653 nm and 666 nm. The chlorophyll a and chlorophyll b contents (mg g<sup>-1</sup> fresh weight) were calculated using the following equations:

$$\text{Chlorophyll } a = 15.56 A_{666} - 7.340 A_{653}$$

$$\text{Chlorophyll } b = 27.05 A_{653} - 11.21 A_{666}$$

#### ***Proline (Pr)***

To measure proline (Pr) content, 5 g of fresh leaves were homogenized in 10 mL of 3% sulfosalicylic acid solution and then centrifuged at 16,000 × g at 4 °C for 15 min. A 2 mL aliquot of the resulting supernatant was mixed with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid. The mixture was heated at 100 °C for 1 h, followed by immediate cooling in an ice bath to halt the reaction. To extract proline from the solution, 4 mL of toluene was added, and the solution was vigorously vortexed for 15–20 seconds. The absorbance of the resulting upper toluene layer

was measured at 520 nm using a spectrophotometer. Proline content was determined using a standard curve generated from proline concentrations ranging from 0 to 20 mg L<sup>-1</sup> (Bates et al., 1973).

#### ***Total sugar content (TSC)***

TSC was measured using the anthrone method, as described by Agrawal et al. (2015). Briefly, 0.02 g of leaf samples were homogenized in 80% ethanol and centrifuged at 8,000 × g for 10 min at room temperature. Subsequently, 0.2 mL of the supernatant was mixed with 3 mL of anthrone reagent, and the mixture was heated in a boiling water bath (100 °C) for 20 min. After cooling to room temperature (25°C), the absorbance of the reaction mixture was measured at 620 nm using a spectrophotometer. The TSC concentration was determined using a standard glucose curve with concentrations of 0, 20, 40, 60, 80, and 100 µg.

#### ***Total protein content (TPC)***

The TPC was determined using the Bradford (1976) method. The absorbance was measured at a wavelength of 595 nm. TPC was calculated using a standard curve with different concentrations of Bovine Serum Albumin (BSA).

#### ***Mineral content***

Leaf samples were oven-dried at 80 °C and ground into a fine powder using a grinding mill. The powdered samples were ashed in a microwave furnace (Model 28, Precision Scientific, Chicago, IL) at 550 °C for 3 h. One gram of ash was dissolved in 5 mL of 2N hydrochloric acid and digested in a hot water bath for 1 h. After cooling to room temperature, the digested solution was diluted to 50 mL with distilled water and filtered through filter paper. The filtered solutions were used to analyze mineral ion content. Potassium (K) and sodium (Na) levels were measured using a flame photometer (Model PFP7, USA), following a method used by Papathanasiou (2012). Calcium (Ca) content was determined using an atomic absorption spectrophotometer. Chlorine (Cl) concentration was analyzed using potassium chromate reagent, based on the Chapman and Goldsmith (1982) method.

#### ***Data analysis***

To analyse the data, the ANOVA procedure of SAS v. 9.4 (Statistical Analysis System, SAS, Cary NC, U.S.) was used. Duncan's Multiple Range Test grouped the traits based on the treatments. Pearson's correlation analysis was also

performed to identify the treatment correlations using R v3.4.3.

## Results

### Morphological traits

Different salinity levels significantly affected primary root diameter (PRD), root fresh weight (RFW), and root dry weight (RDW) in both *C. erectus* and *S. persica*. Although salinity stress (SS) influenced traits such as leaf fresh weight (LFW), leaf dry weight (LDW), and lateral root

diameter (LRD), it did not affect plant height (PH), stem diameter (SD), leaf area index (LAI), leaf length (LL), primary root length (PRL), or lateral root length (LRL) in either species (Table 1). Increasing salinity levels caused reductions in PRD, LL, RFW, and RDW in both species. At 750 mM NaCl, significant decreases were observed: PRD, LL, RFW, and RDW were reduced by 9.73%, 16.96%, and 18.42%, respectively, in *S. persica*, and by 18.91%, 29.46%, and 26.11%, respectively, in *C. erectus*, compared to the control (Table 2).

**Table 1.** ANOVA (mean square) of morphological and biochemical parameters of *C. erectus* and *S. persica* seedlings under salinity.

Factor	Species	Salinity	Species × Salinity	CV (%)
Plant height	559.25*	1536.36**	436.12*	21.30
Stem diameter	14.2*	0.54 <sup>ns</sup>	1.6 <sup>ns</sup>	16.80
Leaf length	638228.3*	58643.4 <sup>ns</sup>	36784.4 <sup>ns</sup>	31.20
Leaf fresh weight	1936.2*	4.73 <sup>ns</sup>	1.52 <sup>ns</sup>	30.80
Leaf dry weight	16.32*	1.01 <sup>ns</sup>	0.64 <sup>ns</sup>	9.10
Chlorophyll <i>a</i>	0.110*	0.001 <sup>ns</sup>	0.02 <sup>ns</sup>	10.90
Chlorophyll <i>b</i>	0.979*	0.169*	0.006 <sup>ns</sup>	9.40
Total chlorophyll	0.864*	0.141*	0.005 <sup>ns</sup>	12.10
Proline	2.59**	0.05*	0.06 <sup>ns</sup>	14.40
Total sugars	3.03*	0.89*	0.26 <sup>ns</sup>	8.80
Total protein	24.16**	2.58 <sup>ns</sup>	0.42 <sup>ns</sup>	17.40
Root diameter	11.12*	7.45**	6.54*	11.76
Root fresh weight	12120.3**	29.57*	9.51 <sup>ns</sup>	9.15
Root dry weight	3964.3**	53.96**	37.24**	7.22

<sup>ns</sup>: indicates not significant; \**P* < 0.05; \*\**P* < 0.01.

**Table 2.** Effect of salinity (0, 250, 500, and 750 mM NaCl) on the morphological and physiological parameters of *C. erectus* and *S. persica* seedlings after six months of vegetative growth.

	NaCl (mM)	PH (cm)	SD (mm)	LAI	LL (cm)	LFW (g)	LDW (g)	PRL (cm)	LRL (cm)	PRD (mm)	LRD (mm)	RFW (g)	RDW (g)
<i>C. erectus</i>	0	55.18 <sup>a</sup>	4.58 <sup>a</sup>	1095 <sup>a</sup>	69.5 <sup>a</sup>	20.32 <sup>a</sup>	0.29 <sup>a</sup>	375.7 <sup>a</sup>	88.2 <sup>ab</sup>	5.0 <sup>b</sup>	0.52 <sup>ab</sup>	50.8 <sup>a</sup>	23.3 <sup>a</sup>
	250	50.53 <sup>ab</sup>	4.93 <sup>a</sup>	1180 <sup>a</sup>	73.9 <sup>a</sup>	19.6 <sup>ab</sup>	0.28 <sup>a</sup>	385.8 <sup>a</sup>	87.6 <sup>ab</sup>	5.29 <sup>a</sup>	0.69 <sup>a</sup>	48.9 <sup>ab</sup>	22.3 <sup>a</sup>
	500	54.09 <sup>ab</sup>	4.58 <sup>a</sup>	1043 <sup>a</sup>	71.6 <sup>a</sup>	17.83 <sup>bc</sup>	0.29 <sup>a</sup>	347.8 <sup>a</sup>	77.52 <sup>b</sup>	4.81 <sup>bc</sup>	0.63 <sup>ab</sup>	44.6 <sup>b</sup>	19.9 <sup>b</sup>
	750	41.50 <sup>b</sup>	4.50 <sup>a</sup>	955 <sup>b</sup>	74.8 <sup>a</sup>	16.90 <sup>c</sup>	0.31 <sup>a</sup>	372.4 <sup>a</sup>	91.77 <sup>a</sup>	4.61 <sup>c</sup>	0.56 <sup>b</sup>	42.9 <sup>b</sup>	19.0 <sup>b</sup>
	Sig.	0.026*	0.22 <sup>ns</sup>	0.04*	0.39 <sup>ns</sup>	0.02*	0.35 <sup>ns</sup>	0.78 <sup>ns</sup>	0.016*	0.001**	0.04 <sup>ns</sup>	0.001**	0.004**
<i>S. persica</i>	0	63.94 <sup>a</sup>	2.36 <sup>a</sup>	202 <sup>a</sup>	40.8 <sup>a</sup>	2.17 <sup>a</sup>	0.14 <sup>c</sup>	112.1 <sup>a</sup>	62.48 <sup>a</sup>	3.49 <sup>a</sup>	0.47 <sup>a</sup>	5.43 <sup>a</sup>	2.03 <sup>a</sup>
	250	60.64 <sup>ab</sup>	2.30 <sup>a</sup>	201 <sup>a</sup>	39.6 <sup>a</sup>	2.02 <sup>a</sup>	0.16 <sup>bc</sup>	94.0 <sup>b</sup>	52.79 <sup>ab</sup>	3.33 <sup>a</sup>	0.44 <sup>ab</sup>	5.06 <sup>a</sup>	1.95 <sup>ab</sup>
	500	57.46 <sup>ab</sup>	2.33 <sup>a</sup>	177 <sup>a</sup>	37.25 <sup>a</sup>	1.82 <sup>b</sup>	0.18 <sup>b</sup>	91.8 <sup>b</sup>	49.64 <sup>b</sup>	2.76 <sup>b</sup>	0.35 <sup>b</sup>	4.55 <sup>b</sup>	1.77 <sup>b</sup>
	750	53.01 <sup>b</sup>	2.35 <sup>a</sup>	183 <sup>a</sup>	40.6 <sup>a</sup>	1.53 <sup>c</sup>	0.22 <sup>a</sup>	99.9 <sup>ab</sup>	52.88 <sup>ab</sup>	2.83 <sup>b</sup>	0.34 <sup>b</sup>	3.83 <sup>c</sup>	1.50 <sup>c</sup>
	Sig.	0.021*	0.96 <sup>ns</sup>	0.58 <sup>ns</sup>	0.33 <sup>ns</sup>	0.001**	0.001**	0.03*	0.028*	0.002**	0.037*	0.001**	0.001**

PH= plant height, SD= stem diameter, LA= leaf area, LL=leaf length, LFW= leaf fresh weight, LDW= leaf dry weight, PRL= primary root length, LRL= lateral root length, PRD= primary root diameter, LRD= lateral root diameter, RFW= root fresh weight, RDW= root dry weight, LAI=Leaf area index. Means with the same letters indicate non-significant difference at 5% level according to Duncan's test. <sup>ns</sup>: indicates not significant; \**P* < 0.05; \*\**P* < 0.01.

In *C. erectus*, the highest primary root diameter (PRD) was observed at 250 mM NaCl, whereas no

significant difference was found between 250 mM and the control in *S. persica*. In *S. persica*, leaf

fresh weight (LFW) decreased with increasing salinity, while leaf dry weight (LDW) increased. The 750 mM NaCl treatment caused a 29.49% reduction in LFW and a 57.14% increase in LDW compared to the control. Additionally, the highest lateral root diameter (LRD) in *S. persica* was recorded in the control group, with no significant differences observed among the salinity treatments (Table 2).

### Biochemical parameters

Salinity treatments significantly affected chlorophyll a/b ratios, proline (Pr) content, and total protein content (TPC) in both *C. erectus* and *S. persica*, as well as the total soluble carbohydrate (TSC) in *S. persica*. Chlorophyll b

content increased with rising salinity levels, though the 750 mM treatment exhibited the lowest chlorophyll b content, with reductions of 46.09% and 33.83% in *C. erectus* and *S. persica*, respectively, compared to the control. Salinity stress caused a significant increase in Pr content, particularly at 750 mM and 500 mM in *C. erectus* (10% and 11% increases, respectively) and at 750 mM in *S. persica* (12% increase). Conversely, TPC decreased under salinity, with reductions of 11.11% in *C. erectus* and 15.62% in *S. persica* at 750 mM compared to the control. In *S. persica*, TSC also declined under salinity treatments relative to the control, although no significant differences were observed among the salinity levels (Table 3).

**Table 3.** Effect of salinity (0, 250, 500, and 750 mM NaCl) on the chlorophyll a and b, proline, total sugars and protein in *C. erectus* and *S. persica* seedlings after six months of vegetative growth.

	Chlorophyll a (mg g <sup>-1</sup> FW)		Chlorophyll b (mg g <sup>-1</sup> FW)		Total Chlorophyll (mg g <sup>-1</sup> FW)		Proline content (mg L <sup>-1</sup> )		Total sugars (%)		Total protein (mg 100g <sup>-1</sup> FW)	
	C.	S.	C.	S.	C.	S.	C.	S.	C.	S.	C.	S.
	<i>erectus</i>	<i>persica</i>	<i>erectus</i>	<i>persica</i>	<i>erectus</i>	<i>S. persica</i>	<i>erectus</i>	<i>persica</i>	<i>erectus</i>	<i>persica</i>	<i>erectus</i>	<i>persica</i>
0	0.97 <sup>a</sup>	1.10 <sup>a</sup>	1.56 <sup>a</sup>	1.75 <sup>a</sup>	2.57 <sup>a</sup>	2.83 <sup>a</sup>	1.00 <sup>c</sup>	1.42 <sup>b</sup>	8.33 <sup>a</sup>	8.12 <sup>a</sup>	6.66 <sup>a</sup>	8.51 <sup>a</sup>
250	0.86 <sup>a</sup>	0.98 <sup>a</sup>	1.40 <sup>a</sup>	1.65 <sup>a</sup>	2.26 <sup>a</sup>	2.63 <sup>a</sup>	1.07 <sup>b</sup>	1.56 <sup>ab</sup>	8.21 <sup>a</sup>	7.66 <sup>a</sup>	6.40 <sup>a</sup>	8.09 <sup>ab</sup>
500	0.75 <sup>ab</sup>	0.90 <sup>a</sup>	0.97 <sup>ab</sup>	1.50 <sup>ab</sup>	1.69 <sup>ab</sup>	2.39 <sup>ab</sup>	1.1 <sup>ab</sup>	1.61 <sup>ab</sup>	7.12 <sup>ab</sup>	7.35 <sup>ab</sup>	6.16 <sup>b</sup>	7.68 <sup>ab</sup>
750	0.61 <sup>b</sup>	0.89 <sup>a</sup>	0.88 <sup>b</sup>	1.41 <sup>b</sup>	1.46 <sup>b</sup>	2.28 <sup>b</sup>	1.21 <sup>a</sup>	1.68 <sup>a</sup>	6.05 <sup>b</sup>	7.32 <sup>b</sup>	5.62 <sup>b</sup>	7.18 <sup>b</sup>
Sig.	0.001 <sup>**</sup>	0.47 <sup>ns</sup>	0.001 <sup>**</sup>	0.021 <sup>*</sup>	0.001 <sup>**</sup>	0.023 <sup>*</sup>	0.014 <sup>*</sup>	0.012 <sup>*</sup>	0.011 <sup>**</sup>	0.019 <sup>*</sup>	0.014 <sup>*</sup>	0.018 <sup>*</sup>

Means with the same letters indicate non-significant difference at 5% level according to Duncan's test. <sup>ns</sup>: indicates not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ .

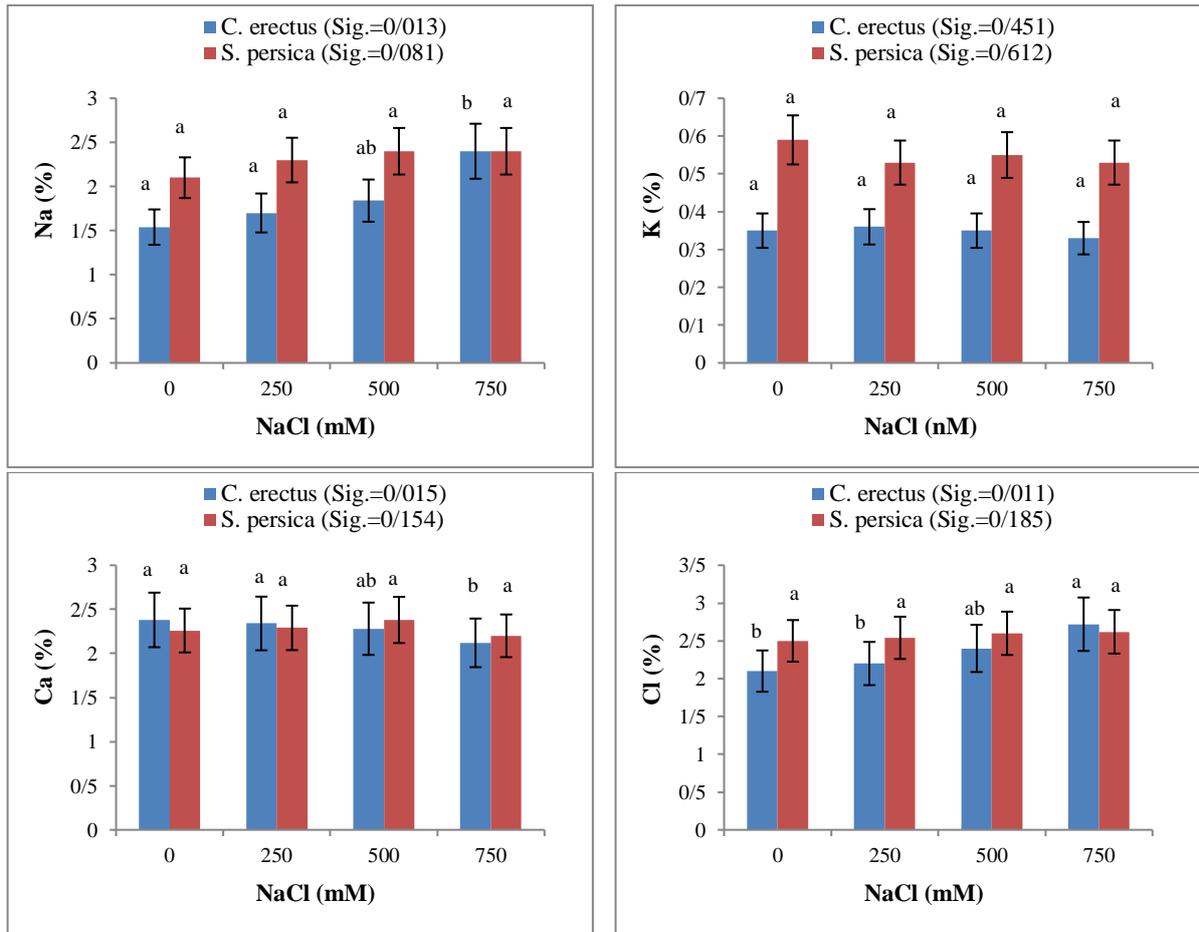
### Plant minerals content

The concentrations of Na and Cl in *S. persica* were significantly influenced by varying levels of NaCl, whereas the levels of K and Ca remained unchanged under salinity stress in both species. Under salinity stress, Na concentrations increased, while Cl concentrations decreased in both *C. erectus* and *S. persica*. Overall, after six months of the salinity treatment, the change in mineral content was more pronounced in *C. erectus* compared to *S. persica* (Fig. 1).

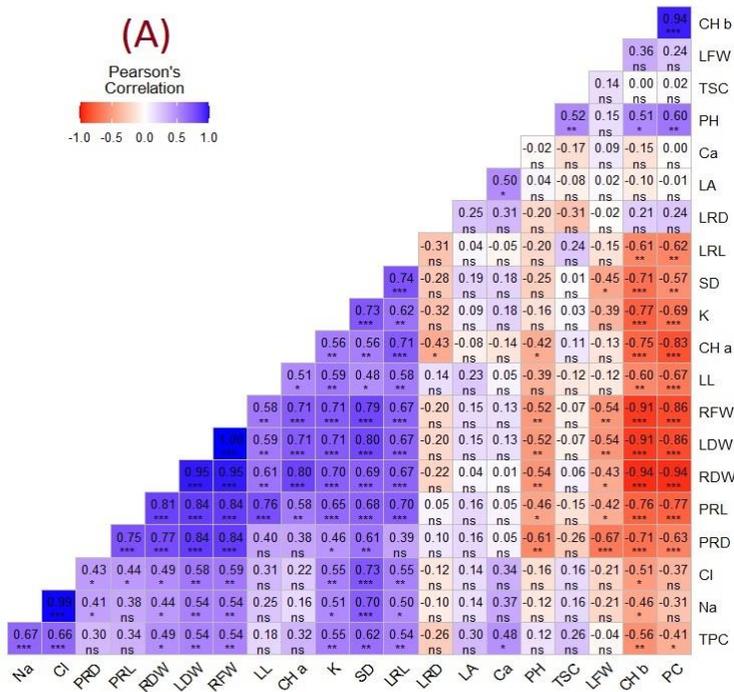
Also, the evaluation of the correlation between the characteristics of the evaluation mode showed that some factors have a significant relationship with each other. For example, there was a significant relationship between chlorophyll content and characteristics such as height, diameter and dry or fresh weight of the plant. In fact, the correlations were identified between i) LDW, chlorophyll b, Pr content, and Ca content, and ii) PH, SD, leaf area, leaf length, LFW, PRL, LRL, PRD, LRD, LL, RFW, RDW, chlorophyll a, TSC, TPC, Na content, K content, and Cl content in both *S. persica* and *C. erectus*. In both species, chlorophyll and the amount of dry and fresh weight of the plant showed the highest correlation (Fig. 2).

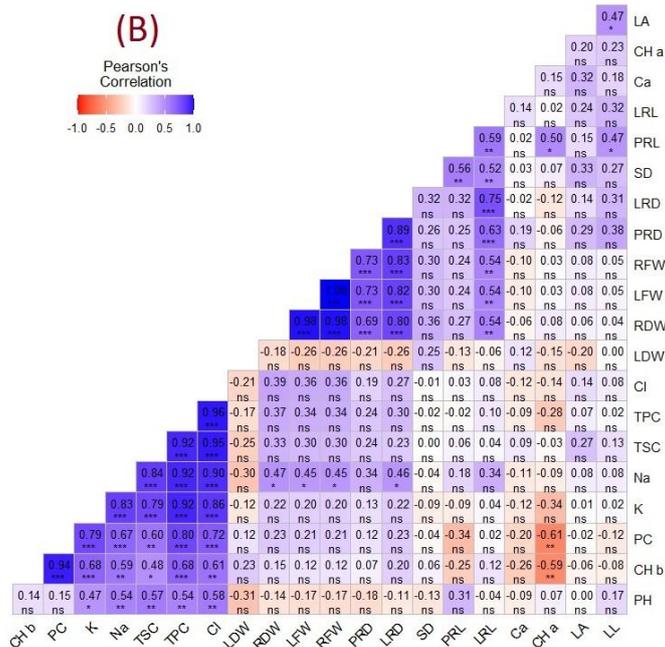
### Discussion

The morphological, physiological, and mineral characteristics of *C. erectus* and *S. persica* exhibited notable differences under salinity stress (SS). At lower stress levels, these characteristics remained relatively unchanged; however, severe salinity caused significant alterations in traits such as leaf fresh weight (LFW), primary root diameter (PRD), lateral root diameter (LRD), lateral root fresh weight (LLRFW), root dry weight (RDW), chlorophyll content, and total phenolic content (TPC). The detrimental effects of salinity on these traits can be attributed to factors such as ionic imbalance, osmotic stress, and reduced nutrient uptake (Alzahrani et al., 2019; Abrar et al., 2020). The osmotic effects induced by SS elevate abscisic acid (ABA) levels in stomatal guard cells, leading to stomatal closure, reduced CO<sub>2</sub> assimilation, and decreased photosynthetic rates. Consequently, plant growth and performance are adversely impacted (Sarker and Oba, 2020). Similar findings have been reported by Patel and Parida (2022), who observed that increasing salinity stress and osmotic pressure caused significant changes in the characteristics of *S. persica*.



**Fig. 1.** Changes in sodium (Na), calcium (Ca) chlorine (Cl) and potassium (K) of *C. erectus* and *S. persica* seedlings under salinity (0, 250, 500, and 700 mM NaCl) after six months. Mean values showing the same letter are not statistically different at  $P \leq 0.05$  according to Duncan's test.





**Fig. 2.** Pearson’s correlation analysis of the morpho-physiological properties in *C. erectus* (A) and *S.persica* (B), depicting correlation coefficients (r) ranging from  $-1.0$  to  $+1.0$ . Positive and negative values are represented in red and blue, respectively. PH: plant height, SD: stem diameter, LA: leaf area, LL: leaf length, LFW: leaf fresh weight, LDW: leaf dry weight, PRL: primary root length, LRL: lateral root length, PRD: primary root diameter, LRD: lateral root diameter, RFW: root fresh weight, RDW: root dry weight, CH *a*: chlorophyll *a*, CH *b*: chlorophyll *b*, PC: proline content, TSC: total sugar content, TPC: total protein content, Na: Na content, K: K content, Ca: C content, Cl: Cl content. ns  $P > = 0$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; and \*\*\*  $P < 0.001$ .

Salinity stress can also result in cellular dehydration, reducing water potential, impairing cell expansion, and hindering cell wall synthesis. This chain of events negatively impacts cell division and elongation, contributing to reduced growth parameters (Kumar et al., 2021). Moreover, SS suppresses the expression of key regulatory genes responsible for cell cycle progression, such as cyclins and cyclin-dependent kinases. This suppression leads to a decline in cell numbers within the meristem, thereby inhibiting growth and compromising the plant’s ability to efficiently absorb nutrients and water (Balasubramaniam et al., 2023). The observed reduction in morphological parameters may also be attributed to the diversion of energy from growth processes toward maintaining salt homeostasis and carbon balance. This survival strategy minimizes biomass production due to reduced carbon assimilation (Pompeiano et al., 2017). Under these conditions, plants prioritize carbon allocation to salinity homeostasis, an anti-stress mechanism, rather than to growth and development (Kumar et al., 2021).

Interestingly, an increase in leaf dry weight (LDW) in *S. persica* under SS may be due to the accumulation of osmolytes, such as proline (Pr), which are known to increase under stress

conditions. These osmolytes facilitate osmotic adjustment, helping maintain cell turgor and water balance, thereby contributing to increased LDW (Hossain et al., 2019). Additionally, energy redirection toward the synthesis of osmoprotectants and antioxidants, along with metabolic restructuring under stress, could further explain this increase (Balasubramaniam et al., 2023). Significant changes were also observed in the roots of *C. erectus* and *S. persica* under SS. Root elongation, a process involving cell division and expansion in the root apical meristem, can be influenced by salinity, either promoting or inhibiting elongation depending on stress levels. The studied species displayed varying responses to soil salinity and moisture deficiencies, altering their root system architecture and expansion. Root plasticity enables plants to adapt to stress by favoring deeper root growth, accessing less saline soil layers (Shelden and Munns, 2023).

Under mild SS, an increase in PRD of *C. erectus* at 250 mM NaCl treatment likely reflects root system plasticity. Conversely, a decrease in PRD in *S. persica* at 500 and 750 mM NaCl concentrations may result from reduced root growth due to lower photoassimilate allocation to roots. This reduction in root diameter may

represent a coordinated mechanism to enhance water flux from the soil to the leaves by creating a favorable osmotic gradient (Ullah et al., 2022). Similar results were observed in other studies, where lateral root elongation was promoted under SS (Sarker and Oba, 2020). Additionally, salinity may cause root thickening and a reduction in terminal roots, contributing to increased root weight (Soda et al., 2017).

Numerous studies have demonstrated that exposure to NaCl stress leads to reductions in plant height (PH), stem length (SL), root diameter (RD), primary root diameter (PRD), leaf fresh weight (LFW), leaf dry weight (LDW), root dry weight (RDW), and root fresh weight (RFW) (e.g., Rahnesan et al., 2018; Kumar et al., 2021). However, contrasting findings were reported by Wang et al. (2023), who observed an overall increase in biomass with escalating SS. The effect of salinity on chlorophyll content has been a subject of debate. While several studies have reported a significant decline in chlorophyll levels under SS (e.g., Sharif et al., 2018), other findings suggest a different trend. For instance, in *Oenanthе javanica*, an increase in chlorophyll content under SS was linked to a rise in chloroplast numbers (Kumar, 2021). In the present study, higher chlorophyll *b* content was observed in *S. persica*, which may contribute to its superior performance compared to *C. erectus* under SS.

Proline (Pr) levels also increased with rising salinity, which can be attributed to enhanced proline biosynthesis and/or inhibited proline catabolism (Sellami et al., 2019). This observation aligns with findings by Tounekti et al. (2018), who reported elevated proline accumulation in *S. persica* under SS. Conversely, TPC exhibited a relative decline with increasing salinity, particularly in *C. erectus*. The reduction in protein content under SS may result from several factors, including diminished protein synthesis, increased proteolysis, limited amino acid availability, and enzyme denaturation associated with protein synthesis (Patel and Parida, 2022).

Salinity also led to a reduction in TSC. This decline may be due to the accumulation of soluble sugars, such as glucose and sucrose, as part of the plant's osmotic adjustment mechanisms. The increased utilization of these sugars for stress adaptation may contribute to the observed reduction in TSC (Sellami et al., 2019). Moreover, SS can alter sugar metabolism, leading to varying levels of sugar compounds that impact overall TSC (Wang et al., 2022). Excessive salinity stress can further disrupt membrane permeability, inhibiting sugar accumulation. This aligns with the observed

inhibition of TSC at higher salinity levels (500–750 mM) in this study.

SS disrupts various aspects of plant growth and development, primarily due to imbalances in ionic concentrations. This disruption often leads to reduced uptake of essential mineral nutrients, adversely affecting cellular metabolism, photosynthesis, and root architecture. To counteract these effects, higher plants have evolved mechanisms to maintain Na<sup>+</sup> and Cl<sup>-</sup> homeostasis, thereby mitigating the negative impacts of NaCl stress (Tounekti et al., 2021). The significance of this mechanism in reducing salinity-induced damage has been emphasized in several studies (Balasubramaniam et al., 2023). To tolerate salinity, most plants must limit the uptake of Na<sup>+</sup> and Cl<sup>-</sup> while ensuring the continued absorption of macronutrients such as K<sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and Ca<sup>2+</sup> (Yassin et al., 2019; Kumar et al., 2021). The findings of this study suggest that *S. persica* exhibits a higher Na<sup>+</sup> content and lower fluctuation in ion uptake levels compared to *C. erectus*. This may be attributed to the presence of antiporters and membrane transporters, which enhance *S. persica*'s ability to withstand salinity stress. Additionally, the observed decrease in Na<sup>+</sup> and Cl<sup>-</sup> content under SS likely reflects the plants' need to restrict their uptake while maintaining the absorption of essential nutrients, as excessive concentrations of these ions can impair plant growth and development.

The evaluation of morphological and physiological changes further indicates that *S. persica* is more adaptable to SS than *C. erectus*. However, to corroborate these findings and achieve more precise insights, future research should assess additional physiological traits, including enzyme activities, stomatal characteristics, transpiration rates, cell membrane stability, and elemental distribution in leaves and roots. Such investigations would provide a more comprehensive understanding of the mechanisms underlying salinity tolerance.

## Conclusions

The present research examined the effect of salinity on some morpho-physiological properties of *C. erectus* and *S. persica*, species commonly used for UGSs. The results indicated that increasing salinity negatively impacted several traits, such as PH, RD, LL, RFW, RDW, chlorophyll, and Pr in both plants, with the most significant changes observed at 750 mM NaCl. Although the interaction of stress and species (species × salinity) influenced the studied characteristics, RDW, RD, and PH exhibited the most notable changes. Under salinity levels of 0–

500 mM NaCl, PH, TSC, and TPC did not show significant changes. The findings also revealed that species had a greater effect than SS on certain traits; for instance, PH, SD, and LAI did not exhibit significant changes in *S. persica*. With the exception of Ca, the concentrations of Na and Cl in the leaves increased with rising SS after six months in both species. Considering both the morphological and physiological characteristics studied, *S. persica* demonstrated greater resistance to salinity compared to *C. erectus*, making it more suitable for cultivation in UGSs under saline conditions.

### Conflict of Interest

The authors indicate no conflict of interest in this work.

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