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Original Article

Comparative assessment of metabolic, ionic and molecular responsiveness of four facultative halophytes to habitat salinization in the southwest of Jeddah Governorate, Saudi Arabia

Avaliação comparativa da capacidade de resposta metabólica, iônica e molecular de quatro halófitas facultativas à salinização do habitat, no sudoeste da província de Jeddah, Arábia Saudita

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Abstract

This study explores the influence of salinity on some physiological and biochemical pathways of four facultative halophytes (*Abutilon pannosum*, *Indigofera oblongifolia*, *Senna italica*, and *Tetraena coccinea*) along the southwest coast of Jeddah Governorate. Through a comparative analysis of these plants in both saline and non-saline environments, the study investigates chlorophyll levels, ion concentrations within the plants, the correlation with the SOS1 gene, and the impact of salinity on metabolic compounds. The overarching goal is to gain insights into the adaptive mechanisms of these specific plants to salt stress, providing valuable information for addressing global agricultural challenges associated with salinity. Throughout the study, metabolic, ionic, and molecular responses of these plants were scrutinized in both environments. The findings revealed elevated levels of Na^+, K^+, Ca^{2+}, and Mg^{2+} in saline habitats, except for Na^+ in *I. oblongifolia*. Despite increased concentrations of Chl b, variations were noted in Chl a and carotenoids in plants exposed to salt. Osmoregulatory patterns in *A. pannosum* and *I. oblongifolia* exhibited reversible changes, including heightened protein and proline levels in *A. pannosum* and decreased levels in *I. oblongifolia*, accompanied by alterations in amino acids and soluble carbohydrates. *Senna italica* displayed higher levels of osmolytes, excluding proline, compared to salinized environments, while *T. coccinea* exhibited lower levels of amino acids. The accumulation of Na^+ emerged as the primary mechanism for ionic homeostasis in these plants, with non-significant decreases observed in K^+, Mg^{2+}, and Ca^{2+}. Notably, an overexpression of the SOS1 gene (plasma membrane Na^+/H^+ antiporter) was observed as a response to maintaining ionic balance. Understanding these halophytes will be critical in addressing salinity challenges and enhancing crop tolerance to salinity.

Keywords: halophytes, osmomodulators, metabolic homeostasis, ionic balance, SOS1 gene expression.

Resumo

Este estudo explora a influência da salinidade em algumas vias fisiológicas e bioquímicas de quatro halófitas facultativas (*Abutilon pannosum, Indigofera oblongifolia, Senna italica* e *Tetraena coccinea*) ao longo da costa sudoeste da província de Jeddah. Através de uma análise comparativa dessas plantas em ambientes salinos e não salinos, o estudo investigou os níveis de clorofila, as concentrações de íons nas plantas, a correlação com o gene SOS1 e o impacto da salinidade nos compostos metabólicos. O objetivo geral consistiu em obter informações sobre os mecanismos adaptativos destas plantas específicas ao stress salino, fornecendo informações valiosas para enfrentar os desafios agrícolas globais associados à salinidade. Ao longo do estudo, as respostas metabólicas, iônicas e moleculares dessas plantas foram examinadas em ambos os ambientes. Os resultados revelaram níveis elevados de Na^+, K^+, Ca^{2+} e Mg^{2+} em habitats salinos, exceto Na^+ em *I. oblongifolia*. Apesar do aumento das concentrações de Clorofila B (Chl), foram observadas variações em Chl a e carotenóides em plantas expostas ao sal. Os padrões osmorregulatórios em *A. pannosum* e *I. oblongifolia* exibiram alterações reversíveis, incluindo níveis elevados de proteína e prolina em *A. pannosum* e níveis diminuídos em *I. oblongifolia*, acompanhados por alterações em aminoácidos e carboidratos solúveis. *Senna italica* apresentou níveis mais elevados de osmólitos, excluindo prolina, em comparação com ambientes salinizados, enquanto *T. coccinea* exibiu níveis mais baixos de aminoácidos. O acúmulo de Na^+ emergiu como o principal mecanismo para a homeostase iônica nessas plantas, com diminuições não significativas observadas em K^+, Mg^{2+} e Ca^{2+}. Notavelmente, uma superexpressão do gene SOS1 (antiportador Na^+/H^+ da membrana plasmática) foi observada como resposta à manutenção do equilíbrio iônico. Compreender estas halófitas será fundamental para enfrentar os desafios da salinidade e aumentar a tolerância das culturas à salinidade.

Palavras-chave: halófitas, osmomoduladores, homeostase metabólica, balanço iônico, expressão do gene SOS1.

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1. Introduction

By 2050, the population is predicted to reach 9.5 billion (Lafarga and Acién, 2022), implying that farming development must increase in response to food security issues. Environmental stresses, such as salinity, have emerged as a major concern challenging plant productivity over the past years, and agricultural practices have exacerbated this problem. Over one-third of all irrigated land around the world is influenced by saltiness (Shrivastava and Kumar, 2015). Human activities are responsible for the salt damage of about 80 million hectares of the estimated billion hectares of irrigated land (Khasanov et al., 2022). In semi-arid regions, soil salinization adversely affects economic progress (Sidike et al., 2014). About 6 million hectares of irrigated land are currently afflicted by soil salinization, and more than 40% of all agricultural land is degraded as a result of unsustainable reclamation practices, which portends irrigated land that is inappropriate for agriculture (Khasanov et al., 2022; Kulmatov, 2014). One of the major environmental issues affecting plant growth and development is the occurrence of high levels of salts in the soil and irrigation water, particularly in arid areas (Himabindu et al., 2016; Mansha et al., 2023). Excessive levels of sodium (Na^+) and chloride (Cl^-) in soils and water are the most frequent causes of salinity. The term "saline" refers to soils whose electrical conductivity (EC) is more than 4 dS/m (Kargas et al., 2020).

Numerous processes, including growth, photosynthesis, protein synthesis, enzyme activity, ionic balance, protein folding, gene expression, and lipid metabolisms are adversely impacted by salt stress (Hamouda et al., 2023; Parida and Das, 2005). In addition, plants experience ionic, osmotic, and oxidative stresses as a result of salt exposure (Fu and Yang, 2023). Plants have developed many salt tolerance strategies, which depend to a large extent on the capability of their roots to regulate both Na^+ and potassium (K^+) absorption and delivery to the shoot system. For instance, Tian et al. (2021) reported that plants with salinity tolerance accumulate K^+ rather than Na^+ in their shoot system. Another salt tolerance approach involves an increase in intracellular Ca^{2+} level, initiating a phosphorylation cascade response, that modulates proteins associated with cellular defense or transcription factors. These transcriptional factors then modulate the expression of specific genes related to the stress response, allowing plants to progressively adapted to their environment (Greenway and Munns, 1980). Further defenses against salt damage include stomatal closure, osmolyte accumulation, and increased Na^+/H^+ antiporter activity (Greenway and Munns, 1980). Accordingly, plants can be categorized into halophytes, which are salt tolerant, and glycophytes, which are salt sensitive.

Halophytes are plants that can thrive and reproduce in soil that contains 200 mM of salt or more. These halophytes constitute about 1% of the world's flora (Mohamed et al., 2023) Halophytes require salt concentrations for proper growth; otherwise, their growth becomes subpar, while glycophytes do not grow well in saline soils despite low salt concentrations (Greenway and Munns, 1980). A unique adaptation of some halophytes, such as salt glands or salt bladders, reduces the harmful effects of high salt concentrations (Mann et al., 2023). The salt tolerance related to particular molecules and specific metabolic activities may be increased by biochemical pathways that are active simultaneously and maybe in synergy (Ben Abdallah et al., 2018). Many biochemical techniques have been identified regarding salt tolerance, including selective ion accumulation or exclusion, regulation of root and leaf ion transport, compartmentalization of ions at the cellular and plant level, synthesis of compatible solutes, modifications to the photosynthetic pathway, alterations in membrane structure, activation of antioxidative enzymes, and implementation of plant hormones (Ahmad et al., 2017).

On the molecular level, the majority of genes in most halophytes are only expressed during situations of stress as opposed to normal growth (Yang et al., 2009). For example, *Limonium gmelinii* (Willd.) Kuntze SOS1 (Salt overly sensitive), *and Kosteletzkya virginica* L., *Membryanthemum crystallinum* L. *NHXs and MnSODs* have only been isolated, characterized, and overexpressed in halophytes, suggesting these genes function as salt tolerance genes (Himabindu et al., 2016). Additionally, these genes support crosstalk signaling, osmotic solute synthesis, and ROS suppression (Shi et al., 2000). Plasma membrane Na^+/H^+ antiporter (SOS1) was identified as one of the first plant proteins to be comprehensively characterized (Shi et al., 2000). Approximately 1146 amino acids make up this protein. A total of approximately 450 amino acids are found in the membrane domain, while 696 amino acids are found in the regulatory cytosolic domain (Ullah et al., 2016). SOS1 has been reported to exist in many plant species including *Arabidopsis thaliana*, *Solanum lycopersicum*, *Oryza sativa*, and *Thellungiella salsuginea* (Sathee et al., 2015). The SOS1 protein maintains a favorable K^+/Na^+ ratio in the leaves, the places of the principal metabolic processes, by mediating Na^+ efflux at the root surface and regulating Na^+ transport between the roots and shoots (Tester and Davenport, 2003). The SOS2/SOS3 kinase complex activates SOS1 through protein phosphorylation (Pardo et al., 2006). The SOS2 protein kinase belongs to the SnRK3 family of serine/threonine protein kinases (Liu et al., 2000). As a Ca^{2+} sensor, SOS3 belongs to the calcineurin B-like (CBL) protein family, which is highly similar to the calcium sensor found in neuronal cavities of animals (Kolukisaoglu et al., 2004). When SOS3 detects Ca^{2+} oscillations as a result of salinity stress, it binds to FISL motif on SOS2 and activates its function. Among its functions is activating the Na^+/H^+ antiport activity of SOS1 and controlling the expression of the SOS1/SOS2 complex (Sánchez-Barrena et al., 2005). The current study's objective was to compare and contrast the growth performance of four plant species in both saline and non-saline habitats by monitoring their biochemical and molecular response as compared to their counterparts in the non-saline habitats, in order to take the benefits of their adaptation strategy in improving the performance and yield of crop plants confronting salt stressful conditions.

2. Materials and Methods

2.1. Samples collection

Four plant species belonging to various families were collected from different spots along the southwestern coast of Jeddah governorate. The plant samples were collected throughout January 2021 from eight locations, including four saline sites alongside the southern corniche's coastline and four non-saline sites distributed across Jeddah's southern region (Figure 1). Each species was collected from two different localities: one saline and one non-saline on succession. The collection coordinates of the investigated species are listed in Table 1. The four collected species included *Abutilon pannosum*, *Indigofera oblongifolia*, *Senna italica*, and *Tetraena coccinea* (Figure 2).

2.2. Physical and chemical properties of the collected soil samples

Within each location, three composite soil samples (0-30 cm in depth) were collected. Samples were air-dried, passed through a 2 mm screen, and stored in paper bags. The Boujoucos hydrometer method was used to assess the texture of the soil (Allen et al., 1974). The pH and electrical conductivity of the preprepared soil water extract (1:5 w/v) were measured using a pH and electrical conductivity meter (WTW-LF-91, UK). Total Na^{+}, K^{+}, Ca^{+2}, and Mg^{+2} contents were determined by using Inductively coupled plasma optical emission spectrometry (ICP-OES). A chloride meter (EIL selective ion electrode, Orion, UK) was used to measure the chlorides in the soil extracts. Additionally, we used the Estefan method to quantify the amount of organic matter (OM) in the soil samples (Estefan, 2013). Sulfate ions in soil samples were detected using the Hach DR 5000 UV-Vis Laboratory Spectrophotometer following the manufacturer's instructions at 450 nm (Estefan, 2013).

2.3. Estimation of photosynthesis pigments

Fresh leaves of the four plant species under investigation were pulverized in 0.1 g for photosynthetic pigments extraction using 25 mL of 80% cold acetone, centrifuged at 7000 rpm, and the optical density was recorded by a spectrophotometer (NovaSpec® II, Pharmacia, England, UK) at 646, 663, and 453 nm (Arnon, 1949). Pigment fractions were calculated according to the following Formulate 1 to 3:

$$\text{Chl a} = 12.21\ A663 - 2.81\ A646 \quad (1)$$

$$\text{Chl b} = 20.13\ A646 - 5.03\ A663 \quad (2)$$

$$\text{Carotenoids} = 1000\ A470 - 3.27\ \text{Chl a} - 104\ \text{Chl b} / 229 \quad (3)$$

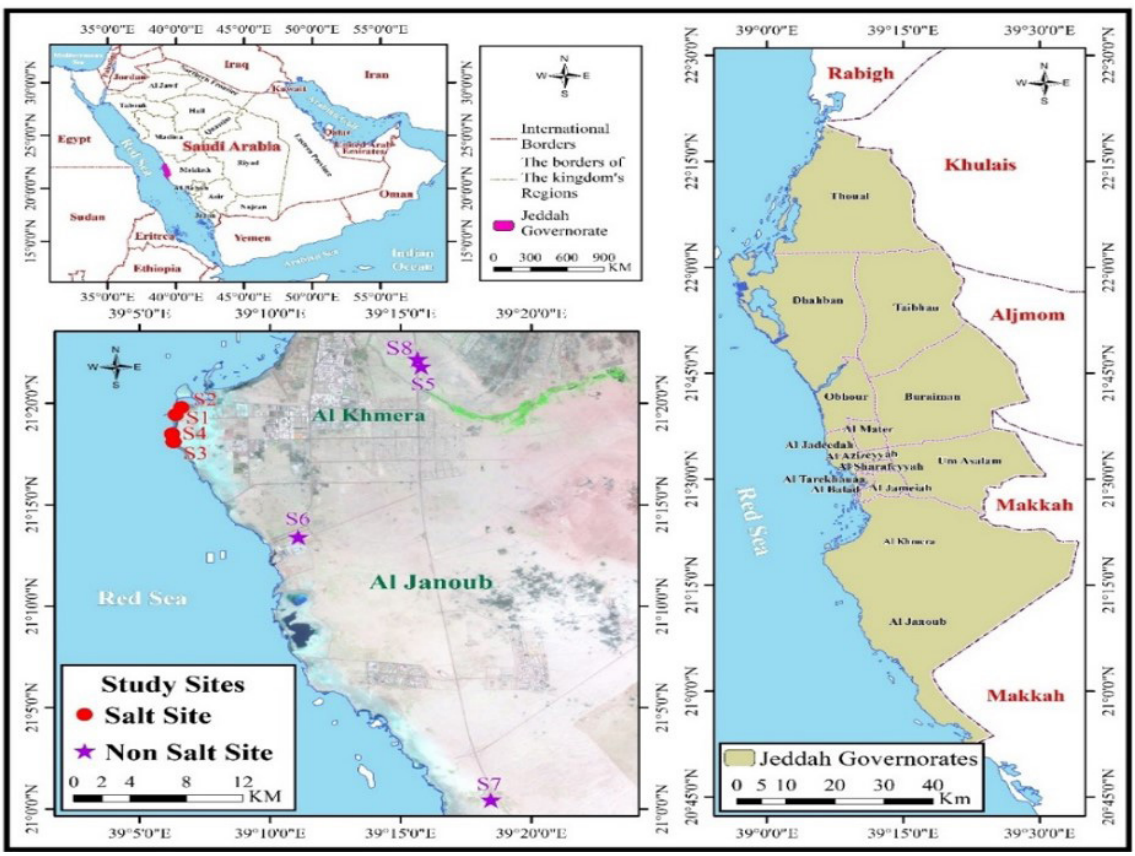

Figure 1. Map of south-western coast of Jeddah governorate showing the study area.

Table 1. Research coordinates, family name and life form of the plant species collected from the southwestern coast of Jeddah governorate.

Plant species	Family	Life form	Research coordinates	
			Saline location	Non-saline location
Abutilon pannosum (G. Forst.) Schltdl.	Malvaceae	Perennial shrub	(21.3079861, 39.1041308)	(21.3635629, 39.263091)
Indigofera oblongifolia Forssk.	Fabaceae	Perennial shrub	(21.3283425, 39.1086010)	(21.2233105, 39.1847697)
Senna italica Mill.	Fabaceae	Perennial shrub	(21.3237930, 39.1065404)	(21.3634036, 39.2631951)
Tetraena coccinea (L.) Beier and Thulin	Zygophyllaceae	Perennial shrub	(21.3078468, 39.1041582)	(21.0073970, 39.3075750)

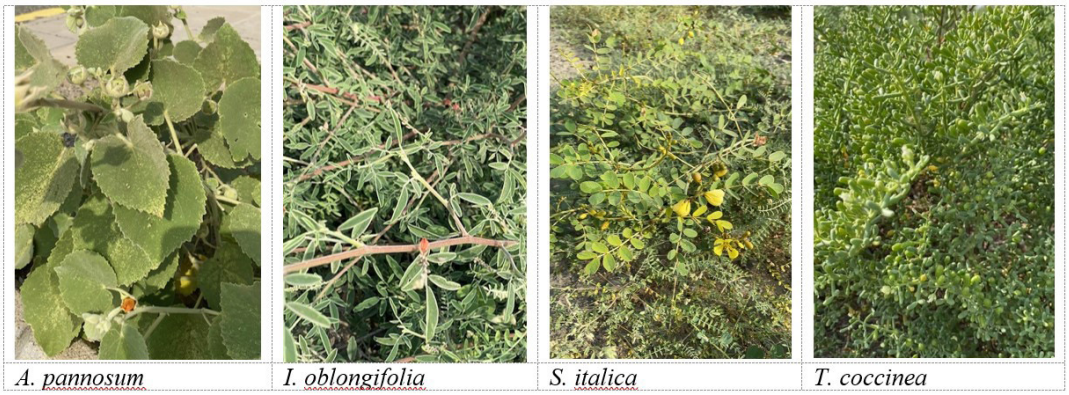

Figure 2. Photos of the investigated plant species taken by iPhone 11.

2.4. Preparation of plant extracts

A known weight of fresh leaves (1 g) was pulverized using a porcelain mortar and pestle alongside 15 mL of deionized water, filtered by Whitman No. 1 filter paper, then the volume was then adjusted into 20 mL using deionized water (Kennedy and Chaplin, 1994). Proline, free amino acids, soluble carbohydrates, and soluble protein concentrations were estimated in the resultant filtrates.

2.5. Estimation of amino acids

Aliquots of 2 mL extract, 1 mL ninhydrin (1%), 2.4 mL glycerol (55%), and 0.4 mL citrate buffer (0.5 M) were mixed. After thoroughly shaking the mixture, it was incubated in a boiling water bath for 12 min. before cooling under running water. The mixture's absorbance was determined spectrophotometrically at 570 nm against a blank generated by replacing the plant extract with distilled water (Lee and Takahashi, 1966). Utilizing a standard curve created using glycine, the concentration of amino acids was represented as mg/g f.wt.

2.6. Estimation of proline

An aliquot of the leaves filtrate (0.5 mL) was combined with 2.5 mL of sulfosalicylic acid (3%), 2 mL of acetic acid, and 1 mL of the acid ninhydrin reagent (1.25 g of ninhydrin + 30 mL glacial acetic acid + 20 mL 6 M phosphoric acid) and boiled for 1 h at 100 °C. The resultant chromatophore was extracted in 4 mL of toluene and the optical density was measured at 520 nm. Based on a standard curve by proline, the proline content was expressed as mg/g f.wt. (Bates et al., 1973).

2.7. Estimation of soluble carbohydrates

A total of 0.4 mL of plant filtrates were added to 3.4 mL of blue tetrazolium reagent (1 g of blue tetrazolium in 200 mL of distilled water and 300 mL of 3 M sodium hydroxide). The mixture was subsequently boiled for 30 sec., cooled, and then mixed with 4 mL of toluene and stirred by the vortex. The samples' absorbance was determined at 570 nm, and the concentration of soluble carbohydrates (mg/g f.wt.) was determined using glucose as a standard (Kennedy and Chaplin, 1994).

2.8. Estimation of soluble protein

The amount of total soluble protein in the plant samples was estimated by mixing 0.2 mL of the extract with 5 mL of the Coomassie Brilliant Blue-G250 reagent and measuring the color intensity at 595 nm. Bovine Serum Albumin (BSA) was used as a standard to determine the protein concentration (mg/g f.wt.) (Bradford, 1976).

2.9. Ionic content of plant samples

Plant leaves were dried at 60 °C till constant weight, ground using an electric mixer, passed through a 2 mm sieve, and stored within paper bags until use. In adherence to the technique of Anjorin et al. (2010), samples were wet digested to determine their ionic contents. Briefly, in a digestion flask, 2 g of leaf powders and 40 mL of nitric acid were combined and heated in a sand bath until clear digestate developed. After filtering, distilled water was used to bring the volume to 200 mL. The mineral content (Na^+, K^+, Ca^{2+}, Mg^{2+}) of the obtained digestates was reported by Inductively coupled plasma optical emission spectrometry (ICP-OES) (Yunus and Abdullahi, 2021).

2.10. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of SOS1 gene

Leaf samples were ground in liquid nitrogen, then RNA was isolated using the modified CTAB procedure (Kiefer et al., 2000). 1 µg RNA was used for making cDNA utilizing QuantiTect® Reverse Transcription Kit (Qiagen, Germany). The sequence of the primer used in this work, SOS1(NHX7), and β-actin as a reference gene are listed in Table 2. The reaction mixture comprised 5 µL of the Power SYBR Green PCR Master Mix, 1 µL of 3 nm of primers, and 4 µL of cDNA template to a final volume of 10 µL. The ViiA7 cycling conditions were as follows: 50 °C for 2 min., 95 °C for 10 min., followed by 40 cycles of 95 °C for 15 s, and then 60 °C for 1 min. The PCR primer efficiency (E) of each primer pair in each reaction was calculated from the changes in fluorescence values (ΔRn) of each amplification plot, using Lin Reg PCR software (Ramakers et al., 2003). E values were averaged across all samples, except in cases where linear regression of amplification plots yielded R^2 value of less than 0.99, in which case the derived E value for that sample was excluded from the calculation of the mean E value. Amplification plots were analyzed using a threshold of 0.20 to give a cycle threshold (Ct) value for the target gene and cDNA combination. Each sample was reproduced three times, and the $^{2-}\Delta\Delta CT$ method was used to calculate the relative expression (Livak and Schmittgen, 2001).

2.11. Statistical analysis

Using SPSS software (V 20.0), the analysis of variance (ANOVA) was carried out to compare the means of the studied variables across the different collection sites. The Tukey post hoc test was used to conduct pairwise comparisons at the 5% significance level. The two normally distributed quantitative variables were correlated using Pearson's coefficient. The data was presented as the mean of three replicates ± SD (standard deviation).

Table 2. Primer sequences for the qRT-PCR expression analysis.

Primer		Sequence (5'-3')
SOS1(NHX7)	Forward	TGATGAATGATGGGACGGCG
	Reverse	TCAGAGTTTTGCCCCATAGCC
β-actin	Forward	TTGCAGGTCGTGACCTAACC
	Reverse	TGACAATCTCACGCTCAGCA

3. Results

3.1. Soil texture

In this study, the texture of soil samples was assessed in both saline and non-saline settings, where the investigated plant species (*A. pannosum*, *I. oblongifolia, S. italica*, and *T. coccinea*) could flourish (Table 3). The assessment of soil texture allows for better comprehension of the relationship between texture and a variety of both chemical and physical variables. According to the study's findings, the soil samples that were collected from sites where plants are growing in non-saline habitats exhibit a sandy texture. By comparison, the soil texture of *S. italica* and *T. coccinea* was loamy sandy in the saline habitat, but the soil texture of *A. pannosum* and *I. oblongifolia* was sandy.

3.2. Physical properties of soil samples collected from saline and non-saline habitats

The physical characteristics of the soil samples collected throughout the current study, as measured by pH, electrical conductivity (EC), chloride ions (Cl^-), total dissolved solutes (TDS), bicarbonate (HCO_3^-), sulfate (SO_4^{2-}), and organic matter (OM) are listed in Table 4. The statistical analysis revealed highly significant differences in the measured variables among the collected soil samples in both salinized and non-salinized habitats, except for OM in the non-salinized habitat, which displayed a non-significant variation within the various sites of the collected plant samples.

The analysis results of the soil samples taken from saline areas, *S. italica* and *T. coccinea* grew in a slightly acidic (6.68-6.88) environment, while *A. pannosum* and *I. oblongifolia* preferred a slightly alkaline (7.0-7.17) environment. The *A. pannosum* growth site and the *T. coccinea* site possessed the highest salinity indices

Table 3. Soil texture of the soil samples at saline and non-saline sites.

Soil	Soil particles	*A. pannosum*	*I. oblongifolia*	*S. italica*	*T. coccinea*	F test
S	Sand (%)	$94.0^{a} \pm 0.58$	$92.33^{a} \pm 0.33$	$89.0^{b} \pm 0.58$	$89.33^{b} \pm 0.33$	26.167* (<0.001*)
	Slit (%)	$2.33^{c} \pm 0.88$	$4.67^{bc} \pm 0.33$	$8.0^{a} \pm 0.58$	$5.33^{ab} \pm 0.67$	13.044* (0.002*)
	Clay (%)	$3.67^{a} \pm 0.67$	$3.0^{a} \pm 0.0$	$3.0^{a} \pm 0.58$	$4.33^{a} \pm 1.20$	0.733^{ns} (0.561)
	Soil texture	Sandy	Sandy	Loamy sandy	Loamy sandy	
NS	Sand (%)	$96.0^{ab} \pm 0.58$	$94.67^{ab} \pm 0.67$	$94.0^{b} \pm 0.0$	$97.0^{a} \pm 0.58$	6.500* (0.015*)
	Slit (%)	$2.0^{a} \pm 0.58$	$2.0^{a} \pm 0.58$	3.0a ± 0.0	$1.0^{a} \pm 1.0$	1.600^{ns} (0.264)
	Clay (%)	$2.0^{b} \pm 0.0$	$3.33^{a} \pm 0.33$	$3.0^{ab} \pm 0.0$	$2.0^{b} \pm 0.58$	4.250* (0.045*)
	Soil texture	Sandy	Sandy	Sandy	Sandy	

S = saline soil; NS = non-saline soil; F = Fisher test. *Significant at 0.05 level, ns = non-significant. Different superscripts within the same row donate significant differences at 0.05 level.

(8550.0 and 8133.0 µS/cm, respectively), according to the EC results, which measure soil salinity and are related to the concentration of free ions in the soil. However, the soil of *I. oblongifolia* had the lowest salinity level (5703.0 µS/cm) among the saline sites. The collection site of *T. coccinea* showed the highest concentration of chloride ions (2033.3 mg/L), whereas the site of *A. pannosum* possessed the second-highest concentration (1701.7 mg/L). Also, the site where *T. coccinea* was collected experienced the highest TDS value (5303.0 mg/L), followed by the site where *A. pannosum* was collected (4092 mg/L). The site of *S. italica* showed the greatest HCO_3^- concentration (3.11 meq/l), followed by the sites of *A. pannosum* and *I. oblongifolia* (1.11 meq/l). Sulfate ion concentration, on the other hand, peaked (77.0 mg/L) at the site of *I. oblongifolia* collection, but the collection site of *A. pannosum* ranked second (38.33 mg/L). Additionally, the *T. coccinea* collection site owned the highest OM value (3.18%), while *A. pannosum* collection site was in the second order (1.68%).

The pH of the growth habitats of *A. pannosum*, *I. oblongifolia*, and *T. coccinea* in the non-saline locations were slightly alkaline (7.11-7.47), but that of *S. italica* was in the slightly acidic range (6.95). As a result, *T. coccinea* survives in weakly acidic areas in the saline habitat but survives in weakly alkaline areas in the non-saline habitat. Similar to the saline habitat, *A. pannosum* sites showed the highest EC value (3337.0 µS/cm), while *T. coccinea* site was in the second position (3281.0 µS/cm). The growth sites of *T. coccinea* and *A. pannosum*, showed the highest concentration of chloride (748.3 mg/L) and the next-highest concentration (723.3 mg/L), respectively. The soil samples from the *A. pannosum* location recorded the highest TDS value (2257.0 meq/l), whereas the soil samples from the *T. coccinea* site recorded a value that was quite close to it (2205.3 meq/l). The HCO_3^- level in soil samples for *S. italica* was the highest (3.55 mg/L), however, it was approximately the same for *A. pannosum* and *T. coccinea* (1.11 mg/L). The SO_4^{2-} content peaked in the soil of *T. coccinea* (67.0 mg/L), but it only reached 10.0 mg/L in the soil of *A. pannosum* (second rank). A non-significant difference was attained in OM content within the soil samples of the non-saline habitats, where it was 2.65% in the soil of *A. italica* and 2.35% in the soil of *I. oblongifolia*.

As a general remark, the physical properties of both habitats; saline and non-saline, were non-significantly different in terms of pH, HCO_3^-, and OM, but the saline habitat overpassed the non-saline one in terms of EC, Cl^-, TDS and SO_4^{2-}. Additionally, *I oblongifolia* grows better in areas with lower EC, Cl^-, and TDS, whether in saline or non-saline environments, but *S. italica* would rather grow in places with lower SO_4^{2-} and OM if grown in saline environments.

3.3. Mineral composition of the soil samples used in this investigation

Table 5 lists the findings of the ionic contents of the collected soil samples from both saline and non-saline-habitats, where the studied plant species were collected. Statistically, the ionic content varied significantly amongst the different collection sites with saline nature. According to the findings, the soil where *A. pannosum* was collected had the highest concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, and Na^+/K^+ ratio (96.47, 8.08, 66.39, and 11.39, respectively) among the collected soil samples in the saline habitats. The soil of *I. oblongifolia* exhibited the highest Ca^{2+}/Mg^{2+} ratio observed in these habitats. Therefore, *A. pannosum* collection site was the most salinized, but *I. oblongifolia* collection site was the least salinized. Despite its high salinity, the *A. pannosum* collection site was the most nutrient-rich site out of all the study sites in the saline habitats.

Table 4. Physical properties of the collected soil samples in this investigation.

Soil	Parameters	*A. pannosum*	*I. oblongifolia*	*S. italica*	*T. coccinea*	F test
S	pH	$7.17^{a} \pm 0.02$	$7.06^{ab} \pm 0.04$	$6.68^{c} \pm 0.05$	$6.88^{b} \pm 0.06$	24.5*
	EC (µS/cm)	$8550.0^{a} \pm 1220.8$	$5703^{b} \pm 48.07$	$5900^{b} \pm 11.54$	$8133^{b} \pm 67.41$	41.8*
	Chloride (mg/L)	$1701.7^{a} \pm 2.73$	$936.7^{d} \pm 21.85$	$1413^{c} \pm 14.52$	$2033.3^{b} \pm 23.3$	122754.8*
	TDS (mg/L)	$4092.0^{a} \pm 18.93$	$3717^{c} \pm 13.3$	$3813^{c} \pm 14.5$	$5303^{b} \pm 21.9$	36642.0*
	Bicarbonate (meq/l)	$1.11^{ab} \pm 0.22$	$1.11^{ab} \pm 0.22$	$3.11^{a} \pm 0.89$	$0.88^{b} \pm 0.22$	4.6*
	Sulfate (mg/L)	$38.33^{b} \pm 0.67$	$77.0^{a} \pm 0.58$	$4.33^{d} \pm 0.33$	$13.67^{c} \pm 0.33$	4212.7*
	OM (%)	$1.68^{b} \pm 0.14$	$1.35^{bc} \pm 0.12$	$0.68^{c} \pm 0.23$	$3.18^{a} \pm 0.20$	35.1*
NS	pH	$7.11^{b} \pm 0.05$	$7.47^{a} \pm 0.09$	$6.95^{b} \pm 0.04$	$7.18^{b} \pm 0.03$	14.3*
	EC (µS/cm)	$3337^{c} \pm 136$	$1815^{b} \pm 4.91$	$2317^{c} \pm 18.55$	$3281^{a} \pm 101$	803.6*
	Chloride (mg/L)	$723.3^{c} \pm 17.6$	$325.3^{b} \pm 3.71$	$406.7^{d} \pm 14.52$	$748.3^{a} \pm 11.29$	2919.7*
	TDS (mg/L)	$2257^{c} \pm 53.6$	$1126.7^{b} \pm 24.31$	$1537^{d} \pm 56.1$	$2205.3^{a} \pm 5.21$	5434.5*
	Bicarbonate (meq/l)	$1.11^{b} \pm 0.22$	$0.88^{b} \pm 0.22$	$3.55^{a} \pm 0.45$	$1.11^{b} \pm 0.22$	18.3*
	Sulfate (mg/L)	$10.0^{b} \pm 0.0$	$8.0^{c} \pm 0.0$	$8.0^{c} \pm 0.0$	$67.0^{a} \pm 0.58$	10219.0*
	OM (%)	$2.12^{a} \pm 0.37$	$2.35^{a} \pm 0.59$	$2.65^{a} \pm 0.20$	$1.24^{a} \pm 0.27$	2.483^{ns}

S = saline soil; NS = non-saline soil; F = Fisher test. *Significant at 0.05 level; ^{ns}non-significant. Different superscripts within the same row donate significant differences at 0.05 level

Though its less saline nature, the nutrient-poor site which contained the least concentrations of Na^+, K^+, and Mg^{2+} (6.18, 0.93, and 1.41 mg/L, respectively) could support the survival of *I. oblongifolia*. Intriguingly, the soil from which *T. coccinea* was collected ranked second in terms of the concentrations of Na^+, K^+, Ca^{2+}, and Mg^{2+} (27.90, 5.33, 32.71, and 2.83 mg/L, respectively).

Concerning the concentrations of the measured ions in the non-saline habitats, the maxima of these ions were reported for Na^+, Ca^{2+}, and Mg^{2+} in the collection sites of *I. oblongifolia* (8.73, 3.79, and 1.08 mg/L, respectively), however, the maximum concentration of K^+ was non-significantly varied among the study sites. The highest Na^+/K^+ ratio (9.16) was reported in the collection site of *T. coccinea*, but the highest Ca^{2+}/Mg^{2+} ratio (9.22) was reported in *A. pannosum* collection site. It can be asserted that the site of *S. italica* collection ranked second in Na^+, Ca^{2+}, and Mg^{2+} ions content (8.73, 2.16, and 0.78, respectively), however, the collection site of *T. coccinea* ranked last in K^+ content (0.52 mg/L). Except for K, which showed non-significant statistical variation, the concentration of the determined ions significantly differed among the collection sites. Regarding the nutritional value of the study sites, the saline habitats exhibited higher K, Ca, and Mg levels than the non-saline habitats for all the studied plant species, though their excessively saline nature.

3.4. Plant analysis

3.4.1. Photosynthesis pigments

Table 6 details the variations in the photosynthetic pigments; Chl a, Chl b, and carotenoids detected in the leaves of the four studied plant species which are thriving in two differentially salinized habitats; saline and non-saline sites. In general, *I. oblongifolia* growing in the saline habitat had the highest levels of Chl a and carotenoids (11.55 and 2.35 mg/g f.wt., respectively), but *A. pannosum* growing in the same habitat had the highest levels of Chl b (5.43 mg/g f.wt.). However, in the non-saline habitat the highest *S. italica, A. pannosum*, and *I. oblongifolia* exhibited the highest Chl a, Chl b, and carotenoids contents (10.09, 2.69, and 2.21 mg/g f.wt., respectively). Interestingly, *T. coccinea* exhibited the lowest pigment fractions in the two habitats.

Table 5. Mineral composition (mg/L) of the soil samples used in this investigation.

Soil	Parameters	*A. pannosum*	*I. oblongifolia*	*S. italica*	*T. coccinea*	F test
S	Na^+	$96.47^{a} \pm 0.36$	$6.18^{d} \pm 0.69$	$15.17^{c} \pm 0.07$	$27.90^{b} \pm 0.79$	5486.3*
	K^+	$8.08^{a} \pm 0.03$	$0.93^{d} \pm 0.03$	$1.81^{c} \pm 0.01$	$5.33^{b} \pm 0.10$	3548.1*
	Na^+/K^+	$11.93^{a} \pm 0.01$	$6.64^{c} \pm 0.74$	$8.38^{b} \pm 0.01$	$5.24^{c} \pm 0.16$	57.4*
	Ca^{2+}	$66.39^{a} \pm 0.11$	$35.65^{b} \pm 0.11$	$6.49^{d} \pm 0.22$	$32.71^{c} \pm 0.06$	31891.2*
	Mg^{2+}	$10.22^{a} \pm 0.04$	$1.41^{c} \pm 0.13$	$1.62^{c} \pm 0.25$	$2.83^{b} \pm 0.06$	825.4*
	Ca^{2+}/Mg^{2+}	$6.50^{bc} \pm 0.02$	$25.67^{a} \pm 2.24$	$4.13^{c} \pm 0.46$	$11.58^{b} \pm 0.27$	70.0*
NS	Na^+	$3.29^{c} \pm 0.89$	$9.05^{a} \pm 1.16$	$8.73^{ab} \pm 0.07$	$4.85^{bc} \pm 0.96$	10.6*
	K^+	$0.63^{a} \pm 0.02$	$0.68^{a} \pm 0.05$	$0.63^{a} \pm 0.03$	$0.52^{a} \pm 0.05$	2.8^{ns}
	Na^+/K^+	$5.21^{b} \pm 1.27$	$13.22^{a} \pm 0.76$	$13.87^{a} \pm 0.49$	$9.16^{b} \pm 0.82$	20.8*
	Ca^{2+}	$2.28^{b} \pm 0.15$	$3.79^{a} \pm 0.07$	$2.16^{b} \pm 0.26$	$1.65^{b} \pm 0.14$	30.3*
	Mg^{2+}	$0.25^{c} \pm 0.01$	$1.08^{a} \pm 0.06$	$0.78^{b} \pm 0.07$	$0.31^{c} \pm 0.01$	69.3*
	Ca^{2+}/Mg^{2+}	$9.22^{a} \pm 0.90$	$3.52^{bc} \pm 0.14$	$2.86^{c} \pm 0.56$	$5.37^{b} \pm 0.30$	26.6*

S = saline soil; NS = non-saline soil; F = Fisher test. *Significant at 0.05 level; ^{ns}non-significant. Different superscripts within the same row donate significant differences at 0.05 level.

Table 6. Variability in the pigment fractions (mg/g f.wt.) within the studied species leaves in response to the characteristics of the growth habitat.

Soil	Parameters	*A. pannosum*	*I. oblongifolia*	*S. italica*	*T. coccinea*	F test
S	Chl a	$6.46^{b} \pm 0.17$	$11.55^{a} \pm 0.86$	$7.62^{b} \pm 0.33$	$6.11^{b} \pm 0.49$	22.24*
	Chl b	$5.43^{a} \pm 0.63$	$3.81^{ab} \pm 0.39$	$1.97^{bc} \pm 0.16$	$1.64^{c} \pm 0.58$	13.62*
	Carotenoids	$1.42^{bc} \pm 0.19$	$2.35^{a} \pm 0.10$	$2.12^{ab} \pm 0.19$	$1.24^{c} \pm 0.23$	8.54*
NS	Chl a	$9.87^{a} \pm 0.40$	$9.70^{a} \pm 0.19$	$10.09^{a} \pm 0.51$	$4.28^{b} \pm 0.71$	32.76*
	Chl b	$2.69^{a} \pm 0.43$	$2.41^{a} \pm 0.59$	$1.73^{a} \pm 0.35$	$0.96^{a} \pm 0.33$	3.13^{ns}
	Carotenoids	$1.75^{a} \pm 0.06$	$2.21^{a} \pm 0.23$	$2.40^{a} \pm 0.15$	$0.83^{b} \pm 0.23$	15.22*

S = saline soil; NS = non-saline soil; F = Fisher test. *Significant at 0.05 level; ^{ns}non-significant. Different superscripts within the same row donate significant differences at 0.05 level.

As an adaptive strategy to the imposed salinity, all the studied plant species showed a pronounced increment in Chl b fraction within their leaves. However, it was found that in response to the imposed salinization, Chl a fraction decreased in the leaves of *A. pannosum* and *S. italica* but increased in those of *I. oblongifolia* and *T. coccinea*. Also, the carotenoids fraction was increased in the leaves of *I. oblongifolia* and *T. coccinea* but decreased in the leaves of *A. pannosum* and *S. italica* in response to salinization.

3.4.2. Osmoregulatory compounds

The change in the content of the osmotic pressure modulators within the leaf cells of the four studied plant species in two contrasting habitats; salinized and non-salinized, is illustrated in Table 7. According to the study's findings, *I.* oblongifolia possessed the most soluble protein and the least soluble carbohydrate levels (8.267 and 0.129 mg/g f.wt., respectively) in the salinized habitat. The highest free proline level (0.553 mg/g f.wt.) was indeed found in *T. coccinea*, but the highest free amino acids and soluble carbohydrates contents (0.007, and 1.447 mg/g f.wt., respectively) were found in *A. pannosum*. Among the species in the same habitat, *S. italica* and *A. pannosum* revealed the presence of the lowest soluble proteins and free proline, (3.343 and 0.094, respectively), whereas *I. oblongifolia* and *S. italica* shared the lowest level of free amino acids (0.004 mg/g f.wt.).

In the non-salinized collection sites, soluble proteins reached their peak (4.080 mg/g f.wt.) in *A. pannosum*, but the lowest content (2.910 mg/g f.wt.) was attained in *S. italica*. *T. coccinea* exhibited the greatest levels of free proline and soluble carbohydrates (0.337 and 0.111 mg/g f.et., respectively), although it shared the highest level of free amino acids (0.012 mg/g f.wt.) with *S. italica*. *I. oblongifolia* was determined to have the least free proline (0.090 mg/g f.wt.), whereas *A. pannosum* showed the lowest levels of free amino acids and soluble carbohydrates (0.004 and 0.045 mg/g f.wt., respectively).

In general, the determined plants' responses to salinization varied in terms of the levels of osmomodulatory compounds. Whereas the exposure of *A. pannosum* resulted in decreasing the levels of soluble proteins and free proline. Contrastingly, salinization significantly raised soluble protein and free proline levels in *I. oblongifolia* while significantly lowering levels of free amino acids and soluble carbohydrates. Both *S. italica* and *T. coccinea* displayed increased levels of soluble proteins and carbohydrates, although they opposed in their levels of free proline, and both demonstrated a decrement in the pool of free amino acids in response to salinization.

3.4.3. Mineral composition of the investigated species in two salt-contrasted habitats

The results of the mineral composition (Na^+, K^+, Ca^{2+}, and Mg^{2+}), as well as the ratios of Na^+/K^+ and Ca^{2+}/Mg^{2+} of the four examined plant species in two contrasted habitats, salinized and non-salinized, are presented in Table 8. Statistically, the mineral composition varied significantly amongst the different plant species with saline nature in the two habitats, except K^+ concentration in the saline habitat which was non-significantly different among the studied species.

The findings showed that among the examined plant species, *T. coccinea* was the most significant mineral ion accumulator in both salinized and non-salinized environments. In the salinized habitat, *T. coccinea* showed the highest levels of Na^+, and Ca^{2+} (22.10, and 77.69 mg/L, respectively). The greatest K^+ concentration, however (8.55 and 8.39 mg/L, respectively), was shared by *A. pannosum* and *T. coccinea*. On the other hand, *A. pannosum* and *I. oblongifolia* possessed the same highest concentration of Mg^{2+} in their leaf tissues (5.64 mg/L). Nevertheless, the greatest values for the ratios of Na^+/K^+ and Ca^{2+}/Mg^{2+} were found in *T. coccinea* (5.65 and 28.25 mg/L, respectively). In contrast, *S. italica* exhibited the lowest concentrations of most of the determined elemental ions, as it displayed the smallest concentrations of Na^+, Ca^{2+}, and Mg^{2+} (2.94, 25.67, and 2.14 mg/L, respectively), whereas *A. pannosum* encompassed the lowest level of K^+ (8.55 mg/L).

In the typical (non-salinized) habitat, *T. coccinea* was also the major ion accumulator within its tissues, where it appeared to be the most prominent accumulator for Na^+, Ca^{2+}, and Mg^{2+} (36.45, 96.45, and 8.21 mg/L, respectively), but it contained the lowest level of K^+ (6.73 mg/L).

Table 7. Variability in the osmomodulator compounds level (mg/g f.wt) within the studied species leaves in response to the characteristics of the growth habitat.

Soil	Metabolic compounds	*A. pannosum*	*I. oblongifolia*	*S. italica*	*T. coccinea*	F test
S	Proteins	$3.953^{b} \pm 0.172$	$8.267^{a} \pm 0.220$	$3.343^{c} \pm 0.070$	$3.953^{b} \pm 0.172$	551.7*
	Proline	$0.094^{c} \pm 0.012$	$0.289^{b} \pm 0.010$	$0.107^{c} \pm 0.005$	$0.553^{a} \pm 0.006$	1826.8*
	Amino acids	$0.007^{a} \pm 0.001$	$0.004^{b} \pm 0.001$	$0.004^{b} \pm 0.001$	$0.006^{a} \pm 0.002$	5.7*
	Carbohydrates	$1.447^{a} \pm 0.315$	$0.129^{b} \pm 0.015$	$0.160^{b} \pm 0.050$	$0.187^{b} \pm 0.051$	47.8*
NS	Proteins	$4.080^{a} \pm 0.538$	$3.280^{ab} \pm 0.040$	$2.910^{b} \pm 0.255$	$3.350^{ab} \pm 0.157$	7.5*
	Proline	$0.096^{b} \pm 0.006$	$0.090^{b} \pm 0.003$	$0.117^{b} \pm 0.006$	$0.337^{a} \pm 0.055$	54.4*
	Amino acids	$0.004^{b} \pm 0.001$	$0.005^{b} \pm 0.001$	$0.012^{a} \pm 0.001$	$0.012^{a} \pm 0.002$	36.8*
	Carbohydrates	$0.045^{b} \pm 0.011$	$0.104^{a} \pm 0.007$	$0.079^{ab} \pm 0.020$	$0.111^{a} \pm 0.015$	13.6*

S = saline soil; NS = non-saline soil; F = Fisher test. *Significant at 0.05 level, ns = non-significant. Different superscripts within the same row donate significant differences at 0.05 level.

However, K^+ was reported with its maximum level (23.12 mg/L) in *A. pannosum*. Furthermore, *T. coccinea* showed the highest ratios of Na^+/K^+ and Ca^{2+}/Mg^{2+} (5.43 and 11.75, respectively) among the investigated species. Contrastingly, *S. italica* showed the lowest concentrations of Na^+ and Mg^{2+} (0.22 and 4.85, respectively), however *A. pannosum* comprised the lowest concentration of Ca^{2+} (25.97 mg/L).

As a general remark, the capacity of the studied species to survive in saline habitats is contingent upon their ionic equilibrium strategies, which may vary depending on the species. In contrast to the strategy used by the other investigated plant species, *T. coccinea* thrives in the saline atmosphere by maintaining lowered Na^+ concentrations but elevated K^+ concentrations within its cells. In addition, the investigated plant species showed lower Mg^{2+} and Ca^{2+} concentrations in the salinized habitats compared to the non-salinized, with the exception of *A. pannosum*, whose Ca^{2+} concentration revealed a trend in the opposite direction.

3.4.4. The expression level of SOS1 gene

In this study, qRT-PCR was used to examine SOS1 gene expression in four plant species (*A. pannosum*, *I. oblongifolia*, *S. italica*, and *T. coccinea*) in two salinity-contrasting growth habitats. Under salt stress, the activity of SOS1 gene modulates the proportions of Na^+ and K^+ in plants, permitting efficient osmotic adjustment. In the current study, the salinization of the growth habitat resulted in the increased expression of SOS1 gene to facilitate their survival in the harsh environment. Under the non-saline conditions, SOS1 expression level was in the following order: *I. oblongifolia* > *S. italica* > *T. coccinea* > *A. pannosum* (0.6, 0.4, 0.3, and 0.2 folds, respectively). When compared to its level in a non-saline environment, SOS1 was highly expressed under saline conditions in *T. coccinea* (17.9 folds), *A. pannosum* (3.7 folds), *S. italica* (1.6 folds), and *I. oblongifolia* (1.5 folds), in that order (Table 9).

3.4.5. Principal component analysis (PCA)

Using PCA, the relationship between soil and plant characteristics for the four studied plant species was assessed in both saline and non-saline conditions. According to Table 10, the growing habitat had an impact on the PCA1, PCA2, and PCA3 for soil and plant variables. In both salinized and non-salinized soils, the three PCAs showed eigenvalues greater than one (Eigenvalue > 1), and they described 100% of the overall variance in soil and plant variables. PCA1 explains 46.73 and 58.16% of the total variability of the measured data for the four plant species in the two growth environments, PCA2 describes 32.03 and 25.97%, and PCA3 describes 21.24 and 15.87% of the variability in salinized and non-salinized sites. Therefore, PCA1 and PCA2 could be utilized to assess the relationship between the variables under investigation in both salinized and non-salinized habitats. Furthermore, in both habitats, the three PCAs showed a strong positive correlation with the majority of the studied soil and plant characteristics.

Table 8. Variability in the mineral composition (mg/L) within the studied species leaves in response to the characteristics of the growth habitat.

Soil	Parameters	*A. pannosum*	*I. oblongifolia*	*S. italica*	*T. coccinea*	F test
S	Na^+	$7.04^{b} \pm 0.95$	$3.46^{b} \pm 0.08$	$2.94^{b} \pm 0.14$	$22.10^{a} \pm 2.83$	36.1*
	K^+	$8.55^{a} \pm 1.22$	$6.87^{a} \pm 0.17$	$7.52^{a} \pm 0.44$	$8.39^{a} \pm 1.29$	0.72^{ns}
	Na^+/K^+	$0.82^{b} \pm 0.024$	$0.50^{c} \pm 0.003$	$0.39^{c} \pm 0.007$	$2.65^{a} \pm 0.10$	398.8*
	Ca^{2+}	$30.46^{c} \pm 2.69$	$58.10^{b} \pm 1.38$	$25.67^{c} \pm 1.88$	$77.69^{a} \pm 3.81$	87.7*
	Mg^{2+}	$5.64^{a} \pm 1,18$	$5.64^{a} \pm 0.03$	$2.14^{b} \pm 0.14$	$2.84^{ab} \pm 0.42$	8.51*
	Ca^{2+}/Mg^{2+}	$5.76^{b} \pm 0.90$	$10.30^{b} \pm 0.18$	$11.97^{b} \pm 0.13$	$28.25^{a} \pm 2.97$	39.7*
NS	Na^+	$1.13^{b} \pm 0.05$	$1.19^{b} \pm 0.08$	$0.22^{b} \pm 0.01$	$36.45^{a} \pm 1.58$	508.8*
	K^+	$23.12^{a} \pm 1.52$	$11.07^{b} \pm 0.22$	$11.08^{b} \pm 0.71$	$6.73^{c} \pm 0.12$	69.1*
	Na^+/K^+	$0.05^{b} \pm 0.0$	$0.11^{b} \pm 0.01$	$0.02^{b} \pm 0.0$	$5.43^{a} \pm 0.31$	291.0*
	Ca^{2+}	$25.97^{c} \pm 2.08$	$76.80^{b} \pm 1.61$	$30.01^{c} \pm 1.84$	$96.45^{a} \pm 3.72$	201.2*
	Mg^{2+}	$6.41^{b} \pm 0.18$	$7.33^{ab} \pm 0.09$	$4.85^{c} \pm 0.34$	$8.21^{a} \pm 0.12$	47.9*
	Ca^{2+}/Mg^{2+}	$4.04^{d} \pm 0.21$	$10.48^{b} \pm 0.10$	$6.20^{c} \pm 0.07$	$11.75^{a} \pm 0.33$	307.9*

S = saline soil; NS = non-saline soil; F = Fisher test. *Significant at 0.05 level, ns = non-significant. Different superscripts within the same row donate significant differences at 0.05 level.

Table 9. Relative expression of SOS1 gene using qRT-PCR technique in the leaves of the studied plant species in saline and non-saline habitats.

Soil	*A. pannosum*	*I. oblongifolia*	*S. italica*	*T. coccinea*	F test
S	$3.7^{b} \pm 0.5$	$1.5^{c} \pm 0.6$	$1.06^{d} \pm 0.1$	$17.9^{a} \pm 8.2$	1.179*
NS	$0.2^{bc} \pm 0.1$	$0.6^{a} \pm 0.3$	$0.4^{b} \pm 0.1$	$0.3^{c} \pm 0.1$	1.187*

S = saline soil; NS = non-saline soil; F = Fisher test. *Significant at 0.05 level. Different superscripts within the same row donate significant differences at 0.05 level.

Table 10. Principal component analysis (PCA) of the soil and plant variables at salinized and non-salinized habitats.

Variables	Salinized habitats			Non-salinized habitats		
	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3
Soil variables						
pH	0.23	-0.09	0.29	0.06	0.23	0.44
EC	0.31	-0.04	-0.04	0.26	0.13	0.09
Cl	0.31	-0.02	-0.05	0.27	0.09	0.05
TDS	0.31	-0.03	-0.04	0.27	0.12	0.07
HCO_3^-	-0.13	-0.12	-0.39	-0.13	0.04	-0.47
SO_4^{2-}	0.03	-0.23	0.37	0.27	-0.04	-0.10
OM	0.04	0.33	0.22	-0.27	0.11	-0.06
Na^+	0.31	0.04	-0.07	-0.10	0.38	-0.11
K^+	0.27	0.18	-0.03	-0.24	0.14	0.21
Na^+/K^+	0.25	-0.15	-0.19	-0.06	0.38	-0.20
Ca^{2+}	0.28	0.00	0.20	-0.12	0.26	0.35
Mg^{2+}	0.31	0.01	-0.04	-0.12	0.37	0.05
Ca^{2+}/Mg^{2+}	-0.10	-0.12	0.41	0.02	-0.38	0.23
Plants variables						
Na^+	0.00	0.36	0.12	0.28	-0.03	-0.09
K^+	0.21	0.26	-0.11	-0.16	-0.30	0.21
Na^+/K^+	-0.01	0.36	0.13	0.27	-0.02	-0.10
Ca^{2+}	-0.10	0.23	0.34	0.23	0.21	0.12
Mg^{2+}	0.18	-0.19	0.29	0.23	0.04	0.29
$C^{2+}a/Mg^{2+}$	-0.14	0.33	0.08	0.21	0.27	0.06
Chl a	-0.14	-0.28	0.24	-0.28	0.02	0.07
Chl b	0.25	-0.20	0.12	-0.21	-0.07	0.34
Carotenoids	-0.17	-0.31	0.01	-0.26	0.15	-0.03
Eigenvalue	10.28	7.05	4.67	12.80	5.71	3.49
Variance %	46.73	32.03	21.24	58.16	25.97	15.87
Cumulative %	46.73	78.76	100.00	58.16	84.13	100.00

The first two PCAs were used to create a biplot and the correlations between soil and plant variables (Figure 3). In both salinized and non-salinized habitats, *A. pannosum* and *T. coccinea* showed a positive correlation with the PCA1 (Figure 3A and B, respectively). In contrast, *T. coccinea* and *I. oblongifolia* were positively correlated with PCA2 in both habitats. The majority of the study's variables showed acute angles during the contribution of the soil and plant variables, showing a positive correlation between them, but their magnitude and regularity varied between salinized and non-salinized sites. In terms of the relationships between soil variables, salinized sites demonstrated a substantial and profitable relationship between Na^+, Ca^{2+}, Mg^{2+}, Cl^-, TDS, EC, pH, and Na^+/K^+, while non-salinized sites showed a correlation between pH, EC, TDS, and Cl^-, as well as between OM, Na^+, Mg^{2+}, Ca^{2+}, K^+, and Na^+/K^+. In terms of relationships between plant variables, salinized sites showed the highest positive correlations between K^+, Na^+, Na^+/K^+, Ca^{2+}, and Ca^{2+}/Mg^{2+}, and between Mg^{2+}, Chl a, Chl b, and carotenoids, but in the non-salinized sites the highest positive correlations were reported between Na^+, Na^+/K^+, Ca^{2+}, and Mg^{2+}, and between Chl a, Chl b, and carotenoids.

In both environments, PCA1 and PCA2 predominantly dispersed and separated the soil and plant variables into two groups in accordance with their interrelationships with the four studied plant species in the biplot analysis (Figure 3). In salinized habitats, the first group was correlated to PCA1 and comprised all soil variables (except HCO_3^- and Ca^{2+}/Mg^{2+}) as well as some plant variables, including K^+, Mg^{2+}, and Chl b, which are positively associated with *A. pannosum* (fourth quarter). According to PCA2, all plant variables (except Mg^{2+}, Chl, and carotenoids), as well as some soil variables (OM, Na^+, K^+, and Mg^{2+}), were positively correlated with *T. coccinea* (second quarter), which is included in the second group.

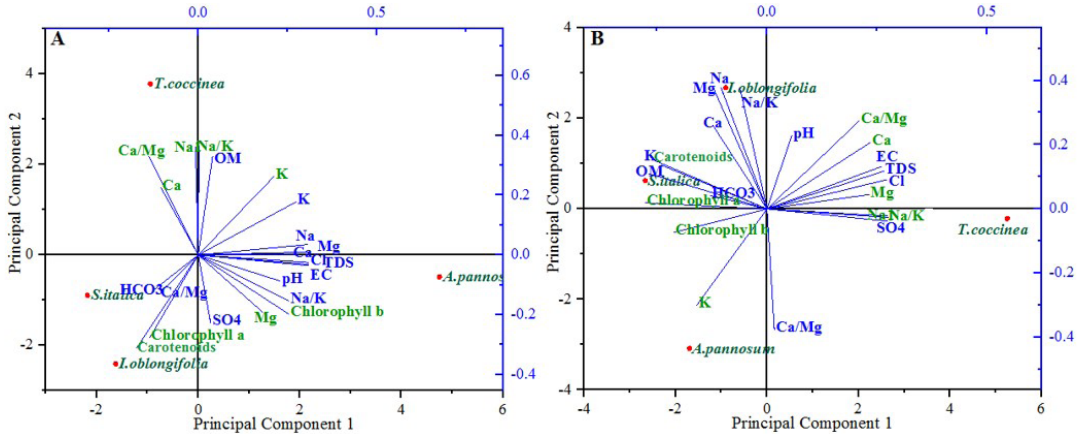

Figure 3. Biplot diagram based on PC1 and PC2 displaying similarities and differences between soil (blue) and plant (green) characteristics in the four studied plant species (red circles) in saline and non-saline habitats. Site under salinity conditions (A), site under non-saline conditions (B).

In the non-salinized habitats, the first group has related to PCA1 and included soil variables (pH, EC, Cl^-, TDS, SO_4^{2-} and Ca^{2+}/Mg^{2+}) and plant variables (Na^+, Na^+/K^+, Ca^{2+}, Mg^{2+}, and Ca^{2+}/Mg^{2+}), which had a positive association with *T. coccinea* (fourth quarter). However, *S. italica* and *I. oblongifolia* were assigned to the second group (second quarter) as they positively correlated with all soil variables except SO_4^{2-} and some plant variables including Ca^{2+}, Mg^{2+}, Ca^{2+}/Mg^{2+}, Chl a, and carotenoids according to PCA2.

4. Discussion

Some plants can thrive in both saline and non-saline soils, therefore considered habitat-indifferent halophytes (Al-Shamsi et al., 2018). Habitat-indifferent halophytes possess appropriate adaptation mechanisms depending on the degree of soil salinity enabling them to survive effectively in saline and non-saline soils (Parida and Jha, 2010). This study investigated the metabolic, ionic and SOS1 gene responses of four salinity-indifferent plant species (*A. pannosum*, *I. oblongifolia*, *S. italica*, and *T. coccinea*) in two salinity-contrasting growth habitats along the southwestern coast of Jeddah governorate. In this context, our study findings demonstrated that *S. italica* and *A. pannosum* growing in saline habitats exhibited lower concentrations of Chl a than their counterparts in the non-saline habitats. However, we found that *I. oblongifolia* and *T. coccinea* in the saline habitats showed higher levels of Chl a than their counterparts in the non-saline habitats. Furthermore, our findings demonstrated that all studied species increased their Chl b contents in response to saline habitats, while the levels of carotenoids varied. For example, compared to the same plants growing in non-saline habitats, *A. pannosum* and *S. italica* showed decreased levels of carotenoids, while *I. oblongifolia* and *T. coccinea* showed increased levels.

It has been established that salt-tolerant species exhibit increased or constant content of chlorophyll under salinity conditions, but chlorophyll levels decline within salt-sensitive species, indicating that this attribute can be used as a biochemical indicator of salt tolerance (Ashraf and Harris, 2013). According to Ghanem et al. (2021), different halophytes respond differently in their chlorophyll content to soil salinity in the same growing habitats. The elevated level of Chl a in saline environments might suggest an alteration in the overall structure of the leaf photosystems to reduce the danger of photoinhibition through enhancing pigment-protein complexes with a comparatively higher Chl a/b ratio (Rabhi et al., 2012). Furthermore, according to Yin et al. (2013), there are no apparent differences between extreme salt stress and control treatments in terms of the amount of Chl b in *Suaeda salsa*. Furthermore, some scholars concluded that the increase in chloroplast abundance may be responsible for the salt-induced increase in chlorophyll concentrations in some plant species (Jamil et al., 2007; Kumar et al., 2021). It might therefore be suggested that PSII is an important aspect in the capability of plants to tolerate salt as a result of the higher levels of chlorophyll in plants grown in saline habitats (Ullah et al., 2022).

It is conceivable that carotenoids act as antioxidants in cases of salt stress, whereas some plant species (*I. oblongifolia* and *T. coccinea*) demonstrated increased concentrations of carotenoids in saline habitats. The increased concentration of carotenoids may be an effective strategy used by plants in salt-stress situations to sustain the concentration of chlorophyll rather than decreasing it (Ghanem et al., 2021). Another investigation on *Nitraria retusa* (Boughalleb and Denden, 2011) corroborated the increase in carotenoids and the enhanced salt tolerance. In contrast, carotenoids in *A. pannosum* and *S. italica* showed decreases in saline habitats. Such decreases in carotenoids in salt-affected plants were reported in different plant species (Aghaleh et al., 2009; Akcin and Yalcin, 2016). Accordingly, in the light of our study *A. pannosum* and *S. italica* were more salt-sensitive than *I. oblongifolia* and *T. coccinea*.

In this study, *I. oblongifolia, S. italica*, and *T. coccinea* were shown to have high soluble protein concentrations in saline habitats, however *A. pannosum* exhibited lowered concentration when compared to their non-salt-affected counterparts.

The increased protein content in response to salt stress was reported in Bermuda grass (Hameed and Ashraf, 2008). In saline environments, proteins may build up because they help restore a more negative water potential that enables plants to endure challenging conditions. According to Xu et al. (2001), salt stress in mangroves caused an increase in the biosynthesis of proteins and translatable mRNA. Stress-induced proteins increase in plant cells may result from constitutively present low-level proteins or from de novo protein synthesis in response to the imposed stress (Dasgupta et al., 2010). Furthermore, protein accumulation in saline circumstances has been reported to offer a form of nitrogen reserve that can then be utilized in addition to its assistance in osmotic homeostasis (Kasim et al., 2016). Nonetheless, the decreased protein accumulation in *A. pannosum* in salinized habitats could be attributed to the interruption of the key routes providing the carbon skeleton for the synthesis of proteins, such as glycolysis, and hexose monophosphate pathway by salinity (Li et al., 2015). Reduced levels of protein may also be linked to altered gene expression and signaling pathways, which may hamper the biosynthetic process and/or cause protein breakdown through improper protein folding and assembling (Saad-Allah and Ragab, 2020).

Except for the opposite trend observed in *S. italica*, free proline as an osmoregulatory displayed a pattern similar to that of free soluble proteins in the investigated plants in response to salt stress. As a survival mechanism, proline accumulates in plant tissues as an osmoregulatory molecule that helps halophytes tolerate high salt concentrations (Ghanem et al., 2021). Increased proline buildup in salinized habitats compared to non-salinized habitats may be crucial to maintaining osmotic balance and acting as a neutralizer of free radicals in *I. oblongifolia* and *T. coccinea*. Additionally, because of its role in preserving cellular components, proteins, and membranes under osmotic stress in plants, an increased proline concentration in salt-imposed plants has been reported (Zhang and Shi, 2018). The decreased accumulation of proline in *A. pannosum* and *S. italica* reported in this study was previously documented in olive (Regni et al., 2019).

In this study, free amino acid analysis displayed a substantial increase in the content of free amino acids in the leaves of *A. pannosum*, whereas a decrease in their content was observed in the leaves of *I. oblongifolia*, *S. italica*, and *T. coccinea* in response to their habitat's salinization. According to Tawfik et al. (2017), the salt stress-induced increases in free amino acids boost plant cells' tolerance by raising both the relative water content required to promote plant growth and the level of osmotic pressure in the cytoplasm. The reduced availability of nitrogen and the altered carbon structure necessary for amino acids production as a result of habitat salinization may be the cause of the decreased concentration of free amino acids (Saad-Allah and Ragab, 2020).

In this study, the plant species *I. oblongifolia*, which accumulates more soluble proteins and proline in salinized habitats, showed decreased levels of soluble carbohydrates, whereas the other plant species showed increased soluble sugar levels in their tissues in comparison to non-salinized counterparts. As a defensive strategy that is essential for osmoprotection, osmotic adjustment, and radical scavenging is the accumulation of soluble carbohydrates in salinized habitats (Singh and Jha, 2016). As a result, the increase in soluble sugar in the current study supports the concept that these compounds are crucial for reducing salt deterioration, either by adjusting the osmotic pressure or by giving plant cells a certain level of dehydration resistance. According to Hajiboland et al. (2014), in conditions of salinity stress, the allocation of assimilates for osmotic homeostasis and their partitioning into roots along with other solutes contributes to osmotic homeostasis. The observed reduction in total soluble sugars in *I. oblongifolia* in the salinized habitats could be ascertained to the conversion of these compounds into non-soluble polysaccharides to contribute to the formation of new protoplasm, formation of lignin, and other stress-defensive metabolites. It is possible to attribute the decrease in total soluble sugars in *I. oblongifolia* in the salinized habitats to the conversion of these sugars into non-soluble carbohydrates and/or lignin for strengthening cell walls, and the synthesis of other structural and stress-defensive metabolites.

In the current study, three plant species, *A. pannosum, I. oblongifolia*, and *S. italica*, displayed increased Na^+ accumulation in their leaf tissues while displaying decreased K^+ accumulation as a response to habitat salinization. *T. coccinea*, in contrast, responds to habitat salinization by increasing K^+ accumulation and decreasing Na^+ accumulation. Due to the ionic stress caused by salt exposure in saline circumstances, plants have developed various mechanisms for salt tolerance. These mechanisms largely depend on the roots' potential to control Na^+ and K^+ absorption and transport to the shoot system. Shabala (2013) demonstrated that high salinity could decrease cytoplasmic K^+ concentration by causing depolarization of the plasma membrane and ROS-induced opening of membrane channels permitting K^+ outflow. It is being demonstrated that some K^+ activities can be replaced by highly accumulated Na^+ in the leaf tissues of plants living in salinized settings (Kronzucker et al., 2013). Sodium can replace some of the K^+-dependent activities that plants use to regulate osmotic adjustment, membrane potential, cell development, enzyme activity, and protein synthesis, however, it is thought that the main function of Na^+ is to control turgor pressure and cell expansion (Adams and Shin, 2014).

The accumulation of K^+ observed in *T. coccinea* in response to habitat salinization is consistent with the findings of Tian et al. (2021), who showed that plants with salinity tolerance accumulate K^+ rather than Na^+ in their shoot system. Ruiz-Lozano et al. (2012) reported that plants use a variety of coping mechanisms for confronting elevated soil salinity, including hindering Na^+ entrance into the root, transportation to and allocation inside the leaf, and sequestration within the vacuole. Accordingly, it can be inferred from the current study that *T. coccinea* has a unique salt tolerance mechanism that differs from that used by the other plant species in this study because it has a mechanism that allows it to avoid salt and that allows it to maintain higher K^+ concentrations while compensating for ionic homeostasis in saline habitats. To pinpoint the precise mechanism used by *T. coccinea*, this hypothesis has to be thoroughly researched in the future.

Except for *A. pannosum*, which demonstrated enhanced Ca^{2+} accumulation, the investigated plants in the current study showed decreased Mg^{2+} and Ca^{2+} contents in response to saline habitats. The decreased Ca^{2+} and Mg^{2+} contents in response to salt exposure reported in this study are in accordance with the previous findings of Ahmad et al. (2008); Cabot et al. (2009); Evelin et al. (2012). According to Michele et al. (2009), the salt-induced nutritional imbalance caused by the plant's decreased Mg^{2+} concentration could damage macromolecules like chlorophyll and cause the loss of photosynthetic activity, which in turn causes early leaves senescence. The decreased concentration of Ca^{2+} in the leaf tissues of salt-exposed plants has been attributed to high Na^+ concentrations in the root zone, which impedes Ca^{2+} uptake and transport. Na^+ can replace Ca^{2+} in both the cell wall and plasma membrane whenever Na^+ levels are high as a result of the unbalanced Na^+/Ca^{2+} ratio, thereby lowering cellular turgidity and hydraulic conductivity in addition to interfering with Ca^{2+} signaling (Evelin et al., 2012).

The results of the qRT-PCR demonstrated that salt stress significantly raised the gene expression level of SOS1 in the studied species, particularly *T. coccinea*. A crucial component of the adaptive response to salt tolerance is the exclusion of Na^+ from the apoplast, which is accomplished by the plasma membrane Na^+/H^+ antiporter encoded by the SOS1 gene (Rolly et al., 2020). Furthermore, it has been reported that SOS1 is necessary for plants to thrive under salt stress by preserving ion homeostasis and regulating long-distance Na+ transport through the xylem. The highest level of Na^+ was found in the leaf tissues of *S. italica*, despite having the highest level of SOS1 expression in the current investigation. This finding supports the hypothesis that this plant species has a different mechanism for reducing Na^+ toxicity. The induced overexpression of SOS1 gene has been reported in saline habitats by many workers (El-Dakak et al., 2021; Lu et al., 2023; Rolly et al., 2020).

5. Conclusion

Salt-tolerant plants possess distinct adaptations that enable them to survive in saline conditions, and they are present in many regions. Among the salt-tolerant species, *S.italica* and *T.coccinea* exhibited the greatest tolerance to salt, while *I.oblongifolia* and *A.pannosum* showed varying responses to salt stress. The high protein content of *S.italica* is not affected by salt accumulation due to the multiple mechanisms used to regulate Na^+ accumulation. Due to high *SOS1* expression, *T. coccinea* showed optimal photosynthetic pigment, protein, and proline levels. Na^+ concentrations in *I.oblongifolia* competed with K^+ and Ca^{2+} concentrations. When compared to non-saline environments, *A. pannosum* exhibits lower levels of specific pigments, proteins, carbohydrates, and ions. The study recommends paying attention to *S.italica* and *T.coccinea* for salt-tolerant landscaping and suggests employing molecular biology approaches for improving salt tolerance in forage grasses using salt-responsive genes such as *SOS1*.

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