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Differential Shoot Nutrient Stoichiometry in *Sulla carnosa* Ecotypes Exposed to Salt Stress under Greenhouse Conditions

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Abstract: Soil salinity is a major environmental constraint in arid and semi-arid regions, impairing plant growth by inducing osmotic and ionic stress and disrupting nutrient balance. Understanding how halophytic species regulate nutrient stoichiometry under salinity is crucial for improving both their ecological and agronomic use. This study investigated the effects of salt stress on growth and shoot nutrient stoichiometry in four Tunisian ecotypes of *Sulla carnosa*, a halophytic forage species of major ecological and economic significance in saline ecosystems. Salinity significantly reduced growth in all ecotypes, although the decrease magnitude was ecotype dependent. Based on the sensitivity index, Kalbia was identified as the most tolerant ecotype, whereas Sidi Khlif was the most sensitive. As hypothesized, salt stress triggered marked changes in nutrient stoichiometry; however, the extent and type of these changes varied among ecotypes: Kalbia maintained relatively stable nutrient ratios and ionic balance under salinity, indicating strong homeostatic regulation. In contrast, Sidi Khlif and Thelja exhibited strong alteration in nutrient relationships, reflecting the occurrence of ionic imbalance concomitant to low salt tolerance. Thus, our findings highlight ecotype-specific strategies of nutrient regulation under salt stress and demonstrate that stoichiometric stability is closely associated with improved growth and salt tolerance. In addition, we provide new insights into the mechanisms of ionic homeostasis in *S. carnosa* and emphasize the ecological significance of selecting tolerant ecotypes for sustainable management and rehabilitation of saline environments.

Key words: Salinity; *Sulla carnosa*, halophyte adaptation, ecotypes; stoichiometric variation; ionic interactions, ionic homeostasis.

Salinity is a major environmental constraint that reduces soil fertility and threatens ecosystem sustainability (Singh, 2022). Elevated sodium chloride (NaCl) concentrations

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impose osmotic and ionic stress on plants, disrupt nutrient uptake and photosynthesis, and ultimately limit growth and productivity (Seeda *et al.*, 2022; Alharbi *et al.*, 2022). Beyond these physiological effects, salinity alters plant elemental stoichiometry and ionic balance by modifying the uptake, transport, and homeostasis of essential nutrients. Such changes influence metabolic functioning at the plant level and nutrient cycling at the ecosystem scale (Sun *et al.*, 2017). Consequently, understanding how plants maintain stoichiometric and ionic equilibrium under saline conditions has become a central focus in ecological and physiological research. Increasing research efforts have therefore been directed toward elucidating plant salt tolerance (Fu and Yang, 2023; Hao *et al.*, 2021; Pandit *et al.*, 2024) and the stoichiometric strategies that regulate nutrient allocation and elemental balance in plants (Sun *et al.*, 2017; Hidri *et al.*, 2019; Li *et al.*, 2021; Zhao *et al.*, 2022; Luo *et al.*, 2022; Zhang *et al.*, 2023;; Zhang *et al.*, 2025).

Plant growth depends on approximately 30 essential elements. Some, such as H, C, N, O, Mg, P, S, K, and Ca, are required in large amounts and are classified as macronutrients, whereas others, including Mn, Zn, Fe, and Mo, are needed only in trace quantities (White and Brown, 2010). These elements have been extensively studied and have generally been well-characterized regarding their specific roles in plants. Salinity influences plant functioning through disruptions in elemental stoichiometry, especially with regard to C, N, and P (Zhao *et al.*, 2022; Zhang *et al.*, 2023; Zhang *et al.*, 2025). In saline environments, variations in plant C:N:P ratios can alter litter chemistry and decomposition (Enríquez *et al.*, 1993). Such stoichiometric shifts can disrupt nutrient cycling, as imbalances in one element influence the availability of others, ultimately affecting soil fertility and ecosystem functioning (McGroddy *et al.*, 2004). Elevated soil salinity can reduce leaf phosphorus while increasing nitrogen and the N:P ratio (Song *et al.*, 2015; Jiang *et al.*, 2023). However, these responses are species-specific, with some plants showing decreased leaf N (Ramoliya *et al.*, 2006) or variable P changes (Loupassaki *et al.*, 2002). Notably, variation in halophyte leaf N:P stoichiometry is driven primarily by soil nutrient availability rather than salinity, highlighting the dominant role of nutrient supply over salt stress (Wang *et al.*, 2015), and emphasizing how species- and site-specific nutrient dynamics shape ecosystem nutrient cycling under saline conditions. Sun *et al.* (2017) showed that depending on the relative growth rate, soil salinity can either increase or decrease the optimal N:P ratio. Although other elements play important roles, their precise quantitative requirements are still not accurately defined. Several studies have shown that salinity stress reduces the accumulation of K, Ca, and Mg, as well as the K:Na ratio in leaves, while increasing Na concentration (Loupassaki *et al.*, 2002; Ashraf *et al.*, 2023). In contrast, other studies have shown that rising salinity can cause K, Ca, and Mg levels to increase or remain stable (Ramoliya *et al.* 2004; Hidri *et al.*, 2019). Salinity can also disturb the absorption or the balance of a given micronutrient, ultimately resulting in quantitative and qualitative yield decrease. Micronutrient concentrations in plants vary with species and the severity of salt stress (Hu and Schmidhalter, 2001). Optimal nutrient levels are vital for sustaining plant growth and metabolic function (Blasco *et al.*, 2018). Maintaining ionic homeostasis ensures balanced nutrient absorption and storage (Faran *et al.*, 2019), while equilibrium in micronutrient uptake is key to sustaining plant health and maximizing yield (Das and Green, 2016). Salinity leads to nutrient deficiencies, especially in Fe, B, Al, Cu, Mn, and Zn (Amini *et al.* 2016), hence affecting the nutritional status and stoichiometry within plants. The contents of available Mn, available Fe, and available Mo in the soil are positively correlated with organic matter content.

Halophytes possess high physiological plasticity, enabling them to tolerate high salinity through effective ion compartmentalization and nutrient regulation (Mohamed *et al.*, 2023). However, despite extensive research on salt tolerance mechanisms, the integrated responses of nutrient stoichiometry and multi-element ionic balance in halophytes remain insufficiently understood. In particular, little is known about how different ecotypes of *S. carnosa* adjust elemental allocation and ion homeostasis under saline conditions, and whether these adjustments reflect coordinated adaptive strategies. Therefore, this study investigates (i) changes in stoichiometric ratios (C, N, P, and K), (ii) patterns of ionic

Table 1. Climatic characteristics of the regions of the sample provenances of Sulla carnosa

Region	Provenance	Climatic region
Kairouan	Kalbia	Semi-arid
Mahdia	Kerker	Semi-arid
Sidi Bouzid	Sidi khlif	Semi-arid
Metlaoui	Thelja	Arid

homeostasis (K, Ca, Mg, S, Zn, and Mn), and (iii) their coordination across different *S. carnosa* ecotypes exposed to salinity. By integrating macronutrient stoichiometry with multi-element ion balance, this work aims to clarify the physiological strategies that enable *S. carnosa* to maintain metabolic and ionic balance when coping with salt stress.

Materials and Methods

Sulla carnosa is a facultative halophyte capable of growing in both saline and non-saline soils. This Tunisian endemic extremophile thrives in semi-arid and arid regions with alkaline-saline soils. *S. carnosa* seeds were collected from the localities of 'Sidi Khlif' (35°08′31′′N 09°42′55′′E); Kalbia (35°48′26′′N 10°08′29′′E); Kerker (35°30′24.7′′N 10°38′26.5′′E); and Thelja (27°35′68.8′′N 07°45′76.5′′E) in Tunisia (Table 1). The experiment was carried under green-house conditions, where plants were cultivated at 24/20°C (day/night), with 16 h/8 h photoperiod, a relative humidity of 50-60%, and an average photosynthetic photon flux density of 800 µmolm^{-2} s^{-1}, as measured with a light meter (LICOR, Lincoln, NE, USA, model LI-188B). First, seedlings of each *S. carnosa* ecotype were transplanted into pots filled with 500 g of this soil/sand mixture, with one plant per pot and five repetitions for each ecotype. The soil used was loamy (Alfisol Xeralf Rhqdoxeralf) (Soil Survey Staff, 1975) collected from the province of Granada (Spain). The soil characteristics were: a pH of 8.1 (measured in water, 1:5, w:v), contained 15 g kg^{-1} of organic matter, and carried the following nutrients: total N, 1.9 g kg^{-1}; total P 1 g kg^{-1}; available P 0.027 g kg^{-1}; and a total K 6.9 g kg^{-1}. The soil was sieved (2 mm) and diluted with quartz sand (< 1 mm) (1:1, v:v).

One month after transplanting, salt stress treatments were applied, with plants exposed to either 0 mM NaCl (control) or 200 mM NaCl. Combining all conditions resulted in eight treatments (control and salt stress for each ecotype) distributed across a total of 40 pots. Treatments were arranged in a randomized complete block design with five replicates, and plants were watered three times per week with 50 mL of the respective NaCl solution.

Plants were harvested after ten weeks of growth. Shoots and roots were first separated and were then oven dried (70°C) for 48 h. Biomass production was determined after weighing samples of dried shoots and roots.

The salinity tolerance index (STI), was determined as follows Bouslimi *et al*. (2021):

STI (%) = (Dry weight of treated plants/ Biomass of control plant) × 100.

Shoot concentrations of nitrogen (N) and carbon (C) were determined using a CHN analyzer (ELEMENTAL LECO TruSpec CN, Instituto de Nutrición Animal, CSIC, Granada, Spain).

Macronutrients including calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), sulfur (S), and phosphorus (P) as well as micronutrients copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were analyzed by using inductively coupled plasma 160 mass spectrometry (ICP-MS; Thermo Electron Corporation Model IRIS intrepid II XDL) and were performed by the Analytical Service of the Centro de Edafologı´a y Biologı´a Aplicada del Segura, CSIC, Murcia.

Results were statistically analysed using SPSS 20 software and were subjected to one-way general linear model ANOVA (analysis of variance), to determine the effect of each treatment. The Duncan's (Duncan, 1955) multiple range test was used to compare differences between treatment means at a significant level of P < 0.05.

Results and Discussion

Plant growth

Figure 1 illustrates a significant reduction in shoot length across all *S. carnosa* ecotypes in response to salinity. Under salt-free conditions, Thelja ecotype exhibited the highest shoot length, and also maintained the highest shoot length value compared to the other ecotypes under salt stress. In contrast, root length remained unaffected by both salinity and ecotype (Fig. 1).

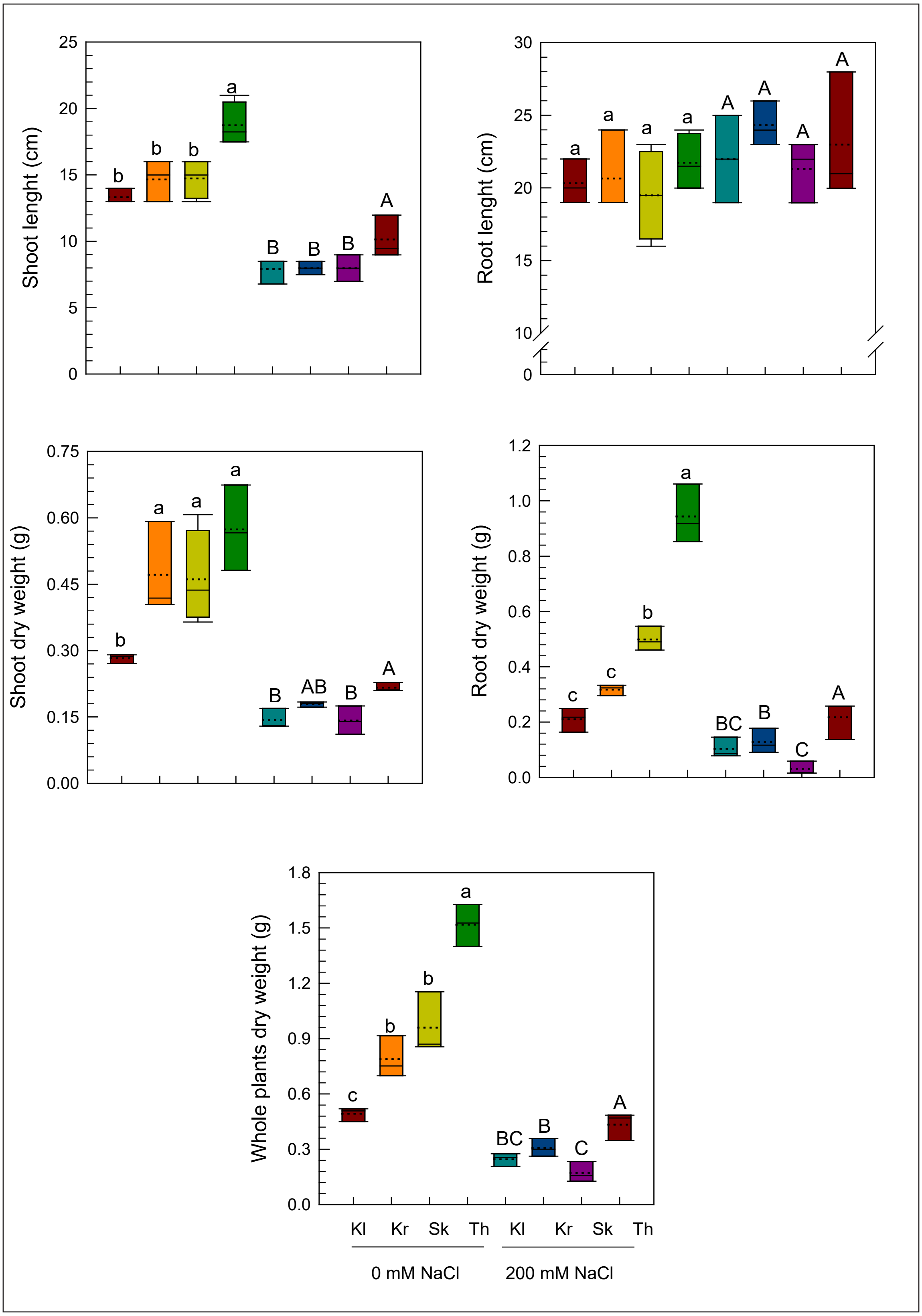

Fig. 1. Effect of salinity on the growth performance of different S. carnosa ecotypes (Kalbia (Kl); Kerker (Kr); Sidi Khlif (SK) and Thelja (Th)). Values are means of five replicates ±SE and different letters indicate a significant difference ($p < 0.05$) using Duncan's test.

Table 2. Tolerance index (TI) of different S. carnosa ecotypes (Kalbia (Kl); Kerker (Kr); Sidi Khlif (SK) and Thelja (Th)) in response to salt stress

	Shoots TI (%)	Roots TI (%)	Whole plants TI (%)
Kalbia	50.69	49.35	50.12
Kerker	37.97	40.59	39.03
Sidi Khlif	30.88	7.16	19.28
Thelja	37.79	25.75	28.67

Plant shoot dry weight of the different *S. carnosa* ecotypes was significantly affected by salinity. Under control conditions, the highest shoot dry weight was observed in Thelja, Kerker, and Sidi Khlif, whereas the lowest was observed in Kalbia (Fig. 1). For all ecotypes, shoot dry weight decreased with increasing salinity levels. Compared with the control plants, shoot dry weight decreased by 49% in Kalbia, 62% in Kerker, 69% in Sidi Khlif, and 62% in Thelja (Fig. 1).

Figure 1 shows also that the root dry weight of different *S. carnosa* ecotypes was also affected by salinity. Under control conditions, the Thelja ecotype exhibited the highest root dry weight, whereas the lowest values were observed in Kalbia and Kerker (Fig. 1). The greatest reductions in root dry weight were observed in Sidi Khlif (92%) and Thelja (74%), followed by Kerker (59%) and Kalbia (50%). These results suggest that the effect of salinity on *S. carnosa* growth varied among ecotypes, likely reflecting differences in their genetic potential (Fig. 1). Sidi Khlif exhibited the lowest TI values across whole plants, followed by Kerker and Thelja, whereas Kalbia showed the highest TI among the ecotypes (Table 2).

Effect of salinity on element: C ratios

Figure 2 shows that salinity significantly increased the N:C and P:C ratios in all studied ecotypes, except in Kalbia for both ratios and in Sidi Khlif for the P:C ratio, where salinity had no significant effect. The K:C ratio decreased in Kalbia and Kerker, whereas it was not significantly affected in Thelja and Sidi Khlif. Under salt-free conditions, Kalbia exhibited the highest K:C ratio. The Ca:C ratio was slightly reduced by salinity in Kalbia and Sidi Khlif, with the highest value observed in Sidi Khlif under salt-free conditions (Fig. 2). The S:C ratio decreased in all studied ecotypes, while Kalbia maintained the highest value under salt-free conditions. For Mn:C, neither salinity nor ecotype had a significant effect (Fig. 2).

Effect of salinity on element: N ratios

Salinity significantly decreased the K:N, Ca:N, and Mg:N ratios in all studied ecotypes, except in Kalbia, where the reduction was minimal for K:N and not significant for Ca:N and Mg:N. Under salt-free conditions, the highest K:N and Mg:N ratios were observed in the Thelja and Sidi Khlif ecotypes, with Sidi Khlif also exhibiting the highest Ca:N ratio (Fig. 3). Ecotypic differences had no significant effect on the P:N ratio; however, salinity significantly decreased this ratio only in Sidi Khlif (Fig. 3). Under salt-stress conditions, no significant differences were observed among ecotypes for the Ca:N, Mg:N and P:N ratios. Salinity also reduced the S:N and Mn:N ratios in all studied ecotypes, except for Mn:N in Kalbia, where the effect was not significant (Fig. 3). Overall, these findings indicate that nutrient stoichiometry under salinity stress is ecotype-dependent, with Kalbia maintaining greater stability, while Sidi Khlif appears more sensitive to ionic imbalance (Fig. 3).

Effect of salinity on element: P ratios

Figure 4 shows that salinity had no effect on the N:P ratio in the studied S. *carnosa* ecotypes, except in Sidi Khlif, where it increased under stress condition. Salinity markedly decreased the K:P, Mg:P, Ca:P, S:P, and Mn:P ratios in all studied ecotypes, except in Kalbia, where its effect on Mg:P and Ca:P ratios was not significant, and the Mn:P ratio showed only a slight reduction (Fig. 4).

Effect of salinity on element: K ratios

Under salt stress conditions, the P:K ratio increased in all *S. carnosa ecotypes* compared to their control plants (Fig. 5). The Mg:K ratio increased in Kalbia, whereas it decreased in Thelja and Sidi Khlif (Fig. 5). The Ca:K ratio increased in Kalbia and Kerker but decreased in Thelja. Salinity strongly increased the Na:K ratio in all ecotypes; Thelja showed the lowest value under salt stress, while under salt-free conditions no significant differences were observed among ecotypes (Fig. 5). Overall, neither ecotype nor salinity had a significant effect on the S:K ratio, except in Thelja, where salinity reduced its value. Under salt stress, the Mn:K ratio increased in Kalbia and Kerker but decreased in Thelja, whereas in Sidi Khlif it did not differ significantly from the control (Fig. 5).

Effect of salinity on element: Mn ratios

Under salinity stress, the Mg:Mn ratio increased in most *S. carnosa* ecotypes, except in Kalbia, which was unaffected (Fig. 6). The Ca:Mn and Zn:Mn ratios were not significantly affected by salinity in the studied ecotypes, except in Kerker, where Ca:Mn slightly decreased and Zn:Mn increased (Fig. 6). Salinity also increased the Fe:Mn ratio in Kerker and Sidi Khlif, and particularly in Kalbia, while it decreased in Thelja (Fig. 6).

Sulla carnosa is an indifferent halophyte whose growth is influenced by high soil salinity. It is mainly distributed in North Africa, particularly in Algeria and Tunisia. This leguminous species plays an important role in

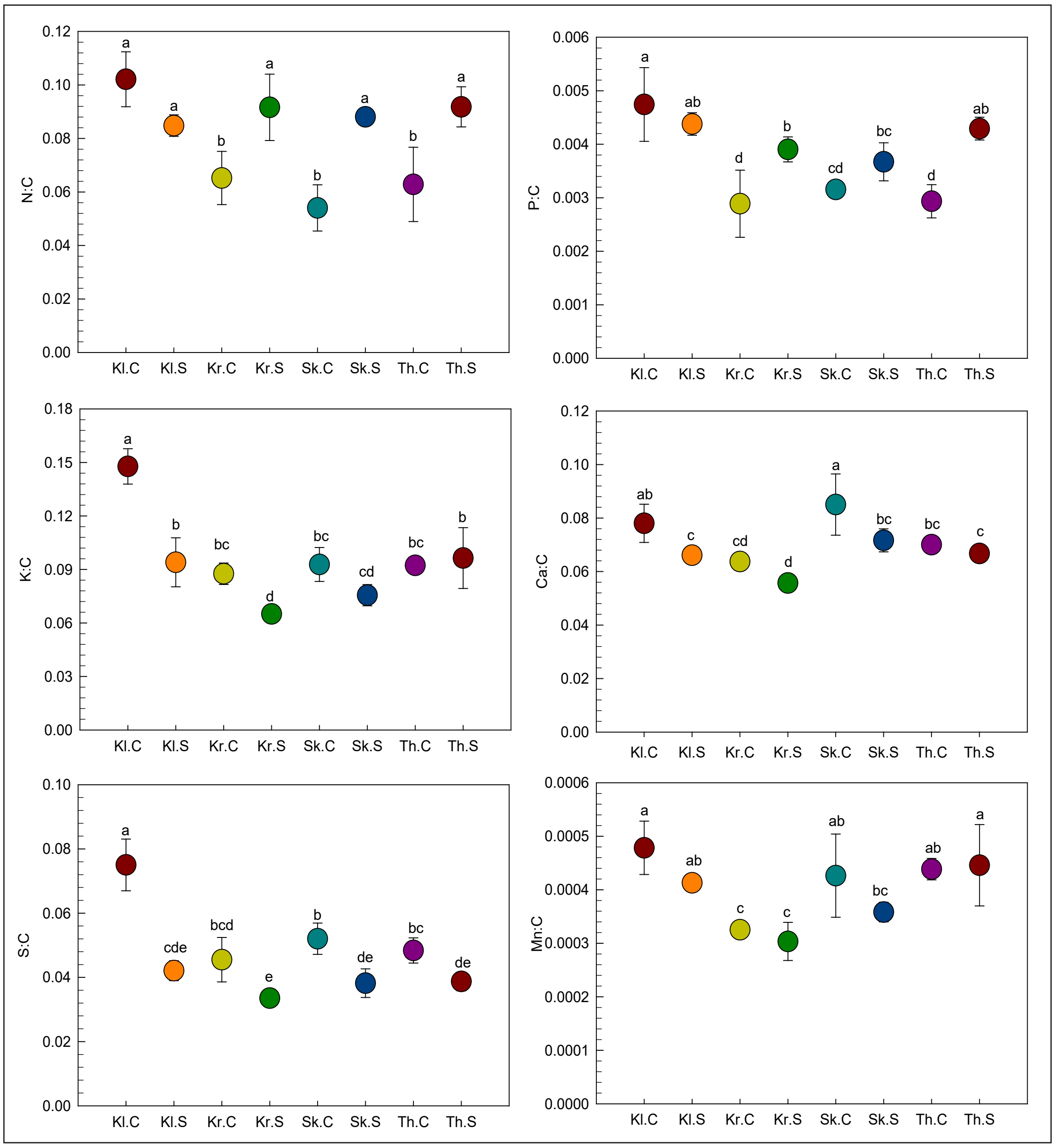

Fig. 2. Effect of salinity on element:C ratios in the shoots of different S. carnosa ecotypes (Kalbia (Kl); Kerker (Kr); Sidi Khlif (SK) and Thelja (Th)). Values are means of five replicates ±SE and different letters indicate a significant difference ($p < 0.05$) using Duncan's test.

soil restoration and serves as a forage resource in salt-affected areas (Bouzidi *et al.*, 2021). In our study, salinity stress (200 mM NaCl) induced a significant reduction in shoot and root dry weight across all *S. carnosa* ecotypes compared to the control. Growth inhibition of *S. carnosa* at high salt concentrations has also been reported in earlier studies (Hmaeid *et al.*, 2019; Hidri *et al.*, 2025), which may result from a considerable reduction in photosynthetic activity (Pandit *et al.*, 2024). The extent of this reduction varied considerably, with Kalbia displaying the greatest resilience and Sidi Khlif being the most adversely affected, indicating significant genetic variability among the ecotypes. Gandour *et al*. (2014) also showed that both population and genotype significantly influence the salt stress tolerance of *S. carnosa*.

According to Li *et al*. (2021), nutrient availability and environmental conditions are major determinants of plant growth and development. Salinity may influence how plants allocate nutrients to support optimal growth and survival in their specific environments. High salt concentrations inhibit essential physiological processes such as photosynthesis, leading to osmotic stress and disrupting nutrient uptake (Liu *et al.*, 2024). In our study, salinity was found to alter plant nutrient balance, increasing some elemental ratios while reducing others, depending on the studied ecotypes. Under these circumstances, plants need to adjust the allocation of elements like carbon (C), nitrogen (N), and phosphorus (P) to maintain homeostasis and adapt to their harsh environment, which in turn directly

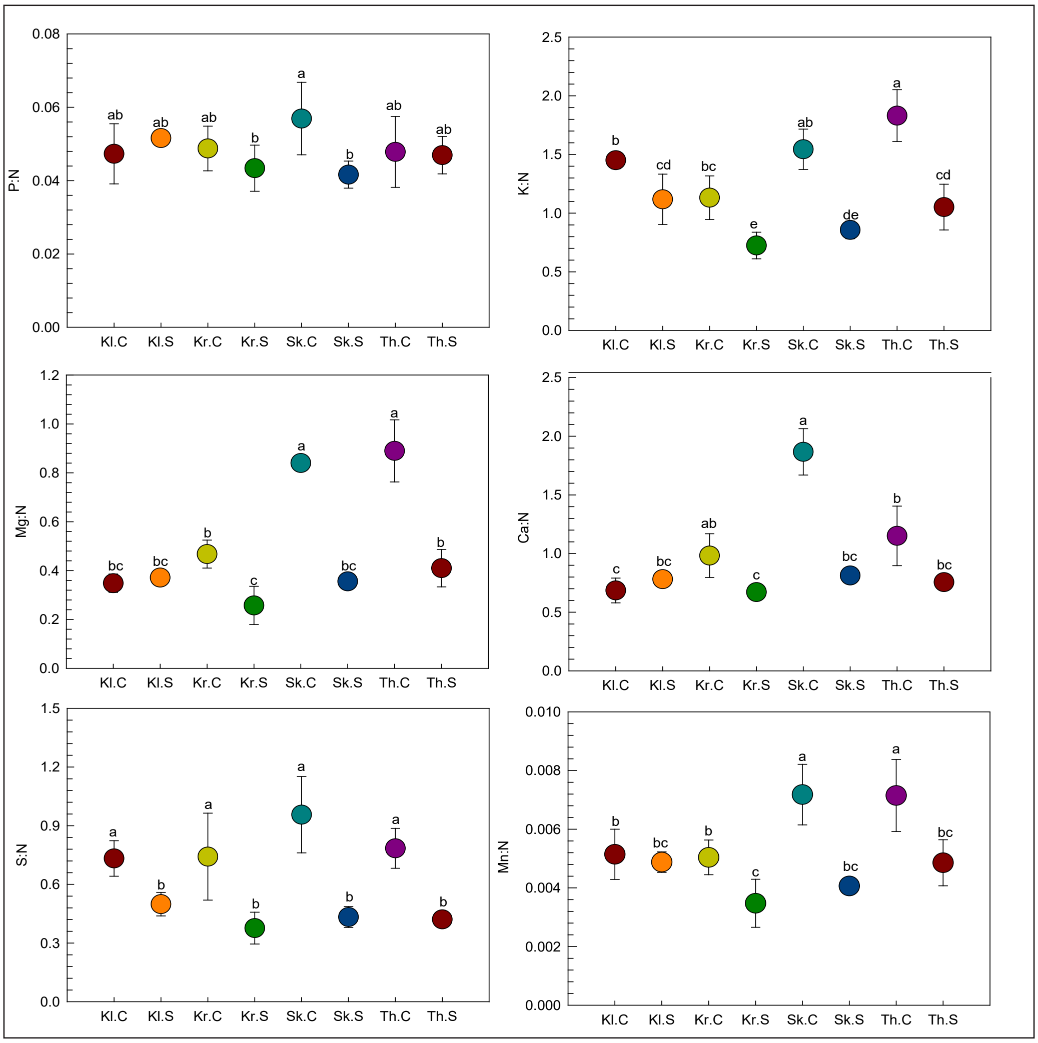

Fig. 3. Effect of salinity on element:N ratios in the shoots of different S. carnosa ecotypes (Kalbia (Kl); Kerker (Kr); Sidi Khlif (SK) and Thelja (Th)). Values are means of five replicates ±SE and different letters indicate a significant difference ($p < 0.05$) using Duncan's test.

affects the C:N:P ratios in plant tissues (Sun *et al.*, 2017; Wang *et al.*, 2024). The C:N:P ratio is the most extensively studied elemental relationship, as nitrogen (N) and phosphorus (P) are often the primary factors limiting plant growth (Sterner and Elser, 2003; Ågren 2004; Wang *et al.*, 2024; Zhao *et al.*, 2024). Although carbon (C) constitutes the structural basis of plants, representing roughly 50% of their dry mass, it can also become a limiting element under certain conditions. Their biochemical functions are highly interdependent, resulting in significant interactions within biological systems (Wang *et al.*, 2024).

The variability of element:C ratios is primarily determined by the proportion of carbon-rich structural components in tissues,

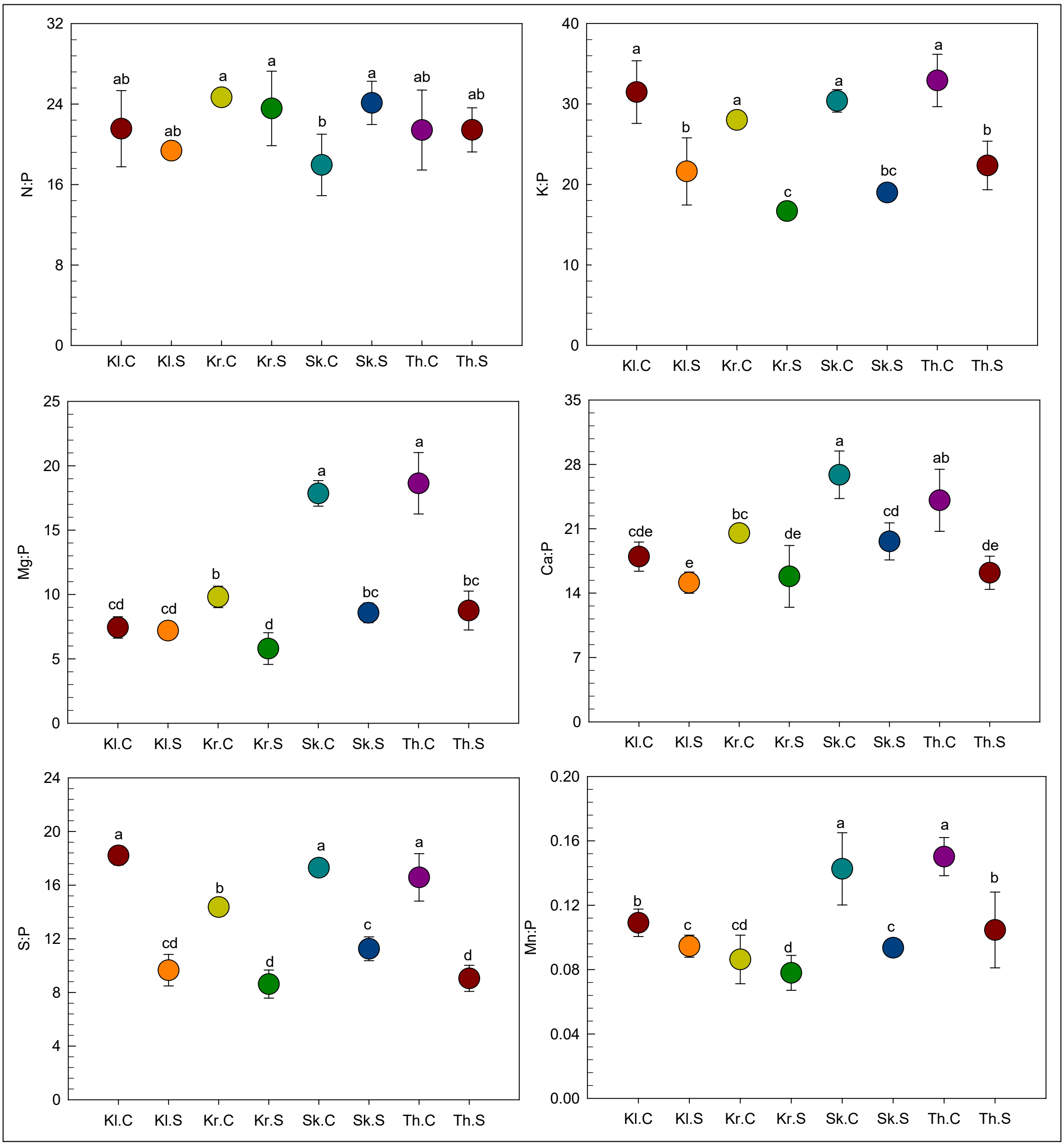

Fig. 4. Effect of salinity on element:P ratios in the shoots of different S. carnosa ecotypes (Kalbia (Kl); Kerker (Kr); Sidi Khlif (SK) and Thelja (Th)). Values are means of five replicates ±SE and different letters indicate a significant difference ($p < 0.05$) using Duncan's test.

while the concentrations of other elements are largely associated with metabolic activities. The decline in the S:C ratio across all *S. carnosa* ecotypes under salt stress suggests sulfur sensitivity, likely due to reduced sulfur uptake or assimilation in the presence of competing ions like Na. This may indicate a shift in nutrient allocation, where nitrogen and phosphorus take priority for osmotic regulation over sulfur, which is less critical in salinity stress. The N:C and P:C ratios in the shoots of all ecotypes, except Kalbia, were elevated under salt stress, likely due to reduced growth rate and limited nutrient-use efficiency (Aronsson and Elowson, 1980). In contrast, Kalbia showed the highest N and P contents, indicating better nutrient uptake efficiency. It also maintained stable N:C and P:C ratios, reflecting higher shoot construction efficiency and greater salt tolerance. This suggests better ability of Kalbia

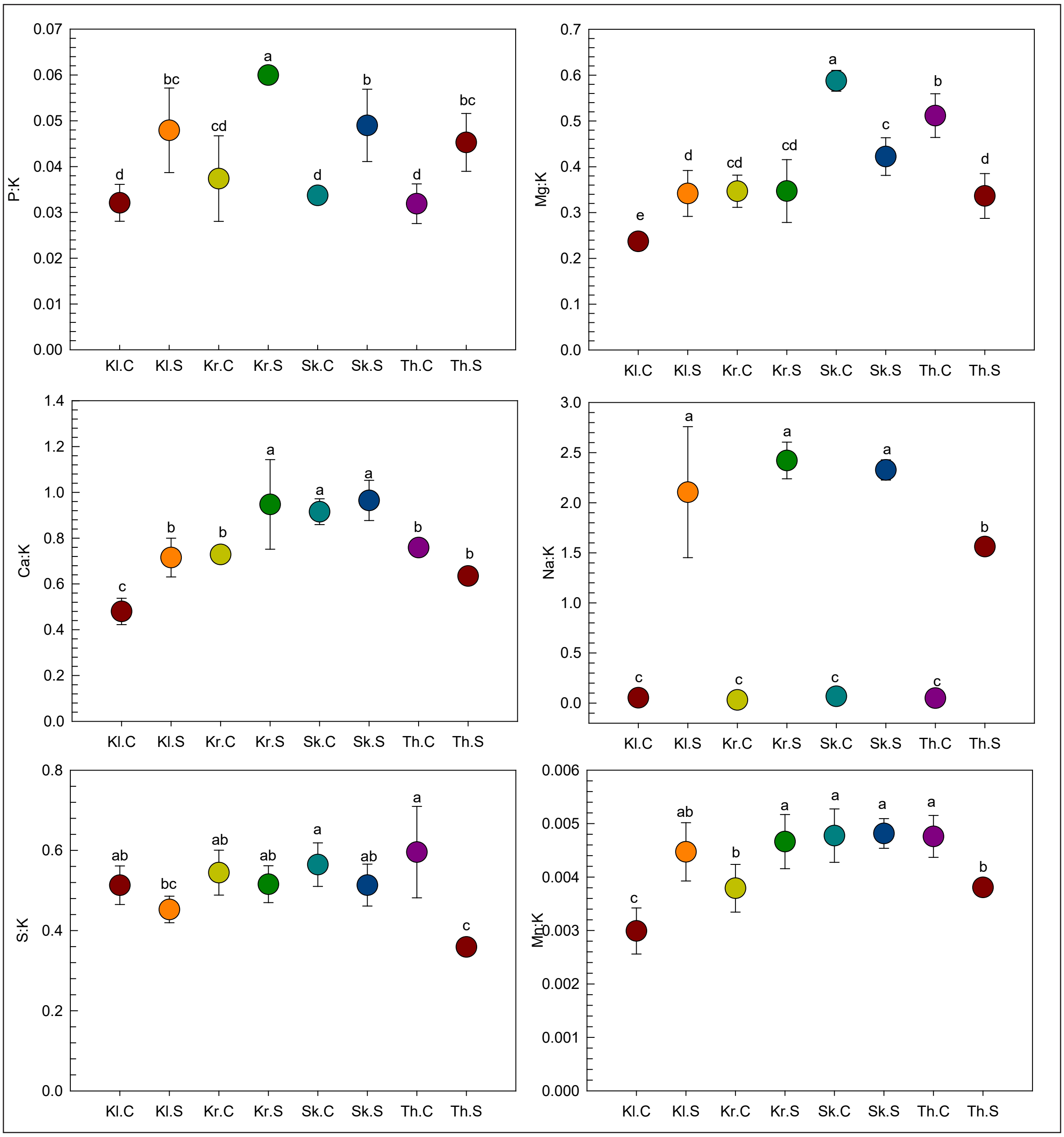

Fig. 5. Effect of salinity on element:K ratios in the shoots of different S. carnosa ecotypes (Kalbia (Kl); Kerker (Kr); Sidi Khlif (SK) and Thelja (Th)). Values are means of five replicates ±SE and different letters indicate a significant difference ($p < 0.05$) using Duncan's test.

to maintain nutrient balance under salinity compared to the other ecotypes.

Leaf N:P ratio is widely recognized as a key indicator of nutrient limitation and is extensively used in ecological stoichiometry studies (Güsewell, 2004; Zhang *et al.*, 2021). In our study, salinity increased the N:P ratio only in the sensitive Sidi Khlif ecotype, suggesting the onset of phosphorus limitation under salt stress. This pattern aligns with the framework proposed by Sterner and Elser (2003) in the productivity hypothesis, which states that highly productive organisms tend to exhibit lower N:P ratios, whereas elevated N:P ratios may reflect nutrient imbalance or growth constraints. The increase in N:P in Sidi Khlif was primarily driven by a marked rise in shoot N content, which was positively correlated with Na accumulation, indicating a possible synergistic effect between salt ions and nitrogen uptake.

Because N and P are the principal limiting nutrients in most terrestrial ecosystems, shifts in their balance provide important insight into how salinity alters nutrient allocation and may exacerbate phosphorus limitation in sensitive ecotypes. This study aims to explore methodologies for incorporating additional elements into stoichiometric analyses. Sardans *et al.* (2021) emphasized that integrating additional bio-elements such as potassium (K), magnesium (Mg), and calcium (Ca) into ecological analyses has substantially improved our understanding of how resource availability in complex ecosystems shapes key physiological processes, including growth and stress adaptation. Although potassium plays essential roles in enzymatic activation, stomatal regulation, turgor maintenance, membrane potential, homeostasis, and photosynthesis (Costa *et al.*, 2023), maintaining balanced K ratios relative to other nutrients is often more critical than its absolute concentration for

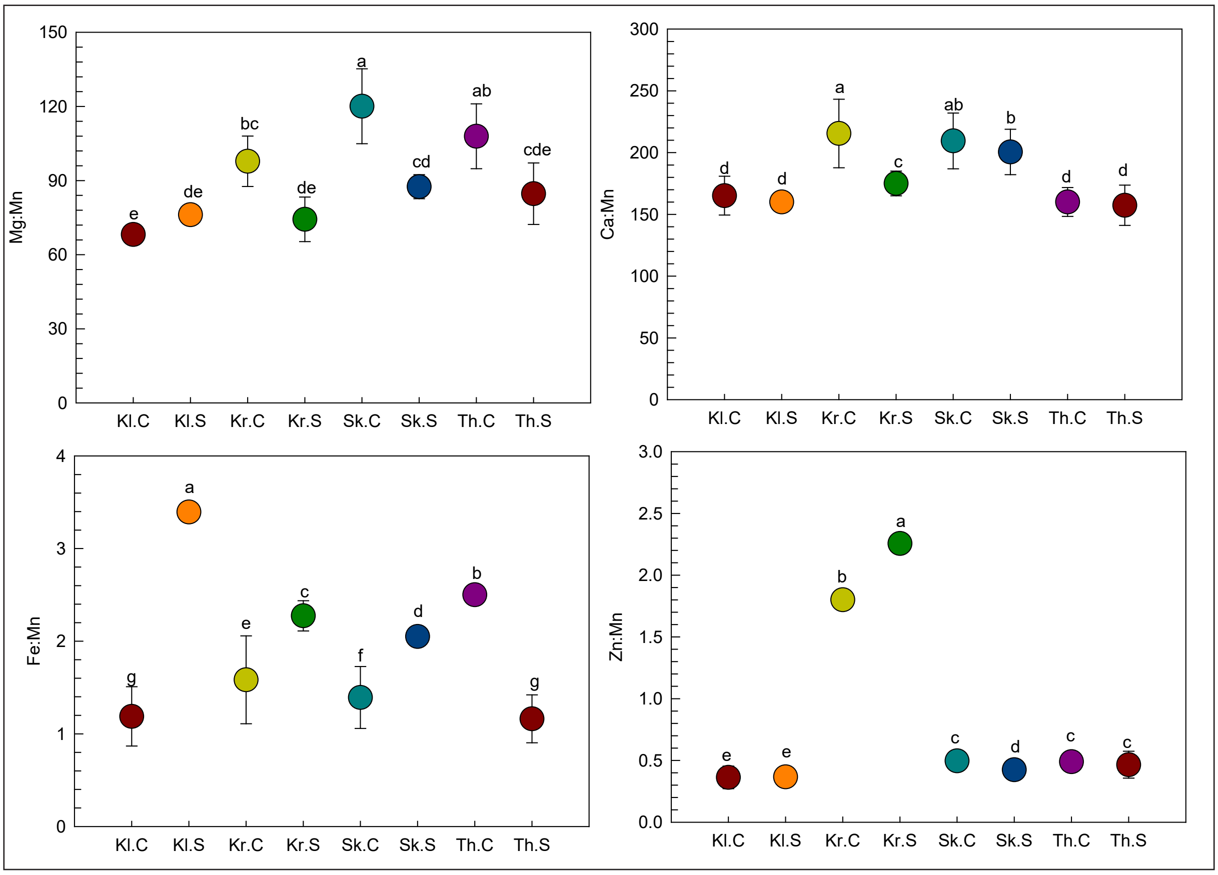

Fig. 6. Effect of salinity on element:Mn ratios in the shoots of different S. carnosa ecotypes (Kalbia (Kl); Kerker (Kr); Sidi Khlif (SK) and Thelja (Th)). Values are means of five replicates ±SE and different letters indicate a significant difference ($p < 0.05$) using Duncan's test.

optimal plant growth and productivity (Ali *et al*., 2019).

In the present study, the contrasting Mg:K and Ca:K ratios among ecotypes represent a central finding, reflecting divergent ion partitioning strategies. The increase in Mg:K and Ca:K ratios in Kalbia indicates a relative enrichment of divalent cations (Mg and Ca), which may enhance structural stability, metabolic efficiency, and stress signaling. Conversely, the lower ratios observed in Thelja (and the reduced Mg:K ratio in Sidi Khlif) suggest preferential K accumulation, potentially supporting osmotic adjustment and stomatal control under local environmental conditions. Salinity decreased the K:N ratio in all ecotypes; however, Kalbia ecotype exhibited the lowest reduction, indicating a relatively higher capacity to maintain potassium-to-nitrogen homeostasis under saline conditions. While salinity did not significantly affect the K:C ratio in Sidi Khlif and Thelja ecotypes, a marked decline was observed in Kalbia and Kerker, suggesting that salinity primarily alters potassium accumulation and distribution rather than carbon in these ecotypes.

For the K:P ratio, all *S. carnosa* ecotypes responded similarly to salt stress, showing a decrease mainly due to reduced potassium content. Under saline conditions, it is well established that plants often restrict K uptake and translocation because of competition with Na ions, which share similar transport pathways (Wakeel, 2013; Ameen *et al*., 2024). Therefore, *S. carnosa* ecotypes may prioritize maintaining osmotic stability and minimizing Na toxicity over sustaining high K accumulation. Overall, our results highlight that differences in potassium allocation strategies alone do not fully explain the variation in salt tolerance among *S. carnosa* ecotypes.

The Mg:N and Ca:N ratios decreased in all studied *S. carnosa* ecotypes except Kalbia, mainly due to a sharp decline in Mg and Ca concentrations. An imbalance in one of those nutrients, whether deficiency or excess, could lead to the accumulation or reduction of other elements (McGroddy *et al*., 2004). According to Ågren and Weih (2012), the uptake of Mg and Ca may be limited by a maximal concentration threshold in plant biomass. Both cations are essential for plant growth and development (He *et al*., 2024). Mg is a key component of chlorophyll and plays a central role in photosynthetic energy transfer, enzyme activation, and carbon fixation (Zhu *et al*., 2025). Ca is crucial for maintaining cell wall integrity, membrane stability, and signalling under stress conditions (Bisht *et al*., 2025). Salinity induced strong nitrogen uptake in Kerker, Sidi Khlif, and Thelja ecotypes, which may lower foliar Mg:N and Ca:N ratios, thereby disrupting ionic homeostasis and potentially causing nutrient imbalances or deficiencies that, in turn, impair metabolic processes, reduce photosynthetic efficiency, and ultimately limit plant growth and productivity. Kalbia ecotype maintained stable Mg:N and Ca:N ratios under salt stress condition, reflecting efficient nutrient regulation and balanced ion uptake. This stability suggests better physiological adaptation and stronger tolerance to salt stress compared with other ecotypes.

According to Hu and Schmidhalter (2001), salinity effects on concentrations of micronutrients such as Mn, Zn, Fe, and B in plants are complex to decipher, as they are influenced by macronutrient status and the intensity of salinity. Excessive soil salinity often leads to decreased Mn accumulation; however, the intensity of this response depends on the plant species or ecotype, the severity of salt stress, and the relative availability of other essential elements, particularly calcium, magnesium, iron and zinc. In our study, salinity increased the Mg:Mn ratio in *S. carnosa* ecotypes, except in Kalbia, where no significant effect was observed. The Kalbia ecotype also maintained a stable Ca:Mn and Zn:Mn ratios, while the Fe:Mn ratio increased markedly under salt stress. These results suggest that Kalbia exhibits a more efficient ionic regulation, maintaining Ca:Mn, Mg:Mn and Zn:Mn homeostasis under saline conditions. The observed increase in the Fe:Mn ratio may reflect an adaptive mechanism aimed at protecting the photosynthetic machinery, since Fe is essential for electron transport and Mn plays a key role in the oxygen-evolving complex (OEC) of photosystem II, protecting the OEC from photoinhibition (Hakala *et al*., 2005).

Conclusions

This study revealed ecotype-specific adjustments in growth, nutrient stoichiometry, and ionic balance in *S. carnosa* when challenged with salinity. Among the investigated ecotypes, Kalbia exhibited higher salt tolerance, which was associated with its capacity to maintain ionic homeostasis and coordinated nutrient regulation under salt stress. Our findings highlight the importance of integrated stoichiometric and ionic strategies in determining plant performance in saline environments. Beyond advancing our understanding of halophyte adaptive mechanisms, at the applied level; the identification of salt-tolerant ecotypes such as Kalbia provides valuable material for saline agriculture, rangeland restoration, and rehabilitation of salt-affected soils. Moreover, the traits associated with improved ionic regulation and nutrient balance may serve as useful selection criteria in breeding programs aimed at enhancing salt tolerance. Overall, our findings underscore the ecological significance of intraspecific variation in shaping plant resilience to salinity and emphasize the potential of ecotype-based approaches for sustainable management and utilization of saline ecosystems.

Author Contributions

RH and RAZ conceived and designed the study, performed the experiments, and analyzed the data. RH and FB wrote the manuscript. RAR, AD, WZ, OMBM, and CA revised the manuscript. All authors contributed to the article and approved the submitted version.

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