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Optimizing salt tolerance in carob plants (*Ceratonia siliqua* L.) by cultivating in association with the halophyte *Spergularia salina*

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ABSTRACT

Salinity poses a major challenge to agriculture, underscoring the urgent need to find alternative solutions. Exploiting halophytic plants represents a promising biological adaptation to this challenge. This article presents a comparative study on the effectiveness of the association between *Ceratonia siliqua* and the halophytic plant *Spergularia salina* in counteracting the effects of saline stress. The study was conducted in a greenhouse for two months. Carob seeds were scarified and planted at the age of 6 months in pots. Four levels of NaCl concentration were applied: 0 mM/L (non-saline control), 85 mM/L, 171 mM/L, and 257 mM/L. *Spergularia salina* was co-cultivated with the carob plants at concentrations of 171 mM/L and 257 mM/L. The findings revealed that salt adversely affected the growth of carob plants. Growth reduction was more pronounced at higher concentrations of NaCl (reductions of 19%, 25%, and 56% respectively at salt concentrations of 85 mM/L, 171 mM/L, and 257 mM/L). Growth in the presence of *Spergularia salina* mitigated the negative consequences of salt stress on the carob. *Ceratonia siliqua* co-cultivated with *Spergularia salina* had the highest growth rate, better water content, and improved photosynthesis. Association cultivation showed that carob plants did not exhibit signs of stress in fact, proline, total sugars, and polyphenol concentrations were low compared to plants cultivated without halophytic plants, indicating better adaptation to saline stress. These results highlight the promising potential of association in saline-prone soils, planting carob when halophytic plants are present, particularly with *Spergularia salina*, this helps agriculture cope with climate change.

Keywords: salt stress, *Spergularia salina*, halophyte, association cultivation, *Ceratonia siliqua* L.

INTRODUCTION

Salt stress is a major obstacle to the growth, yield, and quality of crops, posing significant ecological and environmental challenges to global agriculture (Melino and Tester, 2023; Liu et al. 2024). Salinity affects over 20% (more than 45 million hectares) of the world's irrigated soils, making it a worldwide problem (Dou et al., 2024). By 2050, it is projected that half of the world's irrigated areas will be affected by salinization (Wang et al., 2022). The MENA region is particularly hard hit, losing a billion dollars a year due to soil salinity, reducing agricultural productivity by 30–35% compared with its potential (Choukr-Allah et al., 2023). Traditional monoculture methods demonstrated challenges associated with soil salinity management, and generally a gradual upward trend for the accumulation of salts in the surface (0–20 cm) with increased sodium (Na^+) content, increased pH, and a deterioration of the physicochemical properties of the soil (Zhao et al., 2024). As a resolution of these challenges, examples of recent practices included intercropping systems that utilize halophytic plants, which exhibit desirable results as they can dissipate soil salinity, may prevent salt accumulation, added porosity and organic carbon % while decreasing bulk density, sodium % and pH (Yasseen and Al-Thani 2022). In accordance with these results halophytic plant intercrop systems could be encouraged as a long-term agronomic remediation practice that could ameliorate salinization of the soil profile for improved crop yield (Liang and Shi 2021). Growth in research on soil salinity control has been in the form of halophytic plants in conjunction with cereal and vegetable crops (Navarro-Torre et al., 2023). This line of research has a relevance to Mediterranean climates that are dry or semi-arid, where salinization of the soil limit sustainable agriculture (Navarro-Torre et al., 2023). These halophytes, being innate in terms of their salty habitat, may reduce the detrimental effects of salt stress on crops. Furthermore, halophytes as saline agriculture presents feasible alternatives for many functions such as food, forage, bioenergy, ornamental and pharmaceutical, to give way to sustainable development within these challenging landscapes (Duarte et al., 2021). These results suggest that the inclusion of halophytes with conventional crops can contribute to the reduction in soil salinity, while also enhancing the plants' resistance to salt stress. Again, there are caveats to consider in this phenomena, namely variable responses dependent on plant species, the concentration of salt used and environmental conditions investigated (Kaushik et al., 2024).

Carob (*Ceratonia siliqua* L.) is a legume of the Fabaceae family, which is mainly cultivated for its edible fruit and its capacity to flourish under arid situations (Haq, 2008). It performs an important role in soil rectification and fire prevention due to its ability to thrive in thermal environments (Tous and Antoni, 2013; Cavallaro et al., 2016). In addition to its ecological benefits, it's also treasured economically, with cappotential programs in agrifood and prescribed drugs because of its dietary residences and bioactive compounds (López-Sánchez et al., 2018; Brassesco et al., 2021). Carob seems to grow well in saline soils, with the ability to tolerate soil salt concentrations of up to 3% NaCl (Batlle and Tous 1997; Cruz et al., 1996). *Spergularia salina* was initially documented by Holkema (1870) and later reconfirmed by Heimans et al., (1960). This plant is taken into consideration an obligate halophyte, that means that it's miles especially tailored to live on in environments with immoderate salt concentrations (Svensson and Persson 1994). It prospers on specific sorts of soil. However, commonly prefers people with immoderate salinity (Telenius and Torstensson 1999). Given the cappotential of many halophytic vegetation to eliminate salt from their tissues, the usage of those salt-resistant species to mitigate soil salinity represents a cost-effective and ecological phytoremediation technique for enhancing productiveness in regions laid low with this problem (Wang et al., 2022).

This study aims not only to understand the response of carob seedlings (*Ceratonia siliqua* L.) to salt stress but also to evaluate phytoremediatory impact of the halophyte *Spergularia salina* to improve the root-zone environment (microenvironment) by reducing soil salinity. To our knowledge, this is the first study investigating the co-cultivation of *Ceratonia siliqua* L. with a native obligate halophyte (*Spergularia salina*) under saline conditions as a sustainable phytoremediation strategy. Our aim was to determine how this association affects different morphological, physiological and biochemical parameters of carob plants under different concentrations of sodium chloride. Unlike previous studies, which focused on other crops, our study focused specifically on carob seedlings, an economically important crop in arid regions. Our research proposes innovative agronomic practices to improve crop adaptability to soil salinity. The results of this study will provide practical advice to farmers in areas affected by soil salinity, contributing to agricultural sustainability and productivity.

MATERIALS AND METHODS

Plant Material and cultivation conditions

The experiment was realized at Ibn Tofail University, Faculty of Sciences Kenitra in Morocco. (34°14′49″N; 6°35′13″W), in an experimental greenhouse in July and August 2023. Certified *Ceratonia siliqua* L. seeds were purchased from a commercial nursery in the Agadir region. For scarification, the seeds were dipped in sulphuric acid (H_2SO_4) for 20 minutes and then soaked in water for one day to maximise germination. The carob seedlings were grown in 12 litre plastic pots with a mixture of Maamoura soil, compost and peat, in a 2:1:1 ratio, for six months. The halophyte plant *Spergularia salina* was collected from near a salty river called Oued Malah in the Ouezzane region (El-Khadir et al., 2024).

Saline stress treatments applied

Four levels of NaCl concentration (Table 1), with six plants used for each treatment. According to Tanji (2002), soils with more than 12 g/L of salts are considered highly saline. Since 171 and 257 mM/L (NaCl) correspond to moderate and high salinity levels (≈10 and 15 g/L), *Spergularia salina* (halophytic plant) was used only at these concentrations to assess its effect under critical stress conditions within a period of 60 days.

Experimental design

The experiment was conducted with four salt concentration treatments (C0, C1, C2, C3) as described in Table 1. For each treatment, six carob plants (n = 6) were grown, with a total of 24 carob plants in the monoculture group. In addition, to assess the effect of cultivation in association with the halophyte plant (*Spergularia salina*) other carob plants were grown in association with *Spergularia salina* at concentrations C2 and C3, also with six plants per treatment. This brought the total number of plants to 36. Each plant was grown individually in a pot and considered as an experimental unit.

Parameters studied

Electrical conductivity of soil

Electrical conductivity is a direct indicator of soil salinity, allowing for a simultaneous assessment of soil salinity. Electrical conductivity was measured from soil samples collected at the root zone after 60 days of treatment. 20 g sol was blended with 100 ml distilled water, agitation for 30 minutes, decantation, and finally measurement of electrical conductivity using a conductivity meter (Model AD3000) at 19.1 °C. (Derouiche et al., 2023).

Morphological parameters

Various morphological parameters were evaluated to investigate the response of carob plants to saline stress. Total biomass was determined by separating above-ground and root parts after harvesting, then weighing them immediately. The fresh masses of the above-ground and root parts were then added together to obtain the total biomass. Height of aerial part was measured from ground level to the apical bud of the main stem, using a graduated ruler. The total number of fallen leaves was counted for each plant at the end of the experiment to assess leaf fall. Finally, the length of the main root was measured from the collar to the root tip using a graduated ruler.

Figure 1. Carob cultivation experiment: (a) cultivation without halophyte plant; (b) cultivation in association with *Spergularia salina*

Table 1. Salt treatments applied in the experiment: NaCl concentrations (g/L and mM/L) and corresponding soil electrical conductivity (mS/cm)

Concentration	NaCl (g/l)	NaCl (mM/l)	EC (ms/cm)
C_0	0	0	0.61
C_1	5	85	8.30
C_2	10	171	15.38
C_3	15	257	22.2

Physiological parameters

The relative water content (RWC) was established according to Barrs (1966). We took leaf samples from all treatments and weighed them to record fresh weight (FW) using a digital balance. Following this, all samples were floated in petri dishes containing distilled water for a period of 24 hours. It also had the saturated weight (SW) of the leaves measured and then they were placed at 70 °C for 24 hours or until reaching constant mass to be weighed again as dry weight (DW).

$$RWC\ (\%) = \frac{(FW - DW)}{(SW - DW)} \times 100 \quad (1)$$

The photosynthetic pigments in carob leaves were analyzed by extracting 0.5 grams of fresh leaves in 10 milliliters of 80% acetone and storing them in darkness at 4 °C for the whole night. Following five minutes at 4 °C and 10.000 rpm of centrifugation, absorbance was measured at 470, 645, and 663 nm. Chlorophyll contents were computed using Arnon's formulas (1949) and Lichtenthaler's equations (Wellburn, 1994) for carotenoids.

$$\text{Chlorophyll a}\left(\frac{\text{mg}}{\text{g}}\text{FW}\right) = (12.7 \times A663nm - 2.69 \times A645nm) \times \frac{V}{1000 \times m} \quad (2)$$

$$\text{Chlorophyll b}\left(\frac{\text{mg}}{\text{g}}\text{FW}\right) = (22.9 \times A645nm - 4.48 \times A663nm) \times \frac{V}{1000 \times m} \quad (3)$$

$$Total\ chlorophyll\left(\frac{\text{mg}}{\text{g}}\text{FW}\right) = (20.2 \times A645nm + 8.02 \times A663nm) \times \frac{V}{1000 \times m} \quad (4)$$

$$Carotenoids\left(\frac{\text{mg}}{\text{g}}\text{FW}\right) = \frac{(1000 \times A470nm) - (3.27 \times \text{Chlorophyll a}) - (104 \times \text{Chlorophyll b})}{198} \quad (5)$$

where: V – total volume of extract, m – mass of plant material.

Biochemical parameters

Proline concentration was assessed at 520 nm, following the protocol described by Bates et al. (1973). A mixture of 0.5g of plant material and 10mL of 3% sulfosalicylic acid was macerated. Following a 10-minute centrifugation at 3000× g, 2 mL of the supernatant was mixed with 2 mL of glacial acetic acid and ninhydrin reagent. The mixture was then incubated for 1 hour at 100 °C, and the reaction was halted in an ice bath. Following the reaction, 4 ml of toluene was used to separate the proline, and toluene was used as a blank to measure optical density at 520 nm.

The method described by Dubois et al. (1956) was used to quantify the amount of soluble sugars in the carobs leaves. For the determination, a 0.1 g finely ground sample was extracted using 80% ethanol and was centrifuged for 10 minutes at 5000 rpm. A mixture of 1.25 ml concentrated sulfuric acid, 0.25 ml phenol (5%) and 0.25 ml supernatant were used to measure the optical density at 485 nm.

The procedure outlined by He et al. (2011) was used to ascertain the dried leaves' polyphenol content. Each sample was diluted using 0.5 ml of Folin-Ciocalteu reagent, 1.0 ml of distilled water, and 0.5 ml of each sample. The tubes were filled with 0.5 ml of 10% Na_2CO_3 after two to five minutes had passed. Using a spectrophotometry set to 760 nm, the absorbance was measured after an hour of incubation at room temperature for 30 min.

Determination of Na, K, Mg and Ca in leaves

Leaf samples were incinerated at 550 °C and then digested in concentrated nitric acid (HNO_3).

The concentrations of sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca) were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (X) Series, Thermo Electron Corporation) (Munter et al., 1984).

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 23. The results are expressed as mean ± standard deviation. Each treatment group included six biological replicates (n = 6). The effects of salt treatment (NaCl), presence of *Spergularia salina* (halophyte plant) and their interaction were assessed using the multivariate general linear model (multivariate GLM). This approach enables the simultaneous analysis of several dependent variables by integrating cross experimental factors. Pillai's trace test was used to interpret multivariate effects, due to its robustness to violations of the homogeneity of variance-covariance matrices. The associated univariate analyses (tests of between-subjects effects) were interpreted only when the hypothesis of equality of variances was met (Levene's test, $p > 0.05$). The significance threshold was set at $\alpha = 0.05$. A Pearson correlation analysis was conducted to explore relationships between parameters. Additionally, principal component analysis (PCA) was used to visualize multivariate patterns.

RESULTS

Soil electrical conductivity

The electrical conductivity of soil increases significantly as the quantity of salt increases. In the absence of *Spergularia salina*, conductivity increases from 1.1 µS/cm to 3.78 µS/cm (at 257 mM/l NaCl), demonstrating a direct relationship between salinity and soil conductivity. However, the presence of *Spergularia salina* has a significant moderating effect. At 171 mM/l NaCl (C2), conductivity fell by 28.57% in the presence of the plant, from 2.24 µS/cm to 1.6 µS/cm. Similarly, at 257 mM/l (C3), a 30.95% reduction is observed, with conductivity dropping from 3.78 µS/cm to 2.61 µS/cm. These results suggest that *Spergularia salina* has an important significant role in regulating soil electrical conductivity under saline conditions.

Impact of salt stress on carob morphological traits

The impact of saline stress on the height of aerial part of cultivated carob plants varies significantly depending on the presence of *Spergularia salina* (Figure 2a). Without *Spergularia salina*, salt concentrations C1, C2, and C3 result in reductions of 19%, 25%, and 56% in height of aerial part, respectively. However, when carob plants are cultivated in the presence of *Spergularia salina*, there is an increase of 12.5% and 44% in aboveground length at concentrations C2 and C3, respectively, compared to absence of halophytic plant. The application of salt stress by NaCl a decrease in total biomass by 35%, 43%, and 82%, respectively, in plants subjected to stress (C1, C2, C3) in the absence of the halophytic plant. Carob plants cultivated with *Spergularia salina* at concentrations C2 and C3 showed a total biomass 38% and 25% higher, respectively, than carob plants cultivated without *Spergularia salina* (Figure 2.b). In the absence of *Spergularia salina*, the average number of fallen leaves gradually increases with salt (NaCl) concentration (Figure 2.c). The mean

Table 2. Electrical conductivity (mS/cm à 19.1 °C) of soil

Spergularia salina	NaCl (mM/l)	Electrical conductivity (µS/cm)
Absence	0	1.1 ± 0.04 [a]
Absence	85	1.45 ± 0.05 [b]
Absence	171	2.24 ± 0.07 [c]
Presence	171	1.6 ± 0.06 [d]
Absence	257	3.78 ± 0.12 [e]
Presence	257	2.61 ± 0.06 [f]
Source of variation	Factor A (NaCl)	*** p < 0.001
	Factor B (*Spergularia salina*)	*** p < 0.001
	A*B	*** p < 0.001

values escalate from 2 to 32 as the salt concentration varies from 0 to 257 mM/l. However, in the presence of *Spergularia salina*, although the average values also rise with salt concentration, this increase is less pronounced. For concentrations C2 and C3, the average values are 6 and 11, respectively. In the absence of *Spergularia salina*, a gradual decrease in the average length of the root portion is observed with increasing salt concentration (NaCl). Mean values decrease from 19 cm to 11 cm as the salt concentration varies from 0 to 257 mM/l. Conversely, in the presence of *Spergularia salina*, the average length of the root portion appears to vary without following a clear trend based on salt concentration. Mean values are 17 cm and 13 cm for concentrations C2 and C3, respectively (Figure 2.d).

The results of the ANOVA (Table 3) highlight the significant impact of saline stress on the studied morphological variables. The p-values for NaCl and *Spergularia salina* both indicate significant differences in aerial height, total biomass, leaf fall, and root length. The interaction between NaCl and *Spergularia salina* is also significant for aerial height and leaf fall. However, it is noteworthy that the interaction between the halophytic plant (*Spergularia salina*) and saline stress was not found to be statistically significant for total biomass and root length ($p > 0.05$), indicating an absence of significant effect of the interaction between these two factors on these observed measures.

Impact of salt stress on carob physiological parameters

Relative water content decreases drastically as salt concentration (NaCl) increases. In the absence of *Spergularia salina*, RWC decreased drastically from 82.56% to 55.14% at salt concentrations from 0 to 257 mM/l. In the presence of *Spergularia salina*, however, RWC decreased less drastically, decreasing from 78.13% to 62.23% in concentrations C2 and C3 respectively. Similarly, the interaction between the presence of *Spergularia salina* and increased salt concentrations (NaCl) affects RWC. In the absence of

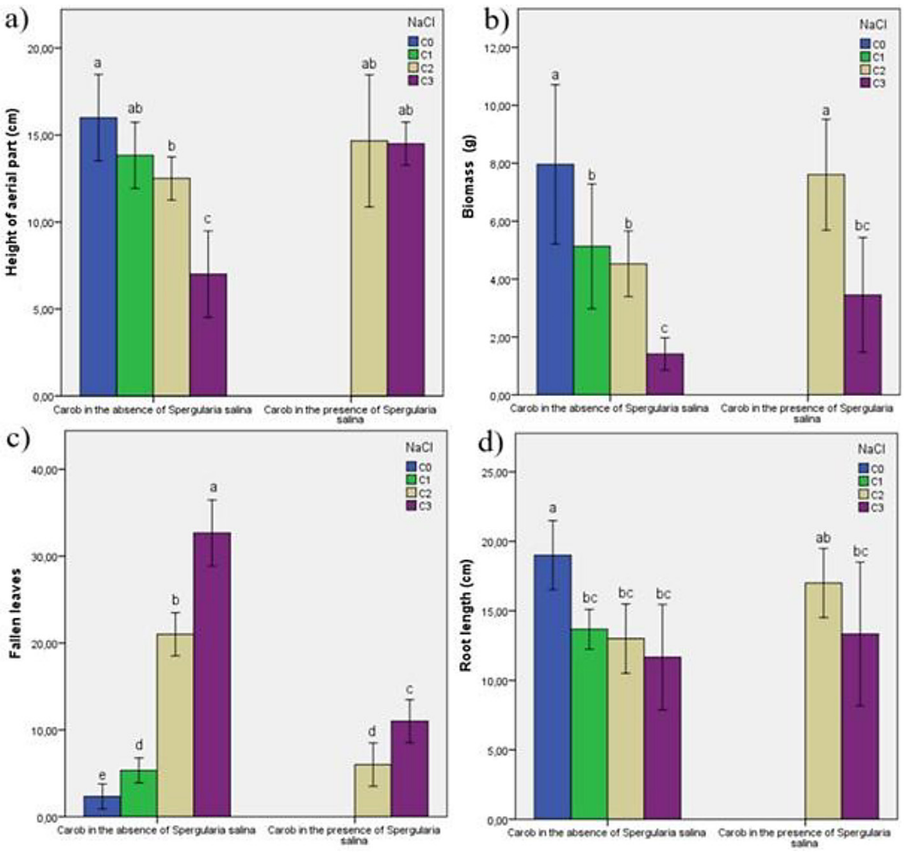

Figure 2. Effect of salt stress in the absence and presence of halophyte on: (a) height of aerial part; (b) total biomass; (c) fallen leaves; (d) root length (values with a different letter in the column have statistical significance at the 5% level)

Table 3. Analysis of variance (ANOVA) of morphological parameters

Morphological parameters	Length of aerial part	Total biomass	Leaves fall	Root length
Source of variation	*p*-value	*p*-value	*p*-value	*p*-value
NaCl (A)	< 0.001	< 0.001	< 0.001	< 0.001
Spergularia salina (B)	< 0.001	< 0.001	< 0.001	< 0.001
A x B	< 0.001	> 0.05	< 0.001	> 0.05

Spergularia salina, chlorophyll and carotenoid values decreased significantly as salt concentration increased. In the presence of *Spergularia salina* however, the decrease was less drastic, with values of 1.7 mg/g and 1.2 y mg/g for chlorophyll and 0.59 mg/g and 0.41 mg/g for carotenoids in concentrations C2 and C3 respectively. The interaction between the presence of *Spergularia salina* and increased salt concentrations (NaCl) similarly affects chlorophyll and carotenoid content.

Impact of salt stress on biochemical parameters

These trends are supported by the results of the analysis of variance (ANOVA), which confirm that both NaCl and *Spergularia salina* exert highly significant effects on proline, total sugar, and polyphenol contents. Furthermore, the interaction between NaCl and *Spergularia salina* is statistically significant for proline and total sugar content, suggesting a synergistic influence on their accumulation. However, this interaction does not significantly affect polyphenol content, indicating that their combined effect does not influence this parameter.

The comparative analysis of biochemical responses in carob plants, cultivated with or without *Spergularia salina* under varying salt concentrations, reveals notable differences. Proline accumulation is significantly higher in the absence of *Spergularia salina*, with increases of 217%, 274%, and 294% for salt concentrations C1, C2, and C3, respectively. In contrast, in the presence of the halophytic plant, the increase in proline levels is more moderate, reaching 175% and 235% for concentrations C2 and C3. Similarly, total sugar content exhibits substantial accumulation in carob plants grown without *Spergularia salina*, with increases of 150.72%, 320.32%, and 761.97% at C1, C2, and C3, respectively. However, when cultivated alongside *Spergularia salina*, the increase is less pronounced, with respective values of 185% and 567.8% at C2 and C3. As for total polyphenol content, it rises from 9.77 to 15.38 mg/g in response to increasing NaCl concentration (0 to 257 mM/l) in the absence of the halophyte, whereas in its presence, the content reaches only 12.2 and 13.34 mg/g at C2 and C3, respectively.

Effect of salt stress on Na, K, Mg and Ca content in carob leaves

There is a significant increase in Na, K, Mg and K concentration under salt stress conditions when carob is grown without *Spergularia salina* (monoculture), but this increase is lower in the case of coculture, particularly in concentrations C2 and C3 (Figure 9). Our results indicate that the concentration of Na, K, Mg and K in carob leaves is influenced by NaCl concentration and the presence of *Spergularia salina*. The interaction between these two factors is also significant (Table 6).

Correlation analysis

Several significant associations were revealed in the correlation analyses between the parameters examined. The correlation analysis revealed a strong significant correlation ($P < 0.05$) for electrical conductivity to sugar content ($r = 0.979$), leaf fall ($r = 0.918$), polyphenol ($r = 0.879$), and proline content ($r = 0.821$). The sodium (Na) content had a significant negative correlation ($P < 0.05$) with chlorophyll content ($r = -0.945$), relative water content ($r = -0.906$), carotenoid ($r = -0.903$), biomass ($r = -0.864$) and aerial height of the plant ($r = -0.852$).

Principal component analysis

Principal component analysis (PCA) indicated that the first two principal components, F1 (77.9%) and F2 (8.1%), accounted for variance in morphological, physiological, and biochemical responses to salt stress in carob plants with and without the halophyte plant, *Spergularia salina*. For flowering carob plants that have not grown under salt stress

Table 4. Impact of saline stress on physiological metrics

Spergularia salina	NaCl (mM/l)	Relative water content (%)	Chlorophyll content (mg/g of fresh weight)	Carotenoid content (mg/g of fresh weight)
Absence	0	82.25 ± 5.61 [a]	2.245 ± 0.066 [a]	0.725 ± 0.024 [a]
Absence	85	71.76 ± 2.14 [b]	1.936 ± 0.051 [b]	0.695 ± 0.020 [b]
Absence	171	62.10 ± 4.28 [c]	1.103 ± 0.028 [c]	0.385 ± 0.012 [c]
Presence	171	77.62 ± 2.44 [d]	1.721 ± 0.057 [d]	0.5935 ± 0.012 [d]
Absence	257	55.66 ± 1.71 [e]	0.998 ± 0.029 [e]	0.3699 ± 0.008 [e]
Presence	257	64.79 ± 3.78 [f]	1.205 ± 0.056 [f]	0.4104 ± 0.014 [f]
Source of variation	Facteur A (NaCl)	<0.001	<0.001	<0.001
	Facteur B (*Spergularia salina*)	< 0.001	< 0.001	< 0.001
	A*B	<0.05	< 0.001	< 0.001

Table 5. Effect of salt stress on the content of proline (µg/mg); polyphenol (mg/g); total sugars (µg/g) in soil according to salt treatments and in the absence and presence of *Spergularia salina* in concentrations 10 and 15 NaCl g/l

Spergularia salina	NaCl g/l	Proline	Polyphenol	Total sugar
Absence	0	36.28 ± 1.24 [d]	9.61 ± 0.48 [e]	154.44 ± 3.45 [e]
Absence	5	77.19 ± 4.74 [b]	11.70 ± 0.25 [d]	228.55 ± 4.93 [d]
Absence	10	97.27 ± 3.77 [a]	14.67 ± 0.59 [a]	483.95 ± 17.33 [c]
Presence	10	60.56 ± 2.08 [c]	12.03 ± 0.54 [c]	279.32 ± 10.79 [d]
Absence	15	105.04 ± 4.52 [a]	15.09 ± 0.52 [a]	1160.52 ± 33.06 [a]
Presence	15	83.13 ± 2.67 [b]	13.15 ± 0.57 [b]	786.56 ± 21.15 [b]
Source of variation	NaCl (A)	<0.001	<0.001	<0.001
	Spergularia salina (B)	< 0.001	< 0.001	< 0.001
	A x B	< 0.001	> 0.05	< 0.001

Table 6. Analysis of variance (ANOVA) of ion content

Ion content	Na	K	Mg	Ca
Source of variation	*p*-Value	*p*-Value	*p*-Value	*p*-Value
NaCl (A)	<0.001	<0.001	<0.001	<0.001
Spergularia salina (B)	<0.001	<0.001	<0.001	<0.001
A x B	<0.001	<0.001	<0.001	<0.05

(monoculture), they had a pronounced upward growth, larger above ground and deeper rooting systems, higher total biomass, higher relative water content, and higher amounts of photosynthetic pigments (chlorophyll and carotenoids). Carob plants with some salt stress had greater levels of soil electrical conductivity and distinctly high secretion for proline, polyphenol, and total sugars and tended towards dropping leaves along with higher levels of Na, K, Mg, and Ca in the leaves of the carob plant indicating an explicit nature of values for the effect of salt stress on growing flowering carob plants. The flowering carob plants associated with the halophyte plant, *Spergularia salina*, and grown under multiple concentrations of NaCl (C2 and C3), with slightly reduced soil electrical conductivity, appeared to have some intermediate attributes but with moderate degree of morphological, physiological and biochemical response. This response may have indicated an enhancement of the association with a halophyte plant helped alleviate some stress levels due to salt stress.

DISCUSSION

Salt in the soil presents a significant challenge for agriculture worldwide, as it greatly

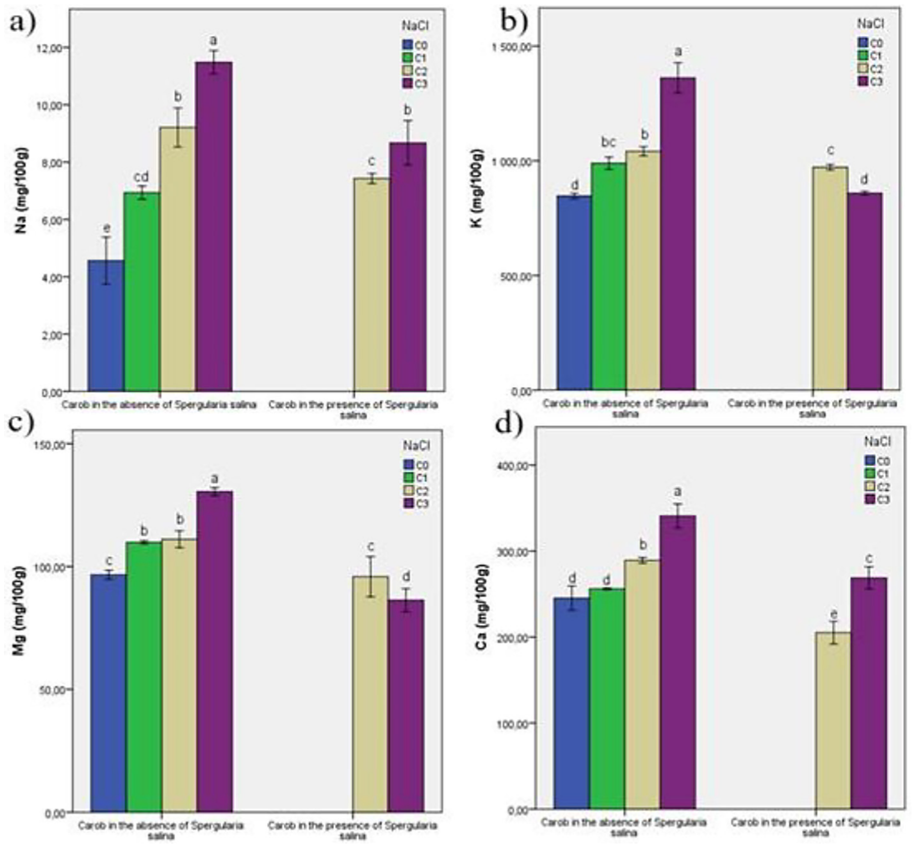

Figure 3. Consequence of salt stress in the absence and presence of halophyte on a : Na ; b : K ; c: Mg; d : Ca

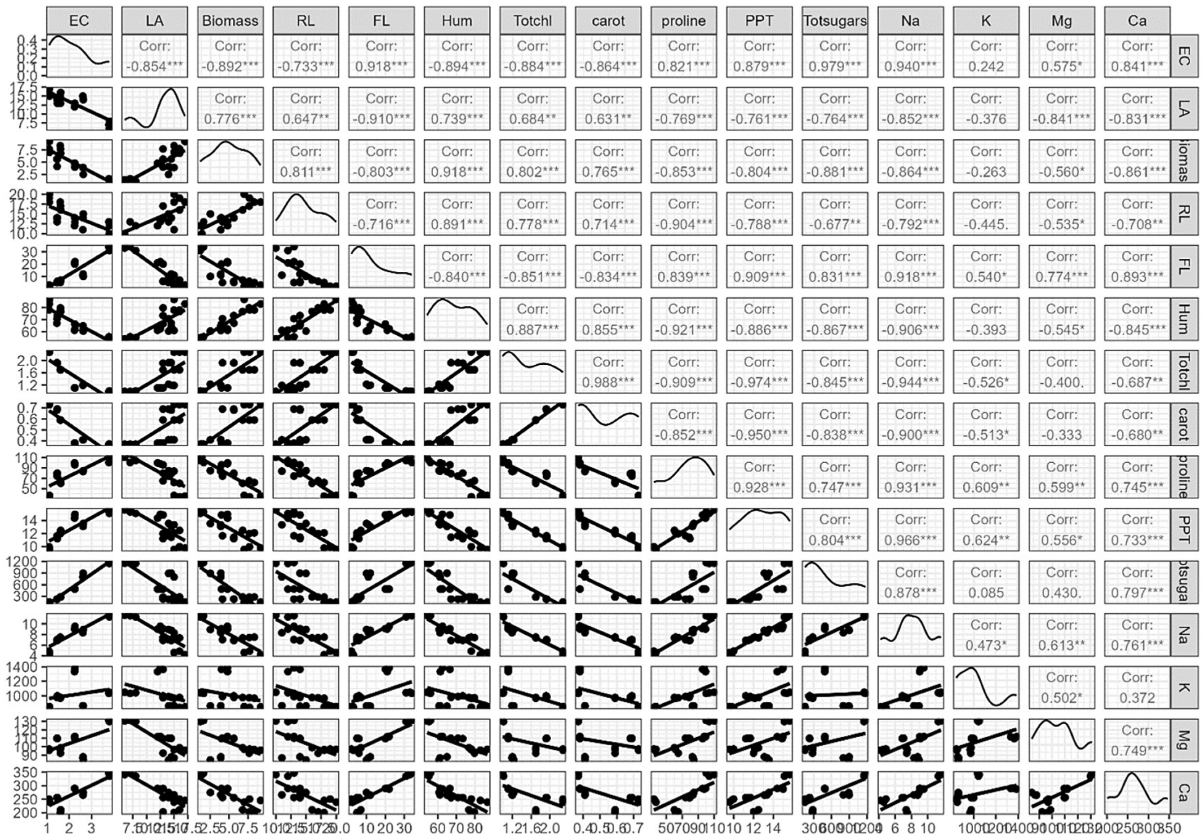

Figure 4. Pearson correlation between the parameters studied (EC, electrical conductivity; LA, length of aerial part; RL, root length; FL, leaf fall; Hum, relative water content; Totchl, chlorophyll; Carot, carotenoids; PPT, polyphenols; Na, sodium; K, potassium; Mg, magnesium; Ca, calcium.)

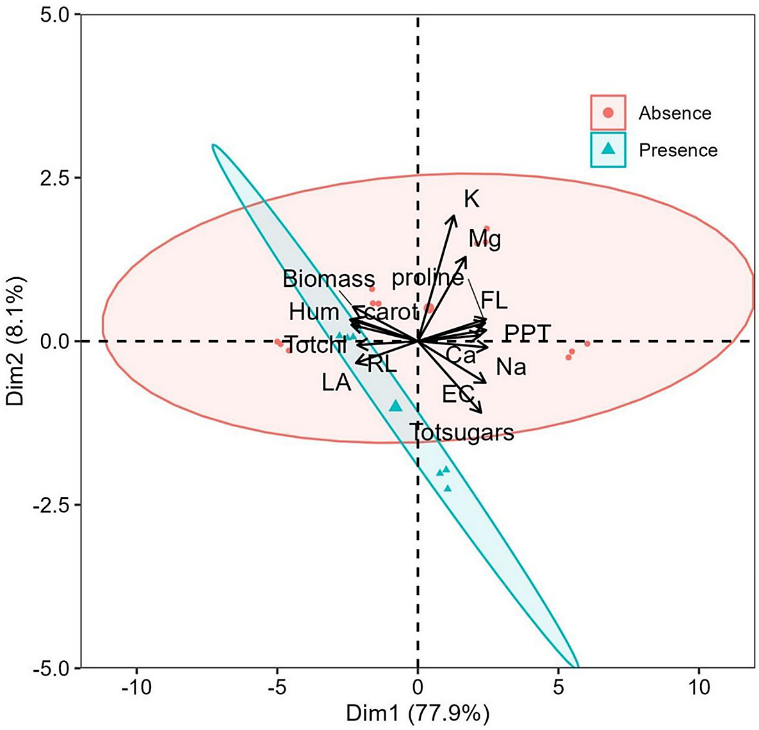

Figure 5. Principal component analysis of the variables studied (EC, electrical conductivity; LA, length of aerial part; RL, root length; FL, leaf fall; Hum, relative water content; Totchl, chlorophyll; Carot, carotenoids; PPT, polyphenols; Na, sodium; K, potassium; Mg, magnesium; Ca, calcium.)

reduces crop productivity and crop development (Liu et al., 2022). Soil salinity is defined as the amount of salt contained in water that is taken from saturated soil. If the saturation extract from soil contains greater than 12 g/l of salt, it is considered extremely saline, and if it has less than 3 g/l of salt, it is considered non-saline (Tanji, 2002). The effects of salt stress on plants have been widely studied and it is well established that this stress produces major morphological, physiological, and biochemical changes that negatively impact growth and development (Rajput et al., 2022). These changes include decreases in height, biomass, and chlorophyll and carotenoid contents (Zulfiqar et al., 2023). In addition, salt stress affects the rate of photosynthesis and promotes the accumulation of ions such as $Na+$ and $Cl-$, affecting the plant's nutritional balance (Safdar et al., 2019). Increased evapotranspiration increases the upward movement of groundwater through capillary action, to deliver salt ions to the root zone (Aljabri et al., 2021). Increased salinity will affect plants' photosynthesis, especially because of reduced water availability in saline soil, as well as the toxic effects of the salt, particularly excess sodium ($Na+$) and chloride ($Cl-$) in plant tissues (Xiao and Zhou, 2022). For that reason, plants must develop numerous defense mechanisms for growth and survival in saline conditions, including morphological, biochemical, and physiological modifications (Chang et al., 2019).

Plants use several defenses and responses to saline stress. These include morphological changes like reduced leaf surface area to reduce transpiration loss, and the lateral and/or deep root systems to take advantage of soils with potentially less salinity and better access to nutrients and water (Zeeshan et al., 2020; Hasanuzzaman et al., 2021; Mehta and Vyas 2023). Certain metabolites like proline act mostly as osmoprotectants to regulate osmotic balance and maintain the integrity of the cell membrane; and total sugars can also accumulate in solution to stabilize proteins and membranes, for salt tolerance (Acosta-Motos et al., 2017). Additionally, plants use non-enzymatic defense systems, including the accumulation of polyphenols, which act as antioxidants to protect their cells from oxidative damage from saline stress (Zulfiqar et al., 2020). The occurrence of salt stress has been associated with decreased growth of above and below ground parts of the carob plants and diminished levels of chlorophyll, whereas carob plants have mechanisms for survival and growth in response to such conditions. For example, carob plants use strategies that include accumulation of proline and soluble sugars and retention of more potassium (K^+) in their leaves. The higher concentration of potassium is needed to retain cations and to retain the calcium (Ca_2^+) that is already present in the plant under salt stress, allowing carob plants to survive in saline environments (Correia et al., 2010). Considering these issues, as the main focus is to develop creative solutions to limit or reduce salt stress on crops, there may be a possibility to utilize halophytes or salt suitable plants which may provide some sustainable bioremediation option for saline soil. In fact, salt-tolerating plants would be helpful to not just remove salts in the soil, but would also allow reduction of salinity levels for typically cultivated crops. Most crops are glycophytes, meaning that they grow to produce food normally in a saline free environment, and salted environments induce salt stress on glycophytes that negatively hinder their production. Halophytic plants exhibit specific strategies to allow them to cope with salty environments (Nikalje et al., 2018; Muchate et al., 2018; Barcia-Piedras et al., 2019). Halophytes have a higher tolerance to high saline levels and are more likely to perform better in those conditions (Williams, 1960). Still, the amount of salt that regulate optimal growth within halophytes varies drastically between plant species (Guo et al., 2021). Recent research studies have shown that Spergularia maritima, a halophyte, had a significantly increased impact on the performance of the glycophyte plant Salvia officinalis being grown in salinity (El-Khadir et al., 2024).

Our findings confirm that the association with *Spergularia salina* contributes to improving carob plant resilience under salt stress. This improvement occurs through two complementary mechanisms: a reduction in soil salinity levels, as indicated by lower electrical conductivity, and a stabilization of key physiological and biochemical indicators. These results demonstrate the potential role of *Spergularia salina* as a natural ally for enhancing crop performance in saline environments. This suggests that promoting *Spergularia salina* can be a useful technique to remediate saline soils were salinity constraints soil quality. Further, our findings indicate that *Spergularia salina* presence when cultivating carob has a corollary where this effect seems to reduce the level of salinity in the soil. This suggests that *Spergularia salina* may have a beneficial effect in ameliorating the negative impacts of salt stress in creating a more beneficial environment for carob. These potential strategies for effective salt stress amelioration in parts of the world, where salinity negatively impacts agricultural production, are potential opportunities to use halophytes that ameliorate salt and soil (Simpson et al., 2018). Good prospects for studying desalination of salt-affected soils are provided by succulent halophytes that can accumulate high amounts of sodium in their aerial organs (Pilon-Smits, 2005). These phytoremediation species are effective in ameliorating saline-alkaline soils. The fact that they can redistribute salts in the soil profile due to their desalination potential can mitigate salinization and improve soil productivity, leading toward long-term recovery of saline soils (Wang et al., 2023). This study is of great importance because it targeted young carob plants, which is a pertinent crop in arid regions where agricultural productivity is limited by salinity. Through the agronomic innovation of it growing carob plants with the halophytes of *Spergularia salina*, it is possible to provide another solution toward managing soil salinity while enhancing productivity. This study's practical recommendations and possible solutions toward minimizing salinity effects on productivity will contribute by providing concise instructions for farmer towards agricultural sustainability and productivity.

CONCLUSION

In conclusion, this study demonstrated that the association with a halophytic plant *Spergularia salina* can significantly mitigate the detrimental effects of salt stress on carob plants by enhancing their growth parameters through the provision of a favorable environment for growth. The results of this study may be applied to elaborate strategies for managing saline soils and crops in modified environments. For example, the introduction of halophytic plants into production systems to enhance crop success in saline soils could be just one way of expanding opportunities for a more resilient agriculture in the face of increasingly complex challenges presented by soil salinity and climate change.

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