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ORIGINAL ARTICLE

Salinity modulates morpho-physiology, biochemical and antioxidant defence system in *Tetragonia decumbens* Mill.: a neglected wild leafy vegetable in South Africa

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Abstract

Tetragonia decumbens is an edible halophyte that grows naturally in saline environment; however, its tolerance mechanisms are poorly understood for bio-saline agriculture. So, this research was designed to look into how salinity affects vegetative growth, leaf succulence, chlorophyll content, cation accumulation, oxidative stress indicators, and antioxidative defence mechanisms involved in the salt tolerance of *T. decumbens*. Saline conditions were prepared by dissolving sodium chlorine (NaCl) in the nutritive solution. The control was maintained and only watered with nutrient solution while the tested treatments contained graded NaCl doses (250, 200, 150, 100, and 50 mM). Results revealed a substantial enhancement in shoot length, number of branches, relative water content, as well as total fresh weight in plants irrigated with 50 and 100 mM NaCl in comparison to the control, while higher saline concentrations (150–250 mM NaCl) reduced plant growth and chlorophyll content. Similarly, these high salt concentrations induced more severe oxidative stress indicated by high amounts of superoxide, cell death viability and malondialdehyde, with the most pronounced effect at the highest NaCl concentration (250 mM). Nevertheless, *T. decumbens* modulated various defence mechanisms with increasing salinity stress, these include the upregulation of superoxide dismutase, catalase, polyphenols, flavonoids, proanthocyanidins and the build-up of sodium ions in the leaves. These results show that *T. decumbens* can withstand salinity by modifying its morpho-physiological traits, antioxidant defence systems, and managing ion toxicity and oxidative stress efficiently, since all plants withstand salinity without showing signs of toxicity.

Keywords Antioxidant defence systems · Dune spinach · Edible halophytes · Salinity tolerance · Underexploited vegetables

Introduction

Soil salinity has been reported as one of the contributing drivers of devastating crop production losses around the globe (Manuel et al., 2017; Rehman et al., 2023). Although precise measurements are unattainable, the extent of soil infected by salinity is increasing and is particularly evident in irrigated soils (Zhang et al., 2022). According to estimates, salinity affects about 10% of the world's land mass and around half of all irrigated land (Boamah et al., 2023; Medini et al., 2023). This has been attributed to the overuse of low-quality water, and extensive irrigation coupled with intensive farming and inadequate drainage systems resulting in the built-up of soluble salt ions in the soil (Hassani et al., 2020). These salt ions impede the roots' ability to absorb water, leading to osmotic stress and nutritional imbalances that impair vital physiological functions like photosynthetic activity, tissue hydration, and the initiation of oxidative stress and membrane damage, resulting in reduced plant biomass (Hussain et al., 2023; Mahmood et al., 2021). Moreover, soil salinity and the increasing global climate change will undoubtedly deteriorate food production in the coming years (Mangal et al., 2022; Zuluaga et al., 2023).

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Thus, alternative use of resilient crops is crucial to fulfil the increasing food demand in regions impacted by both salinity and drought. This will then provide needed calories, proteins, fats, and nutrients for inhabitants in these areas (Rasheed et al., 2020). Nevertheless, such species are still underutilised particularly in emerging countries where a 90% increase in population growth predicts that food insecurity may soon become a major concern (Ogunniyi et al., 2020). The underutilisation of these salt-tolerant species is due to limited research on their nutritional composition, growing protocols, as well little urgency shown by plant biologist and crop scientist (Salami et al., 2022). Interestingly, these underutilised potential crops are probably present among edible halophytes, which have been declared neglected in most African countries including South Africa (Mokganya & Tshisikhawe, 2019). These species can tolerate salinity and drought and some even require sodium chloride (NaCl) for optimum growth.

Halophytic species use different metabolic and physiological adaptations to prevent oxidative mutilation, nutritional imbalance, and osmotic stress (Z. Gul et al., 2022; Zhao et al., 2020). Some species increase their roots/ shoot biomass to regulate sodium (Na^+) entry to the xylem or minimize its transport to the shoots, thus minimizing ion toxicity (Parvez et al., 2020). During this process, active nutrients such as potassium, magnesium and calcium protect photosynthetic pigments that enable photosynthetic efficiency throughout the process (Ma et al., 2022). Nevertheless, ion toxicity can still occur when ion homeostasis is disrupted, which in turn affects the photosynthetic process by destroying or altering the biosynthesis of light-trapping pigments, thus reducing plant growth (Azeem et al., 2023).

Excessive build-up of Na^+ and chlorine (Cl^-) within plant cells induce oxidative stress by producing superoxide radicals via the Mehler reaction (Golldack et al., 2014; González-Orenga et al., 2021a, 2021b; Rodrigues de Queiroz et al., 2023). These free radicals disrupt the normal metabolic processes in cytoplasm, peroxisomes, and mitochondria by causing oxidative mutilation to proteins, lipids, and other essential biomolecules, resulting in acute impairment and ultimately, cell death (Flowers & Colmer, 2015; Saleem et al., 2022). To prevent the catastrophic effect of oxidative stress, plants have established defence mechanisms such as antioxidative enzymes followed by the synthesis of non-enzymatic metabolites, which scavenge the excessive reactive oxygen species (ROS). Of these several antioxidative enzymes, Superoxide Dismutase (SOD) is thought to constitute primary defence tool against oxidative stress in plants (Gill et al., 2015; Zulfiqar & Ashraf, 2022). This enzyme incites the dismutation of superoxide radicals into H_2O_2 and O_2. Subsequently, several antioxidant enzymes, such as Catalase (CAT) and Peroxidase (POX), convert H_2O_2 to water. It has been reported in the literature that halophytic plants tend to accumulate high amount of SOD under salt stress, a feature that provides an adaptation advantage over salt-sensitive species (glycophytes) (Bose et al., 2014; Huang et al., 2019; Zhao et al., 2020). Moreover, halophytes also activate the manufacturing of non-enzymatic compounds such as phenols, flavonoids, anthocyanins, and tannins to scavenge these highly toxic species when excessive highly toxic ROS are not eliminated by the antioxidative enzymes (Azeem et al., 2023). These bioactive electron-donor compounds inhibit clusters of oxidative reactions and safeguard membranes and macromolecules from damage, thus making edible halophytes good sources of nutritional antioxidants with various benefits for overall health and illness prevention.

Tetragonia decumbens Mill (Aizoaceae), also referred to as 'dune spinach' is an underutilised leafy vegetable that is mainly dispersed in coastal areas of South Africa (Klak et al., 2017). The salty leaves of this species can be eaten raw in green salads and can also be cooked like spinach and added to soups and stews (Sogoni et al., 2021; Tembo-Phiri, 2019). Earlier reports have shown that this species can withstand salinity stress (Sogoni et al., 2021). To cope with these conditions, *T. decumbens* was reported to have unchanged chlorophyll content and increased ferric reducing antioxidant power under low to moderate salinity (50–100 mM). While the highest salinity (200 mM) increased the phenolic content, nitrogen, phosphorus, and sodium. Nevertheless, in dept studies illuminating the molecular processes and physiological mechanisms responsible for the tolerance of this plant to salt levels are limited, especially on the morpho-physiological features (leaf succulency, relative water content, photosynthetic pigments), oxidative stress markers (MDA, Superoxide radicals and etc.), antioxidant defence systems (superoxide dismutase, Catalase, polyphenols, flavonoids) and managing of ion toxicity. These adaptation mechanisms have been documented in numerous laboratory experiments on halophytic plants cultivated under saline conditions to better understand their salt defence mechanism (Hussain et al., 2015, 2023; Peng et al., 2019). So, this study was conceived to assess the effects of salinity on vegetative growth, leaf pigmentation, leaf succulence, cation build-up, oxidative stress indicators, and antioxidant defence systems, to better elucidate the salt tolerance traits in *T. decumbens* for bio-saline agriculture.

Materials and methods

Growth conditions and experimental location

The study was executed in the research greenhouse of the Cape Peninsula University of Technology. The greenhouse was configured to have daytime temperatures of 21–26 °C and nighttime temperatures of 12–17 °C, with 60% relative

humidity. The photosynthetic photon flux density ranged from 400–600 µmol/m2/s during the experimental period.

Preparation of plant material and saline conditions

T. decumbens plants were generated from cuttings following the propagation method described by Sogoni et al. (2022). Homogeneous established plants (180) were transplanted in black plastic pots (12.5 cm) filled with a combination of peat and sand (1:1) and stationed in the hardening off area to acclimatize. The plants were then watered with a full Nutrifeed™ solution produced by STARKE AYRES Pty. Ltd. South Africa. After sixteen days of plant development, plants were irrigated with reverse osmosis water for six days to eliminate any salt residue before being subjected to saline treatments, each containing ten replicates, and the study was repeated three times. Saline conditions were established on six treatments by elevating the concentration of NaCl in the nutritive solution (0, 50, 100, 150, 200, and 250 mM) as described by Sogoni et al. (2021). Each plant received 300 millilitres of nutrient solution with graded NaCl and were irrigated every two days. The control plants were only irrigated with nutritive solution without salinity. To verify that the right concentration of salinity in each pot was sustained, drain water from each pot was collected and the electrical conductivity was measured. After four months of salt treatment, plant material was harvested, and used for further analysis.

Plant growth evaluation

Shoot increment and the quantity of lateral branches

Shoot increment and the quantity of branches were employed as factors to evaluate growth development. Branch numbers were counted manually while shoot increment was determined weekly with a metre tape placed from the surface of the growth media to the shoot tip.

Plant weight

After four months of saline treatment, plants were thoroughly watered to loosen the soil and harvested cautiously to prevent damage. The harvested plants were rinsed multiple times with RO water and dried using tissue paper. Thereafter the shoots and roots were split with a sterile secateur, and the fresh samples were weighed using a RADWAG® laboratory scale. The fresh material was then air dried in a LABTECH™ oven at 56 °C for 50 h, and the dry weight was determined.

Leaf Hydration

Relative water content (RWC)

To evaluate variability in leaf RWC, three leaves were excised from three distinct plants in each of the six treatments and weighed (FW). Thereafter, were submerged in distilled water and left in the dark for 4 h and the turgid weight (TW) was established. Lastly, the leaves were air dried in an oven at 56 °C for 24 h to acquire the dry weight (DW). The percentage of RWC was computed using the approach provided by (Bistgani et al., 2019) with the equation shown below:

$$\mathrm{RWC}\% = \frac{(\mathrm{FW} - \mathrm{DW})}{(\mathrm{TW} - \mathrm{DW})} \times 100$$

Leaf succulence

Leaf succulence was calculated following Mantovani's (2011) methodology, using the below equation:

$$\mathrm{LS} = \frac{(\mathrm{LFW} - \mathrm{LDW})}{(\mathrm{LA})}$$

,

Where LS is the leaf succulence (mg H_2O cm^2); LFW is the leaf fresh weight (mg); LDW is the leaf dry weight (mg); and LA is the leaf area (cm^2). A portable AM350 leaf area meter manufactured by Bio-scientific limited, London, United Kingdom was used to measure the leaf area.

Photosynthetic pigment

Chlorophyll *a* and chlorophyll *b* were estimated using spectrophotometric procedures as stated by Wang et al. (2023). Concisely, 100 mg of fine grounded fresh leaves was extracted with 5 ml of 99.5% dimethyl sulfoxide (DMSO; Sigma). The determination of the photosynthetic pigment was conducted using the absorbance of the supernatants at 649 and 665 nm, as described in the work of Lichtenthaler and Wellburn, (1983).

Quantification of cations

The concentrations of Sodium, Calcium and Potassium were determined in leaves and roots following the method of Bulawa et al. (2022).

Determination of oxidative stress markers

Cell viability

The cell viability analysis in dune spinach leaves was assessed following the method described by Egbichi et al.

(2014) with slight adjustment. Three leaf sections of 1 cm^2 were excised from three separate plants from each of the six treatments. The removed leaf sections were then stained for 30 min at room temperature in Eppendorf tubes filled with one millilitre of 0.25% (w/v) Evans Blue solution. After washing the leaf samples for 30 min in distilled water, the Evans Blue stain absorbed by dead leaf cells in the leaf tissue was extracted with one % (w/v) sodium dodecyl sulphate (SDS) after 1 h incubation at 55 °C. The level of Evans Blue taken up by the leaf tissue was evaluated by determining the absorbance of the extract at 600 nm with a spectrophotometer.

Superoxide radical and malondialdehyde (MDA)

The superoxide radical content in dune spinach leaves was measured following the technique reported by Gokul et al. (2016) and the extinction coefficient of 12.8 mM cm^{-1} was used to calculate the superoxide radical levels in the leaves. To quantify the MDA content within the tested samples, the procedure explained by González-Orenga et al. (2021b) was used.

Activity of antioxidant enzymes

The method outlined by Gill et al. (2015) was followed to extract protein from the leaf samples. The protein concentration in the extracts was measured using the Bio-Rad reagent and bovine serum albumin (BSA) as the standard. Spectrophotometric experiments were used to assess the activity of the selected antioxidant enzymes in protein extracts.

The activity of superoxide dismutase was determined at 560 nm spectrophotometrically by measuring the suppression and photoreduction of nitro blue tetrazolium (NBT); the reaction solution contained riboflavin as a determinant of superoxide radicals. A unit SOD is labelled as the quantity of enzyme required to block NBT photoreduction by 50% in experimental conditions as outlined in the methodology of Brenes et al. (2020).

Catalase (CAT) activity was measured by observing a decrease in absorbance at 240 nm following the consumption of H_2O_2 added to the extracts (Baureder et al., 2014). One CAT unit was defined as the quantity of enzyme degrading one mmol of H_2O_2 at 25 °C per minute.

Phytochemicals and antioxidant assays

Crude extraction

Tetragonia decumbens leaves were harvested and oven-dried (40 °C) for 7 days. A Junkel & Kunkel type A 10 mill was used to grind the dried plant material into a fine powder. Thereafter, two grams was mixed with 80% ethanol (50 mL) for an hour for crude extraction. The supernatants were subsequently used for all analyses after being centrifuged at 4000 rpm for 5 min.

Total phenol, flavonoid and proanthocyanidins (condensed tannins)

The total phenolic content was assessed using the Folin–Ciocalteu technique, as depicted by Ingarfield et al. (2023) and the flavonoid content was determined using the aluminium chloride spectrophotometric test reported by Jimoh et al. (2019). The Condensed tannins was assessed following the procedure reported by Jimoh et al. (2023).

DPPH

The antioxidant activity of the extract was measured using a slightly modified version of the DPPH free radical scavenging assay as reported by Jimoh et al. (2020). A 0.135 mM DPPH solution was made in a dark bottle using ethanol. The DPPH solution was reacted with 0.2 mg mL^{-1} of the extracts in equal volumes. Equal amount of 0.135 mM DPPH and the solvent were used to make the control solution. The resulting mixture was violently agitated before being incubated at room temperature for 30 min after which the absorbance was measured at 517 nm on a spectrophotometer. The equation below was used to calculate the scavenging activity of the plant extract.

$$\%\text{Scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}$$

Statistical analysis

The experimental data was analysed using STATISTICA, version 13.5.0.17. A one-way ANOVA was conducted to determine substantial differences between treatments, followed by Fisher's (LSD) test at $p \leq 0.05$. The Shapiro–Wilk test was conducted before the analysis of variance to verify the validity of normality, while Levene's test was employed to evaluate the homogeneity of variance. The results gathered were expressed as mean values and standard error.

Table 1 Growth parameters of *T. decumbens* to salinity at the end of treatments

Treatments	SLI (cm)	NB (n)	FWL (g)	FWR (g)	DWL (g)	DWR (g)	TFW (g)	TDW (g)
Control	92.25 ± 2.4 ^{a}	3 ± 0.14 ^{c}	91.51 ± 6 ^{c}	28.44 ± 2.06 ^{a}	20.12 ± 0.65 ^{c}	16.7 ± 0.9 ^{a}	119.9 ± 6.1 ^{c}	36.8 ± 1.1 ^{a}
50 mM	93.46 ± 2.4 ^{a}	4 ± 0.15 ^{a}	153.7 ± 7 ^{a}	28.42 ± 1.42 ^{a}	23.8 ± 0.70 ^{ab}	15.9 ± 0.8 ^{ab}	182.13 ± 7.6 ^{a}	39.7 ± 1.1 ^{a}
100 mM	83.3 ± 1.4 ^{b}	3.5 ± 0.2 ^{b}	140.13 ± 4.8 ^{ab}	23.39 ± 1.18 ^{bc}	21.6 ± 0.71 ^{c}	14.5 ± 0.45 ^{bc}	164.52 ± 5.2 ^{ab}	36.2 ± 0.8 ^{a}
150 mM	76.13 ± 2.3 ^{c}	3 ± 0.18 ^{c}	134.41 ± 7 ^{b}	23.78 ± 0.97 ^{bc}	22.01 ± 0.44 ^{bc}	14.3 ± 0.36 ^{bc}	158.2 ± 6.8 ^{b}	36.3 ± 0.6 ^{a}
200 mM	74.83 ± 1.7 ^{c}	2.8 ± 0.14 ^{c}	133.76 ± 5.2 ^{b}	21.96 ± 1.22 ^{c}	24.76 ± 1.6 ^{a}	13.7 ± 0.37 ^{c}	155.7 ± 5.6 ^{b}	38.5 ± 1.7 ^{a}
250 mM	75.67 ± 1.9 ^{c}	2.9 ± 0.14 ^{c}	109.28 ± 6.6 ^{c}	20.71 ± 1.81 ^{c}	21.01 ± 0.85 ^{c}	13.35 ± 0.7 ^{c}	129.9 ± 6.5 ^{c}	36.3 ± 1.1 ^{a}
F-statistic	15.96*	8.70*	13.77*	3.16*	3.7*	2.73*	12.05*	1.59 ns

The F-statistic asterisks and different letters show that the tested treatments were significantly different at $p \leq 0.05$

SLI Shoot length increment; *NB* Number of branches; *FWL* Fresh weight of leaves; *DWL* Dry weight of leaves; *FWR* Fresh weight of roots; *DWR* Dry weight of roots; *TFW* Total fresh weight; *TDW* Total dry weight

Results

Plant growth response

The growth responses of *T. decumbens* under NaCl concentrations varied among treatments as shown in Table 1. Increment in shoot length and branch number were notably influenced by NaCl, with longer shoots and more branches obtained in plants irrigated with the lowest dosage of salinity (50 mM). The same pattern was also noted in fresh weight of leaves, with plants receiving 50 mM of NaCl irrigation reporting the highest weight. Conversely, the highest dry weight of leaves was noted in plants receiving 200 mM of NaCl irrigation, but this was comparable with those irrigated with 50 mM NaCl. When assessing the roots fresh and dry weights, the control plants had the highest weights. Nevertheless, the total fresh weight was positively influenced by saline treatments with plants receiving 50 mM irrigation registering the highest weight. While there was no discernible variation in total dry weight between the treatments.

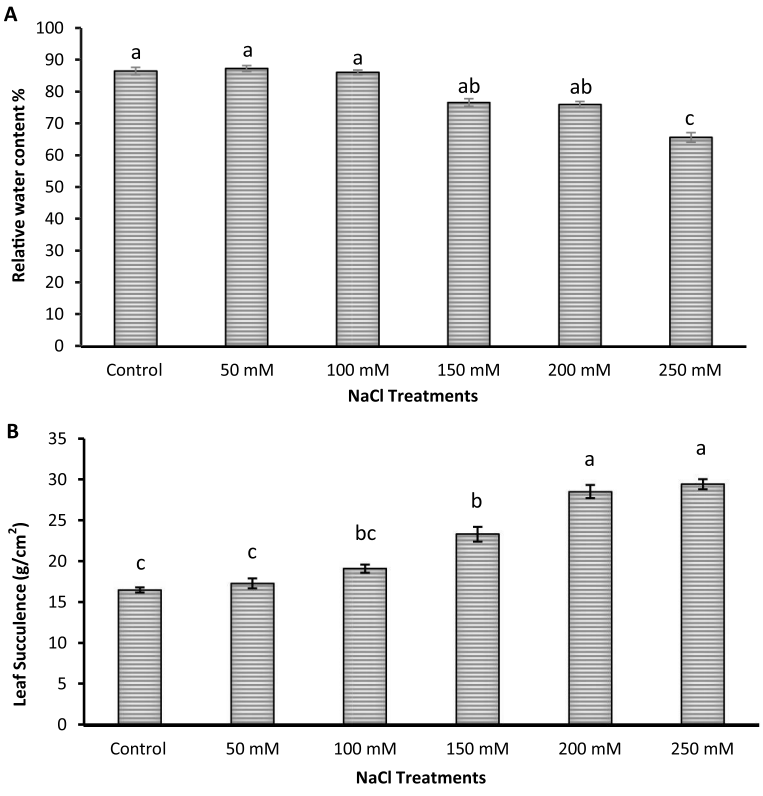

Fig. 1 Relative water content (**A**) and Leaf succulency (**B**) of dune spinach leaves in response to salinity. Means with distinct letters vary substantially from one another ($p \leq 0.05$)

Leaf hydration

Relative water content (RWC) and leaf succulence

Leaf RWC and leaf succulence were notably influenced by NaCl irrigation as shown in Fig. 1. A constant RWC was noted in plants irrigated with 0–100 mM NaCl, then a decline was detected in plants receiving 150–250 mM of NaCl irrigation respectively. Conversely, leaf succulence increased with increasing doses of NaCl. The maximum leaf succulence was noted in plants subjected to 200 and 250 mM of NaCl irrigation and these mean values were greater than most treatments including the control.

Photosynthetic pigment

The effects of NaCl irrigation on chlorophyll content of dune spinach leaves are reported in Fig. 2. Results showed that chlorophyll *a* and *b* were significantly affected and remarkably reduced with elevated NaCl concentrations of 100 up to 250 mM compared with the control. While plants treated with low NaCl concentration (50 mM) had a comparable chlorophyll content with control plants.

Quantification of cations

High amounts of Na^+ were recorded with increasing NaCl irrigation in leaves and roots as indicated in Fig. 3. The highest Na^+ concentration was noted in plants receiving 250 mM of NaCl irrigation and the highest values were obtained in leaves than in roots. All values obtained from other treatments were notably lower than these values. On the contrary, the concentration of K^+ and Ca^{2+} drastically decreased in plants with increasing NaCl irrigation (Fig. 3). The control treatments yielded the maximum values of K^+ and Ca^{2+} in both roots and leaves which were significantly higher than the values obtained in other treatments except the roots values of plants irrigated with 50 mM NaCl which were comparable to the control roots.

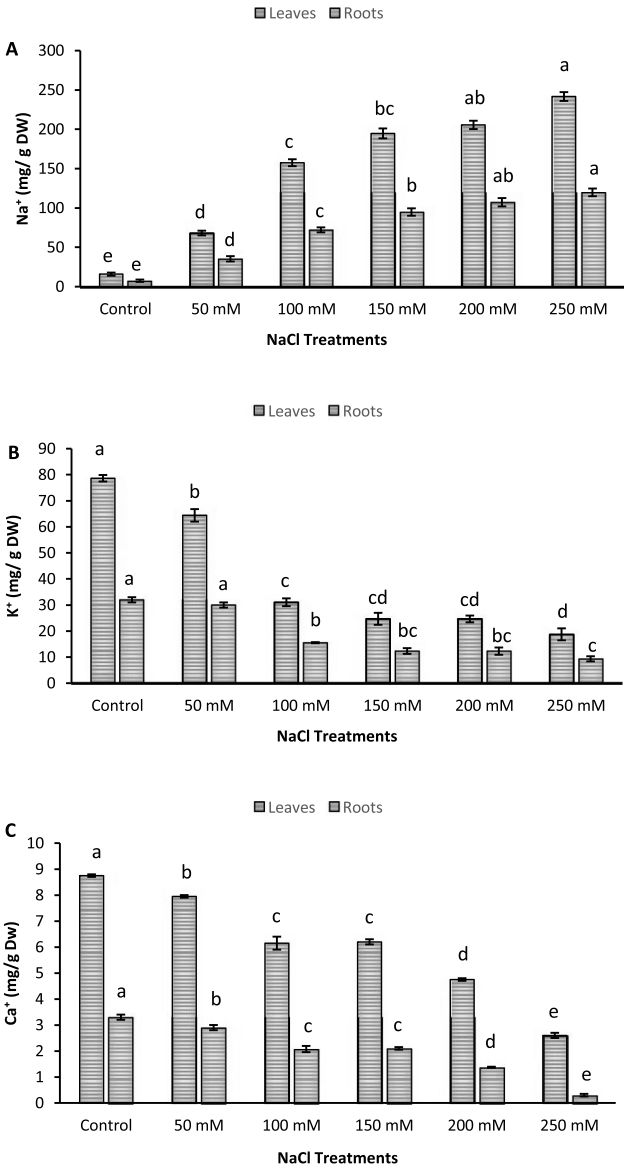

Fig. 3 Sodium (**A**), Potassium (**B**) and Calcium (**C**) of dune spinach leaves and roots in response to salinity. Means with distinct letters vary substantially from one another ($p \leq 0.05$)

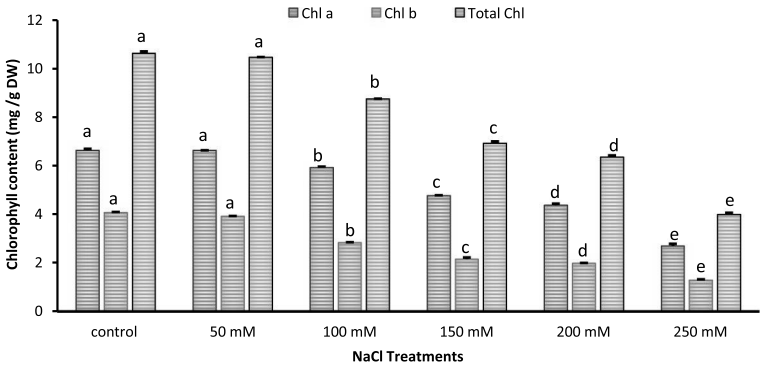

Fig. 2 Chlorophyll content of dune spinach leaves in response to salinity. Means with distinct letters vary substantially from one another ($p \leq 0.05$)

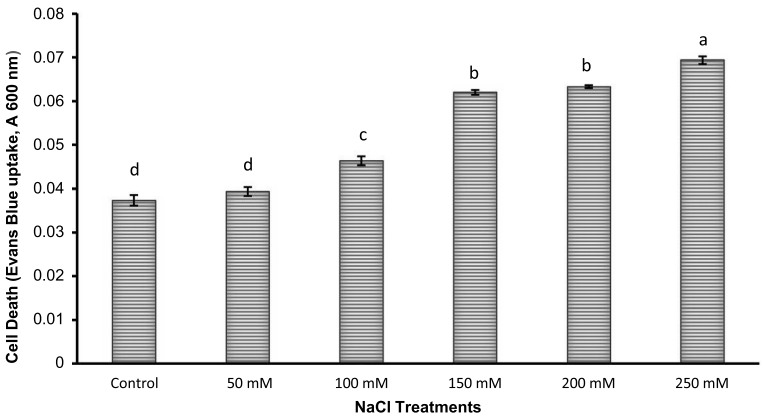

Fig. 4 Cell viability of dune spinach leaves in response to salinity. Means with distinct letters vary substantially from one another ($p \leq 0.05$)

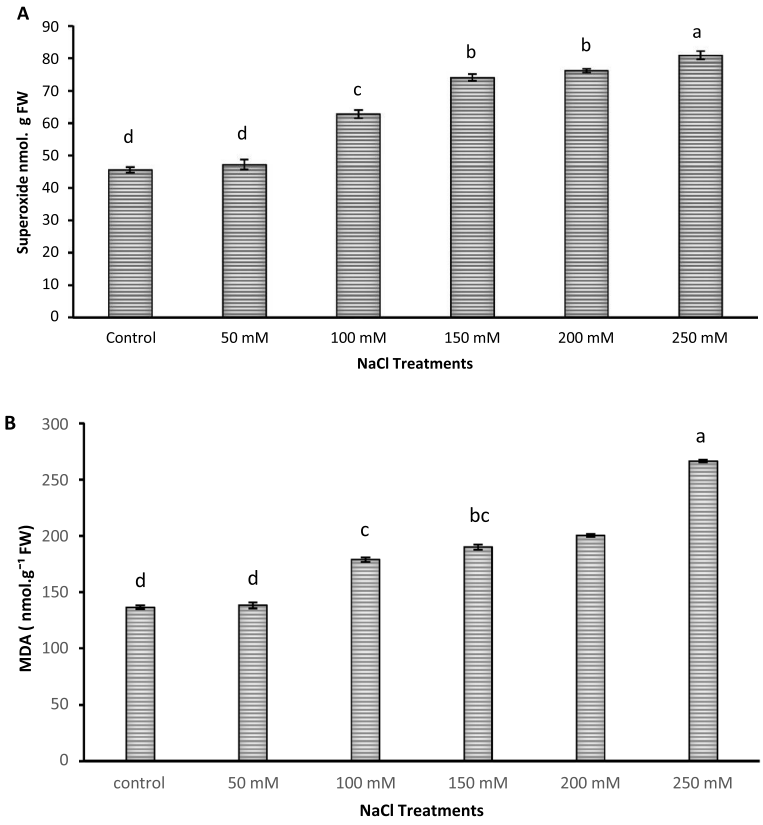

Fig. 5 Superoxide (**A**) and MDA (**B**) of dune spinach leaves in response to salinity. Means with distinct letters vary substantially from one another ($p \leq 0.05$)

Oxidative stress markers

Cell viability, superoxide and malondialdehyde (MDA)

The leaf cell death and relative accumulation patterns of superoxide and MDA in response to salt stress were similar (Figs. 4 and 5). Increasing NaCl treatment caused leaf cell death, with pronounced effect at highest NaCl concentration (250 mM) (Fig. 4). This treatment had a significantly higher cell death than all other treatments. The same pattern was also noted for superoxide and MDA accumulation in the leaves of dune spinach, where 250 mM resulted in significantly higher oxidative stress than other treatments (Fig. 5). However, it must be noted that plants watered with the lowest NaCl (50 mM) had the same leaf cell death, superoxide, and MDA content as the control plants.

Activity of antioxidant enzymes

Superoxide dismutase and catalase

A substantial increase in catalase and superoxide dismutase activity with elevated NaCl concentrations was observed as illustrated in Fig. 6. The highest activities of both enzymes were noted in plants subjected to 250 mM of NaCl irrigation.

Phytochemicals and antioxidant activity

Total phenolic content (TPC)

The phenolic content in dune spinach leaves varied significantly among treatments as presented in Table 2. The highest phenolic content was noted in plants irrigated with 250 mM NaCl and this was considerably higher than the values reported in control, 50, 100 and 150 mM NaCl.

Total flavonoid content (TFC)

Increasing NaCl concentrations optimised the flavonoids in the leaves of dune spinach. Plants irrigated with NaCl at 250 mM had substantially higher flavonoids than other treatments and the control (Table 2). The lowest flavonoid content was obtained in control and was equivalent with values reported for plants irrigated with 50 mM of NaCl.

Total proanthocyanidins (TPD)

Treatments such as the control, 50, 100 and 150 mM of NaCl negatively affected the accumulation of proanthocyanidins since all these treatments had negative values, implying proanthocyanidins were not detected (Table 2). However, proanthocyanidin content was detected in plants subjected to 200 and 250 mM of NaCl irrigation and the recorded values were comparable.

DPPH

When assessing the DPPH scavenging activity within samples, plants irrigated with NaCl had significantly lower scavenging activity in comparison to the control and as such, the highest scavenging activity was noted in control plants.

Discussion

Some plants dedicate substantial energy to multiple metabolic pathways to successfully navigate and mitigate the impact of extreme environmental challenges, such as salinity. *Tetragonia decumbens* has been reported to with stands both drought and salinity (Nkcukankcuka et al., 2021; Sogoni et al., 2021; Tembo-Phiri, 2019), however, its tolerance mechanisms and physiological responses are poorly understood. It therefore became imperative to research the impacts of salinity on growth development, leaf succulence, chlorophyll, cation concentration, oxidative stress markers, and antioxidant defence systems to elucidate its tolerance mechanisms. Furthermore, the antioxidant activity of salt-stressed *T. decumbens* and its therapeutic potential was assessed to obtain insight into the major processes conferring tolerance to salinity in this understudied plant.

Increasing salinity induced growth inhibition

Salinity is known to disrupts respiration, photosynthesis, and mineral uptake, resulting in decreased crop production and quality (Farooq et al., 2022; Rhaman et al., 2024). Nonetheless, halophytic species have been documented to complete their life cycles in saline environments ranging from mild to extreme salinity through osmotic adjustment (Zhao et al., 2020). In the current study, saline treatment enhanced growth development at 50 and 100 mM NaCl, while at higher salinity (200 and 250 mM NaCl), growth parameters reduced substantially in comparison to the control. However, it should be noted that even at these high salt concentrations (200 and 250 mM NaCl), plants stayed alive and showed no signs of toxicity such as chlorosis and leaf necrosis. Growth stimulation at low to moderate salinities have been reported in *T. decumbens* by Sogoni et al. (2021) and in close relative species *Tetragonia tetragoniodes* by Atzori et al. (2020) and Jeeva (2020). These findings suggest that *T. decumbens* can be regarded as a facultative halophyte due to its ability to grow in moderate saline conditions.

Leaf hydration response to salinity

Relative water content (RWC) is an appropriate indicator of plant water level in various environmental conditions such as drought and salinity (Khatami et al., 2022). In this study, the leaf RWC remained constant from 0 to 100 mM of NaCl, then declined drastically at higher salinities (150, 200, and 250 mM). The capacity to maintain unaltered leaf RWC under mild salinity (0–100 mM of NaCl) shows that *T. decumbens* evolved suitable mechanisms to assure adequate water intake in saline soils. Ben Amor et al. (2020) earlier

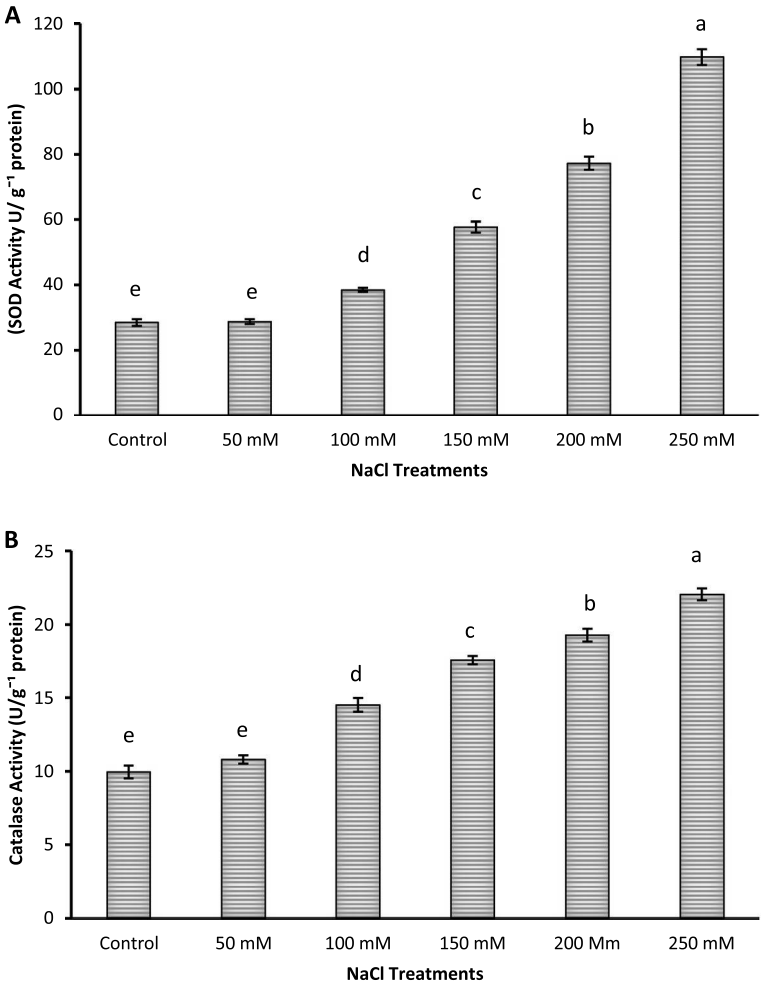

Fig. 6 Superoxide dismutase (**A**) and Catalase (**B**) of dune spinach leaves in response to salinity. Means with distinct letters vary substantially from one another ($p \leq 0.05$)

Table 2 Quantification of phytochemicals and DPPH antioxidants in leaves of *T. decumbens*

Treatments	TPC (mg GAE/g DW^{-1})	TFC (mg GE/g DW^{-1})	TPD (mg CE/g DW^{-1})	DPPH activity (µmol TE/g)
Control	74.66 ± 6.5^{d}	329.8 ± 9.4^{e}	-13.9 ± 2.6^{c}	72.5 ± 1.3^{a}
50 mM	89.32 ± 4.2^{cd}	339.9 ± 7.3^{e}	-17.7 ± 2.5^{c}	62.8 ± 0.53^{bc}
100 mM	102.09 ± 9.7^{bc}	447.1 ± 6.2^{d}	-15.7 ± 1.3^{c}	64.3 ± 0.25^{b}
150 mM	114.42 ± 2^{ab}	581.1 ± 6.2^{c}	-3.1 ± 0.9^{b}	61.2 ± 0.13^{c}
200 mM	125.12 ± 8.5^{a}	639.7 ± 1.9^{b}	2.8 ± 1.1^{a}	59 ± 0.84^{d}
250 mM	128.63 ± 6.1^{a}	798 ± 9.3^{a}	6.46 ± 1.2^{a}	61.5 ± 0.53^{c}
F-statistics	9.84*	170.9*	6.69*	43.17*

The F-statistic asterisks and different letters show that the tested treatments were significantly different at $p \leq 0.05$

reported that the ability of dicotyledonous halophytes to tolerate salt is closely linked to their capacity to accumulate large amounts of osmolytes in their tissues. This allows them to regulate water osmotically by decreasing the osmotic potential in the cytoplasm. Osmotic adjustment is a mechanism that helps plants retain their ability to absorb water via their roots and keep their leaves firm even when the soil has limited water availability (Gul et al., 2024; Paulino et al., 2020).

Moreover, findings from this study revealed that salinity induced an increase in leaf succulency, while specific leaf area was reduced. These findings are corroborated by Atzori

et al. (2020), who stated that enhanced leaf succulence (high water storage) is one of the defence strategies facultative halophytes deploy in response to low external water potential caused by salinity. In *Mesembryanthemum crystallinum, Sarcocornia quinqueflora,* and *Cakile maritima* grown under saline conditions, increased leaf succulence was reported as a mechanism to detoxify sodium ions and maintain cytoplasmic salinity below toxic levels (Ahmed et al., 2022; Yasseen & Al-Thani, 2022; Belghith et al., 2022). Based on these findings it can be inferred that *T. decumbens* plants treated with different salt treatments used this mechanism to avoid the toxic effect of sodium ions since all plants survived even at a higher salinity concentration.

Salinity induced the reduction of photosynthetic pigment

The total chlorophyll content is frequently employed as a salt tolerance index in plant species (Banakar et al., 2022; Sheng et al., 2024). Many plant species have been shown to have decreased photosynthetic activity and leaf pigments when exposed to salinity by means of reducing the leaf area (Khatri & Rathore, 2022). The decrease in chlorophyll content in *T. decumbens* under high salinity implies a method for protecting photosynthetic features from salt-induced damage and avoiding extreme production of reactive oxygen species (ROS). Similar patterns of chlorophyll reduction in halophytes such as *Nitraria schoberi* and *Lobularia maritima* were described by Zilaie et al. (2022) and Hsouna et al. (2020). This decrease could be attributed to disruptions in photosynthetic machinery, pigment malfunction, pigment–protein complex instability in the light-harvesting complex. Furthermore, high salt content causes ROS formation in chloroplasts, which breaks the double bonds of unsaturated fatty acids, causing chloroplast membrane damage and chlorophyll leakage from thylakoids (Azeem et al., 2023).

Leaf and roots cation response to salinity

When plants are exposed to salinity, ion transport and accumulation are altered as a result of nutritional imbalances induced by Na^+ and Cl^- ions competitors, which limit the uptake of nutrients such as Ca^{2+} and K^+ essential for optimal growth (Abbas et al., 2024). The reduction observed in the growth of *T. decumbens* under high salinity could be due to nutritional imbalances (Alabdallah et al., 2024). Increased Na^+ build-up in *T. decumbens* leaves was associated with a considerable decrease in K^+ and Ca^{2+} uptake, which was countered by an increase in K^+ and Ca^{2+} usage efficiency (Hualpa-Ramirez et al., 2024). As a result, when exposed to salt stress, *T. decumbens* was able to economically utilise K^+ for specified tasks, and K^+ could be substituted by Na^+ for osmotic adjustment by accumulating it in the vacuoles. These findings suggest that *T. decumbens* employs an inclusion mechanism in which sodium ions accumulate in the leaves and are sufficiently diluted to keep cytoplasmic salinity below toxic levels. This suggests that efficient K^+ transport systems are also at action in *T. decumbens* cells to sustain mineral nutrition as well as to retain metabolic activity under saline conditions as found in several halophytes (Chen & Wang, 2024).

Oxidative stress and antioxidant system activation

Several biochemical markers, including cell death viability, superoxide, and lipid peroxidation, are commonly employed to determine the extent of oxidative stress in plants growing under adverse conditions (Jameel et al., 2024). In the present study, cell death viability, superoxide and lipid peroxidation were all increased with increasing saline treatment, with a more pronounced influence at 250 mM of NaCl. High production of ROS that exceeds the plant controllable limit is known to damage cellular structures and membranes, which could be the reason for the substantial decline in plant growth with increasing salinity. These findings are respectively corroborated by Azeem et al. (2023) and Parvez et al. (2020) on *Moringa oleifera* and *Chenopodium quinoa* genotypes subjected to salt stress, where increasing saline treatment caused a substantial increase in oxidative stress markers leading to reduced plant growth.

However, plants have evolved defence mechanisms such as antioxidative enzymes followed by the production of non-enzymatic compounds such as phenolic compounds to combat the catastrophic effect of oxidative stress. Among these different types of antioxidative enzymes, superoxide dismutase is considered to be the primary source of defence against oxidative stress in plants (Gill et al., 2015; Zulfiqar & Ashraf, 2022). This enzyme catalyses the transformation of two O_2 radicals into H_2O_2 and O_2. Alternatively, various antioxidant enzymes, such as catalase and peroxidase (POX), convert H_2O_2 to water. Halophytic plants tend to accumulate high amount of SOD under salt stress, a feature that provides an adaptive advantage over salt-sensitive species (Huang et al., 2019; Zhao et al., 2020). In this study, high activity of SOD and CAT with increasing saline treatment was observed, confirming that *T. decumbens* uses these two enzymes as the first line of defence against ROS caused by salinity. The same observation has been reported in other halophytes such as *Bupleurum tenuissimum, Chenopodium quinoa*, *Kandelia obovate* and *Mesembryanthemum crystallinum* (González-Orenga et al., 2021a, 2021b; Hasanuzzaman et al., 2021; Mohamed et al., 2021; Parvez et al., 2020).

Halophytes are said to activate the accumulation of non-enzymatic compounds to scavage these highly toxic species

when excess toxic ROS are not eliminated by the antioxidative enzymes. In this study, *T. decumbens* accumulated high quantities of polyphenols and flavonoids with increasing salinity, while proanthocyanidins were only detected in samples of *T. decumbens* irrigated with the highest salinities (200 and 250 mM NaCl). This response has been reported in many halophytes, where increasing saline irrigation induced the increase of phenols and flavonoids to scavenge highly toxic free radicals (Kumari et al., 2017). In contrast to the rise in polyphenols, flavonoids, and proanthocyanidins, antioxidant activity (DPHH) in *T. decumbens* leaves was substantially lower in saline-treated plants compared to control. These findings substantiate previous results of Sogoni et al. (2021) on *T. decumbens* where increasing NaCl treatment lowered the antioxidant activity.

Conclusion

The results of this study revealed a substantial growth enhancement in plants irrigated with lower or no NaCl dosage up to 100 mM. High concentrations of NaCl reduced growth and total chlorophyll content, but all plants remained alive without signs of toxicity. The reported high quantities of Na^+ in the leaves than in roots of *T. decumbens* indicated an ion inclusion mechanism. In this regard, the absence of dehydration and increased leaf succulence in salt-treated plants could be attributed to efficient salt ion compartmentalization. To curb the oxidative damage in plant tissues at high NaCl, *T. decumbens* regulated the production of antioxidative enzymes (CAT and SOD) and non-enzymatic compounds (polyphenols, flavonoids and proanthocyanidins) to scavenge the reactive oxygen species liable for cellular damage and reduced growth. These findings show that *T. decumbens* can withstand salinity up to 250 mM of NaCl by modulating morpho-physiological features, antioxidant defence systems, and managing ion toxicity and oxidative stress effectively. This connotes its potential use in bio-saline agriculture, particularly in semi-arid and dry regions where seawater could be diluted and used in the cultivation of this species.

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References

Abbas, A., Mansha, S., Waheed, H., Siddiq, Z., Hayyat, M. U., Zhang, Y. J., & Alwutayd, K. (2024). NaCl stress, tissue specific Na+ and K+ up-take and their effect on growth and physiology of *Helianthus annuus* L. and *Solanum lycopersicum* L. *Scientia Horticulturae, 326*, 112454. https://doi.org/10.1016/J.SCIENTA.2023.112454

Ahmed, H. A. I., Shabala, S., Goemann, K., & Shabala, L. (2022). Development of suberized barrier is critical for ion partitioning between senescent and non-senescent tissues in a succulent halophyte *Sarcocornia quinqueflora*. *Environmental and Experimental Botany, 194*, 104692. https://doi.org/10.1016/J.ENVEXPBOT.2021.104692

Alabdallah, N. M., Al-Shammari, A. S., Saleem, K., AlZahrani, S. S., Raza, A., Asghar, M. A., Ullah, A., Hussain, M. I., & Yong, J. W. H. (2024). Unveiling the mechanisms of silicon-induced salinity stress tolerance in Panicum turgidum: Insights from antioxidant defense system and comprehensive metabolic and nutritional profiling. *South African Journal of Botany, 168*, 328–339. https://doi.org/10.1016/J.SAJB.2024.03.006

Atzori, G., Nissim, W., Macchiavelli, T., Vita, F., Azzarello, E., Pandolfi, C., Masi, E., & Mancuso, S. (2020). Tetragonia tetragonioides (Pallas) Kuntz. as promising salt-tolerant crop in a saline agricultural context. *Agricultural Water Management, 240*, 106261. https://doi.org/10.1016/J.AGWAT.2020.106261

Azeem, M., Pirjan, K., Qasim, M., Mahmood, A., Javed, T., Muhammad, H., Yang, S., Dong, R., Ali, B., & Rahimi, M. (2023). Salinity stress improves antioxidant potential by modulating physio-biochemical responses in *Moringa oleifera* Lam. *Scientific Reports, 13*(1), 1–17. https://doi.org/10.1038/s41598-023-29954-6

Banakar, M. H., Amiri, H., Sarafraz Ardakani, M. R., & Ranjbar, G. H. (2022). Susceptibility and tolerance of fenugreek (*Trigonella foenum-graceum* L) to salt stress: Physiological and biochemical inspections. *Environmental and Experimental Botany, 194*, 104748. https://doi.org/10.1016/J.ENVEXPBOT.2021.104748

Baureder, M., Barane, E., & Hederstedt, L. (2014). In vitro assembly of catalase. *Journal of Biological Chemistry, 289*(41), 28411–28420. https://doi.org/10.1074/jbc.M114.596148

Belghith, I., Senkler, J., Abdelly, C., Braun, H.-P., Debez, A., Belghith, I., Senkler, J., Abdelly, C., Braun, H.-P., & Debez, A. (2022). Changes in leaf ecophysiological traits and proteome profile provide new insights into variability of salt response in the succulent halophyte *Cakile maritima*. *Functional Plant Biology, 49*(7), 613–624. https://doi.org/10.1071/FP21151

Ben Amor, N., Jiménez, A., Boudabbous, M., Sevilla, F., & Abdelly, C. (2020). Chloroplast implication in the tolerance to salinity of the halophyte *Cakile maritima*. *Russian Journal of Plant Physiology, 67*(3), 507–514. https://doi.org/10.1134/S1021443720030048/TABLES/3

Bistgani, Z. E., Hashemi, M., DaCosta, M., Craker, L., Maggi, F., & Morshedloo, M. R. (2019). Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of *Thymus vulgaris* L. and Thymus daenensis Celak. *Industrial Crops and Products, 135*, 311–320. https://doi.org/10.1016/J.INDCROP.2019.04.055

Boamah, S., Ojangba, T., Zhang, S., Zhu, N., Osei, R., John Tiika, R., Boakye, T. A., Khurshid, A., Inayat, R., Effah, Z., Essel, E., & Xu,

B. (2023). Evaluation of salicylic acid (SA) signaling pathways and molecular markers in Trichoderma-treated plants under salinity and Fusarium stresses. *A Review European Journal of Plant Pathology, 166*(3), 259–274. https://doi.org/10.1007/S10658-023-02660-9/FIGURES/2

Bose, J., Rodrigo-Moreno, A., & Shabala, S. (2014). ROS homeostasis in halophytes in the context of salinity stress tolerance. *Journal of Experimental Botany, 65*(5), 1241–1257. https://doi.org/10.1093/JXB/ERT430

Brenes, M., Perez, J., Gonzalez-Orenga, S., Solana, A., Boscaiu, M., Prohens, J., Plazas, M., Fita, A., & Vicente, O. (2020). Comparative Studies on the Physiological and Biochemical Responses to Salt Stress of Eggplant (*Solanum melongena*) and Its Rootstock S. torvum. *Agriculture*. https://doi.org/10.3390/AGRICULTURE10080328

Bulawa, B., Sogoni, A., Jimoh, M. O., & Laubscher, C. P. (2022). Potassium application enhanced plant growth, mineral composition, proximate and phytochemical content in Trachyandra divaricata Kunth (Sandkool). *Plants, 11*(22), 3183. https://doi.org/10.3390/PLANTS11223183

Chen, J., & Wang, Y. (2024). Understanding the salinity resilience and productivity of halophytes in saline environments. *Plant Science, 346*, 112171. https://doi.org/10.1016/J.PLANTSCI.2024.112171

Egbichi, I., Keyster, M., & Ludidi, N. (2014). Effect of exogenous application of nitric oxide on salt stress responses of soybean. *South African Journal of Botany, 90*, 131–136. https://doi.org/10.1016/J.SAJB.2013.11.002

Farooq, T. H., Rafay, M., Basit, H., Shakoor, A., Shabbir, R., Riaz, M. U., Ali, B., Kumar, U., Qureshi, K. A., & Jaremko, M. (2022). Morpho-physiological growth performance and phytoremediation capabilities of selected xerophyte grass species toward Cr and Pb stress. *Frontiers in Plant Science, 13*, 3232. https://doi.org/10.3389/FPLS.2022.997120/BIBTEX

Flowers, T. J., & Colmer, T. D. (2015). Plant salt tolerance: Adaptations in halophytes. *Annals of Botany, 115*(3), 327–331. https://doi.org/10.1093/AOB/MCU267

Gill, S. S., Anjum, N. A., Gill, R., Yadav, S., Hasanuzzaman, M., Fujita, M., Mishra, P., Sabat, S. C., & Tuteja, N. (2015). Superoxide dismutase—mentor of abiotic stress tolerance in crop plants. *Environmental Science and Pollution Research, 22*(14), 10375–10394. https://doi.org/10.1007/S11356-015-4532-5/TABLES/3

Gokul, A., Roode, E., Klein, A., & Keyster, M. (2016). Exogenous 3,3′-diindolylmethane increases *Brassica napus* L. seedling shoot growth through modulation of superoxide and hydrogen peroxide content. *Journal of Plant Physiology, 196–197*, 93–98. https://doi.org/10.1016/J.JPLPH.2016.03.013

Golldack, D., Li, C., Mohan, H., & Probst, N. (2014). Tolerance to drought and salt stress in plants: Unraveling the signaling networks. *Frontiers in Plant Science*. https://doi.org/10.3389/FPLS.2014.00151/BIBTEX

González-Orenga, S., Grigore, M.-N., Boscaiu, M., & Vicente, O. (2021a). Constitutive and induced salt tolerance mechanisms and potential uses of limonium mill. *Species. Agronomy, 11*(3), 413. https://doi.org/10.3390/agronomy11030413

González-Orenga, S., Leandro, M. E. D. A., Tortajada, L., Grigore, M. N., Llorens, J. A., Ferrer-Gallego, P. P., Laguna, E., Boscaiu, M., & Vicente, O. (2021b). Comparative studies on the stress responses of two Bupleurum (*Apiaceae*) species in support of conservation programmes. *Environmental and Experimental Botany, 191*, 104616. https://doi.org/10.1016/J.ENVEXPBOT.2021.104616

Gul, B., Hameed, A., Ahmed, M. Z., Hussain, T., Rasool, S. G., & Nielsen, B. L. (2024). Thriving under salinity: Growth, ecophysiology and proteomic insights into the tolerance mechanisms of obligate halophyte *Suaeda fruticosa*. *Plants, 13*(11), 1529. https://doi.org/10.3390/PLANTS13111529/S1

Gul, Z., Tang, Z. H., Arif, M., & Ye, Z. (2022). An insight into abiotic stress and influx tolerance mechanisms in plants to cope in saline environments. *In Biology*. https://doi.org/10.3390/biology11040597

Hasanuzzaman, M., Inafuku, M., Nahar, K., Fujita, M., & Oku, H. (2021). Nitric oxide regulates plant growth, physiology, antioxidant defense, and ion homeostasis to confer salt tolerance in the mangrove species, *Kandelia obovate*. *Antioxidants, 10*(4), 611. https://doi.org/10.3390/ANTIOX10040611

Hassani, A., Azapagic, A., & Shokri, N. (2020). Predicting long-term dynamics of soil salinity and sodicity on a global scale. *Proceedings of the National Academy of Sciences of the United States of America, 117*(52), 33017–33027. https://doi.org/10.1073/PNAS.2013771117/SUPPL_FILE/PNAS.2013771117.SAPP.PDF

Hsouna, A. B., Ghneim-Herrera, T., Romdhane, W. B., Dabbous, A., Saad, R. B., Brini, F., Abdelly, C., Hamed, K. B., Hsouna, A. B., Ghneim-Herrera, T., Romdhane, W. B., Dabbous, A., Saad, R. B., Brini, F., Abdelly, C., & Hamed, K. B. (2020). Early effects of salt stress on the physiological and oxidative status of the halophyte *Lobularia maritima*. *Functional Plant Biology, 47*(10), 912–924. https://doi.org/10.1071/FP19303

Hualpa-Ramirez, E., Carrasco-Lozano, E. C., Madrid-Espinoza, J., Tejos, R., Ruiz-Lara, S., Stange, C., & Norambuena, L. (2024). Stress salinity in plants: New strategies to cope with in the foreseeable scenario. *Plant Physiology and Biochemistry, 208*, 108507. https://doi.org/10.1016/J.PLAPHY.2024.108507

Huang, H., Ullah, F., Zhou, D. X., Yi, M., & Zhao, Y. (2019). Mechanisms of ROS regulation of plant development and stress responses. *Frontiers in Plant Science, 10*, 800. https://doi.org/10.3389/FPLS.2019.00800/BIBTEX

Hussain, T., Asrar, H., Zhang, W., & Liu, X. (2023). The combination of salt and drought benefits selective ion absorption and nutrient use efficiency of halophyte *Panicum antidotale*. *Frontiers in Plant Science, 14*, 1091292. https://doi.org/10.3389/FPLS.2023.1091292/BIBTEX

Hussain, T., Koyro, H. W., Huchzermeyer, B., & Khan, M. A. (2015). Eco-physiological adaptations of Panicum antidotale to hyperosmotic salinity: Water and ion relations and anti-oxidant feedback. *Flora - Morphology, Distribution, Functional Ecology of Plants, 212*, 30–37. https://doi.org/10.1016/J.FLORA.2015.02.006

Ingarfield, P., Sogoni, A., Jimoh, M. O., Rautenbach, F., Kambizi, L., & Laubscher, C. P. (2023). Total phenols and antioxidant potential of Pelargonium reniforme Curtis and Pelargonium sides DC under different watering frequencies and arbuscular mycorrhiza applications. *Acta Horticulturae, 1358*, 107–113. https://doi.org/10.17660/ActaHortic.2023.1358.15

Jameel, J., Anwar, T., Siddiqi, E. H., & Alomrani, S. O. (2024). Alleviation of NaCl stress in tomato varieties by promoting morpho-physiological attributes and biochemical characters. *Scientia Horticulturae, 323*, 112496. https://doi.org/10.1016/J.SCIENTA.2023.112496

Jeeva, S. (2020). Studies on the performance and phytoremediation effect of underutilized leafy vegetables in salt affected soils. *International Journal of Chemical Studies, 8*(2), 1762–1764. https://doi.org/10.22271/chemi.2020.v8.i2aa.9015

Jimoh, M. O., Afolayan, A. J., & Lewu, F. B. (2019). Antioxidant and phytochemical activities of *Amaranthus caudatus* L. harvested from different soils at various growth stages. *Scientific Reports, 9*(1), 1–14. https://doi.org/10.1038/s41598-019-49276-w

Jimoh, M. A., Idris, O. A., & Jimoh, M. O. (2020). Cytotoxicity, phytochemical, antiparasitic screening, and antioxidant activities of *Mucuna pruriens* (Fabaceae). *Plants, 9*(9), 1249. https://doi.org/10.3390/plants9091249

Jimoh, M. O., Jimoh, M. A., Bakare, O. O., Bamigboye, S. O., Senjobi, C. T., Sogoni, A., Okaiyeto, K., Kambizi, L., & Laubscher,

C. P. (2023). Total phenols, flavonoids, proanthocyanidins and antioxidant potential of Justicia secunda Vahl. *Acta Horticulturae, 1358*, 211–217. https://doi.org/10.17660/ActaHortic.2023.1358.28

Khatami, S. A., Kasraie, P., Oveysi, M., Tohidi Moghadam, H. R., & Ghooshchi, F. (2022). Mitigating the adverse effects of salinity stress on lavender using biodynamic preparations and bio-fertilizers. *Industrial Crops and Products, 183*, 114985. https://doi.org/10.1016/J.INDCROP.2022.114985

Khatri, K., & Rathore, M. S. (2022). Salt and osmotic stress-induced changes in physio-chemical responses, PSII photochemistry and chlorophyll a fluorescence in peanut. *Plant Stress, 3*, 100063. https://doi.org/10.1016/J.STRESS.2022.100063

Klak, C., Hanáček, P., & Bruyns, P. V. (2017). Out of southern Africa: Origin, biogeography and age of the Aizooideae (Aizoaceae). *Molecular Phylogenetics and Evolution, 109*, 203–216. https://doi.org/10.1016/j.ympev.2016.12.016

Kumari, A., Parida, A. K., Rangani, J., & Panda, A. (2017). Antioxidant activities, metabolic profiling, proximate analysis, mineral nutrient composition of Salvadora Persica fruit unravel a potential functional food and a natural source of pharmaceuticals. *Frontiers in Pharmacology*. https://doi.org/10.3389/FPHAR.2017.00061/FULL

Lichtenthaler, H. K., & Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions, 11*(5), 591–592. https://doi.org/10.1042/BST0110591

Ma, J., Saleem, M. H., Yasin, G., Mumtaz, S., Qureshi, F. F., Ali, B., Ercisli, S., Alhag, S. K., Ahmed, A. E., Vodnar, D. C., Hussain, I., Marc, R. A., & Chen, F. (2022). Individual and combinatorial effects of SNP and NaHS on morpho-physio-biochemical attributes and phytoextraction of chromium through Cr-stressed spinach (*Spinacia oleracea* L.). *Frontiers in Plant Science, 13*, 2745. https://doi.org/10.3389/FPLS.2022.973740/BIBTEX

Mahmood, U., Hussain, S., Hussain, S., Ali, B., Ashraf, U., Zamir, S., Al-Robai, S. A., Alzahrani, F. O., Hano, C., & El-Esawi, M. A. (2021). Morpho-physio-biochemical and molecular responses of maize hybrids to salinity and waterlogging during stress and recovery phase. *Plants, 10*(7), 1345. https://doi.org/10.3390/PLANTS10071345

Mangal, V., Lal, M. K., Tiwari, R. K., Altaf, M. A., Sood, S., Kumar, D., Bharadwaj, V., Singh, B., Singh, R. K., & Aftab, T. (2022). Molecular insights into the role of reactive oxygen, nitrogen and sulphur species in conferring salinity stress tolerance in plants. *Journal of Plant Growth Regulation, 42*(2), 554–574. https://doi.org/10.1007/S00344-022-10591-8

Mantovani, A. (2011). A method to improve leaf succulence quantification. *Brazilian Archives of Biology and Technology, 42*(1), 9–14. https://doi.org/10.1590/S1516-89131999000100002

Manuel, R., Machado, A., Serralheiro, R. P., Alvino, A., Freire, M. I., & Ferreira, R. (2017). Soil Salinity: Effect on vegetable crop growth management practices to prevent and mitigate soil salinization. *Horticulturae, 3*(2), 30. https://doi.org/10.3390/HORTICULTURAE3020030

Medini, W., Ellouzi, H., Farhat, N., Alharbi, A., Aggag, A. M., Zorrig, W., Smaoui, A., Abdelly, C., & Rabhi, M. (2023). Monoculture and coculture of *Sesuvium portulacastrum* and Sulla carnosa under saline and non-saline conditions: Plant vigour and soil phytodesalination. *Water, Air, & Soil Pollution, 234*(7), 1–10. https://doi.org/10.1007/S11270-023-06461-Y

Mohamed, E., Ansari, N., Yadav, D. S., Agrawal, M., & Agrawal, S. B. (2021). Salinity alleviates the toxicity level of ozone in a halophyte *Mesembryanthemum crystallinum* L. *Ecotoxicology, 30*(4), 689–704. https://doi.org/10.1007/S10646-021-02386-6/FIGURES/1

Mokganya, M. G., & Tshisikhawe, M. P. (2019). Medicinal uses of selected wild edible vegetables consumed by Vhavenda of the Vhembe District Municipality, South Africa. *South African Journal of Botany, 122*, 184–188. https://doi.org/10.1016/J.SAJB.2018.09.029

Nkcukankcuka, M., Jimoh, M. O., Griesel, G., & Laubscher, C. P. (2021). Growth characteristics, chlorophyll content and nutrients uptake in *Tetragonia decumbens* Mill. cultivated under different fertigation regimes in hydroponics. *Crop and Pasture Science, 73*(2), 67–76. https://doi.org/10.1071/CP20511

Ogunniyi, A. I., Mavrotas, G., Olagunju, K. O., Fadare, O., & Adedoyin, R. (2020). Governance quality, remittances and their implications for food and nutrition security in Sub-Saharan Africa. *World Development, 127*, 104752. https://doi.org/10.1016/j.worlddev.2019.104752

Parvez, S., Abbas, G., Shahid, M., Amjad, M., Hussain, M., Asad, S. A., Imran, M., & Naeem, M. A. (2020). Effect of salinity on physiological, biochemical and photostabilizing attributes of two genotypes of quinoa (*Chenopodium quinoa* Willd.) exposed to arsenic stress. *Ecotoxicology and Environmental Safety, 187*, 109814. https://doi.org/10.1016/J.ECOENV.2019.109814

Paulino, M. K., Souza, S., Rodrigues, E., de Souza, C., Lins, M. T., Dourado, P. R. M., de Carvalho Leal, L. Y., Monteiro, D. R., de Andrade Rego Junior, F. E., & de Carvalho Silva, C. U. (2020). Influence of vesicular trichomes of *Atriplex nummularia* on photosynthesis, osmotic adjustment cell wall elasticity and enzymatic activity. *Plant Physiology and Biochemistry, 155*, 177–186.

Peng, C., Chang, L., Yang, Q., Tong, Z., Wang, D., Tan, Y., Sun, Y., Yi, X., Ding, G., Xiao, J., Zhang, Y., & Wang, X. (2019). Comparative physiological and proteomic analyses of the chloroplasts in halophyte *Sesuvium portulacastrum* under differential salt conditions. *Journal of Plant Physiology, 232*, 141–150. https://doi.org/10.1016/J.JPLPH.2018.10.028

Rasheed, R., Ashraf, M. A., Iqbal, M., Hussain, I., Akbar, A., Farooq, U., & Shad, M. I. (2020). Major Constraints for Global Rice Production: Changing Climate, Abiotic and Biotic Stresses. In Aryadeep Roychoudhury (Ed.), *Rice Research for Quality Improvement: Genomics and Genetic Engineering: Volume 1: Breeding Techniques and Abiotic Stress Tolerance* (pp. 15–45). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-15-4120-9_2

Rehman, M., Mubeen, S., Wang, Y., Ma, W., Fu, H., Li, L., Ruan, X., & Zhang, X. (2023). Effects of salinity stress on growth and physiological parameters and related gene expression in different ecotypes of *Sesuvium portulacastrum* on Hainan Island. *Genes, 14*(7), 1336. https://doi.org/10.3390/GENES14071336

Rhaman, M. S., Rauf, F., Tania, S. S., Bayazid, N., Tahjib-ul-Arif, M., Robin, A. H. K., Hoque, M. A., Yang, X., Murata, Y., & Brestic, M. (2024). Proline and glycine betaine: A dynamic duo for enhancing salt stress resilience in maize by regulating growth, stomatal size, and oxidative stress responses. *Plant Stress, 14*, 100563. https://doi.org/10.1016/J.STRESS.2024.100563

Rodrigues de Queiroz, A., Hines, C., Brown, J., Sahay, S., Vijayan, J., Stone, J. M., Bickford, N., Wuellner, M., Glowacka, K., Buan, N. R., & Roston, R. L. (2023). The effects of exogenously applied antioxidants on plant growth and resilience. *Phytochemistry Reviews, 2023*, 1–41. https://doi.org/10.1007/S11101-023-09862-3

Salami, S. O., Adegbaju, O. D., Idris, O. A., Jimoh, M. O., Olatunji, T. L., Omonona, S., Orimoloye, I. R., Adetunji, A. E., Olusola, A., Maboeta, M. S., & Laubscher, C. P. (2022). South African wild fruits and vegetables under a changing climate: The implications on health and economy. *South African Journal of Botany, 145*, 13–27. https://doi.org/10.1016/J.SAJB.2021.08.038

Saleem, A., Zulfiqar, A., Ali, B., Naseeb, M. A., Almasaudi, A. S., & Harakeh, S. (2022). Iron sulfate ($FeSO_4$) improved physiological attributes and antioxidant capacity by reducing oxidative stress of

Oryza sativa L. *Cultivars in Alkaline Soil Sustainability, 14*(24), 16845. https://doi.org/10.3390/SU142416845

Sheng, W., Liu, L., Wu, Y., Yin, M., Yu, Q., Guo, X., Song, H., & Guo, W. (2024). Exploring salt tolerance and indicator traits across four temperate lineages of the common wetland plant, *Phragmites australis*. *Science of the Total Environment, 912*, 169100. https://doi.org/10.1016/J.SCITOTENV.2023.169100

Sogoni, A., Jimoh, M. O., Kambizi, L., & Laubscher, C. P. (2021). The impact of salt stress on plant growth, mineral composition, and antioxidant activity in *Tetragonia decumbens* mill.: An underutilized edible halophyte in South Africa. *Horticulturae, 7*(6), 140. https://doi.org/10.3390/horticulturae7060140

Sogoni, A., Jimoh, M. O., Laubscher, C. P., & Kambizi, L. (2022). Effect of rooting media and IBA treatment on rooting response of South African dune spinach (Tetragonia decumbens): an underutilized edible halophyte. *Acta Horticulturae, 1356*, 319–325. https://doi.org/10.17660/ActaHortic.2022.1356.38

Tembo-Phiri, C. (2019). *Edible Fynbos Plants: A soil types and irrigation regime investigation on Tetragonia decumbens and Mesembryanthemum crystallinum* (Issue April) [Msc, University of Stellenbosch]. https://scholar.sun.ac.za/handle/10019.1/105974%0Ahttps://www.agroecologyconference.co.za/wp-content/uploads/2019/02/Chimwemwe-Tembo-Phiri-Adoption-of-edible-fynbos-plants-in-the-Western-Cape.pdf%0Ahttps://www.semanticscholar.org/paper/Edible-fynbos-plant

Yasseen, B. T., & Al-Thani, R. F. (2022). Endophytes and halophytes to remediate industrial wastewater and saline soils: Perspectives from Qatar. *Plants, 11*(11), 1497. https://doi.org/10.3390/PLANTS11111497/S1

Zhang, Y., Hou, K., Qian, H., Gao, Y., Fang, Y., Xiao, S., Tang, S., Zhang, Q., Qu, W., & Ren, W. (2022). Characterization of soil salinization and its driving factors in a typical irrigation area of Northwest China. *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2022.155808

Zhao, C., Zhang, H., Song, C., Zhu, J. K., & Shabala, S. (2020). Mechanisms of plant responses and adaptation to soil salinity. *The Innovation, 1*(1), 100017. https://doi.org/10.1016/J.XINN.2020.100017

Zilaie, M. N., Arani, A. M., Etessami, H., Dinarvand, M., & Dolati, A. (2022). Halotolerant plant growth-promoting rhizobacteria-mediated alleviation of salinity and dust stress and improvement of forage yield in the desert halophyte Seidlitzia rosmarinus. *Environmental and Experimental Botany, 201*, 104952. https://doi.org/10.1016/J.ENVEXPBOT.2022.104952

Zulfiqar, F., & Ashraf, M. (2022). Antioxidants as modulators of arsenic-induced oxidative stress tolerance in plants: An overview. *Journal of Hazardous Materials, 427*, 127891. https://doi.org/10.1016/J.JHAZMAT.2021.127891

Zuluaga, M. Y. A., Monterisi, S., Rouphael, Y., Colla, G., Lucini, L., Cesco, S., & Pii, Y. (2023). Different vegetal protein hydrolysates distinctively alleviate salinity stress in vegetable crops: A case study on tomato and lettuce. *Frontiers in Plant Science, 14*, 485. https://doi.org/10.3389/FPLS.2023.1077140/BIBTEX