Acta Botanica Brasilica, 2024, 38: e20230243
doi: https://doi.org/10.1590/1677-941X-ABB-2023-0243

Original Article

High tolerance and adaptive responses to salinity of a valuable medicinal plant *Grangea maderaspatana*

Dan Quang Tran[1,*], Anh Cong Pham[1], Trieu-Giang Thi Tran[1], Tuan Chau Vo[1], Hoang Duc Vu[1], Tho Thi Bui[1], Giap Ta Ho[2], Sayed Mohammad Mohsin[3]

[1]Faculty of Biology and Environmental Science, The University of Danang–University of Science and Education, Da Nang, Vietnam.
[2]NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh, Vietnam.
[3]Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.
*Corresponding author, E-mail: tqdan@ued.udn.vn

ABSTRACT

Grangea maderaspatana is a valuable medicinal plant growing in salt-affected areas, but its tolerance capability, physiological and biochemical responses to salinity is still unclear. To understand these traits, this study examined effects of salinity at different levels (50–400 mM NaCl) on plant growth and its responses. The results shown that the plant's dry biomass decreased with increasing salinity levels of 100–400 mM NaCl, but its growth was maintained at 400 mM NaCl level with a dry biomass equal to 0.45 times that of the control, indicating that *G. maderaspatana* had a tolerance ability to high salinity. The plant also had adaptive responses to the salinity. The content of leaf chlorophylls and carotenoids were retained, even enhanced by 50–200 mM NaCl, suggesting a high adaptation of photosynthesis. Proline, Na^+, and Cl^- was highly accumulated while the accumulation of K^+ and NO_3^- was maintained with 200–400 mM NaCl, indicating that the plant had adaptive mechanisms for osmotic adjustment and ion homeostasis. Antioxidative activities of catalase, superoxide dismutase, and peroxidase were enhanced by the salinity. These findings are useful information for understanding salt tolerance mechanisms and for utilization of this medicinal plant in saline agriculture.

Keywords: Ion accumulation; photosynthetic pigments; salt stress; salt-tolerant plant; salt tolerance mechanism

Introduction

Soil salinization, mainly caused by predominant accumulation of NaCl, is becoming a serious environmental problem affecting global agricultural production as it causes serious reduction in the yield and productivity of most crops (Garcia-Caparros *et al.* 2023). According to a recent estimation, salt-affected land accounts for approximately 1 billion hectares (about 7%) of the earth's land surface, and the area of salt-affected soil is predicted to increase over years due to climate changes and anthropogenic activities (Hopmans *et al.* 2021). This will be a threat to sustainable agriculture production and food security in the world. Thus, both salt tolerance improvement of crops and developing salt-tolerant plants as alternative crops are considered to effective solutions for sustainable agriculture production in saline areas (also referred as saline agriculture) (Panta *et al.* 2014). For developing tolerant crops, it is necessary to understand how plants are affected by salinity and their tolerance mechanisms (Zhao *et al.* 2020).

Received October 22, 2023; Accepted December 22, 2023
Editor-in-Chief: Thaís Elias Almeida; Associate Editor: Bruno Garcia Ferreira

How to cite:
Tran DQ, Pham AC, Tran T-GT *et al.* 2024. High tolerance and adaptive responses to salinity of a valuable medicinal plant *Grangea maderaspatana*. Acta Botanica Brasilica 38: e20230243. doi: 10.1590/1677-941X-ABB-2023-0243

Salinity inhibits growth, physiological and biochemical processes of plants by imposing two major detrimental effects (Safdar *et al.* 2019). First, an abundant amount of salt in the environment will lower the extracellular water potential, leading to an osmotic stress that reduces water uptake of plants. As a result, plants fall into the status of physiological drought that interrupts physiological processes such as transpiration, water uptake, photosynthesis (Sudhir & Murthy 2004; Hniličková *et al.* 2019). The second effect is ionic stress caused by the intrusion of Na^+ and Cl^- through cell membrane channels/transporters (Shabala & Mackay 2011; Flowers *et al.* 2015). These ions will compete with other microelement ions such as K^+ and NO_3^-, leading to the nutrient deficiency and loss of ion homeostasis (Kumar *et al.* 2021; Alharbi *et al.* 2022). In addition, the excessive salt accumulation in cytosol is detrimental to cellular metabolisms, which is attributed to its inhibitory effects on catalytic activity of enzymes (Zhao *et al.* 2020). When physiological functions such as photosynthesis and respiration are disturbed by the salt-induced stresses, reactive oxygen species (ROS) is overproduced, and the ROS accumulation at a certain level will induce cellular oxidative stress (Taïbi *et al.* 2016; Kiani *et al.* 2021). It is reported that salt-tolerant plants possess various sophisticated mechanisms at both tissue and cellular level to deal with salt stress (Liang *et al.* 2018; Van Zelm *et al.* 2020). The cells can trigger osmotic adjustment by osmolyte accumulation for resisting the osmotic stress, while salt sequestration into vacuoles or exclusion from the cells are also utilized to maintain ion homeostasis and avoid ionic stress (Shabala & Mackay 2011). Enhancing non-enzymatic and enzymatic antioxidant activity are also effective strategies to reduce the ROS accumulation (Zhao *et al.* 2020). In tissue level, salt is sequestrated into non-photosynthetic tissues or senescent organs to eliminate the stress on other parts of plants (Munns *et al.* 2016). Despite great advances in understanding salt tolerance mechanisms of salt-tolerant plants, it is a complex process and varies from plant species to species.

It is suggested that salt-tolerant medicinal plants have a high potential to become effectively alternative crops for the development of saline agriculture due to their values (Banerjee & Roychoudhury 2017). *Grangea maderaspatana* (syn. *Artemisia maderaspatana*), belongs to the Asteraceae family, is a hairy, branched, bud-woolly and annual herbaceous species with a heigh of up to 70 cm. Its leaves are alternate, stalkless, and in a tooth-lobed shape; the flower heads are small (6–10 mm) in diameter, with yellow florets (Galani 2015). This species is widely distributed in Africa and Asia's tropical and subtropical regions (Chaudhary *et al.* 2023). The whole plant is commonly utilized as a valuable medicinal herb in traditional medicines for treating diarrhea, antipyretic, headache, paralysis, dyspepsia, hysteria, rheumatics, obstructed menses, snake bikes, and especially earache and eyesore (Dodiya & Jain 2017). The therapeutic values are also established through phytochemical and pharmacological studies. It is reported that the plant extracts exhibited analgesic (Ahmed *et al.* 2001), antiphlogistic and antiarthritic (Rachchh & Galani 2015), cytotoxic (Ruangrungsi *et al.* 1989), antioxidant and antimicrobial (Singh *et al.* 2013), and oestrogenic (Jain *et al.* 1993) activities. The phytochemical screenings also identified a variety of bioactive compounds such as flavonoids, diterpenes, sesquiterpenes, steroids, and essential oils, and their pharmacological activities was documented in a recent review (Aruna & Kumar 2020). In addition, this plant is used as an edible vegetable and spice in raw or cooked preparations (Burkill 1995). Despite its great medicinal values and usages, *G. maderaspatana* has not yet been cultivated on a large scale for economic and commercial purposes. Besides, it is proposed that the plant have high adaptability to salinity due to its natural distribution on coastal sandy and wet lands (Galani 2015), but its capability and salt tolerance mechanisms are still unclear. We proposed that *G. maderaspatana* may become a potential crop and a useful genetic resource for the development of saline agriculture if its salinity adaptation is elucidated.

Thus, the present study aimed to elucidate the salt tolerance capacity of *G. maderaspatana* and its responses in terms of photosynthesis, osmotic adjustment, ion homeostasis, water uptake, and antioxidant activity, to the salt-induced stresses. The findings would be useful to understand salinity adaptation mechanisms of *G. maderaspatana* in particular and of plants in general. For these purposes, the young plants were exposed to different salinity levels (50, 100, 200, and 400 mM NaCl), and the related growth, physiological and biochemical parameters were analyzed.

Materials and Methods

Plant material and culture conditions

G. maderaspatana seeds were collected from the plants growing on coastal sandy areas (N15°56'08.9", E108°18'40.2") in Quang Nam province (Vietnam) in the summer of 2021. The young plants were prepared by culture method as described by Tran and Pham (2023). In brief, the seed was surface-sterilized and sown in a tray filled with a mixture of coco peat, vermiculite, and perlite by a 2:1:1 volume ratio. The two-week-old seedlings were transplanted into a 0.5 L pot contained the same mixture. The pots were placed in a growth chamber (CMP6010-Conviron, Canada) with following growing condition: photoperiod/temperature regime of 14 h light at 28 °C and 10 h dark at 25 °C; relative humidity of 75 %; and light intensity of 10,000 lux. The half-strength Hoagland nutrient solution (No. 2) was used for the seedling irrigation for 4 weeks prior to the salt treatments.

Salt treatment

For the salt treatments, the six-week-old seedlings in uniform sizes were irrigated with the nutrient solutions containing 50, 100, 200, and 400 mM NaCl. The irrigation was carried out according to a procedure as described by Sahin *et al.* (2018), by which the plants were treated with increasing salt concentrations to avoid osmotic shocks. In brief, the plants were irrigated with the salt solutions of increasing concentrations before the treatment of designated level. The plants were under the saline conditions for totally 14 days after the onset of 50 mM NaCl treatment. The irrigation was repeated every day during the period of salt treatment. In order to maintain soil salinity, the salt solutions were applied continuously to the pot until the out-flow from the bottom reached a minimum amount equal to the pot's volume. In addition to the salt treatments, the plants were also irrigated with the nutrient solution without NaCl for the control. For the study's purpose, the growth parameters of the plant were measured on day 14 of the treatment; meanwhile, the physiological and biochemical parameters of young leaves were determined on the day 7.

Growth measurement

The whole plant, aerial parts (assigned as shoots), and roots were determined the fresh weight (FW) after removed from the treatments. Then, the samples were dried at 70 °C for 48 h in a drying oven prior to the dry weight (DW) measurement (Sahin *et al.* 2018). Length of the shoots and roots was also measured using a metric ruler.

Determination of photosynthetic pigment content

The content of chlorophyll *a* (chl *a*), chlorophyll *b* (chl *b*), and carotenoids were determined according to the method established by Wellburn (1994). In brief, the leaf samples (ca. 100 mg) were homogenized in 80 % acetone. The homogeneous mixture was centrifuged at 5,000× g for 10 min. The supernatant's absorbance at 645, 663, and 470 nm was measured using a spectrometer (Jasco V730 UV-VIS) to determine the content of chl *a*, chl *b*, and carotenoids.

Determination of relative water content, proline and total phenolic contents

The relative water content (RWC) was determined according to the method established by González and González-Vilar (2001). In brief, after measured the FW, the leaf discs were floated on deionized water for 4 h at cool temperature before measuring the turgid weight (TW). Then, the DW was measured after drying for 48 h at 70 °C. The FW, TW, and DW were used for the RWC calculation.

The content of proline was determined according to a method as described by Bates *et al.* (1973). In brief, the leaf sample (ca. 50 mg) was homogenized in 10 mL of 3 % sulfosalicylic acid, then centrifuged at 12,000× g at 4 °C, for 15 min. A mixture of each 2 mL of supernatant, ninhydrin acid, and acetic acid was mixed to react at 95–100 °C for 60 min. Then, the reacted mixture was mixed with 4 mL of toluene, and the solvent fraction's absorbance at 520 nm was measured. The content was calculated using a standard curve of concentrated proline solutions.

The total phenolic content (TPC) was determined by the Folin-Ciocalteu method as described by Kiani *et al.* (2021), with minor modifications. In brief,the dried leaf sample (ca. 100 mg) was homogenized in 10 mL of 80 % methanol, and then incubated in an orbital-shaking incubator (150 rpm) at 25 °C for 24 h. A mixture of 0.5 mL of the extract, 2.5 mL of 10-fold-diluted Folin-Ciocalteu reagent, and 2 mL of 7.5 % sodium carbonate was mixed to react at 45 °C for 15 min, and the reacted mixture's absorbance at 765 nm was measured. Gallic acid was used as a standard for the TPC quantification.

Determination of malondialdehyde and hydrogen peroxide content, and electrolyte leakage

The content of malondialdehyde (MDA) was determined according to a method as described by Senthilkumar *et al.* (2021), with some minor modifications. In brief, the leaf sample (ca. 50 mg) was homogenized with 2 mL of 0.1 % trichloracetic acid (TCA) and centrifuged at 12,000× g for 10 min. A mixture of 1 mL of the supernatant and 4 mL of 0.5 % thiobarbituric acid was mixed to react at 95 °C for 25 min, and the reacted mixture's absorbance at 532 and 600 nm was measured. The MDA content was determined with an extinction coefficient of 155 mmol L^{-1} cm^{-1}.

The content of hydrogen peroxide (H_2O_2) was determined as described by Alexieva *et al.* (2001), with some minor modifications. In brief, the leaf sample (ca. 50 mg) were homogenized in 2 mL of 0.1 % TCA on ice and centrifuged at 12,000× g, 4 °C for 15 min. A mixture of 0.5 mL of the supernatant, 0.5 mL of 10 mM potassium phosphate buffer, and 1 mL of 1 M KI was mixed to react for 1 h in darkness at room temperature, and the maximum absorbance at 350 nm was measured (Junglee *et al.* 2014). The content was calculated using a standard curve of concentrated H_2O_2 solutions.

The electrolyte leakage (EL) was measured according to the procedure as described by Sahin *et al.* (2018). In brief, ten leaf discs (ca. 10 mm in diameter) were detached from three plants in a pot and washed with deionized water. Then, the sample was incubated with 30 mL of freshly deionized water in darkness for 24 h at room temperature. After the incubation, the sample was heated to 95 °C for 20 min, and then cooled down to room temperature to determine EL.

Determination of ion content

The content of cations (Na^+, K^+) and anions (Cl^-, NO_3^-) were determined using the ion chromatography as reported by Tran *et al.* (2020). The dried leaf sample (ca. 50 mg) was finely ground and soaked in 10 mL of deionized water for 24 h at room temperature. The extract was filtered using a 0.45-µm syringe filter, and centrifuged at 18,000× g for 20 min to eliminate particles. The supernatant's ion concentration was determined using Dionex ICS-3000 ion chromatography system (USA). The ion concentration was calculated using a standard curve of concentrated ion solutions.

Assays for enzymatic activity of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD)

The crude leaf extracts were prepared according to a procedure as reported by Poli *et al.* (2018), with minor modifications. The frozen leaf sample (ca. 50 mg) was homogenized with 2 mL of an extraction buffer including 0.1 M sodium phosphate buffer (pH 7.5) added with 0.5 mM EDTA. The extract was centrifuged at 12,000× g for 15 min at 4ºC. The supernatant was used for assaying enzymatic activity.

The CAT activity was measured according to a procedure as reported by Poli *et al.* (2018). A mixture of the extract (0.05 mL), 0.1 M sodium phosphate buffer (pH 7.0) (1.5 mL), 30 mM H_2O_2 (0.5 mL), and distilled water (0.95 mL) was mixed to react at room temperature. Decrease of the reacting mixture's absorbance at 240 nm within 30 s was recorded. The CAT activity was expressed on the basic of the absorbance decrease (units) per time and sample weight (units s^{-1} g^{-1} FW).

The POD activity was assayed according to a method as described by Castillo *et al.* (1984). A mixture of the extract (0.1 mL), 60 mM sodium phosphate buffer (pH 6.1) (1 mL), 16 mM guaiacol (0.5 m L), 2 mM H_2O_2 (0.5 mL), and distilled water (0.9 mL) was mixed to react at room temperature. Increase of the reacting mixture's absorbance at 470 nm within 30 s was recorded. The POD activity was expressed on the basic of the absorbance increase (units) per time and sample weight (units s^{-1} g^{-1} FW).

The SOD activity was assayed using a procedure as described by Dhindsa *et al.* (1981). A mixture of the extract (0.1 mL), 100 mM sodium phosphate buffer (pH 7.8) (2.3 mL), 200 mM methionine (0.2 mL), 3 mM EDTA (0.2 mL), 2.25 mM nitroblue tetrazolium (NBT) (0.1 mL), and 60 µM riboflavin (0.1 mL) was mixed to react under the light condition for 15 min at room temperature. The reacted mixture's absorbance at 560 nm was measured. One SOD activity unit was defined as enzyme content inhibiting 50 % the photochemical reduction of NBT. The SOD activity was expressed in units g^{-1} FW.

Experimental design and statistical analysis

Each the treatment was repeated randomly with five pots (replicates). Data was expressed as means and standard deviations ($\alpha = 0.05$). The statistically significant difference between the treatments was determined according to Duncan's multiple range test with p-value ≤ 0.05. The statistical analyses were conducted using the R software (Rstudio Cloud version).

Results and Discussion

Effects of salinity on the growth characteristics

The data shown that the salt treatments had effects on the growth of *G. maderaspatana*. The plant tended to reduce its growth with increasing salinity levels, but it still grown with new young leaves and exhibited no signs of the death (Fig. 1). Both the FW and DW of the salt-treated plants gradually decreased with increasing salinity levels, excepting for the 50 mM NaCl-treated plants that their FW and DW increased by 1.20 and 1.19 times compared to the control, respectively (Fig. 2A, 2B). The plant's biomass started to be reduced by 100 mM NaCl, which their FW and DW decreased by 0.82 and 0.81 times compared to the control, respectively. A maximum reduction of biomass was observed in the 400 mM NaCl-treated plants that their FW and DW were 0.34 and 0.45 times compared to the control, respectively (Fig. 2A, 2B). It was reported that salinity seriously inhibits growth of salt-sensitive plants, even threatens their survival when soil salinity level is greater than 100 mM (Khan *et al.* 2022). Our study showed that *G. maderaspatana* could survive although its growth was significantly reduced by 400 mM NaCl. This trait indicated that the plant had an adaptability to high salinity, and it could be classified as a salt-tolerant plant (Khan *et al.* 2022). Similar adaptability was also reported for salt-tolerant plants such as *Amaranthus tricolor* (Kronzucker *et al.* 2013), *Portulaca* sp. (Borsai *et al.* 2020), and *Beta burgaris* (Yamada *et al.* 2016). Remarkably, *G. maderaspatana* significantly increased its biomass with 50 mM NaCl (Fig. 2A, 2B). This result suggested that salt promoted the plant's growth, which is usually found in halophytes (Flowers *et al.* 2015). With its salinity adaptability, *G. maderaspatana* would be a great potential to become a medicinal crop in saline agriculture. It is hypothesized that salt tolerance capacity of plants varies depending on its stage of life cycle (Van Zelm *et al.* 2020), and the salt stress may reduce their medicinal quality (Banerjee & Roychoudhury 2017). Thus, it is necessary to clarify these hypotheses for *G. maderaspatana* because of its application.

Figure 1. Effect of salinity on appearance of *G. maderaspatana* at 14 days after the onset of NaCl treatments. Bar = 2 cm.

Different parts of plants may have different responses in growth to salinity (Munns *et al.* 2016). To examine whether there was any difference in salinity tolerance between *G. maderaspatana* shoots and roots, changes in their biomass and length were also examined. The data showed that both the shoots and roots had similar trends in their biomass reduction (Fig. 2C–F). Under the 50 mM NaCl treatment, the shoot's FW and DW increased by 1.19 times compared to the control, but decreased by 0.82–0.35 times and 0.82–0.45 times with the higher salinity levels, respectively (Fig. 2C, 2D). Also, the root's FW and DW increased by 1.17–1.20 times under the 50 mM NaCl treatment compared to the control, and decreased by 0.81–0.30 times and 0.84–0.52 times with the higher salinity levels, respectively (Fig. 2E, 2F). However, under the 400 mM NaCl condition, both the root's DW and shoot's DW decreased by 0.45–0.52 times, but their FW reduced only 0.30–0.35 times compared to the control (Fig. 2C–F). This result indicated that the plant's dry matter was less affected by this salinity level than their fresh biomass. The plant might reduce water uptake and suffer an osmotic stress (Safdar *et al.* 2019). Moreover, our data also shown that the shoot's length and root's length decreased in similar pattern to their biomass, except for the roots under the 50 mM NaCl condition (Fig. 2G, 2H). Under this saline condition, the root's biomass increased (Fig. 2E, 2F), but its length remained unchanged (Fig. 2H). It could be explained by an increase in number of the roots (Fig. 1) although it was difficult to exactly measure this parameter due to the hairy roots.

Effects of salinity on photosynthetic pigments

The data shown that the salinity had effects on the contents of chlorophylls and carotenoids (Fig. 3). The chl (*a*+*b*) content increased in the plants under the 50–200 mM NaCl treatments, by 1.10–1.11 times compared to the control; meanwhile, it was unchanged in the plants treated with 400 mM NaCl (Fig. 3C). A similar pattern was observed for the chl *a* content with higher increases (1.01–1.16 times) (Fig. 3A). In contrast, the chl *b* content slightly reduced in the salt-treated plants, by 0.91–0.98 times compared to the control (Fig. 3B). On the other hand, the carotenoid content in the salt-treated plants increased by 1.10–1.18 times compared to the control (Fig. 3D).

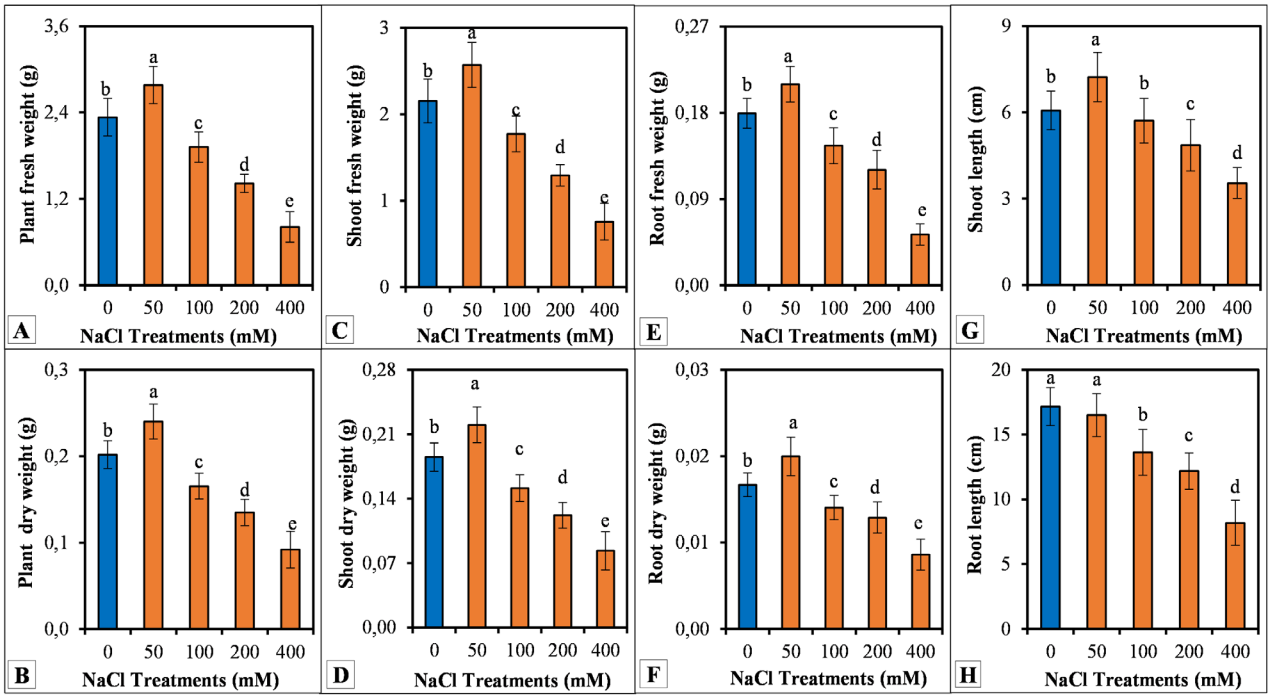

Figure 2. Effect of salinity on growth parameters of *G. maderaspatana* at 14 days after the onset of NaCl treatments. The plant fresh weight (A), plant dry weight (B), shoot fresh weight (C), shoot dry weight (D), root fresh weight (E), root dry weight (F), shoot length (G), and root length (H). Error bars indicate standard deviations.

Chlorophylls and carotenoids play vital roles in absorbing and converting solar energy into chemical energy in plant photosynthesis. As a result, any changes in their accumulation may affect photosynthetic activity of plants (Gong *et al.* 2018). It was reported that salinity can change the accumulation of photosynthetic pigments, depending on plant species and stress level (Sudhir & Murthy 2004; Hnilickova *et al.* 2021). Our study shown that the salinity also had effects on the accumulation of total chlorophylls and carotenoids in *G. maderaspatana* (Fig. 3). The contents of these pigments were significantly increased in the plants treated with 50–200 mM NaCl, and it was maintained with 400 mM NaCl (Fig. 3C, 3D). Many previous studies reported reduction in chlorophyll content of salt-sensitive plants under salt stress, such as *Triticum aestivum* (Saddiq *et al.* 2021), *Phaseolus vulgaris* (Taïbi *et al.* 2016), *Capsicum annuum* (Taffouo *et al.* 2017). However, increased accumulation was found in salt-tolerant plants, such as *Beta vulgaris* (Jamil *et al.* 2007), *Kalidium foliatum* (Gong *et al.* 2018), and *Oenanthe javanica* (Kumar *et al.* 2021). It was suggested that the increase of chlorophylls could be due to an increased number of photosystems in cells (Rabhi *et al.* 2012), and the chlorophyll accumulation is required to enhance energy production for salt tolerance although the mechanism is poorly understood (Gong et al. 2018; Van Zelm *et al.* 2020). These suggestions might also support for *G. maderaspatana*. Among the two assayed chlorophylls, chl *a* showed main contribution to total chlorophyll accumulation (Fig. 3A, 3B), suggesting that chl *a* had an important role in the plant's salt tolerance which could be explained by its prominent presence in photosystems (Gong *et al.* 2018). In addition to being a photon-absorbing pigments, carotenoids are antioxidants that can protect photosynthetic machinery from ROS (Sudhir & Murthy 2004). Due to these roles, increased accumulation of carotenoids was found in salt-tolerant plants under salt stress (Rabhi *et al.* 2012; Kumar *et al.* 2021). It might be due to its similar roles, *G. maderaspatana* increased accumulation of carotenoids under the salt stress (Fig. 3D). Remarkably, although it did not increase, the plant still maintained the accumulation of both pigment types under the 400 mM NaCl condition (Fig. 3C, 3D), an adaptive characteristic of many halophytes (Flowers & Colmer 2008). Together, the pigment accumulation suggested that *G. maderaspatana* might evolve photosynthetic mechanisms adapting to high salinity. Thus, further studies are necessary to examine photosynthetic activity and related factors under the salt stress for understanding the adaptive mechanisms.

Effects of salinity on water status and osmotic adjustment

Leaf RWC is considered as an indicator that reflects water status of plants under salinity (Kotagiri & Kolluru

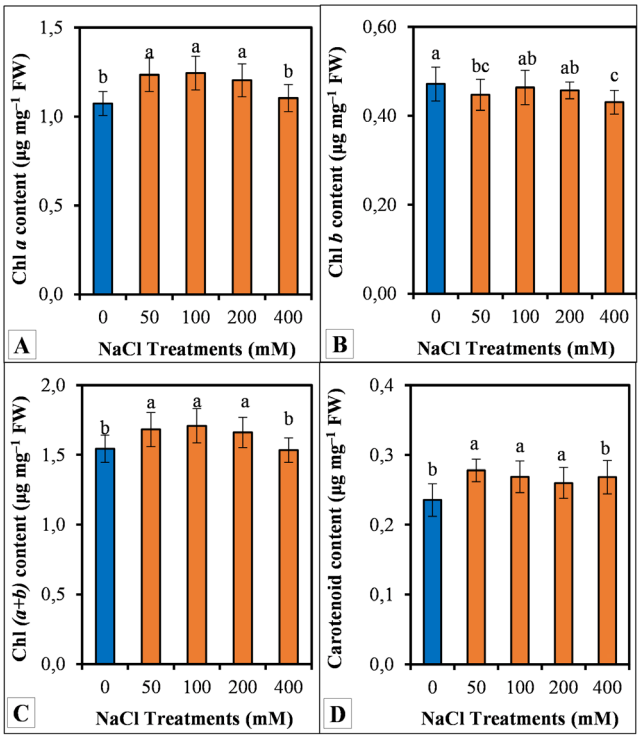

Figure 3. Effect of salinity on photosynthetic pigment contents of *Grangea maderaspatana*. The content of chl *a* (A), chl *b* (B), chl (*a*+*b*) (C), and carotenoids (D) at 7 days after the onset of NaCl treatments. Error bars indicate standard deviations.

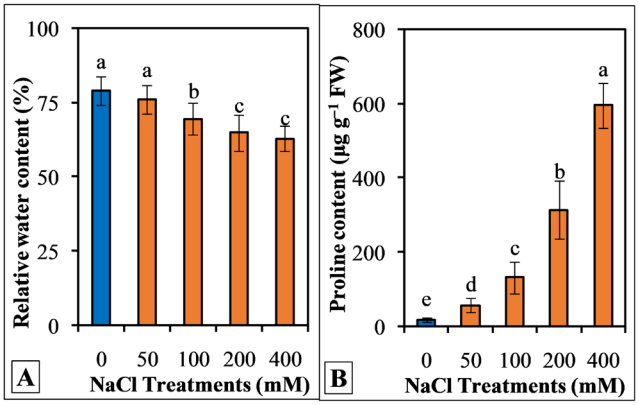

Figure 4. Effect of salinity on content of relative water (A) and proline (B) of *G. maderaspatana* at 7 days after the onset of NaCl treatments. Error bars indicate standard deviations.

2017). Our data shown that the plant treated with 50 mM NaCl did not change its RWC compared to the control, but it gradually decreased 9.28–16.02% with increasing salinity levels of 100–400 mM NaCl (Fig. 4A), indicating that the plant's water content was reduced by the salt stress. It could be explained that abundant accumulation of 100–400 mM NaCl in the soil decreased the soil water potential to the level that interfered the plant's water uptake. The plant might suffer osmotic stress under these saline conditions (Zhao *et al.* 2020). Notably, although the plant's water uptake decreased, the reduction was only 9.28–16.02% (Fig. 4A), suggesting adaptive mechanisms that maintain its water uptake.

It is found that plants have mechanisms of adjusting osmotic pressure in cells, which increases cell's water potential to retain water uptake. In salt-tolerant plants, the osmotic adjustment is achieved by enhanced accumulations of both compatible solutes and salt ions (Shabala & Shabala 2011; Munns *et al.* 2020). Glycine betaine, proline, polyamines, and soluble sugars are compatible osmolytes that are popularly utilized by various plants for the osmotic adjustment under salt stress (Flowers *et al.* 2015; Munns *et al.* 2020). In our study, the proline content in the salt-treated plants was significantly increased with increase of salinity levels, by 3.18–33.00 times compared to the control (Fig. 4B). This result suggested that *G. maderaspatana* triggered an osmotic adjustment, and proline was involved in this response. Proline was highly accumulated in the plants under the 400 mM NaCl condition. In addition to being an osmolyte, it was suggested that proline could also play a role as an osmoprotectant under the salt stress (Adolf *et al.* 2013).

Effects of salinity on ion accumulation

Accumulation of ions Na^+, K^+, Cl^-, and NO_3^- in plant cells was found to be affected by salinity (Shabala & Shabala 2011; Flowers *et al.* 2015). Our data also indicated effects of salinity on the accumulation of these ions in *G. maderaspatana* (Fig. 5A–F). The Cl^- content in the salt-treated plants gradually increased with increasing salinity levels, by 4.10–7.50 times compared to the control (Fig. 5A). Meanwhile, the NO_3^- content also increased by 2.65–2.80 times in the plants treated with 50–100 mM NaCl, but it was significantly unchanged with the higher salinity levels (Fig. 5B). Consequently, the NO_3^-/Cl^- content ratio in the salt-treated plants gradually decreased with increasing salinity levels, by 0.12–0.61 times compared to the control (Fig. 5C). The Na^+ content in the salt-treated plants increased in a similar pattern to the Cl^-, by 2.42–4.79 times compared to the control (Fig. 5D). Meanwhile, the K^+ content shown a similar trend to the NO_3^-. The K^+ content increased by 2.23–2.76 times in the plants treated with 50–100 mM NaCl, but unchanged with 200–400 mM NaCl (Fig. 5E). As a result, the K^+/Na^+ content ratio in the salt-treated plants decreased by 0.19–0.64 times compared to the control, except for an increase by 50 mM NaCl (Fig. 5F).

It is found that Na^+ and Cl^- passively pass through ion channels or transporters in plasma membrane if they present with a certain amount in soils, leading to their accumulation in plant cells (Shabala & Mackay 2011; Flowers

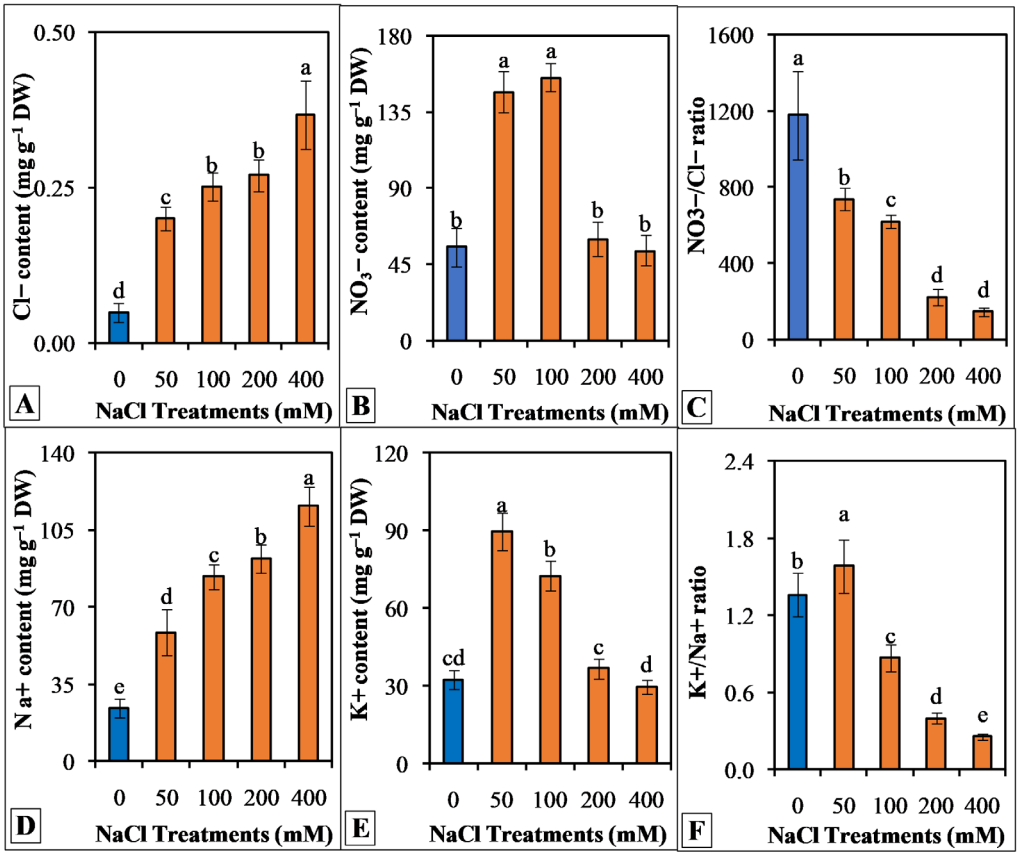

Figure 5. Effect of salinity on ion accumulation of *G. maderaspatana*. The content of Cl^- (A), NO_3^- (B), NO_3^-/Cl^- ratio (C), Na^+ (D), K^+ (E), and K^+/Na^+ ratio (F) at 7 days after the onset of NaCl treatments. Error bars indicate standard deviations.

et al. 2015). The enhanced accumulation of these ions was observed for salt-tolerant plants grown under salinity (Amiri *et al.* 2010; Ashrafi *et al.* 2018; Hnilickova *et al.* 2021). Our study indicated that *G. maderaspatana* also increased the Na^+ and Cl^- accumulations with increasing salinity levels (Fig. 5A, 5D). This result suggested that the plant might suffer the ionic stress and operate adaptive mechanisms at cellular level to withstand the effects of ions. It was explained that salt-tolerant plants have a tolerance to certain level of the salt accumulation in the cytosol. If the cytosolic accumulation is excessive, the salt will be sequestered into the vacuoles to avoid its effects and contribute to the osmotic adjustment (Flowers *et al.* 2015). Similar mechanisms might be involved in the salt tolerance of *G. maderaspatana*, and it is needed to elucidate by further studies.

It is reported that the salt accumulation can interfere the absorption of K^+ and NO_3^- by plants due to their antagonistic competition for membrane channels/transporters. The K^+ and NO_3^- effluxes are also induced to balance membrane potential. These events lead to the decrease of K^+ and NO_3^- in plants (Amiri *et al.* 2010; Shabala & Mackay 2011). However, our study indicated that the K^+ and NO_3^- accumulations of *G. maderaspatana* were not decreased by the salinity, even significantly increased with 50–100 mM NaCl (Fig. 5B, 5E). Similarly, the retention of K^+ and NO_3^- accumulation was observed for salt-tolerant plants, such as *Chenopodium quinoa* (Adolf *et al.* 2013) and *Beta vulgaris* (Kaburagi *et al.* 2014). The enhanced accumulations even occurred in halophytes, such as *Salicornia bigelovii* (Yamada *et al.* 2016) and *Mesembryanthemum crystallinum* (Tran *et al.* 2020). Due to important roles of K^+ and NO_3^- in growth of plants, their retention of accumulation might be an important response required for salt tolerance of *G. maderaspatana*. We propose that further studies are needed to clarify how these plants act to eliminate the antagonistic competition and membrane potential imbalance under salt stress.

Plants need to maintain an optimal ratio of the antagonistic ions for its growth under salt stress (Adolf *et al.* 2013). Previous studies reported that the K^+/Na^+ and NO_3^-/Cl^- ratios were reduced in salt-tolerant plants grown under salt stress (Amiri *et al.* 2010; Hniličková *et al.* 2019; Kumar *et al.* 2021). Also, our study indicated that *G. maderaspatana* decreased its K^+/Na^+ and NO_3^-/Cl^- ratios (Fig. 5C, 5F). These changes suggested that the plant might have ion homeostasis mechanisms for its salt tolerance.

Effects of salinity on oxidative stress and antioxidant activity

The data shown that the MDA content in the salt-treated plants was gradually increased with increasing salinity levels, by 1.13–1.30 times compared to the control (Fig. 6A). Similarly, the H_2O_2 content increased to 1.08–1.13 times with 100–400 mM NaCl, but unchanged with 50 mM NaCl (Fig. 6B). On the other hand, the plants gradually increased its EL with increasing salinity levels, by 1.65–4.91 times compared to the control (Fig. 6C).

The MDA, H_2O_2, and EL accumulation are considered as useful indicators to identify salt-induced oxidative stress and examine its effect on membrane integrity in plants under salt stress (Sarker & Oba 2020). In this study, the salt stress increased the H_2O_2 accumulation in *G. maderaspatana* (Fig. 6B), suggesting that the plant might suffer the oxidative stress. It was reported that salt-tolerant plants are still exposed to oxidative stress despite they have a high ability to control salt-induced ROS formation (Zhao *et al.* 2020). H_2O_2 acts as a signaling molecule in plant cells, but its excessive accumulation can injure membrane structures through lipid peroxidation (Zhao *et al.* 2020; Alharbi *et al.* 2022). Here, the increased accumulations of MDA were found in the salt-stressed plants (Fig. 6A), indicating that the H_2O_2 accumulation also induced membrane injuries in the plant. Notably, the MDA content was also increased in the plants that positively grown with 50 mM NaCl (Fig. 6A), suggesting the lipid peroxidation might not affect the growth of the plant under this saline condition. Previous reports suggested that salt-tolerant plants exhibit less lipid peroxidation than salt-sensitive plants due to their high membrane stability and ROS scavenging capacity (Kumar *et al.* 2021). However, *G. maderaspatana* increased its EL with increasing salinity levels (Fig. 6C), indicating that the plant's membrane integrity was reduced by the salinity (Demidchik *et al.* 2014; Kiani *et al.* 2021).

Maintaining an optimal level of ROS accumulation is required for salt tolerance in salt-tolerant plants, and it can be achieved by non enzymatic and/or enzymatic antioxidant systems (Zhao *et al.* 2020). Our data indicated that the enzymatic activities of POD, SOD, and CAT were increased different levels by the salinity (Fig. 7A–C). The SOD activity in the salt-treated plants was unchanged by 50 mM NaCl, but it was increased by 100–400 mM NaCl with the increase of 0.30–0.41 times compared to the control (Fig. 7A). Similarly, the POD activity increased (0.06–1.01 times) with increasing salinity levels (Fig. 7B). Meanwhile, the CAT activity was increased (0.07–0.23 times) with increasing salinity levels of 50–100 mM NaCl, but it was unchanged by 400 mM NaCl (Fig. 7C).

The enhancement of POD, SOD, and CAT activities are reported to be involved in scavenging salt-induced ROS (Liang *et al.* 2018). The Mehler reaction in the photosystem I and over-reduction of ubiquinone pools allows energy-high electrons moving to O_2 to form superoxide anion ($O_2^{\bullet -}$). The SOD plays a role in converting $O_2^{\bullet -}$ to H_2O_2, while both CAT and POD are required for the conversion of H_2O_2 to O_2 (Zhao *et al.* 2020). Thus, the increased activity of these enzymes might play important roles for reducing the ROS accumulation in *G. maderaspatana* (Fig. 6B). Similar roles of POD, SOD, and CAT were found in many salt-tolerant plants, such as *Chenopodium quinoa* (Adolf *et al.* 2013) and *Portulaca* sp. (Borsai *et al.* 2020). On the other hand, antioxidative metabolites are also effective factors contributing to the salt-induced ROS scavenge. It was reported that phenolic compounds have high capacity in reducing the salt-induced ROS formation (Adolf *et al.* 2013; Kiani *et al.* 2021). However, in our study the phenolic compounds were only increased by 400 mM NaCl (Fig. 7D), suggesting that they might have role in the ROS scavenge when *G. maderaspatana* was under the stressful condition. In addition to the antioxidants tested, the plants could utilize other factors which needs to be identified to better understand the plant's control for the oxidative stress.

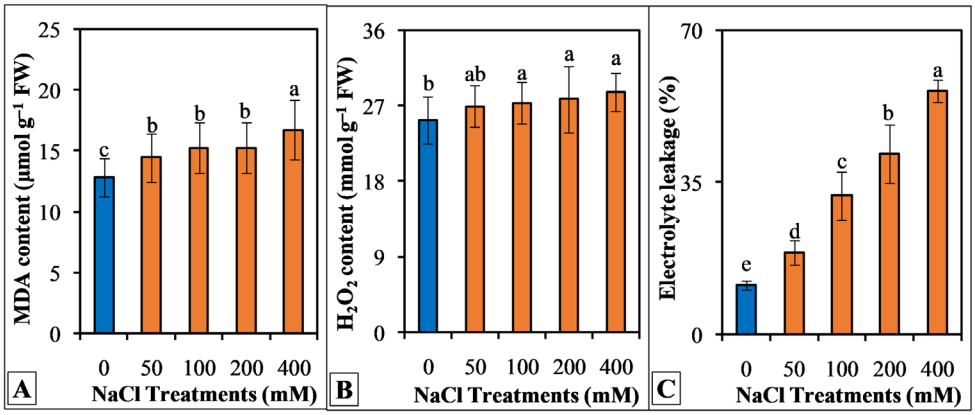

Figure 6. Effect of salinity on accumulation of oxidative stress markers in *G. maderaspatana*. The content of MDA (A), H_2O_2 (B), and electrolyte leakage (C) at 7 days after the onset of NaCl treatments. Error bars indicate standard deviations.

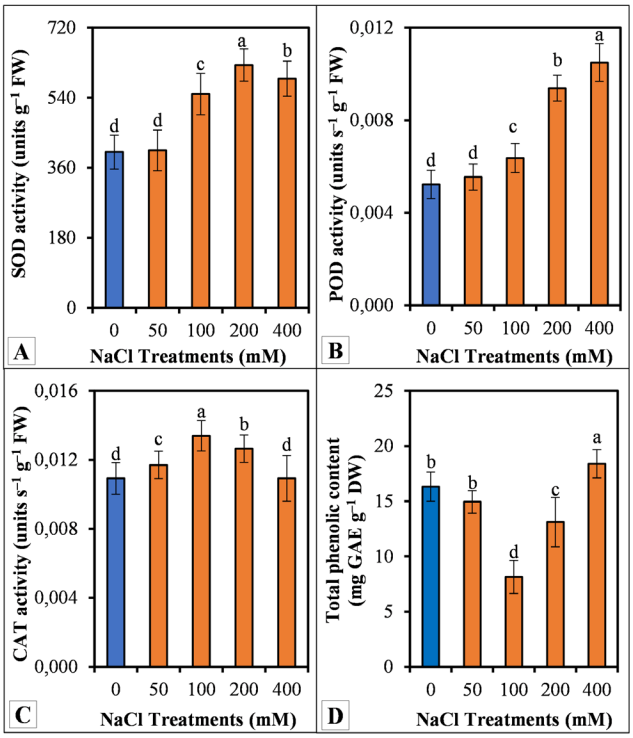

Figure 7. Effect of salinity on enzymatic antioxidant activities of *G. maderaspatana*. The SOD (A), POD (B), CAT (C) activity, and total phenolic content (D) at 7 days after the onset of NaCl treatments. Error bars indicate standard deviations.

Conclusions

For the first time, the present study evidenced that *G. maderaspatana* had a high salt tolerance capacity, and it could be considered as a salt-tolerant plant. The plant could grow and survive under the examined saline conditions, even obtained a promoted growth under 50 mM NaCl. The plant tended to gradually reduce its growth with increasing salinity levels of 100–400 mM NaCl. Under these stress conditions, the plant might suffer osmotic stress, ion imbalance, and oxidative stress, which was indicated by decreased RWC and increased accumulation of salt ions, MDA, H_2O_2, and EL. To reduce the salt-induced stresses, the plant highly accumulated proline, maintained the uptake of K^+ and NO_3^-, and enhanced the antioxidant activities of POD, CAT, and SOD. Notably, the salt stress did not reduce the accumulation of total chlorophylls (*a*+*b*) and carotenoids in the plant, even these pigments were increased under moderate saline conditions. Together, these adaptive responses revealed evolved tolerance mechanisms that are associated with photosynthesis, ion homeostasis, osmotic adjustment, and antioxidant defense. The findings are useful information for future works to elucidate the mechanisms and apply this medicinal plant to saline agriculture.

Acknowledgement

This study was funded by The Ministry of Education and Training (Vietnam) via a research project (code: B2021-DNA-10). The authors thank those who supported us during the experimental works and the manuscript preparation.

Author contributions

DQT, TCV, ACP, TGTT, HDV, TTB, and GTH conceived, designed and performed the experiments. DQT, SMM, and ACP analyzed the data and prepared the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest

References

Adolf VI, Jacobsen S-E, Shabala S. 2013. Salt tolerance mechanisms in quinoa (*Chenopodium quinoa* Willd.). Environmental and Experimental Botany 92: 43-54.

Ahmed M, Islam MM, Hossain CF, Khan OF. 2001. A preliminary study on the analgesic activity of *Grangea maderaspatana*. Fitoterapia 72: 553-554.

Alexieva V, Sergiev I, Mapelli S, Karanov E. 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell & Environment 24: 1337-1344.

Alharbi K, Al-Osaimi AA, Alghamdi BA. 2022. Sodium chloride (NaCl)-induced physiological alteration and oxidative stress generation in *Pisum sativum* (L.): A toxicity assessment. ACS Omega 7: 20819-20832.

Amiri B, Assareh M, Rasouli B, Jafari M, Arzani H, Jafari A. 2010. Effect of salinity on growth, ion content and water status of glasswort (*Salicornia herbacea* L.). Caspian Journal of Environmental Sciences 8: 79-87.

Aruna B, Kumar VS. 2020. Neuropharmacological exploration of *Grangea maderaspatana* (L.). Poir. High Technology Letters 26: 119-130.

Ashrafi E, Razmjoo J, Zahedi M. 2018. Effect of salt stress on growth and ion accumulation of alfalfa (*Medicago sativa* L.) cultivars. Journal of Plant Nutrition 41: 818-831.

Banerjee A, Roychoudhury A. 2017. Effect of salinity stress on growth and physiology of medicinal plants. In: Ghorbanpour M, Varma A (eds.). Medicinal plants and environmental challenges. Cham, Springer. p. 177-188.

Bates LS, Waldren Ra, Teare I. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil 39: 205-207.

Borsai O, Hassan MA, Negrușier C *et al.* 2020. Responses to salt stress in Portulaca: Insight into its tolerance mechanisms. Plants 9: 1660.

Burkill HM. 1995. The useful plants of west tropical Africa. 2nd. edn. Kew, Royal Botanic Gardens. vol. 1.

Castillo FJ, Penel C, Greppin H. 1984. Peroxidase release induced by ozone in *Sedum album* leaves: involvement of Ca^{2+}. Plant Physiology 74: 846-851.

Chaudhary N, Chauhan J, Maitreya B. 2023. Phytochemical analysis and antioxidant activity of *Grangea maderaspatana* (L) Poir. International Association of Biologicals and Computational Digest 2: 100-107.

Demidchik V, Straltsova D, Medvedev SS, Pozhvanov GA, Sokolik A, Yurin V. 2014. Stress-induced electrolyte leakage: The role of K^+-permeable channels and involvement in programmed cell death and metabolic adjustment. Journal of Experimental Botany 65: 1259-1270.

Dhindsa RS, Plumb-Dhindsa P, Thorpe TA. 1981. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. Journal of Experimental Botany 32: 93-101.

Dodiya T, Jain V. 2017. Pharmacognostic, physicochemical and phytochemical investigation of *Grangea maderaspatana*. The Journal of Phytopharmacology 6: 359-363.

Flowers TJ, Munns R, Colmer TD. 2015. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. Annals of Botany 115: 419-431.

Galani VJ. 2015. *Grangea maderaspatana* (L.) Poir. – A comprehensive review. Innoriginal: International Journal of Sciences 2: 4-8.

Garcia-Caparros P, Al-Azzawi MJ, Flowers TJ. 2023. Economic uses of salt-tolerant plants. Plants 12: 2669.

Gong D, Wang G, Si W, Zhou Y, Liu Z, Jia J. 2018. Effects of salt stress on photosynthetic pigments and activity of ribulose-1, 5-bisphosphate carboxylase/oxygenase in *Kalidium foliatum*. Russian Journal of Plant Physiology 65: 98-103.

González L, González-Vilar M. 2001. Determination of relative water content. In: Reigosa Roger MJ (eds.). Handbook of plant ecophysiology techniques. Netherlands, Kluwer Academic Publishers. p. 207-212.

Hniličková H, Hnilička F, Orsák M, Hejnák V. 2019. Effect of salt stress on growth, electrolyte leakage, Na^+ and K^+ content in selected plant species. Plant, Soil and Environment 65: 90-96.

Hnilickova H, Kraus K, Vachova P, Hnilicka F. 2021. Salinity stress affects photosynthesis, malondialdehyde formation, and proline content in *Portulaca oleracea* L. Plants 10: 845.

Hopmans JW, Qureshi A, Kisekka I *et al.* 2021. Critical knowledge gaps and research priorities in global soil salinity. Advances in Agronomy 169: 1-191.

Jain S, Sareen V, Narula A. 1993. Oestrogenic and pregnancy interceptory efficacy of a flavonoid mixture from *Grangea maderaspatana* Poir (*Artemisia maderaspatana*) in the mouse. Phytotherapy Research 7: 381-383.

Jamil M, Rehman S, Rha E. 2007. Salinity effect on plant growth, PSII photochemistry and chlorophyll content in sugar beet (*Beta vulgaris* L.) and cabbage (*Brassica oleracea* capitata L.). Pakistan Journal of Botany 39: 753-760.

Junglee S, Urban L, Sallanon H, Lopez-Lauri F. 2014. Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide. American Journal of Analytical Chemistry 5: 730.

Kaburagi E, Morikawa Y, Yamada M, Fujiyama H. 2014. Sodium enhances nitrate uptake in Swiss chard (*Beta vulgaris* var. cicla L.). Soil Science and Plant Nutrition 60: 651-658.

Khan MO, Irfan M, Muhammad A *et al.* 2022. A practical and economical strategy to mitigate salinity stress through seed priming. Frontiers in Environmental Science 16: 13.

Kiani R, Arzani A, Mirmohammady MS. 2021. Polyphenols, flavonoids, and antioxidant activity involved in salt tolerance in wheat, *Aegilops cylindrica* and their amphidiploids. Frontiers in Plant Science 12: 646221.

Kotagiri D, Kolluru VC. 2017. Effect of salinity stress on the morphology and physiology of five different *Coleus* species. Biomedical and Pharmacology Journal 10: 1639-1649.

Kronzucker HJ, Coskun D, Schulze LM, Wong JR, Britto DT. 2013. Sodium as nutrient and toxicant. Plant and Soil 369: 1-23.

Kumar S, Li G, Yang J *et al.* 2021. Effect of salt stress on growth, physiological parameters, and ionic concentration of water dropwort (*Oenanthe javanica*) cultivars. Frontiers in Plant Science 12: 660409.

Liang W, Ma X, Wan P, Liu L. 2018. Plant salt-tolerance mechanism: A review. Biochemical and Biophysical Research Communications 495: 286-291.

Munns R, James RA, Gilliham M, Flowers TJ, Colmer TD. 2016. Tissue tolerance: An essential but elusive trait for salt-tolerant crops. Functional Plant Biology 43: 1103-1113.

Munns R, Passioura JB, Colmer TD, Byrt CS. 2020. Osmotic adjustment and energy limitations to plant growth in saline soil. New Phytologist 225: 1091-1096.

Panta S, Flowers T, Lane P, Doyle R, Haros G, Shabala S. 2014. Halophyte agriculture: Success stories. Environmental And Experimental Botany 107: 71-83.

Poli Y, Nallamothu V, Balakrishnan D *et al.* 2018. Increased catalase activity and maintenance of photosystem II distinguishes high-yield mutants from low-yield mutants of rice var. Nagina22 under low-phosphorus stress. Frontiers in Plant Science 9: 1543.

Rabhi M, Castagna A, Remorini D *et al.* 2012. Photosynthetic responses to salinity in two obligate halophytes: *Sesuvium portulacastrum* and *Tecticornia indica*. South African Journal of Botany 79: 39-47.

Rachchh RP, Galani VJ. 2015. Evaluation of antinociceptive and antirheumatic activity of *Grangea maderaspatana* (L.) Poir. using experimental models. Ayu 36: 425-431.

Ruangrungsi N, Kasiwong S, Likhitwitayawuid K, Lange GL, Decicco CP. 1989. Constituents of *Grangea maderaspatana*. A new eudesmanolide. Journal of Natural Products 52: 130-134.

Saddiq MS, Iqbal S, Hafeez MB *et al.* 2021. Effect of salinity stress on physiological changes in winter and spring wheat. Agronomy 11: 1193.

Safdar H, Amin A, Shafiq Y *et al.* 2019. A review: Impact of salinity on plant growth. Natural Science 17: 34-40.

Sahin U, Ekinci M, Ors S, Turan M, Yildiz S, Yildirim E. 2018. Effects of individual and combined effects of salinity and drought on physiological, nutritional and biochemical properties of cabbage (Brassica oleracea var. capitata). Scientia Horticulturae 240: 196-204.

Sarker U, Oba S. 2020. The response of salinity stress-induced A. tricolor to growth, anatomy, physiology, non-enzymatic and enzymatic antioxidants. Frontiers in Plant Science 11: 559876.

Senthilkumar M, Amaresan N, Sankaranarayanan A, Senthilkumar M, Amaresan N, Sankaranarayanan A. 2021. Estimation of malondialdehyde (MDA) by thiobarbituric acid (TBA) assay. In: Senthilkumar M, Amaresan N, Sankaranarayanan A (eds.). Plant-microbe interactions. New York, Humana Press. p. 103-105.

Shabala S, Mackay A. 2011. Ion transport in halophytes. In: Turkan I (eds.). Advances in botanical research. Amsterdam, Academic Press. p. 151-199.

Shabala S, Shabala L. 2011. Ion transport and osmotic adjustment in plants and bacteria. Biomolecular Concepts 2: 407-419.

Singh D, Mathela C, Pande V, Panwar A. 2013. Antioxidant and antimicrobial activity of *Grangea maderaspatana* (L.) Poir. Journal of Drug Discovery and Therapeutics 1: 46-52.

Sudhir P, Murthy S. 2004. Effects of salt stress on basic processes of photosynthesis. Photosynthetica 42: 481-486.

Taffouo VD, Nouck AE, Nyemene KP, Tonfack B, Meguekam TL, Youmbi E. 2017. Effects of salt stress on plant growth, nutrient partitioning, chlorophyll content, leaf relative water content, accumulation of osmolytes and antioxidant compounds in pepper (*Capsicum annuum* L.) cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 45: 481-490.

Taïbi K, Taïbi F, Abderrahim LA, Ennajah A, Belkhodja M, Mulet JM. 2016. Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L. South African Journal of Botany 105: 306-312.

Tran DQ, Konishi A, Cushman JC, Morokuma M, Toyota M, Agarie S. 2020. Ion accumulation and expression of ion homeostasis-related genes associated with halophilism, NaCl-promoted growth in a halophyte *Mesembryanthemum crystallinum* L. Plant Production Science 23: 91-102.

Tran DQ, Pham AC. 2023. Responses in growth, photosynthesis, and water uptake of a halophyte (*Tetragonia tetragonoides*) under halophilism condition. The University of Danang–Journal of Science and Technology 21: 107-112.

Van Zelm E, Zhang Y, Testerink C. 2020. Salt tolerance mechanisms of plants. Annual Review of Plant Biology 71: 403-433.

Wellburn AR. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology 144: 307-313.

Yamada M, Kuroda C, Fujiyama H. 2016. Function of sodium and potassium in growth of sodium-loving Amaranthaceae species. Soil Science and Plant Nutrition 62: 20-26.

Zhao C, Zhang H, Song C, Zhu J-K, Shabala S. 2020. Mechanisms of plant responses and adaptation to soil salinity. The Innovation 1: 100017.