


Negative effects of long-term exposure to salinity, drought, and combined stresses on halophyte *Halogeton glomeratus*

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Abstract

Plants are subjected to salt and drought stresses concurrently but our knowledge about the effects of combined stress on plants is limited, especially on halophytes. We aim to study if some diverse drought and salt tolerance traits in halophyte may explain their tolerance to salinity and drought stresses, individual and in combination, and identify key traits that influence growth under such stress conditions. Here, the halophyte *Halogeton glomeratus* was grown under control, single or combinations of 60 days drought and salt treatments, and morphophysiological responses were tested. Our results showed that drought, salinity, and combination of these two stressors decreased plant growth (shoot height, root length, and biomass), leaf photosynthetic pigments content (chlorophyll *a*, *b*, *a* + *b* and carotenoids), gas exchange parameters (Net photosynthesis rate [P_N], transpiration rate [E], stomatal conductance [g_s]), and water potential (ψ_w), and the decreases were more prominent under combined drought and salinity treatment compared with these two stressors individually performed. Similarly, combined drought and salinity treatment induced more severe oxidative stress as indicated by more hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) accumulated. Nevertheless, *H. glomeratus* is equipped with specific mechanisms to protect itself against drought and salt stresses, including upregulation of superoxide dismutases (SOD; EC 1.15.1.1) and catalase (CAT; EC 1.11.1.6) activities and accumulation of osmoprotectants (Na^+ , Cl^- , and soluble sugar). Our results indicated that photosynthetic pigments content, gas exchange parameters, water potential, APX activity, CAT activity, soluble sugar, H_2O_2 , and MDA are valuable screening criteria for drought and salt, alone or combined, and provide the tolerant assessment of *H. glomeratus*.

1 | INTRODUCTION

Soil salinity and drought are regarded as the most critical and adverse environmental factors for plants, leading to enormous losses in agricultural production worldwide and definitely influencing the distribution of wild species (Raza et al., 2019; Zhou et al., 2019). In arid and semi-arid regions, the evaporation and upward water extraction by plant roots lead to the solution's concentration in the soil, which raises the salt level in the near-surface soil. Thus, soil salinity routinely overlaps with drought, and both of these two harsh environmental factors

influence plant growth and productivity together (Alam et al., 2021; Bai et al., 2019). In China, saline soils cover an area of 99 million ha and almost 70% is in arid and semi-arid lands (Zhao et al., 2013). Salinity becomes the major restriction on agriculture, particularly in oasis irrigation regions of Northwestern China. Generally, phytoremediation is considered an economical solution to decrease salinity, which can be defined as the cultivation of plants accumulating high level of salts. (Hasanuzzaman et al., 2014). Recent reports have suggested that the cultivation of halophytes can efficiently reduce soil salinity and is an effective way to reclaim saline soils in arid and semi-arid lands (Wang

et al., 2021). Therefore, to have a successful phytoremediation of saline soils in arid and semi-arid lands, a detailed study of the mechanisms adopted by halophytes in the adaptation to drought and salt stresses is a high priority.

In nature, plants are usually concurrently subjected to environmental abiotic stress, such as salinity, drought, high temperature, and ultraviolet radiations, throughout the growing season (Maaloul et al., 2021; Pandey et al., 2017; Suzuki et al., 2014). However, the interaction of drought (water deficit) and salinity is a common coexisting constraint that has been neglected (Stavridou et al., 2019). Several studies have reported that drought could aggravate the adverse influences of salinity through disturbing photosynthesis and nutrient uptake, resulting in further growth inhibition (Alam et al., 2021; Álvarez & Sánchez-Blanco, 2015). On the contrary, other studies indicated that combining low salinity with drought could alleviate the adverse effects of drought alone on growth, photosynthesis, and water deficit (Hussain et al., 2020). Both salinity and drought induce osmotic stress on plants, restricting their capacity to take up water and therefore reducing tissue turgor (Al-Yasi et al., 2020; Méndez-Alonzo et al., 2016). Additionally, salt stress can exert ion stress through accumulating a large amount of Na^+ and Cl^- (Panda et al., 2019; Wiszniewska et al., 2021). Hence, the physiological responses of plants exposed to salinity and drought can be similar and dissimilar. For example, plants tend to accumulate K^+ under drought stress to achieve osmotic adjustment (Jdey et al., 2014; Lü et al., 2019). However, excessive Na^+ in the growing medium results in a reduction in K^+ content in plants, because both K^+ and Na^+ employ the same transporter (Nounjan et al., 2018). Nevertheless, both salinity and drought stresses can exert oxidative stress in plants, causing a number of changes, such as inhibition of plant growth, photosynthesis, and essential nutrients absorption from soils (Alam et al., 2021; Jamshidi Goharrizi, Amiramhani, & Salehi, 2020). A decrease in photosynthetic pigments content and increase in peroxidation of membrane lipids are the main reasons for plant growth inhibition induced by overproduction of highly reactive oxygen species (ROS), such as hydroxyl radical ($\text{HO}\cdot$), superoxide radical ($\text{O}_2^{\cdot-}$), and hydrogen peroxide (H_2O_2 ; Hossain et al., 2015; Muhammad et al., 2021). ROS can be scavenged by enzymes, including superoxide dismutases (SOD; EC 1.15.1.1), catalases (CAT; EC 1.11.1.6), guaiacol peroxidase (POD; EC 1.11.1.7), and ascorbate peroxidase (APX; EC 1.11.1.11; Blokhina et al., 2003; Mittler, 2002).

Halogeton glomeratus (Chenopodiaceae) is a succulent annual halophyte and is one of the most widely distributed halophytes in Central Asia and arid regions in northwestern China (Wang et al., 2015). It is an ecologically important annual forb on the gravel deserts and alluvial fan. It is mainly distributed between 700 and 800 m a.s.l., but it can be found at 2300 m a.s.l. on the north slopes of the Kunlun Mountains, Xinjiang (Sun et al., 2008). *H. glomeratus* often forms monodominant communities on the Qira gravel desert on the southern edge of the Taklamakan. Soils in this area contain a high level of salinity and plants growing here need to be adapted to high salinity and extreme aridity. *H. glomeratus* is a native plant species in this area and plays an important role in sand stabilization (Zhao & Zhang, 2003). It

was reported that seeds of *H. glomeratus* can germinate at high NaCl concentration (800 mM), and non-germinated seeds in the NaCl pre-treatment solution can germinate after transfer to distilled water (Lu et al., 2012). However, little is reported about the adaptive mechanisms of tolerance to combined salt and drought stress in this halophyte at seedling stage. In this study, we were interested in evaluating the effects of salinity and drought stresses, both individually and combined, on (1) seedling growth, (2) photosynthetic parameters, (3) oxidative stress parameters and antioxidative enzymes activity, (4) water status and compatible solutes content, and (4) ions uptake of *H. glomeratus*. We aimed to analyze the relationships among all tested morphophysiological parameters and assess which parameters might be considered as effective screening criteria for the improvement and selection of salinity and drought tolerance, alone or in combination. Consequently, the results from this research will be very useful for the scientific validation of *H. glomeratus* for phytoremediation purposes.

2 | MATERIALS AND METHODS

2.1 | Plant materials and experimental treatments

Mature seeds were collected in October, from plants of *H. glomeratus* distributing in a desert-oasis ectone near the Cele Research Station of Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences located in the Cele oasis (80°03'24"–82°10'34"E; 35°17'55"–39°30'00" N; 1340–1380 m a.s.l.). Large amounts of seeds were sampled from 20 randomly distributed plants, and the seeds were finally pooled. The mean mass of six groups of 1000 seeds each was 334.7 ± 6.8 mg (means \pm SE).

In mid-April, the seeds were evenly planted in round plastic pots (22 cm diameter and 15 cm depth) filled with 2 kg soil. The soil came from the natural habitat where *H. glomeratus* population grew (sampling depth of 0–20 cm) and passed through a sieve of 2 mm. The soil was homogeneous loose eolian sediments with the field capacity of $18.51 \pm 0.58\%$. Soil physico-chemical parameters had been shown by Lu et al. (2017). The seedlings were grown in a greenhouse. The daily photoperiod, light intensity, day/night temperature, and air relative humidity were 14 h day^{-1} , $1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$, $28 \pm 2.2^\circ\text{C}/20 \pm 1.8^\circ\text{C}$, and 35%–45%, respectively. Seedlings were regularly watered with 380 ml purified water every 3 days. When seedlings had grown for 30 days, seedlings were thinned to maintain 10 uniform plants per plot, and seedlings were exposed to salinity and drought treatments.

In mid-May, we carried out different levels of salinity and drought stresses: For salinity stress, 0, 200, or 400 mM NaCl were applied gradually in aliquots of 100 mM NaCl solution every 3 days until the set concentration was reached to avoid saline shock. Then, all salinity-treated pots were irrigated with different quantities of purified water (100%, 70%, and 40% of field capacity). Pots were weighted every 3 days between 19:00 and 21:00 h, and were irrigated to the designed level. After 60 days of different irrigation treatments, pots

were divided into three levels of drought stress, including 100%, 70%, and 40% of field capacity. As a result of the experiment, we studied the alone and combined effects of salinity and drought stresses in the form of nine levels, as follows: (1) 100% of field capacity + 0 mM NaCl (control), (2) 100% of field capacity + 200 mM NaCl, (3) 100% of field capacity + 400 mM NaCl, (4) 70% of field capacity + 0 mM NaCl, (5) 70% of field capacity + 200 mM NaCl, (6) 70% of field capacity + 400 mM NaCl, (7) 40% of field capacity + 0 mM NaCl, (8) 40% of field capacity + 200 mM NaCl, and (9) 40% of field capacity + 400 mM NaCl. The experiment was performed as a completely randomized experimental design. Each treatment had eight pots, among them four pots for plant biomass determination and four pots for plant physiological and biochemical parameters measurement. Plants were ready for analysis after 100 days from seed emergence, including 60 days of stress exposure.

2.2 | Biomass and ions content

After 60 days of combined salinity and drought stresses treatment, whole plants were harvested. Shoot height and root length were measured by a measuring scale. After quick washing with distilled water, plants were gently dried on blotting paper, shoots and roots were separated immediately, and were individually weighed for fresh mass (FM). Shoots and roots materials were dried in an oven at 75°C for 48 h, and then weighed for dry mass (DM). Then the dried leaves, stems, and roots were ashed in a muffle furnace at 550°C for 24 h. The ash (0.1 g) was digested overnight with 25 ml of 0.1 M HNO₃ (Sibole et al., 2003). Concentrations of K⁺ and Na⁺ were measured by an inductively coupled plasma-optical emission spectrometer (ICP-OES, Agilent 735), and the Cl⁻ content was detected using the same acid extract by a chloride meter (Jenway PC LM3).

2.3 | Photosynthetic pigments and gas exchange characteristics

After 60 days of combined salinity and drought stresses treatment, the photosynthetic pigments were extracted with 80% acetone. The clear supernatant fraction obtained after centrifugation at 480g for 3 min was employed for measuring chlorophyll using the extinction coefficients and equations redetermined by Lichtenthaler (1987).

Net photosynthesis rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO₂ concentration (C_i) were determined by a portable photosynthesis system (LI-6400, LI-CO) between 10:30 and 11:00 h. Four plants were chosen for each treatment in the following atmospheric conditions: PAR 1237 ± 89 μmol m⁻² s⁻¹, temperature 28.7 ± 1.7°C, CO₂ concentration 389.5 ± 5.3 μmol mol⁻¹, RH 38.8 ± 1.6%. After photosynthetic measurement, the leaf (irregularly shaped) was removed and scanned to estimate its surface area using the image analysis system Delta-T Scan (Cambridge, CB50EJ). The gas exchange parameters were then re-calculated based on the actual effective photosynthetic area.

2.4 | Relative water content (RWC) and water potential (ψ_w)

After 60 days of combined salinity and drought stresses treatment, RWC was calculated according to Smart and Bingham (1974) and was obtained by the formula: $RWC = (FM - DM)/(TM - DM) \times 100\%$. Turgid mass (TM) was determined after immersing leaves in distilled water for 4 h at room temperature in darkness. Leaf ψ_w was detected at midday on freshly cut leaves by a WP4 Dewpoint Water Potential Meter (Decagon Devices, Inc.).

2.5 | Total soluble sugars and proline content

After 60 days of combined salinity and drought stresses treatment, the content of soluble sugars was measured according to the anthrone method (Palma et al., 2009). The proline content was measured according to the method of Bates et al. (1972) using a spectrophotometer (UV752 N, Shanghai Precision & Scientific Instrument Co.).

2.6 | MDA and H₂O₂ content

After 60 days of combined salinity and drought stresses treatment, the MDA content was determined according to the method of Kosugi and Kikugawa (1985). H₂O₂ concentration was determined by the method of Sergiev et al. (1997) using a spectrophotometer (UV752 N, Shanghai Precision & Scientific Instrument Co.).

2.7 | Enzyme extraction and assays

After 60 days of combined salinity and drought stresses treatment, the supernatant fraction adopted for determining soluble protein content and SOD, CAT, POD, and APX activities was acquired by the method of Lu et al. (2010). Enzyme activities were measured using a spectrophotometer (UV752 N, Shanghai Precision & Scientific Instrument Co.). SOD activity was measured according to the method of Beauchamp and Fridovich (1971). CAT activity was tested by detecting the consumption of H₂O₂ by the method of Aebi (1984). POD activity was obtained by the method of Chance and Maehly (1955). APX activity was measured in accordance with the method of Nakano and Asada (1981). Proteins were measured according to the method of Bradford (1976) with bovine serum albumin for the standard protein.

2.8 | Statistical analysis

Four independent experiments, each being a single plant, were carried out to evaluate the effects of salinity and drought stresses on selected physiological and biochemical indexes. Data presented here are

means \pm SD. Mean values were separated using Tukey's test at the 0.05 probability level. Analysis was performed with SPSS Inc., Ver. 18. Linear relationships between morphological, physiological and biochemical parameters were analyzed and plotted in R Studio.

3 | RESULTS

3.1 | Plant growth

Both individual and combined stresses had a significant impact on growth compared to control (Figure 1). Shoot height, FM, and DM, as well as root length, FM, and DM significantly decreased by 30.1%, 40.7%, 42.5%, 41.6%, 49.6%, and 50.3%, respectively, under 400 mM NaCl treatment without drought stress compared to plants grown under control level (Figure 1A–G). These six growth parameters also significantly decreased by 39.5%, 49.1%, 59.5%, 10.6%, 19.1%, and 29.9%, respectively, under 40% of field capacity without NaCl addition compared to plants grown under control level. However, combined drought and salinity stress aggravated the negative effect on

plant growth, and the six growth parameters decreased by 57.6%, 59.5%, 74.9%, 50.9%, 65.1%, and 67.2%, respectively, under 40% of field capacity with 400 mM NaCl treatment compared to control level. Furthermore, root/shoot ratio (FM and DM) increased gradually under enhanced drought stress without NaCl addition, and the maximum increase was observed at 40% of field capacity without NaCl treatment (Figure 1D,H). However, the ratio decreased gradually under increased salinity stress without drought stress.

3.2 | Ions uptake

The Na^+ and Cl^- contents in leaves, stems, and roots increased with aggravated drought and salinity stresses, individual or in combination (Figure 2). The maximum content of Na^+ and Cl^- was in plants grown under 40% of field capacity with 400 mM NaCl treatment; the Na^+ and Cl^- increased by 261.1% and 342.4% in leaves, by 545.2% and 271.6% in stems, and by 277.9% and 286.8% in roots compared to plants grown under control level (Figure 2A–H). However, K^+ content increased gradually under enhanced drought stress without NaCl

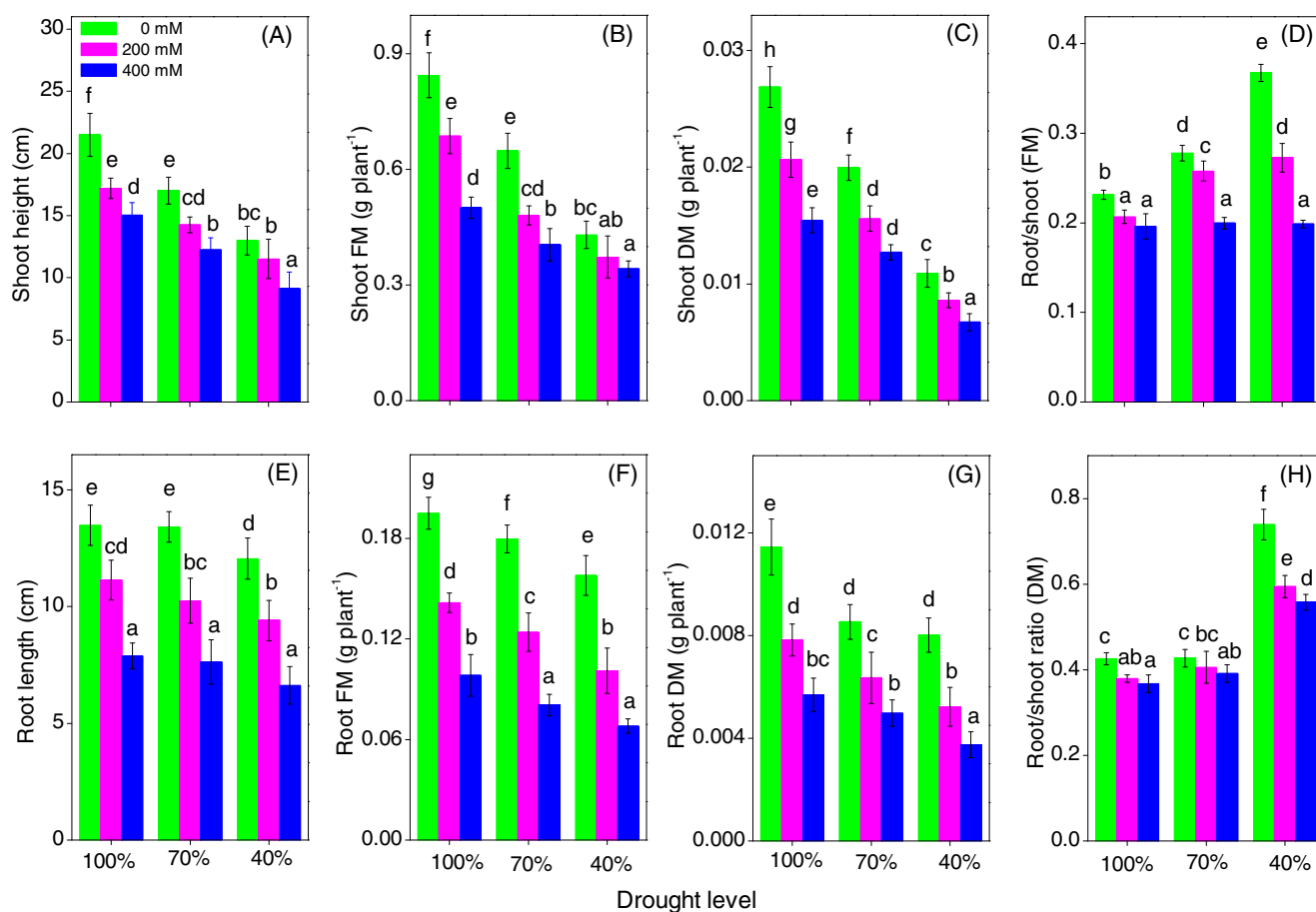


FIGURE 1 (A) Shoot height, (B) shoot fresh mass (FM), (C) shoot dry mass (DM), (D) fresh mass root/shoot ratio, (E) root length, (F) root fresh mass (FM), (G) root dry mass (DM) and (H) dry mass root/shoot ratio of *H. glomeratus* subjected to drought and salinity, individually and in combination. Data represent means \pm SD ($n = 4$ independent replicates, each being a single plant). Different letters above the error bars indicate significant difference between the treatments according to Tukey's test at a significant level of $P < 0.05$. Salinity treatments are as follows: 0, 200, or 400 mM NaCl. Drought levels are as follows: 100%, 70%, or 40% water field capacity

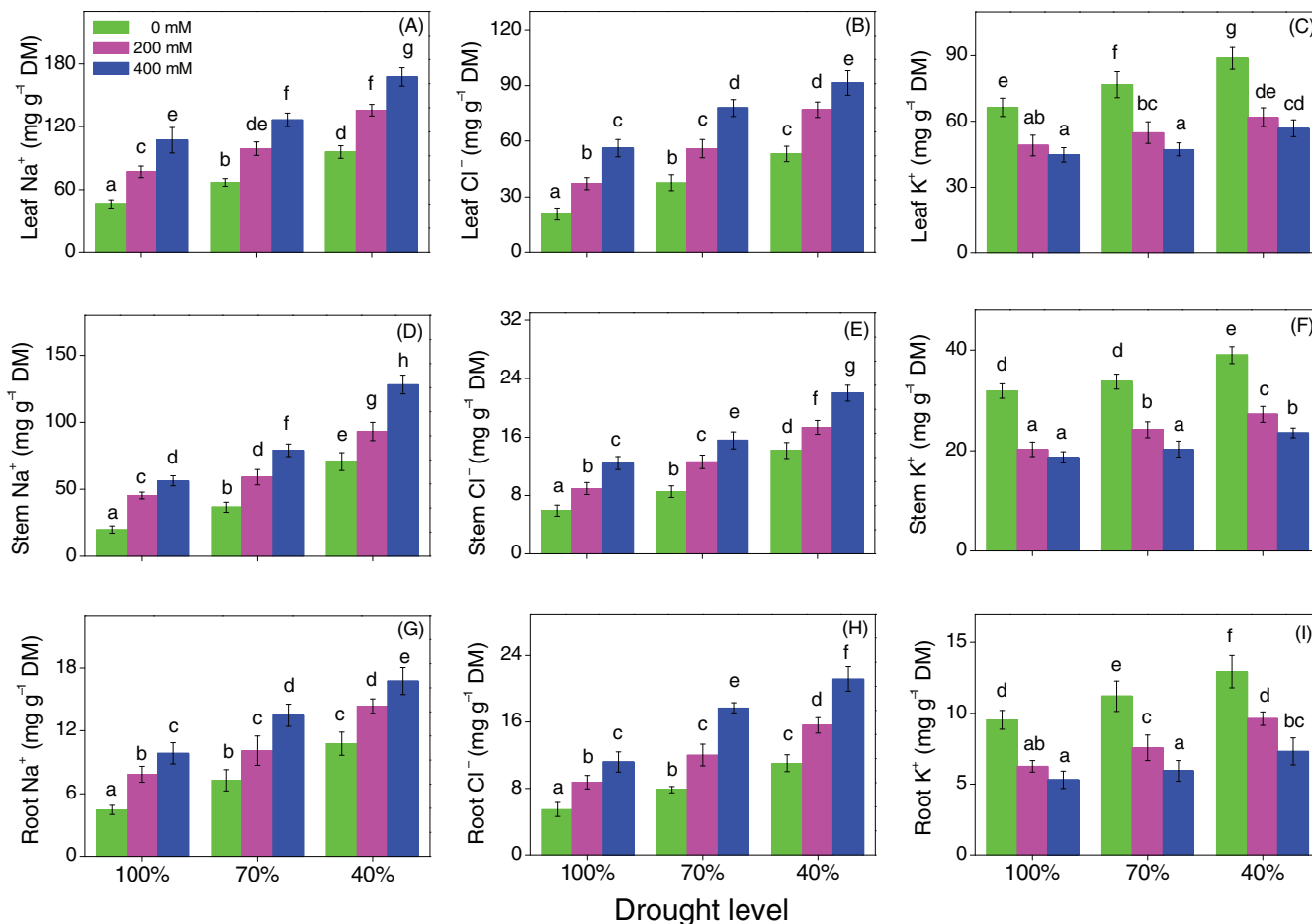


FIGURE 2 (A) Leaf Na⁺, (B) leaf Cl⁻, (C) leaf K⁺, (D) stem Na⁺, (E) stem Cl⁻, (F) stem K⁺, (G) root Na⁺, (H) root Cl⁻, and (I) root K⁺ of *H. glomeratus* affected by drought and salinity, individually and in combination. Data presented here are means ± SD (n = 4 independent replicates, each being a single plant). Different letters above the error bars indicate significant difference between the treatments according to Tukey's test at a significant level of P < 0.05. Salinity treatments are as follows: 0, 200, or 400 mM NaCl. Drought levels are as follows: 100%, 70%, or 40% water field capacity

addition, while it decreased gradually under elevated salinity stress without drought stress (Figure 2C,F,I). The maximum increase in K⁺ was measured in plants grown under 40% WHC without NaCl addition, and K⁺ increased by 33.7%, 22.5%, and 35.6% in leaves, stems, and roots, respectively, compared to control level. Maximum reduction of K⁺ was observed in plants grown in 100% of field capacity with 400 mM NaCl treatment, and K⁺ reduced by 32.8%, 41.4% and 44.3% in leaves, stems, and roots, respectively, compared to control level.

3.3 | Photosynthetic pigments and gas exchange characteristics

Total Chl content, carotenoids (Car) content, P_N, E, and g_s decreased by 14.9%, 14.7%, 31.8%, 26.3%, and 20.3%, respectively, in plants grown under 100% of field capacity with 400 mM NaCl treatment compared to plants grown under control level (Figures 3 and 4). However, the addition of drought stress aggravated the reduction of photosynthetic parameters, and the maximum reduction of total Chl, Car

content, P_N, E, and g_s was observed at 40% of field capacity with 400 mM NaCl treatment, these parameters were reduced up to 35.1%, 44.1%, 56.2%, 50.4%, and 45.5%, respectively, compared to plants grown in control condition. The C_i was not significantly affected across enhanced salt stress but significantly decreased when combined with drought stress. The maximum reduction of C_i was observed in plants grown under 40% of field capacity with 400 mM NaCl treatment.

3.4 | RWC, ψ_w, and compatible solutes content

Both individual and combined salinity and drought stresses significantly impacted RWC, ψ_w, and compatible solutes content (Figure 5). RWC increased gradually under enhanced salinity stress without drought stress, and the maximum increase was observed at 100% of field capacity with 400 mM NaCl treatment (Figure 5A). However, RWC decreased gradually under increased drought stress without NaCl addition, and the maximum reduction was measured at 40% of

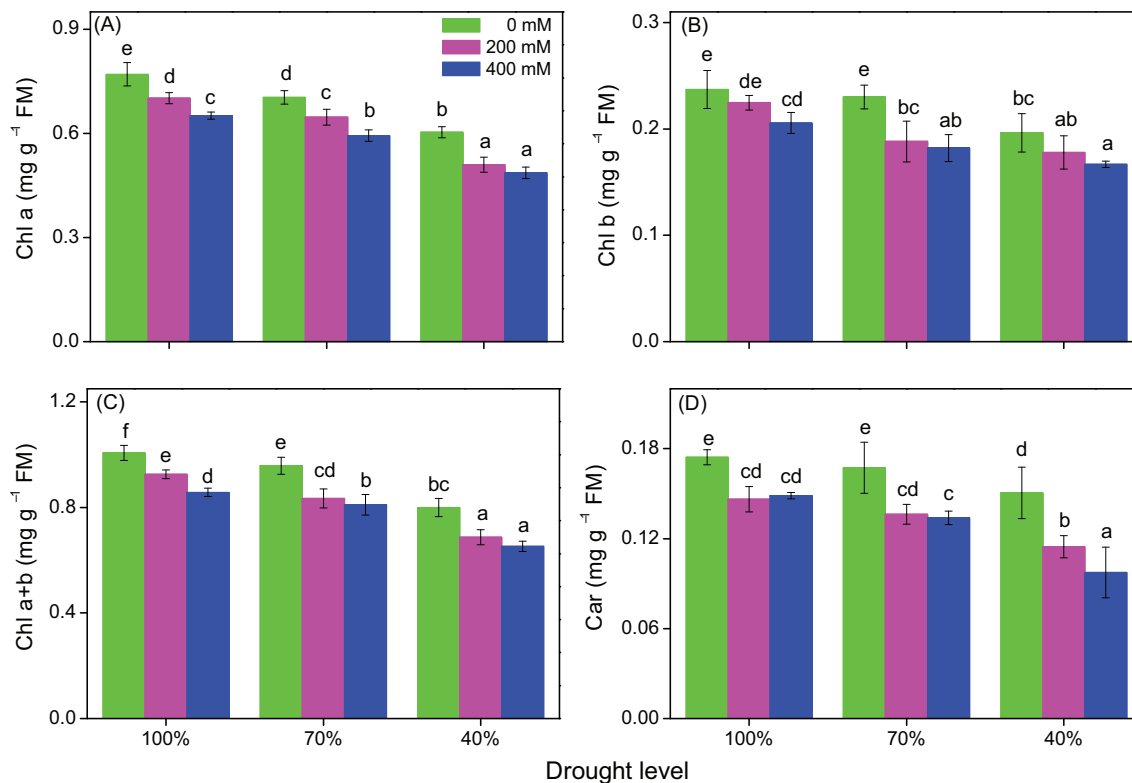


FIGURE 3 (A) Chlorophyll *a* (Chl *a*), (B) chlorophyll *b* (Chl *b*), (C) chlorophyll *a* + *b* (Chl *a* + *b*), and (D) carotenoids (Car) contents in leaves of *H. glomeratus* affected by drought and salinity, individually and in combination. Data presented here are means ± SD ($n = 4$ independent replicates, each being a single plant). Different letters above the error bars indicate significant difference between the treatments according to Tukey's test at a significant level of $P < 0.05$. Salinity treatments are as follows: 0, 200, or 400 mM NaCl. Drought levels are as follows: 100%, 70%, or 40% water field capacity

field capacity with 0 mM NaCl treatment. ψ_w decreased by 151.4% in plants grown under 40% of field capacity without NaCl addition. However, the addition of NaCl in soil reduced ψ_w up to 217.8% compared to plants grown in control condition (Figure 5B).

Proline content significantly increased with increased drought stress (Figure 5C). Soluble sugar content significantly increased by 21.5% in plants grown under 100% of field capacity with 400 mM NaCl but the addition of drought stress (40% of field capacity) increased soluble sugars up to 112.8% compared to plants grown at control level (Figure 5D).

3.5 | Oxidative stress parameters and antioxidative enzymes activity

H_2O_2 and MDA content significantly increased by 35.2% and 39.5% in plants grown under 100% of field capacity with 400 mM NaCl treatment compared to plants grown under control level (Figure 6A, B). However, the addition of drought stress aggravated the accumulation of H_2O_2 and MDA, and the maximum increase of H_2O_2 and MDA was observed at 40% of capacity with 400 mM NaCl treatment; both of them increased up to 94.2% and 112.7%, respectively, compared to plants grown in control condition.

SOD activity increased significantly at all levels of salinity treatment compared to control level (Figure 6C). SOD activity increased up to a drought level of 70% of field capacity, then began to reduce with a drought level of 40% of field capacity, but still was significantly higher than control level. CAT activity increased with enhanced drought level, and significantly increased at all levels of salinity compared to plants grown in control condition (Figure 6D). POD activity increased up to a drought level of 70% of field capacity, and then significantly reduced with a drought level of 40% of field capacity compared to control treatment (Figure 6E). APX activity decreased with an enhanced drought level, it only significantly increased under 100% of field capacity with 200 mM NaCl (Figure 6F).

3.6 | Correlation analysis

According to the correlation analysis, the growth parameters (shoot height, shoot FM, shoot DM, root length, root FM, and root DM) were significantly and positively correlated with the photosynthetic pigments content (Car and Chl content), gas exchange parameters (P_N , E , g_s , and C_i), water potential and APX activities. However, they were significantly and negatively correlated with CAT activities, oxidative stress parameter (H_2O_2 and MDA content), compatible solutes

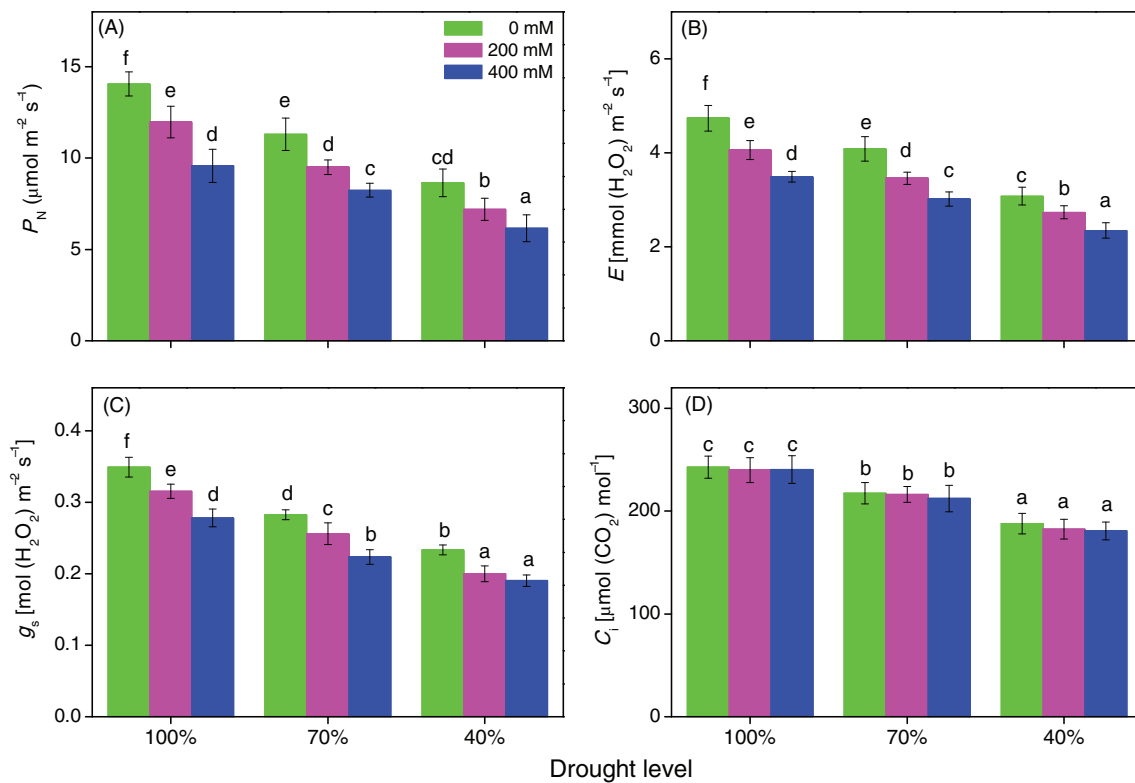


FIGURE 4 (A) Net photosynthesis rate (P_N), (B) transpiration rate (E), (C) stomatal conductance (g_s), and (D) intercellular CO_2 concentration (C_i) in leaves of *H. glomeratus* affected by drought and salinity, individually and in combination. Data presented here are means \pm SD ($n = 4$ independent replicates, each being a single plant). Different letters above the error bars indicate significant difference between the treatments according to Tukey's test at a significant level of $P < 0.05$. Salinity treatments are as follows: 0, 200, or 400 mM NaCl. Drought levels are as follows: 100%, 70%, or 40% water field capacity

(soluble sugar and proline content), and ions uptake (Na^+ and Cl^- ; Figure 7). The correlation analysis elucidated a significant connection between growth and different morphophysiological characteristics of *H. glomeratus*.

3.7 | Principal component analysis of growth and morphophysiological characteristics

The parameters showing a significant correlation in the above-mentioned correlation analysis were further subjected to a principal component analysis (PCA) (Figure 8). The first two components accounted for 98.95% of the total variation, and the first and second PCA accounted for 91.52% and 7.43% of the total variability, respectively. The nine drought-salinity treatments performed in this research were successfully scattered in the whole dataset, indicating that drought and salinity stresses cause a negative effect on the growth of *H. glomeratus*. Plant growth, photosynthetic pigments, gas exchange parameters, water potential, and APX activities were grouped together and positively correlated with each other. Compatible solutes, oxidative stress parameter, CAT activities, and ions uptake (Na^+ and Cl^-) were grouped together and positively correlated with each other.

4 | DISCUSSION

Drought and salinity often occurred simultaneously in nature, especially in arid and semi-arid regions. In this study, the changes in morphological, physiological, and biochemical responses of *H. glomeratus* were studied under individual and combined drought and salinity stresses. *H. glomeratus* exhibited a more severe response under combined stresses than under individual stress. This finding is in accordance with previous studies relating that to high salinity combined with severe drought negatively influenced several physiological processes (Alam et al., 2021; Jamshidi Goharrizi, Amiramhani, & Salehi, 2020; Stavridou et al., 2019).

4.1 | Combined drought and salt stress had the highest impact on growth and the changes in root/shoot ration differed between drought and salt stress

Inhibition of growth is one of the first and most common responses to stress, as plants rearrange their energy from primary metabolism and biomass accumulation to the activation of stress responses (Munns & Tester, 2008). In this study, the growth of *H. glomeratus* significantly reduced upon drought (from 70% of field capacity) and salt (200 mM

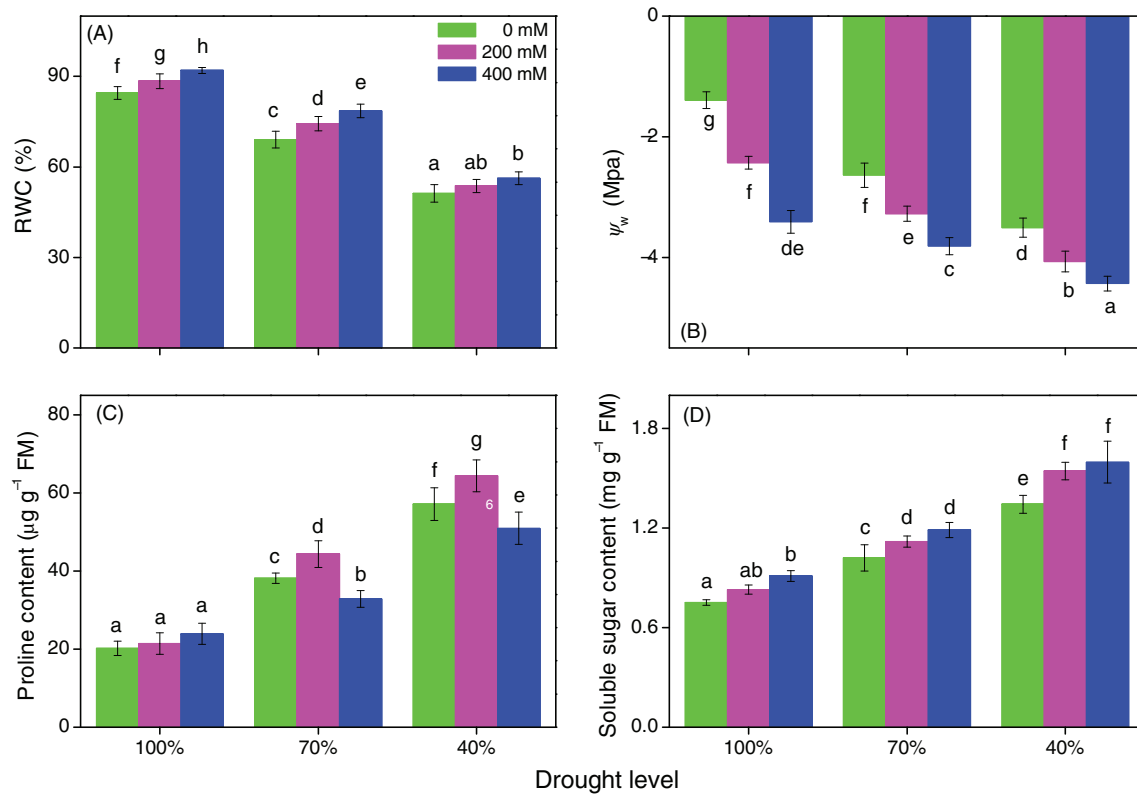


FIGURE 5 (A) Relative water content (RWC), (B) water potential (ψ_w), (C) proline content, and (D) soluble sugar content in leaves of *H. glomeratus* affected by drought and salinity, individual and in combination. Data presented here are means \pm SD ($n = 4$ independent replicates, each being a single plant). Different letters above the error bars indicate significant difference between the treatments according to Tukey's test at a significant level of $P < 0.05$. Salinity treatments are as follows: 0, 200, or 400 mM NaCl. Drought levels are as follows: 100%, 70%, or 40% water field capacity

NaCl) stresses, but the effect of combined stress on growth inhibition is more significant than the single stress. Exposure of plants to combined salinity and drought stress usually leads to a remarkable growth decrease in halophytes, such as *Sarcocornia quinqueflora* and *Sesuvium portulacastrum* (Ahmed et al., 2021; Slama, M'Rabet, et al., 2015). Furthermore, root/shoot ratio reacted differently to drought and salinity stress. While the root/shoot ratio increased with water stress, it decreased with increased salinity stress. Water deprivation results in a reduction in shoot growth, whereas root growth is often maintained and roots elongation often occurs, ultimately leading to an enhanced root/shoot ratio. This could be explained by the capacity of roots to transform their architecture and allocation of resources to "find" more water (Bogeat-Triboulot et al., 2007; Brunner et al., 2015; Hasibeder et al., 2015; Ma et al., 2020). In contrast, long-term salt stress decreased root growth to minimize the entry of toxic ions (Na^+ and Cl^-), ultimately resulting in a reduced root/shoot ratio. Similar results were reported in the halophytes *Atriplex prostrata*, *Plantago coronopus*, *Suaeda glauca*, and *Suaeda salsa* in container-culture study (Bueno et al., 2020; Li, Hussain, et al., 2019). Therefore, decrease and increase of root/shoot ratio were previously found to be an environmental-dependent response in *H. glomeratus*.

4.2 | Contribution of inorganic ions to osmotic adjustment in *H. glomeratus* under drought and salt stress, alone or in combination

Osmotic adjustment is one of the major physiological strategy adopted by plants subjected to osmotic stress. Plants react to reduce the tissue water potential to promote continued water influx and turgor maintenance. The osmotic adjustment could be accomplished by the accumulation of various osmotica, including inorganic ions as well as compatible solutes (Bai et al., 2019; Cui et al., 2020; Slama, Abdelly, et al., 2015). Na^+ toxicity is one of the main factors restricting plant growth exposed to saline environment as it leads to inhibition of photosynthesis and attenuation of metabolic activity (Kronzucker et al., 2013). For most glycophytes and halophytes, a quite high concentration of NaCl is required for optimum growth (Munns & Tester, 2008). The Na^+ tolerance of halophytes is highly related to their capacity to exclude, transport and/or mobilize Na^+ , which they mainly accomplish by extruding Na^+ to the outside of the cell and/or compartmentalize it into the vacuole to decrease its accumulation in the cytoplasm (Munns & Tester, 2008). The intracellular sequestration of Na^+ in particular part of the plant not only relieves the negative

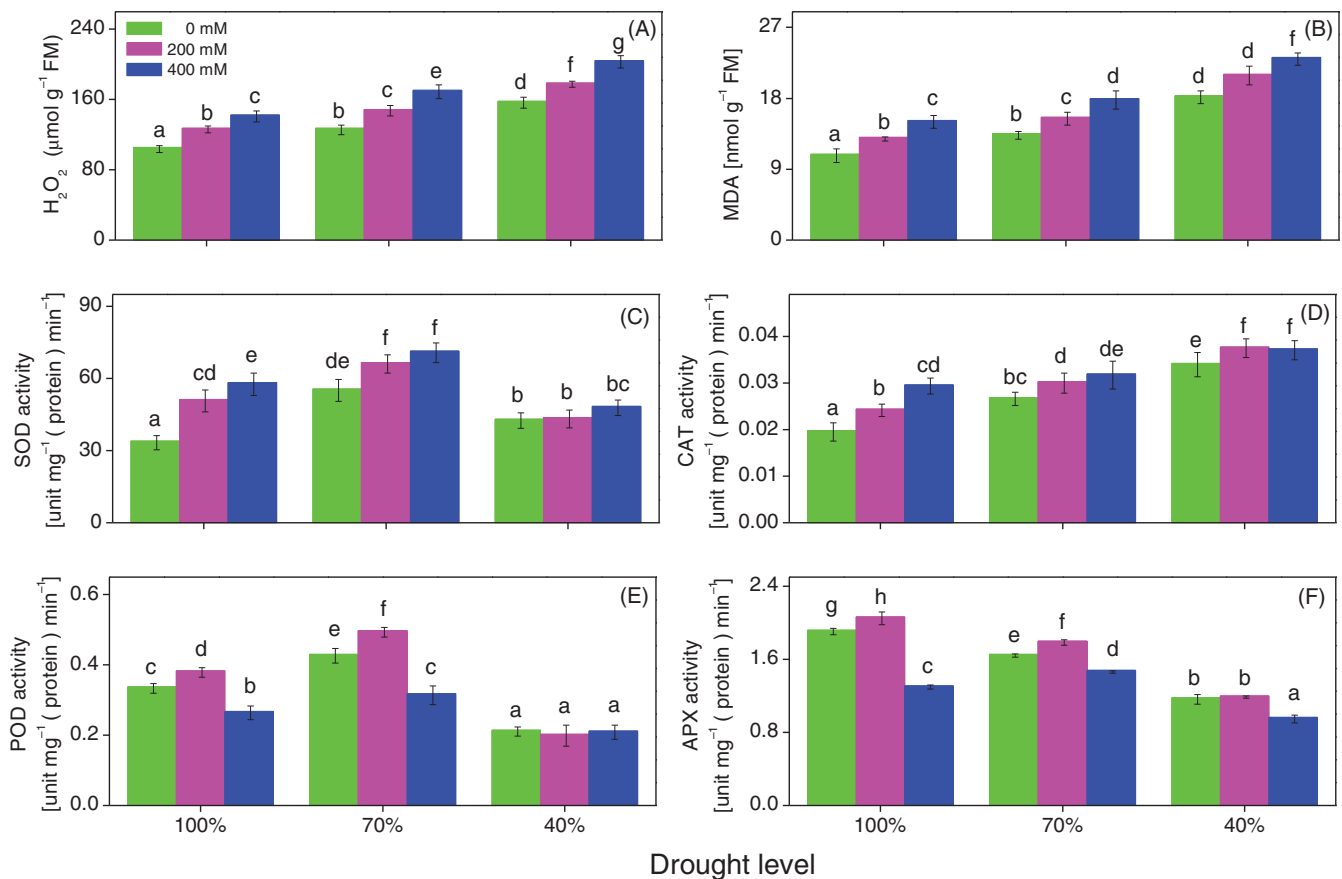


FIGURE 6 (A) Hydrogen peroxide (H₂O₂) content, (B) malondialdehyde (MDA) content, (C) superoxide dismutases (SOD) activity, (D) catalases (CAT) activity, (E) guaiacol peroxidase (POD) activity, and (F) ascorbate peroxidase (APX) activity in leaves of *H. glomeratus* affected by drought and salinity, individually and in combination. Data presented here are means \pm SD ($n = 4$ independent replicates, each being a single plant). Different letters above the error bars indicate significant difference between the treatments according to Tukey's test at a significant level of $P < 0.05$. Salinity treatments are as follows: 0, 200, or 400 mM NaCl. Drought levels are as follows: 100%, 70%, or 40% water field capacity

influence of excess Na⁺ in the cytoplasm, but also maintains the osmotic balance within cells (Munns & Tester, 2008). For instance, in some succulent halophytes subjected to drought and salinity stress, a large amount of Na⁺ can be measured in succulent leaves/assimilating branches, which adjust the osmotic potential and hence contribute to osmotic adjustment (Lü et al., 2019; Ma et al., 2012). In this study, the concentration of Na⁺ in all organs of *H. glomeratus* seedlings increased significantly with enhanced osmotic stress induced by drought, salinity, and combined stresses, and the ions accumulated mostly in the succulent leaves, as reported in many other succulent halophytes (Ahmed et al., 2021; Lu et al., 2021). It had been reported that *H. glomeratus* accumulated huge quantities of sodium in water-storage tissue in leaves to maintain the tissue water content and the succulence of leaves in response to salt stress (Wang et al., 2015). *H. glomeratus* can potentially hyperaccumulate not only salts but also heavy metals, therefore it is a promising candidate for phytoremediation of salt-affected and heavy metal-polluted soils (Li, Wang, et al., 2019).

K⁺ is participating in various metabolic processes in plants, such as in the promotion of protein synthesis and photosynthesis (Gierth & Mäser, 2007). The compartmentalization of K⁺ into vacuoles also a key

role in osmotic adjustment in plants (Gierth & Mäser, 2007; Tang et al., 2015). Usually, loss of K⁺ is usually accompanied by a gradual increase in Na⁺ when plants are under salinity stress (Bueno et al., 2020; Rahman et al., 2019; Wang et al., 2015). In this study, K⁺ reduction was accompanied by increased Na⁺ after salinity stress and combined stress. However, in *H. glomeratus*, K⁺ increase was accompanied by increased Na⁺ after drought stress. Therefore, K⁺ in *H. glomeratus* seedlings may play a crucial role in dealing with the osmotic stress induced by drought, whereas Na⁺ could be a more efficient and effective regulator in coping with the osmotic stress induced by salinity and combined stress.

Cl⁻ is considered an inorganic osmoticum for plants because it promotes the turgor-driven stomatal opening and improvement of leaf turgor pressure (Franco-Navarro et al., 2016, 2019). Besides, Na⁺ and K⁺ sequestration into the vacuole is accompanied by an elevated accumulation of anionic solutes such as Cl⁻ and NO₃⁻ to promote osmotic adjustment and balance the positive electrical charge in the vacuole (Franco-Navarro et al., 2016; Munns & Tester, 2008). In our study, Cl⁻ increased was accompanied by increased Na⁺ and K⁺ upon drought stress, and Cl⁻ increased was accompanied by increased Na⁺ after salinity and combined stress. It

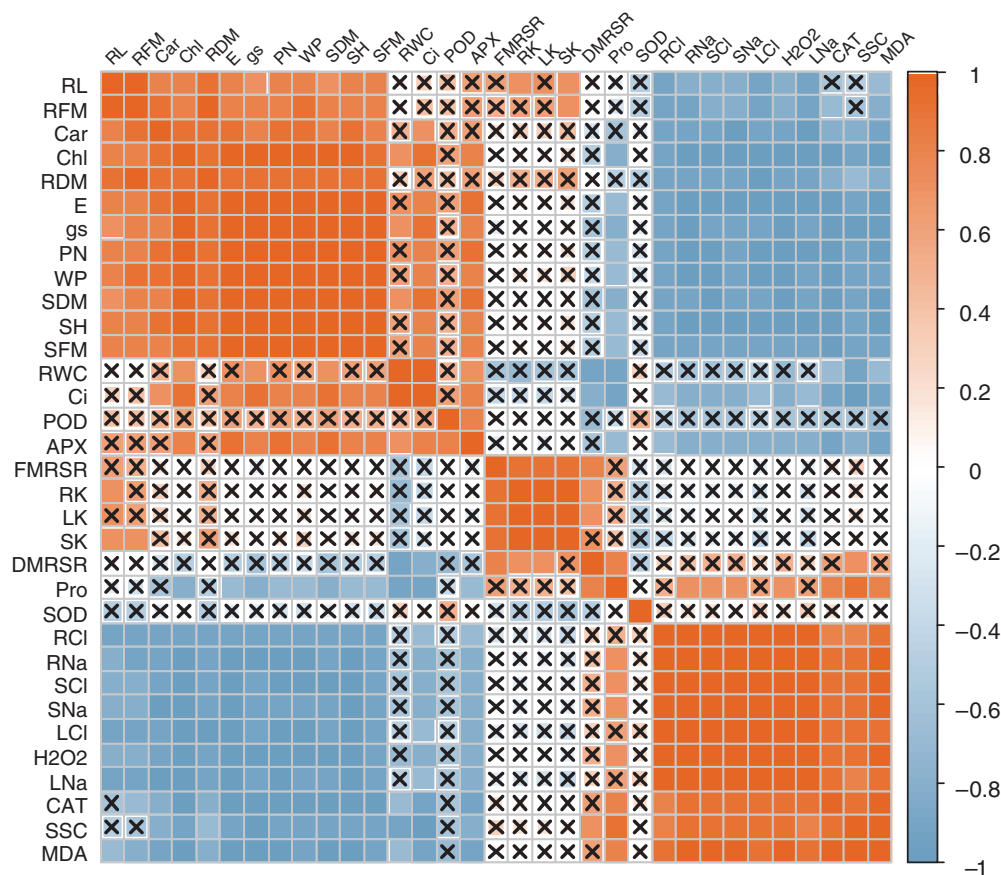


FIGURE 7 Correlation matrix among various morphophysiological parameters of *H. glomeratus* subjected to drought, salinity, and combined stresses. Symbol (×) indicates no significant correlation among the tested morphophysiological parameters. APX, ascorbate peroxidase; Car, carotenoids; CAT, catalase; Chl, chlorophyll *a* + *b*; Ci, intercellular CO₂ concentration; DMRSR, dry mass root/shoot ratio; E, transpiration rate; FMRSR, fresh mass root/shoot ratio; gs, stomatal conductance; H₂O₂, hydrogen peroxide; LCI, leaf Cl⁻; LK, leaf K⁺; LNa, leaf Na⁺; MDA, malondialdehyde content; RCI, root Cl⁻; RDM, root dry mass; RFM, root fresh mass; RK, root K⁺; RL, root length; RNa, root Na⁺; PN, net photosynthesis rate; POD, guaiacol peroxidase; Pro, proline; RWC, relative water content; SCI, shoot Cl⁻; SDM, shoot dry mass; SFM, shoot fresh mass; SH, shoot height; SK, shoot K⁺; SNa, shoot Na⁺; SOD, superoxide dismutases; SSC, soluble sugar content; WP, water potential

had been reported that Cl⁻ contributed to the growth of the xerophytic desert plant *Pugionium cornutum* by promoting the osmotic adjustment capability under salinity and drought stresses (Cui et al., 2020).

4.3 | Photosynthetic pigments content and gas exchange parameters were reduced in *H. glomeratus* under drought and salt stress, alone or in combination

Photosynthetic pigments content and gas exchange parameters were reduced under single and combined drought and salinity stresses in this study. Drought is one of the harshest abiotic factors inhibiting photosynthesis and thereby restricting plant growth and yield (Alam et al., 2021; Panda et al., 2021). Drought stress limits the efficiency of the photosynthetic apparatus, resulting in substantial damage to the thylakoid membranes, and decrease in photosynthetic pigments (Velikova et al., 2016; Wang et al., 2019). To ensure survival under

such an extreme environment, plants employ their own defense system, such as reduced transpiration rate by closing stomata (Rangani et al., 2018; Xi et al., 2018; Yang et al., 2021). Furthermore, intake of CO₂ can be disrupted by stomatal closure, which disturbs enzymatic activities, causing membrane damages and decreases in ATP synthesis and RuBP regeneration, thereby reducing the RUBISCO activity and inhibiting photosynthesis (Sharma et al., 2020). By contrast, salinity stress inhibits photosynthesis by the ionic (Na⁺ and Cl⁻) effect on the structure of subcellular organelles and inhibition of metabolic processes: significant reduction in photosynthetic pigments, damage of the thylakoid membrane, irrecoverable inactivation of photophosphorylation, and inhibition of electron transport in the thylakoid membrane (Manaa et al., 2019; Muhammad et al., 2021; Pan et al., 2021). Moreover, a decrease in plant growth related to the reduction in photosynthetic pigments content and gas exchange parameters was also reported in other plants exposed to individual and combined drought and salinity stresses (Alam et al., 2021; Hussain et al., 2020; Stavridou et al., 2019).

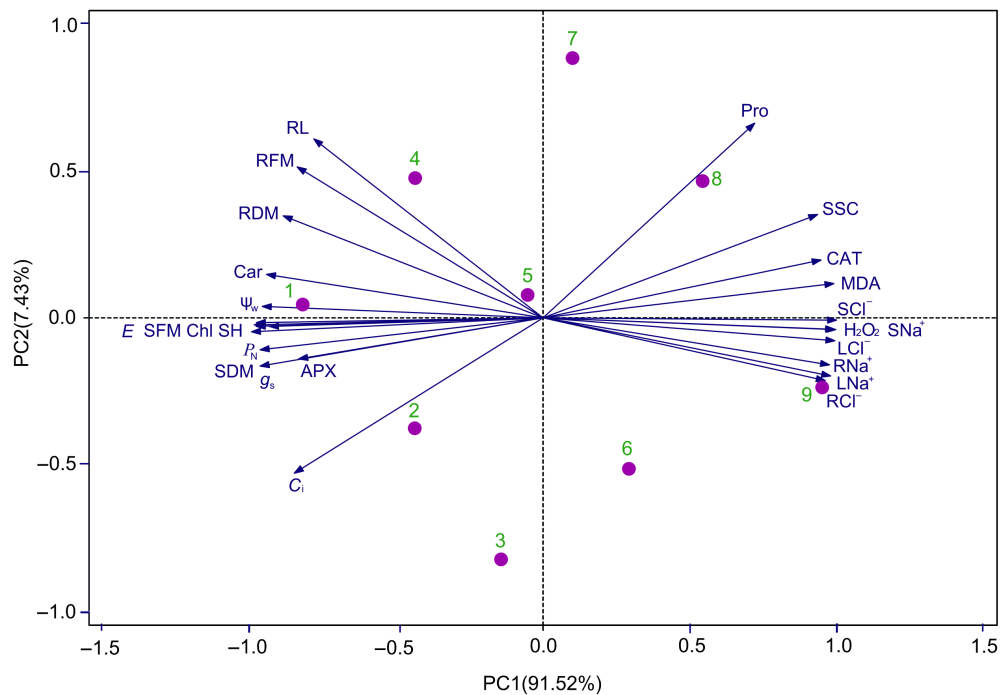


FIGURE 8 Biplot of principal component analysis on different physiological and biochemical parameters with growth of *H. glomeratus* under drought, salinity and combined stresses. (1) 100% of field capacity + 0 mM NaCl (control), (2) 100% of field capacity + 200 mM NaCl, (3) 100% of field capacity + 400 mM NaCl, (4) 70% of field capacity + 0 mM NaCl, (5) 70% of field capacity + 200 mM NaCl, (6) 70% of field capacity + 400 mM NaCl, (7) 40% of field capacity + 0 mM NaCl, (8) 40% of field capacity + 200 mM NaCl, and (9) 40% of field capacity + 400 mM NaCl. Abbreviations are as described in Figure 7

4.4 | Contribution of organic osmotic regulators to osmotic adjustment in *H. glomeratus* under drought and salt stress, alone or in combination

Drought and salinity compromise water uptake, resulting in osmotic adjustment in plants. Water relations and the capacity of regulating the osmotic concentration play a great part in plant development and overall growth (Munns, 2002). In this study, the reduction of leaves ψ_w in individual or combined drought/salt-treated plants indicates an osmotic stress. *H. glomeratus* under drought stress had a reduced capacity to take up water, which was evidenced by a decrease in RWC. On the contrary, salinity exposure caused a significant increase in RWC in leaves of *H. glomeratus*. The ability of *H. glomeratus* to keep relatively high RWC in leaves under salt stress plays a protective role against the deleterious influences of excessive salt. In halophytes, increased leaf succulence can be a crucial adaptive response and is accomplished by enlarging the size of mesophyll cells and also the relative size of vacuoles (Parida et al., 2016; Shabala & Mackay, 2011). An increment in RWC in succulent halophytes exposed to salinity was also reported by other studies (Ahmed et al., 2021; Lu et al., 2021; Wang et al., 2015). In addition, the external addition of NaCl in the growth medium of *H. glomeratus* under drought stress avoided leaf tissue dehydration as reported by Sucre and Suárez (2011). Gimeno et al. (2012) explained that increased Na⁺ and Cl⁻ in the leaves reduced leaf osmotic potential, and that it coincided with a high flux of entering

water to the cells, elevating leaf turgor potential. The water in the leaf cells was not lost through transpiration because the large amount of Na⁺ and Cl⁻ in the leaves led to stomatal closure.

Osmolytes are low-molecular-weight organic solutes accumulated in the cytoplasm of halophytes to balance the osmotic potential of the elevated Na⁺ and Cl⁻ in the vacuole (Slama, Abdelly, et al., 2015). Proline and soluble sugars are essential compatible solutes involved in osmotic adjustment, and both can be accumulated in large amounts in response to drought and salt stresses (Alam et al., 2021; Zaher-Ara et al., 2016). However, not every higher plant synthesizes a considerable amount of proline to prevent the adverse influence of abiotic stress (Lü et al., 2019). In this study, proline accumulation in leaves of *H. glomeratus* is only 20.2–64.4 $\mu\text{g g}^{-1}$ FM, and this concentration is too low to play an effective role in the osmotic adjustment (Ghars et al., 2008; Slama, Abdelly, et al., 2015). Thus, proline was not the major osmotic substances for osmotic adjustment in *H. glomeratus*. In contrast, soluble sugars largely accumulated in leaves of *H. glomeratus* under salt and drought stress compared with control, and accumulated more prominently under drought than salt stress. Therefore, soluble sugars can be important regulators of the osmotic adjustment in response to drought stress and they can play an effective role in membrane protection and radicals elimination (Krasensky & Jonak, 2012). However, in *H. glomeratus*, Na⁺ rather than soluble sugar significantly accumulated upon exposure to salt stress, suggesting that Na⁺ may be the main contributor to osmotic adjustment. At the cellular level, taking compatible solutes as osmolytes is

supposed to be an energy-consuming process (Flowers & Colmer, 2008); thus the synthesis of compatible solutes, such as proline and soluble sugar, consumes more energy in comparison with the uptake of Na^+ . Lü et al. (2019) reported that inorganic ions can be more effective in holding the osmotic pressure of the cell than soluble sugars and they are crucial factors in coping with the low osmotic potential originated from the extreme environment in the succulent *Haloxylon ammodendron*.

4.5 | Increasing antioxidative enzyme activities is an important strategy for *H. glomeratus* under drought and salt stresses, alone or in combination

ROS are natural products in various metabolic processes plant cells. In plants subjected to various abiotic stresses, such as salt and drought, ROS levels can be significantly enhanced, causing redox imbalance and oxidative stress (Haider et al., 2019; Sarker & Oba, 2018). Protonation of $\text{O}_2^{\cdot-}$ produces the hydroperoxyl radical ($\cdot\text{HO}$ and H_2O_2), and it can transform fatty acids into toxic lipid peroxides and damage biological membranes (Weckx & Clijsters, 1996). Furthermore, excessive accumulation of ROS under oxidative stress induces lipid peroxidation, which induces oxidative malfunctions of proteins, DNA and RNA. It also generates altered and disrupted aldehyde substances like malondialdehyde (MDA). MDA is regarded as a oxidative stress indicator in plants (Demidchik, 2015). In our study, H_2O_2 and MDA accumulated significantly in response to drought and salt, individually or in combination, with the highest effects obtained when stresses were combined, which also had been proven by other studies (Alam et al., 2021; Jamshidi Goharrizi, Baghizadeh, et al., 2020; Jin et al., 2020). The positive correlation between H_2O_2 and MDA contents indicates that drought and salt induce the accumulation of H_2O_2 , and eventually induce lipid peroxidation by the $\cdot\text{HO}$ generation in *H. glomeratus*.

To minimize oxidative damage, plants employ the antioxidative defense system, including SOD, POD, CAT, APX, and other enzymes (Abdelal et al., 2018; Jamshidi Goharrizi, Amiramhani, & Salehi, 2020; You & Chan, 2015). The antioxidative defense mechanism maintain a balance between the ROS over-accumulation and their elimination to keep ROS at a signaling level (Hossain et al., 2015). SOD is the primary defense enzyme acting as $\text{O}_2^{\cdot-}$ scavenger in living organisms by promptly changing $\text{O}_2^{\cdot-}$ into O_2 and H_2O_2 , and being regarded as a first-line protector from the oxidative stress induced by ROS (Blokhina et al., 2003; Mittler, 2002). In this study, SOD enzyme activity increased under drought and salinity stress, alone or in combination, compared to the control level, indicating that the higher SOD activity can regulate the appropriate level of $\text{O}_2^{\cdot-}$ in *H. glomeratus*. Similarly, upregulation of SOD activity under salt and drought stress was reported in other halophytes (Lu et al., 2021; Panda et al., 2019). However, the substance generated by SOD activity is H_2O_2 , and H_2O_2 is still toxic and can be scavenged by changing into H_2O in the next process. In plants, CAT, POD, and APX are considered the most important in scavenging H_2O_2 (Blokhina et al., 2003; Mittler, 2002;

Singh et al., 2015). As a very important antioxidant enzyme, CAT scavenges H_2O_2 via its dismutation into H_2O and O_2 . In our study, the CAT enzyme activity was elevated under drought and salinity stresses, alone or in combination, compared to control condition. CAT is related to enhanced resistance in response to salt and drought stresses in plants (Ahmed et al., 2013; Cai & Gao, 2020; Ozfidan-Konakci et al., 2020). POD activity was significantly reduced at extreme salt (400 mM NaCl) and drought (40% of field capacity) alone and extreme drought combine with salt compared to control. Previous studies showed that POD activity is reduced in plants in response to extreme drought and salt stresses (Acosta-Motos et al., 2015; Panda et al., 2019; Zhang et al., 2015). APX regulates the level of ROS by the ascorbate-glutathione cycle that changes H_2O_2 into H_2O and O_2 . In *H. glomeratus*, APX activity only significantly increased at 200 mM NaCl treatment without drought stress, and decreased significantly with enhanced drought stress compared to control. Similarly, APX was reduced in response to salt and drought stress in other species (Acosta-Motos et al., 2015; Hassan et al., 2017; Panda et al., 2019). According to our results, in *H. glomeratus*, H_2O_2 was mainly eliminated by POD and CAT at moderate drought and salt, individually or in combination, and only by CAT under higher individual and combined drought and salt stresses. Because CAT does not need a supply of reducing equivalents to maintain its function, it might be insensitive to the cell redox status and its function might not be influenced by stress (Mittler, 2002).

4.6 | Effective criteria were selected for assessing the tolerant capacity of *H. glomeratus* to drought and salt stresses, alone or in combination

PCA and correlation analysis present an overall explanation of the interrelationships between all tested indexes, and they indicate which parameters can be regarded as an individual, interchangeable, or supplementary criteria for assessing the tolerant capacity to drought and salt stresses, single or combined (El-Hendawy et al., 2017). According to the data from this study, growth parameters (shoot height, shoot FM, shoot DM, root length, root FM, and root DM), photosynthetic pigments content (Chl and Car content), gas exchange parameters (P_n , E , g_s , and C_i), water potential, and APX activities were grouped together. On the other hand, CAT activity was grouped with oxidative stress parameters (H_2O_2 and MDA content), compatible solutes (soluble sugar) and ions uptake (Na^+ and Cl^-). Therefore, the parameters in each group are significantly correlated and can be taken as complementary screening criteria for assessing tolerant capacity of *H. glomeratus* to drought and salt stresses, alone or in combination. Moreover, the significant negative correlation between these two groups indicated that drought and salinity, individually or in combination, can cause osmotic and oxidative stress through the reduction of water potential and excessive accumulation of H_2O_2 and MDA, which affect photosynthesis and growth of *H. glomeratus*. However, *H. glomeratus* can cope with these stresses by the accumulation of soluble sugars and upregulation of CAT activity. Therefore,

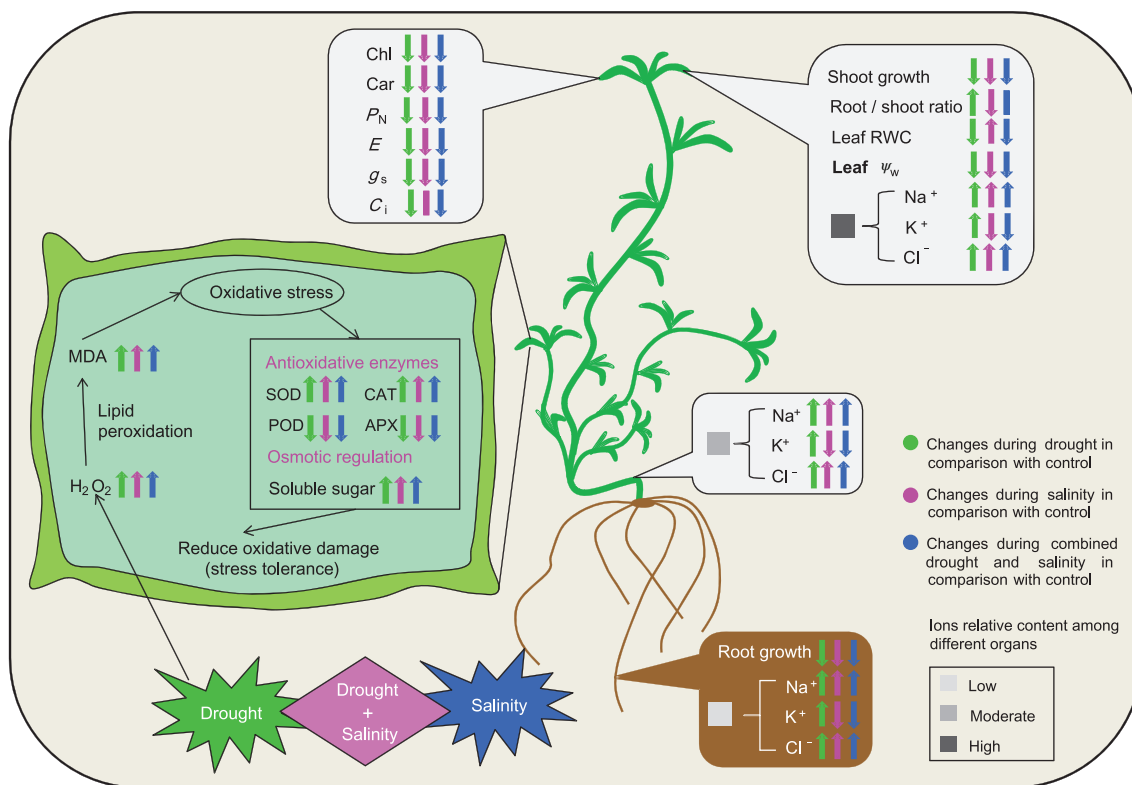


FIGURE 9 Schematic diagram showing the changes of morphophysiological parameters in response to drought, salinity and combined stresses in *H. glomeratus*. Symbols such as (↑), (↓), and (|) represent upregulation, downregulation, and no significant changes of various parameters, respectively

photosynthetic pigments content, gas exchange parameters, water potential, CAT activity, soluble sugar, H₂O₂, and MDA content can be taken as interchangeable or individual screening criteria for drought and salt tolerant assessment of *H. glomeratus*.

5 | CONCLUSIONS

In conclusion, growth inhibition and reduction of photosynthetic pigments, gas exchange parameters, water potential were observed under individual and combined drought and salt stresses. Their reduction was intensified under combined salt and drought stress compared to drought or salinity individually performed. Furthermore, accumulation of H₂O₂ and MDA was detected under individual and combined drought and salt stress, and the accumulation was intensified under combined stresses compared to individual stresses. Therefore, under combined drought and salt stresses, the higher growth and photosynthetic inhibition were due to higher osmotic and oxidative stresses compared to drought and salt stress individually performed. However, *H. glomeratus* is equipped with specific adaptation mechanisms to protect itself against those stresses. These adaptations include upregulating the SOD and CAT activities and accumulation of osmoprotectants, such as Na⁺, Cl⁻, and soluble sugar. Moreover, root/shoot ratio and RWC responded differently to the two stresses. Root/shoot ratio increased under drought and decreased under salt

stress, whereas RWC reduced under drought stress and increased under salt stress. A schematic diagram showing the physiological and biochemical mechanisms in response to drought and salt stress is presented in Figure 9.

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AUTHOR CONTRIBUTIONS

Yan Lu, Fanjiang Zeng, and Xiangyi Li conceived and designed the research. Yan Lu cultured the plant and performed the morphophysiological parameters analyses. Yan Lu and Bo Zhang performed the statistical analyses. Yan Lu interpreted the results and wrote the manuscript. Fanjiang Zeng and Xiangyi Li reviewed the manuscript. All authors have read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as all new obtained data are already presented within this article.

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