Morphological and Physiological Responses of Suaeda salsa under Drought and Salt Stress

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Abstract

Drought and salt stress are major constraints for agricultural survival and development. *Suaeda salsa* is one of the hot spots plants in the study of drought- and salt-tolerant with important soil improvement and ecological restoration roles. Therefore, it is significant to understand the effects of water shortage and salt stress on physiological and biochemical properties of plants. In the study, two different horizontal gradients were set up to measure the phenotype, physiological and biochemical characteristics of *S. salsa* under drought and salt stress. The results showed that drought and salt stress inhibited the growth of *S. salsa*. Different salt concentrations significantly increased the activities of catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD). With increasing concentration of salt stress, malondialdehyde (MDA), soluble protein and soluble sugar contents showed an upward trend, while chlorophyll b and carotenoid contents first decreased, and then increased by 28.21% and 45.45% due to the accumulation of proline. As drought stress concentration increased, the POD activity first decreased and then increased, while soluble protein contents showed an opposite trend. Under severe drought, chlorophyll a/b and carotenoid contents decreased by 52.0%, 57.01%, and 20.00%, respectively. Our results indicated that drought and high salinity inhibited the growth, morphology, and most physiological and biochemical characteristics of *S. salsa*, while the activities of antioxidant enzymes, content of osmotic adjustment substances and photosynthetic pigments might be related to the accumulation of proline.

Introduction

Plants are constantly challenged by various abiotic stress in the changing environment, including drought, salinity, extreme temperatures, high light intensity, nutrient deficiency, and heavy metal toxicity (Bhatnagar-Mathur et al. 2008). Drought stress causes water scarcity in plants, and salt stress causes osmotic stress (Li et al. 2017), which adversely affect physiological and biochemical processes of plants. Drought and salt stress are considered the primary environmental factors that affect the natural distribution of plants and reduce crop yield (Zhu 2016), affecting their morphological, physiological, biochemical, and molecular characteristics (Salehi-Lisar and Bakhshayeshan-Agdam 2016).

Drought and salt stress can hinder growth and physiological, biochemical processes, and productivity of plants (Cao et al. 2017). Under drought conditions, plants reduce the ability of their roots to transport ions and other nutrients to other parts of the ground, impeding plant growth. Salt stress affects root absorption, distribution and assimilation of mineral ions, thus affecting seed germination and fruit development (Li et al. 2017). Yu et al. (2015) found that the biomass, plant height, and root length of non-halophyte *Mentha canadensis* seedlings were significantly inhibited under salt treatment. Observing the changes in plant growth under adverse circumstances is one of the most intuitive ways to measure plant stress tolerance (Fu et al. 2021).

Plants can respond to drought and salt stress by accelerating the production of reactive oxygen species (ROS), However, the harmful effects of ROS can be reduced by enzymatic and non-enzymatic

detoxification pathways, including POD, CAT, SOD, and non-enzymatic compounds (Hasanuzzaman et al. 2012). ROS include oxidative stressors like hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), superoxide anion ($O_2^{\bullet-}$), and singlet oxygen ($^1O_2$), which result in increased toxic oxygen compound levels in plant systems (Sharma et al. 2012). Plants continuously produce ROS through various metabolic pathways, and the excessive production of ROS under abiotic stress can lead to oxidative stress.

Proline is a potent nonenzymatic antioxidant that prevents ROS from causing oxidative damage through its accumulation in plants (Rejeb et al. 2014). Rady et al. (2019) studied the effects of soaking seeds with proline or MLE on hydrogen peroxide content in wheat plants and found that proline effectively decreased the accumulation of H_2O_2 and MDA. The change in proline contents contributes to plant tolerance to environmental stress. In general, plants respond to abiotic stress by reducing ROS accumulation, or by increasing the content of osmotic adjustment substances such as proline, soluble sugars, and soluble proteins, to relieve the damage caused by abiotic stress (Fang and Xiong 2015; Li et al. 2020). In addition, the changes in the above osmotic adjustment substances, the degree of change in chlorophyll content in plants is closely related to drought resistance (Saha et al. 2020; Pushpalata et al. 2015). Chlorophyll, an important pigment located on the thylakoid membranes, is closely associated with photosynthesis and can convert light energy into stable chemical energy (Zhang et al. 2022). The lack of water in plants will affect the synthesis of chlorophyll, accelerate the decomposition of chlorophyll, and inhibit photosynthesis (Li et al. 2018). Iturbe-Ormaetxe et al. (1998) found that in the case of severe water shortage, plants inhibited photosynthesis by changing chlorophyll content or damaging photosynthetic organs.

Suaeda salsa L., commonly known as saltwort, is an annual herb belonging to the Chenopodiaceae family, specifically the *Suaeda* Forsk ex Scop. Genus (Wang 2020). It is mainly distributed in deserts, lakeside, coastal wetlands, and other saline-alkali areas in northeastern and northwestern China (Zhang 2003). *S. salsa* is an ideal plant resource for salt and drought tolerance research, and plays a vital role in improving the ecological environment of saline-alkali regions. *S. salsa* can absorb harmful ions (especially Na^+ and Cl^-) which have toxic effects on plants in saline soil. It is widely cultivated in saline-alkali lands for soil reclamation, contributing to the improvement of the ecological environment in saline-alkali areas (Zhao 2013). Drought or salt stress not only affects the root and leaf systems of *S. salsa* as well as seed germination and seedling growth. Guo et al. (2017) found that under hydroponic conditions, the total root length, surface area, and volume of *S. salsa* showed a trend of first increasing and then decreasing trend with increasing NaCl concentration. Although several studies have been conducted to evaluate the growth changes of *S. salsa* under abiotic stress, the effects of drought and salt stress on the antioxidant enzyme protective mechanism and osmotic adjustment substances have not been thoroughly studied, and the relevant studies on the response of various antioxidant enzymes to drought and salt stress in the enzymatic clearance pathway have hardly been reported. Therefore, most of the important physiological and biochemical mechanisms under drought and salt stress have not been clarified.

We established four levels of water gradients: well-watered (D1) at 60 ± 5% of field capacity, mild stress (D2) at 50 ± 5%, moderate stress (D3) at 40 ± 5%, and severe stress (D4) at 30 ± 5% and five levels of pure NaCl gradients: (S1) at 5 $g \cdot kg^{-1}$, (S2) at 10 $g \cdot kg^{-1}$, (S3) at 15 $g \cdot kg^{-1}$, (S4) at 20 $g \cdot kg^{-1}$, and (S5) at 25 $g \cdot kg^{-1}$. Therefore, the purposes of this study are as follows: (1) To study the effects of drought and salt stress on the growth and morphological characteristics of *S. salsa*; (2) Measure and compare the physiological and biochemical characteristics of plants under drought and salt stress; (3) Explore the response and influence mechanism of *S. salsa* on changes of photosynthetic pigments. The results can provide an experimental basis for further research on the mechanism of salt resistance and drought resistance of *S. salsa*, and contribute to the effective development and utilization of *S. salsa* resources.

Materials and methods

Experimental materials

Plant preparation

In the sunlight greenhouse of Xinjiang Urumqi Agricultural High-tech Park, approximately 200 mature *S. salsa* seeds from plants of the same developmental stage were harvested. Disinfection was performed with a 0.01% NaClO solution before the seeds were sown in a salt-free peat soil substrate. Germination took place in a controlled environment, a plant cultivation room maintained at 25°C. The soil moisture was preserved through regular irrigation. A photoperiod of 13 hours of light and 11 hours of darkness was applied to the seedlings. To minimize evaporation and sustain consistent temperature conditions, each cultivation container was enclosed with black film. Once the seeds had germinated and the seedlings had achieved a height of approximately 10 cm, a selection of three similarly developed seedlings was transplanted into each pot. They were cultivated to reach an approximate height of 15 cm, after which they were subjected to salt stress and drought treatment.

Reagents and instruments

All experimental reagents and equipment used in this study are respectively listed in Table 1 and Table 2.

Table 1
Reagent kits used in experiments

Reagent kits	Item No.	Brands
Superoxide dismutase (SOD) reagent-WST-S method	G0101F	Grace Biotechnology
Catalase (CAD) Kit-Visible Colorimetric Method	G0105F	Grace Biotechnology
Malondialdehyde (MDA) kit	G0109F	Grace Biotechnology
Proline (PRO) Contents Kit	G0111F	Grace Biotechnology
Soluble sugar contents (SS) kit	G0501F	Grace Biotechnology
Chloroplast pigments (chlorophyll a,b and carotenoids) content kit	G0613F	Grace Biotechnology
Peroxidase (POD) kit	G0107F	Grace Biotechnology
Protein contents (SP) kit (double condensed pulse method)	G0432F	Grace Biotechnology

Table 2
Experimental instruments

Instrument Type	Instrument Type	Brands
Micro Plate Spectrophotometer	MuLtiskan SkyHigh	Thermo Scientific
Cryogenic centrifuge	Neofug 23R	Shanghai Lingyi
Pipettes	SOYOO Genex Beta	Shanghai Suyang
thermostat water bath	DZKW-4	Beijing ever bright
Electronic Balance	5003A	Shanghai Hengji
Electric Thermostatic Drying Oven	DHG-9141A	TAISITC

Applied treatments

In this study, we constructed salt and drought stress gradients informed by the survival rate of *S. salsa* under these conditions. NaCl was fractioned into five gradient levels and administered six times per day to incite salt stress. Any excess solution was reabsorbed into the soil to facilitate plant growth monitoring. Four levels of water tension were established using a gravimetric method. Upon achieving the stipulated water content, the daily short-term water supply was calculated via evaporation measurement. For each treatment group, two containers were allocated for extended water monitoring. The soil in these containers was subjected to a drying and weighing process every fifteen days. Consequently, soil water content was re-evaluated and the daily water supply was recalculated.

Measurement of morphological parameters

Using direct measurement methods under varying conditions of drought and salt stress, five *S. salsa* plants were selected for each of the following measurements. Plant height was determined by straightening the plants, measuring from the base of the above-ground stem to the top of the plant with a ruler, with an average of three repeat measurements recorded. Leaf length was measured by straightening the second leaf from the top of each plants and using calipers to measure from the base to the tip of the leaf. The average of three repeat measurements was recorded. Root length was determined by carefully washing the soil from the roots and drying them, then measuring their length with a ruler. The average of three repeat measurements was recorded. The stem diameter was measured at the base of the above-ground stem using calipers. The average of three repeat measurements was recorded. Fresh and dry weights were measured by cleaning the leaves and stems, washing the soil from the roots and drying them, and weighing the plants on an electronic scale. The average of three repeat measurements of fresh weight was recorded. The plants were then dried in an oven at 75°C for 12 hours, then at 60°C until fully dehydrated. After 24 hours, the dry weight of the plants was measured and the average of three repeat measurements was recorded.

Determination of physiological and biochemical indices in response to salt and drought stress

Antioxidant enzyme activities and lipid peroxidation determination

Enzyme liquid extraction: The leaves (about 0.1g) and 1 mL of phosphoric acid buffer were homogenized. Then the homogenates were centrifuged at 12000 rpm for 10 min at 4°C. The supernatants were used to determine the antioxidant enzyme activities as described below.

POD activity (EC 1.11.1.7): POD was determined by the catalysis of the oxidation of hydrogen peroxide (H_2O_2) according to Wang (2010). The reaction mixture contained 40 μL supernatant, 160 μL guaiacol, 560 μL acetic acid-sodium acetate solution, and 40 μL of 30% H_2O_2. The reaction mixtures were placed in a spectrophotometer and the absorbance at 560 nm was recorded. One unit of SOD was defined as an increase in absorbance value of 0.5 per gram of fresh leaf tissue per minute at 470 nm in the reaction system.

CAT activity (EC 1.11.1.6): CAT was determined by the catalysis of the oxidation of H_2O_2. The reaction mixture contained 10 μL of the mixtures (80 μL sodium phosphate, 20 μL H_2O_2, and 100 μL dilute sulfuric acid), 900 μL sodium phosphate, and 290 μL chromogenic agent. The reaction mixtures were placed under 25°C for 5 min. Absorbance at 510 nm was recorded.

SOD activity (EC 1.15.1.1): SOD was determined according to the color reaction of WST-8. The reaction mixture contained 60 μL xanthine oxidase, 60 μL sample, and 630 μL of mixture (sodium phosphate, WST-8, and xanthine reagent were mixed in a ratio of 280 μL:30 μL:320 μL). Absorbance at 450 nm was recorded.

Lipid peroxidation determination: Lipid peroxidation was estimated by measurement of MDA using thiobarbituric acid (TBA) according to Yang (2018). The reaction mixture was homogenized in a centrifuge tube containing 600 μL mixture (TBA and trichloroacetic acid (TCA)) and 400 μL supernate. The mixture was placed in 90–95 ℃ boiling water for 30 min and centrifuged for 10 min at 12000 rpm after cooling. The supernatant was measured and absorbance was recorded at 532 nm and 600 nm.

Osmotic adjustment substances

Proline contents: Proline was measured according to Bates et al.(1973). Briefly, the supernatant was mixed with the acid ninhydrin with glacial acetic acid and sulfosalicylic acid. This mixture was incubated in a 95℃ boiling-water bath for 30 min. Absorbance was read at 520 nm.

Soluble sugars: soluble sugars were determined according to Zhao (2006). Reaction mixtures contained 50 μL supernate, 150 μL distilled water, 60 μL anthrone, and 500 μL concentrated sulphuric acid. The mixtures were heated in a 95℃ boiling-water bath for 10 min. After cooling, absorbance was recorded at 620 nm.

Soluble proteins: soluble sugars were determined by using the biuret method. BCA reagent (bovine serum albumin), copper sulphate, and ultrapure water were mixed in a ratio of 5:3:2 and used to prepare the reaction mix. Then, 900 μL of the mixed reagent and 100 μL BCA were mixed at 37℃ for 10 min. And absorbance was recorded at 540 nm.

Estimation of photosynthetic pigments

Approximately 0.1 g of finely chopped *S. salsa* leaves were measured and ground under dim light conditions with 1 mL of 95% ethanol and 50 mg of calcium carbonate. The resultant solution was then transferred into a 15 mL Eppendorf tube and topped up to 10 mL using 95% ethanol, producing the extraction solution. Aliquots of 200 μL of the extraction solution and 200 μL of 95% ethanol were separately placed into the wells of a 96-well microplate of a microplate reader, corresponding to the test and blank tubes, respectively. Absorbance values were then measured at 649 nm, 665 nm, and 470 nm wavelengths using a spectrophotometer. The change in absorbance at each wavelength (ΔA665, ΔA649, and ΔA470) was calculated by subtracting the absorbance of the blank from that of the test tube at the corresponding wavelength. Following empirical formulas, Ca (mg L^{-1}) = 13.95 × ΔA665 − 6.88 × ΔA649; Cb (mg L^{-1}) = 24.96 × ΔA649 − 7.32 × ΔA665; and Cc (mg L^{-1}) = (1000 × ΔA470 − 2.05×Ca − 114.8×Cb)/245, Chlorophyll a (mg g^{-1}) = Ca× $\frac{V \times D}{1000 \times W}$, Chlorophyll b (mg g^{-1}) = Cb× $\frac{V \times D}{1000 \times W}$,Carotenoid (mg g^{-1}) = Cc× $\frac{V \times D}{1000 \times W}$. In these formulas, V represents the volume of the extraction solution, D the dilution factor, and W the sample weight in grams.

Statistical analyses

In R 4.2.2, the homogeneity of variance was first assessed using the 'bartlett.test' function. If the resulting p-value is greater than 0.05, it indicates the presence of homogeneous variances, fulfilling the assumption required for subsequent one-way analysis of variance (ANOVA). The 'aov' and 'anova'

functions were employed to determine the significance of differences in the impact of various stress treatment conditions on *S. salsa*. Subsequently, the 'LSD.test' function from the 'agricolae' package was utilized to perform multiple comparisons using the LSD method (Fisher's Least Significant Difference), with a significance level set at $\alpha = 0.05$. The letter notation method was utilized to display significant differences between different treatments. The relevant letter annotations were placed above the bars in the column chart or above the boxes in the box plot. Treatments sharing the same letter were considered non-significantly different, while those with different letters were deemed significantly different.

All bar and box plots for this study were completed using the ggplot2 package.

Results

Effects of drought and salt stress on morphological characteristics of S. salsa.

Drought and salt stress have certain inhibitory effects on the growth of *S. salsa*. Under the experimental conditions, multiple morphological indices of *S. salsa* decreased with the increase of stress intensity under drought and salt stress (Fig. S1). Specifically, under drought stress conditions, the plant height of *S. salsa* decreased by 11.77%, 25.74%, and 36.45%, respectively under D2, D3, and D4 treatments, compared with the control group (Fig. 1A). Under salt stress conditions, the plant height decreased by 14.58%, 18.04%, 29.94%, and 35.47% in S2, S3, S4, and S5 conditions, respectively (Fig. 1G). Abiotic stress had a certain effect on leaf growth. Under drought stress, the leaf length of the plants was lower by 8.33%, 29.52%, and 44.15% in D2, D3, and D4 treatments, respectively, compared with that under full water supply (Fig. 1B). Under salt stress, the leaf length was also lower by 4.89%, 11.31%, 21.41%, and 45.27% in S2, S3, S4, and S5 conditions, respectively (Fig. 1H). The leaf length of *S. salsa* decreased gradually with the increasing concentration of stress.

Drought and salt stress also affected the variability of root length and stem thickness of *S. salsa*. Under drought stress conditions, the root length was significantly lower by 39.77%, 52.56%, and 61.86% in D2, D3, and D4 conditions, respectively, compared with the control group (Fig. 1C). With increasing concentration of salt stress, the root length was also significantly lower by 31.34%, 11.66%, 50.18%, and 66.62% under S2, S3, S4, and S5 treatments, respectively (Fig. 1I). The stem thickness of *S. salsa* was slightly higher, by 5.32% in D2 condition, while it was lower by 18.11% and 37.87%, respectively, in D3 and D4 conditions, under drought stress (Fig. 1D). This indicates that mild drought stress can promote the growth of stems, and appropriate water conditions are conducive to the growth of stems. The stem thickness showed no significant change in S2 and S3 treatments, but it was also significantly lower by 28.66% and 41.44%, respectively, in S4 and S5 treatments, respectively, under salt stress (Fig. 1J). This indicates that the growth of *S. salsa* stems can be promoted under appropriate conditions, but under extreme drought or high salt conditions, ion toxicity may result in inhibiting the growth of stem diameter of *S. salsa*, and causing the stem of the plants to become increasingly slender.

Under the influence of abiotic stress, the fresh and dry weight of *S. salsa* also showed a decreasing trend. The fresh weight significantly decreased by 87.44% and 84.37% in D3 and D4 conditions, respectively,

under drought stress (Fig. 1E). The fresh weight showed no significant change under S2 treatment, while significantly decreased by 38.13%, 56.34%, and 74.64% in S3, S4, and S5 treatments, respectively, under salt stress (Fig. 1K). Under drought stress conditions, the dry weight of *S. salsa* showed no significant change under in D2 treatment compared with the control group, but significantly decreased by 78.76% and 81.35% in D3 and D4 treatments, respectively (Fig. 1F). The change of dry weight was similar to that of fresh weight, and both began to decrease significantly after moderate drought stress treatment. Under salt stress conditions, the dry weight was lower by 8.70%, 10.87%, and 17.39% in S2, S3, and S4 treatments, respectively (Fig. 1L), showing a decreasing trend. The change in dry weight was relatively small compared with the fresh weight, possibly due to the lower dry matter contents, but the change in dry weight still showed a decreasing trend. Almost all of the morphological indexes showed a decreasing trend under salt stress, indicating that salt stress significantly inhibited the normal growth of *S. salsa*, and the effects of different concentrations of salt stress on the morphology of *S. salsa* were different.

Effects of drought and salt stress on antioxidant enzyme activities and lipid peroxidation

High amount of ROS produced by plants under abiotic stress, can lead to an imbalance between the production and removal of ROS. In order to reduce the damage of ROS to plant cells, the activities of antioxidant enzymes in plants are increased. (Fan et al. 2021; Cao et al. 2022). Under drought stress, the POD activity in *S. salsa* was lower, by 33.40%, but POD activity significantly higher by 33.93% and 64.85% in D3 and D4 conditions, respectively (Fig. 2A). The POD activity decreased from 6.85 U g^{-1} (D1) to 4.56 U g^{-1} (D2), and then increased to 11.29 U g^{-1} (D4). It indicated that POD activity was first inhibited, and increased with the increasing concentration of drought stress. Under salt stress (S1-S5), the POD activity was higher in the range of 11.73 U g^{-1} to 26.14 U g^{-1} (Fig. 2F), indicating that different concentration of salt stress could enhance POD activity in *S. salsa*.

Under drought stress (D1-D4), CAT activity was significantly higher in the range of 253.69 U g^{-1} to 551.08 U g^{-1} (Fig. 2B). And CAT activity significantly increased from 80.95 U g^{-1} to 437.23 U g^{-1} under salt stress (S1-S5), showing an upward trend, indicating that drought and salt stress significantly enhanced the CAT activity (Fig. 2G). Under drought stress (D1-D4), SOD activity was higher with the increasing concentration of stress, significantly increased from 70.64 U g^{-1} to 226.8 U g^{-1} (Fig. 2C). Under salt stress (S1-S5), SOD activity significantly increased from 26.26 U g^{-1} to 202.78 U g^{-1}, showing an upward trend (Fig. 2H). As salt stress concentration increased, the SOD activity gradually increased, especially with a significant increase under S5. In this study, it was found that the increase in the content of protective enzymes such as SOD, CAT, and POD under salt stress was higher than that under drought stress, and SOD activity was significantly higher than CAT and POD activities under drought (Fig. 2E) and salt stress (Fig. 2J). In this study, it was found that the activities of SOD, CAT, and POD increased gradually with the increasing concentration of salt stress, and CAT activity was much higher than SOD and POD activities under drought and salt stress, especially under S5 condition.

MDA, as a final product damage of cell membrane, usually as plants respond to adversity is an important index of the strength (Li 2021). There were significant differences in MDA content under drought and salt stress ($P < 0.05$). Under drought stress, MDA content in D2, D3, and D4 treatments was significantly higher by 38.37%, 95.32%, and 236.37%, respectively, compared with the control group (Fig. 2D). Different salt concentrations significantly increased MDA, and MDA content was significantly higher by 29.93%, 48.63%, 54.61%, and 67.08%, respectively, under S2, S3, S4, and S5 conditions (Fig. 2I). Under salt stress (S1-S5), MDA content increased from 1.34 nmol g^{-1} to 2.23 nmol g^{-1}, indicating that the salt and drought stress would promote the accumulation of MDA in *S. salsa.*

Effects of drought and salt stress on osmotic adjustment substances

Proline, as one of the most important osmotic adjustment substances in plants, has strong water solubility and can remove ROS, for the integrity of the plant cell membrane plays a protective role. Under drought stress, the proline contents in *S. salsa* was significantly lower by 31.56%, 82.35% and 108.19%, under D2, D3 and D4 treatments, respectively, compared with the control group. The proline contents showed an upward trend (D1-D4) (Fig. 3D), greatly increasing from 15.55 μg g^{-1} to 32.37 μg g^{-1} (Fig. 3A). Under salt stress (S1-S5), proline contents showed a significant increasing trend, from 9.43 μg g^{-1} to 130.37 μg g^{-1} (Fig. 3E). As salt stress concentration increased, proline contents continued to increase, especially under S5 condition, indicating that large quantities of proline were synthesized in the cells of the plant to resist salt stress.

The soluble proteins and soluble sugars in plants are also important osmotic adjustment substances. Total soluble protein contents first increased and then decreased under drought stress (D1-D4). The soluble protein contents increased from 8.56 mg g^{-1} (D1) to 13.93 mg g^{-1} (D2), and then decreased to 8.36 mg g^{-1} (D4) (Fig. 3B). The amounts of reduction were related to the concentration and duration of drought stress. Under salt stress, soluble protein contents significantly increased by 62.68%, 73.65% and 105.59% in S3, S4 and S5 conditions, respectively (Fig. 3F). The soluble proteins have a certain capacity to hold water (Li et al. 2019). Under salt stress, *S. salsa* can adapt to stress environment by increasing the soluble protein contents.

Under drought stress, soluble sugar contents under D2, D3 and D4 treatments increased by 1.75%, 21.93% and 42.11%, respectively, compared with the control group (Fig. 3C). In arid environments, *S. salsa* can protect the integrity of cell membrane by increasing the soluble sugar contents. Under salt stress, soluble sugar contents significantly increased by 44.29%, 65.71%, 79.29% and 100.01% in S2, S3, S4, and S5 conditions, respectively (Fig. 3G), indicating that salt stress would promote the accumulation of soluble sugar contents in *S. salsa*. In general, proline and soluble sugars showed an upward trend under drought and salt stress, and the increase of proline under salt stress was much higher than that of soluble proteins and soluble sugars (Fig. 3H). Under stress conditions, plants play an important role in resisting osmotic stress by regulating the contents of small molecules such as proline, soluble proteins and soluble sugars.

Effects of drought and salt stress on photosynthetic pigments

Photosynthesis is an important metabolic process for the source of energy and material required for plant development. Photosynthetic pigments, mainly including chlorophyll a, chlorophyll b and carotenoids, are involved in absorption, transmission of light energy and cause primary photochemical reactions in photosynthesis (Zhang 2021). Under drought stress conditions, chlorophyll a content in *S. salsa* was significantly lower by 37.80% and 52.0% in D3 and D4 treatments, respectively, compared with the control group (Fig. 4A), showing a downward trend (Fig. 4H). Under salt stress conditions, chlorophyll a content increased from 0.24 mg g^{-1} (S1) to 0.26 mg g^{-1} (S3), decreased to 0.16 mg g^{-1} (S4), but increased to 0.27 mg g^{-1} (S5). Under salt stress (S1-S5), chlorophyll a content in *S. salsa* showed an increasing and then decreasing trend, which then increased again (Fig. 4A).

Under drought stress conditions, chlorophyll b content in D2, D3, and D4 treatments was significantly lower by 10.28%, 36.45%, and 57.01%, respectively, compared with the control group (Fig. 4B). It showed a decreasing trend (D1-D4) (Fig. 4H). Under salt stress conditions, chlorophyll b content was significantly lower by 21.15%, 25.00%, and 25.00% in S2, S3, and S4 treatments, respectively. Chlorophyll b content was significantly decreased in the range of 0.17 mg g^{-1} (S1) to 0.13 mg g^{-1} (S4), but increased to 0.16 mg g^{-1} (S5). Chlorophyll b content under S5 condition increased by 28.21% compared with S4 condition. Under salt stress (S1-S5), chlorophyll b content in *S. salsa* showed a trend of first decreasing and then increasing (Fig. 4F).

Under drought stress, carotenoid content decreased by 52.00%, 37.60%, and 20.00% in D2, D3, and D4 treatments, respectively (Fig. 4C). There was an overall downward trend in carotenoid content compared with the control group (Fig. 4H). Under salt stress, carotenoid content was lower in the range of 0.09 mg g^{-1} (S1) to 0.03 mg g^{-1} (S4), and higher to 0.05 mg g^{-1} (S5), showing a trend of decline and then increase (Fig. 4G). Carotenoid content under S5 condition increased by 45.45% compared with S4 condition. In general, under moderate and severe drought stress, chlorophyll a/b and carotenoid contents decreased, while it decreased and the photosynthetic rate decreased under heavy salt conditions.

Discussion

Drought and salt stress are the main factors limiting growth and development in plants (Sikuku et al. 2010). In this study, we found that drought and salt stress significantly inhibited the growth of *S. salsa* as well as most of its physiological and biochemical characteristics. Under drought and salt stress, most of the phenotypic indexes such as plant height, leaf length, root length, dry weight, and fresh weight decreased with the increase of stress degree. Ou et al. (2019) reported that drought and salt stress had inhibitory effects on various growth indexes of Platycladus orientalis seedlings. Furthermore, saline-alkali conditions have been reported to cause growth retardation and reduced dry matter accumulation in several cereal legumes (Taibi et al. 2013a, b), and growth retardation can be attributed to the inhibition of

cell elongation by abiotic stresses (Bandeoğlu et al. 2004). Munns (2002) reported that salinity causes plant cells to lose water and shrink, as well as decreasing cell elongation rates. Under salt stress, the growth and morphology of plant were mostly inhibited. In this study, we found that as drought stress concentration increased, the soluble proteins in *S. salsa* increased first and a reduction was observed, which was consistent with previous studies. For example, Mohammadkhani and Heidari (2008) found that the total soluble protein contents in the roots and leaves of two maize cultivars increased first and then decreased under drought stress. The initial increase of soluble protein contents under drought stress may be related to the expression of new stress proteins, but later decrease in soluble protein contents with the increasing concentration of stress is most likely related to the decrease of photosynthesis (Riccardi et al. 1998). Under salt stress, soluble protein contents in *S. salsa* increased greatly with the concentration of stress. Gao et al. (2018) reported the impact of salt stress on agronomic traits, antioxidant activities, and osmotic adjustment substance contents of *Arachis hypogaea*, and found that plant growth and pod production were significantly reduced in all stress treatment groups. Furthermore, soluble protein levels in *A. hypogaea* increased with the increase of salt stress severity under salt stress. Similarly, we found that soluble proteins showed an increasing trend with the increase of salt stress intensity, which may be due to that *S. salsa* maintains osmotic balance by synthesizing a large number of osmotic adjustment substances, thus preventing the dehydration of proteins and cell membranes under salt stress (Sawhney and Singh 2002). Under abiotic stress, plants can reduce cell osmotic potential and maintain the stability of intracellular proteins by the accumulation of soluble sugars (Xie 2021). Mild drought stress can also increase soluble sugar contents and activate the osmotic regulation of plants (Zhou and Yu 2009). This study found that under drought and salt stress, soluble sugar contents in *S. salsa* showed an increasing trend, indicating that plants had affinity with the external environment.

Under long-term abiotic stress, plants will produce amounts of ROS, which can lead to nucleic acid damage, protein oxidation, enzyme inhibition, activation of programmed cell death (PCD) pathways, and ultimately cell death (Mittler 2002; Sharma and Dubey 2005, 2007). Liu et al. (2013) reported that ROS metabolism in apple seedling leaves under drought conditions, and found that the contents of H_2O_2 and MDA gradually increased with the extension of stress time and the aggravation of stress degree. And the relationship between H_2O_2 and MDA content was extremely significant, which indirectly confirmed the view that drought stress caused the accumulation of ROS and led to the peroxidation of membrane lipids. Plants can regulate intracellular ROS levels and employ antioxidant mechanisms to control the increase of ROS during abiotic stress (Rasti Sani et al. 2018). In this study, SOD and CAT activities in *S. salsa* showed an increasing trend with increasing stress intensity under drought and salt stress, and their antioxidant activities were significantly enhanced, which was consistent with the results of previous studies. Xu et al. (2022) reported that the antioxidant enzymes in *Heimia myrtifolia* were first increased and then remained stable with the increasing concentration of salt stress. Our study found that POD activity in *S. salsa* exhibited a continuously increasing trend as salt stress concentration increased. Wang et al. (2020) reported the physiological mechanisms of exogenous ABA regulation of drought stress in ginger, observed increased activities of SOD, POD, and CAT in plant leaves upon early application of ABA to the roots. In our study, POD activity in *S. salsa* initially decreased insignificantly and then increased

with the increasing severity of drought stress, which may be because different antioxidant enzymes have different responses to drought stress (Anjum et al. 2011; Bai et al. 2021). Protective enzymes that play a dominant role in plants may also differ under stress (Seki et al. 2007). Zhang (2022) investigated the response of two *Clematis* plants to varying degrees of drought stress, and found that SOD and POD activities exhibited an initial increase, and exhibited decrease trend. Our study found proline and MDA contents increased with extension treatment time under the same level of drought stress, which indicated the important role of antioxidant enzymes in combating abiotic stress. In our study, CAT activity was much higher than SOD and POD activities under drought and salt stress, and the activities of SOD, CAT and POD increased gradually with the increasing concentration of salt stress, especially under S5 condition, which may be due to the accumulation of proline under salt stress.

To mitigate the physiological damage caused by abiotic stress, antioxidant enzyme activities and osmotic regulation can be enhanced by inducing proline accumulation. Ma et al. (2023) studied the effects of exogenous IAA (indole-3-acetic acid) soaking on seed germination and early seedling growth of *Onobrychis viciifolia* under drought stress, and found that seed soaking at 25 mg L^{-1} IAA could improve antioxidant enzyme activities and proline contents of *O. viciifolia* seedlings under drought stress. It can also reduce the contents of hydrogen peroxide and MDA, and alleviate the oxidative damage caused by drought stress. Our results showed that proline contents in *S. salsa* increased significantly as stress concentration increased under drought and salt stress. Heidari and Moaveni (2009) studied ABA (abcisic acid) and proline in different varieties of maize and found a close correlation between proline accumulation and drought stress. Under abiotic stress, plants accumulate large quantities of proline to protect cell membrane and protein contents in leaves (Mi 2018). Zhang et al. (2020) studied the changes in metabolic components of perennial ryegrass during salt stress. The results showed that proline contents increased and MDA content decreased under 50 mmol L^{-1} NaCl stress, while relative water content gradually decreased and MDA content gradually increased under 100–400 mmol L^{-1} NaCl stress, which is different from the results of our study. We found that drought and salt stress can promote MDA accumulation in *S. salsa*, which was due to the the intensification of membrane lipid peroxidation under abiotic stress, leading to the increase of MDA content. In addition, proline, a non-enzymatic reaction product, participates in plant in response to different stress conditions. In this study, we found that proline contents in *S. salsa* increased significantly under drought and salt stress conditions, indicating that *S. salsa* can adapt to drought and salt stress by increasing proline contents. Proline also acts as an electron acceptor for photosynthesis, preventing damage to the photosystem when dealing with ROS (De Ronde et al. 2004).

ROS produced under abiotic stress conditions contribute to chlorophyll degradation and pigment loss, so changes in chlorophyll content are also considered as a measure of oxidative damage (Ghorbanli et al. 2013). Our study showed that chlorophyll a/b content was reduced under moderate and severe drought stress, which is consistent with the results of previous studies. Nyachiro et al. (2001) found that water deficit caused a significant decrease in chlorophyll a/b content in six different varieties of wheat. The decrease in chlorophyll content may be due to the inhibition of certain chlorophyll synthesis processes

and the increase of chlorophyll degrading enzymes under stress conditions (Zhao et al. 2020). Boyer et al. (1970) reported that under drought conditions, plants have reduced stomatal conductance, decreased fixed carbon dioxide and photosynthetic rate, which hinder plant growth. Heidari et al. (2014) studied the effects of different salt concentrations on chlorophyll and fluorescence in 12 sunflowers, and found that salt stress increased chlorophyll content at the beginning, and then declined with the increase of time, which was consistent with our study. We found that chlorophyll a content increased (S1-S3), but decreased again (S4), which may be due to the initial accumulation of proline was conducive to the increase of photosynthesis under salt stress. As salt stress concentration increased, the chlorophyll content in *S. salsa* decreased, which may be due to the decomposition of chloroplasts and the disappearance of thylakoid structure under high salt conditions (Conroy et al. 1988). We also found that under salt stress, chlorophyll a/b and carotenoid contents increased under S5 condition, which may be due to the accumulation of proline (Fig. 4H). Proline is one of the main substances for chlorophyll synthesis in plants. Under salt stress, the accumulation of proline provides material conditions for chlorophyll synthesis (Zhao 1993). In this study, the changes and mechanisms of photosynthetic pigments in *S. salsa* under drought and salt stress were investigated. In addition, we found that chlorophyll b and carotenoids contents in *S. salsa* decreased and then increased due to large quantities of proline with the increasing concentration of salt stress, indicating that the accumulation of proline may contribute to the increase in photosynthesis under salt stress. The increased photosynthesis of proline under stress may be due to its effective reduction of hydrogen peroxide content (Rady 2019), as well as increases the activities of enzymatic and non-enzymatic defense systems.

Conclusion

Generally, the growth and morphological characteristics of *S. salsa* are inhibited by drought and salt stress, and physiological indexes such as plant height, leaf length, root length, fresh weight and dry weight decreased with the increase of stress intensity. Antioxidant activities, osmotic adjustment substances and photosynthetic pigment contents were related to the accumulation of proline under salt stress. Under drought and salt stress, the content of MDA and activities of CAT and SOD were significantly increased with the increasing concentration of stress. As stress concentration increased, the POD activity first decreased and a increase was observed under drought stress. Osmotic adjustment substances such as proline and soluble sugar contents increased significantly under drought and salt stress conditions. However, the soluble protein contents decreased under moderate and severe drought stress, which may be related to the decrease of photosynthesis under drought stress. As salt stress concentration increased, chlorophyll a content showed a trend of first increasing, then decreasing and then increasing, while chlorophyll b and carotenoid contents decreased and then increased.

Declarations

Author contribution statement X-MT and Q-YW designed and supervised the research, J-LL, and Y-YW conceived the conception, performed the experiments, analyzed the data, and wrote the original draft

manuscript; Z-JD and J-BB were involved in some of the data analysis tasks. Z-JD, J-BB, Gulbar Y, and Z-ZC performed the experiments and revised the draft. All authors contributed to editing and approving the final version of the manuscript.

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Data availability Not applicable.

Conflicts of Interest All other authors declare no conflicts of interest.

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Figures

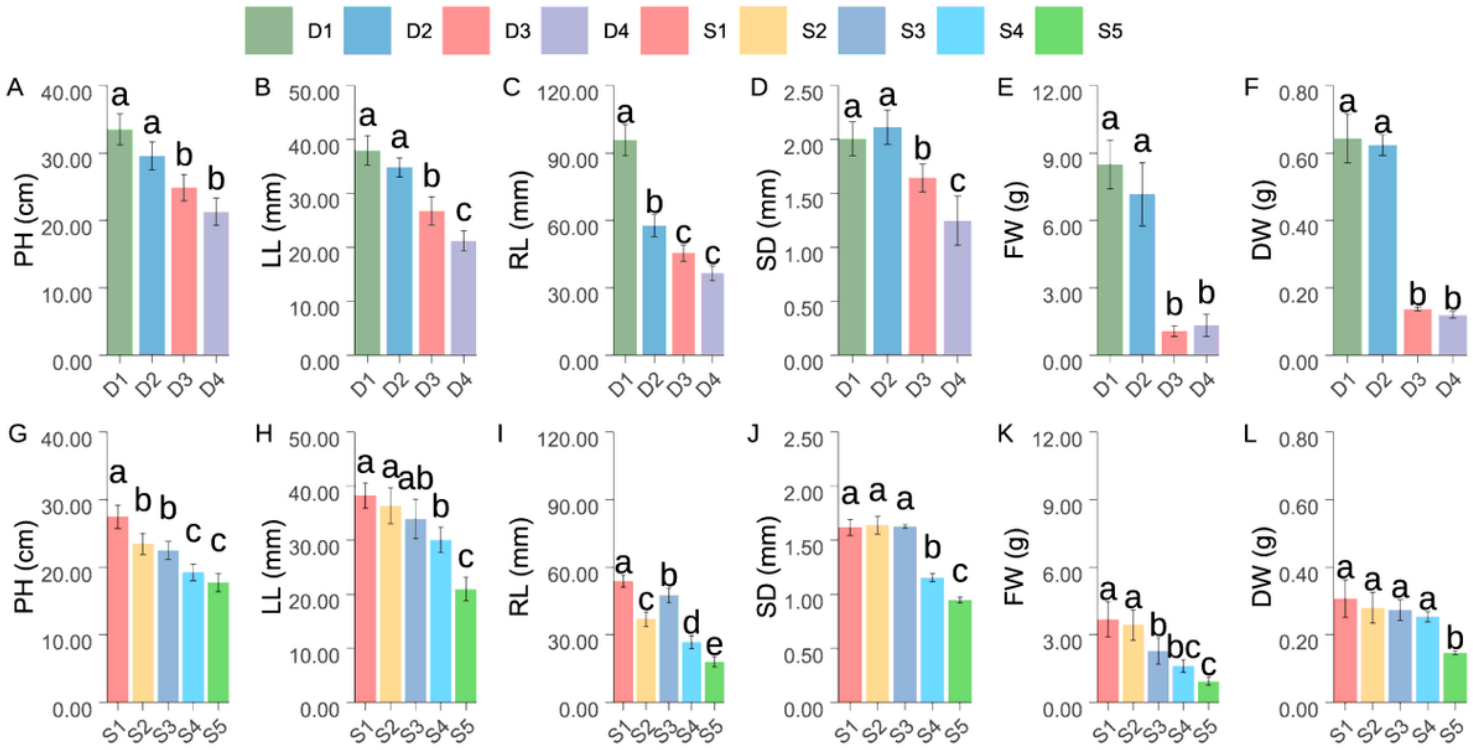

Figure 1

Effects of drought stress and salt stress on the morphology of *S. salsa*. Panels A to F show the effects of drought stress on the morphology of *S. salsa*, while panels G to L show the effects of salt stress on the morphology of *S. salsa*. The morphological parameters include plant height (PH), leaf length (LL), root length (RL), stem diameter (SD), fresh weight (FW), and dry weight (DW).

Note: In the bar graphs, error bars are displayed above the bars, calculated as the mean (± standard deviation), reflecting the variation within the treatment groups. Different letters above the bars indicate statistically significant differences between groups ($P<0.05$).

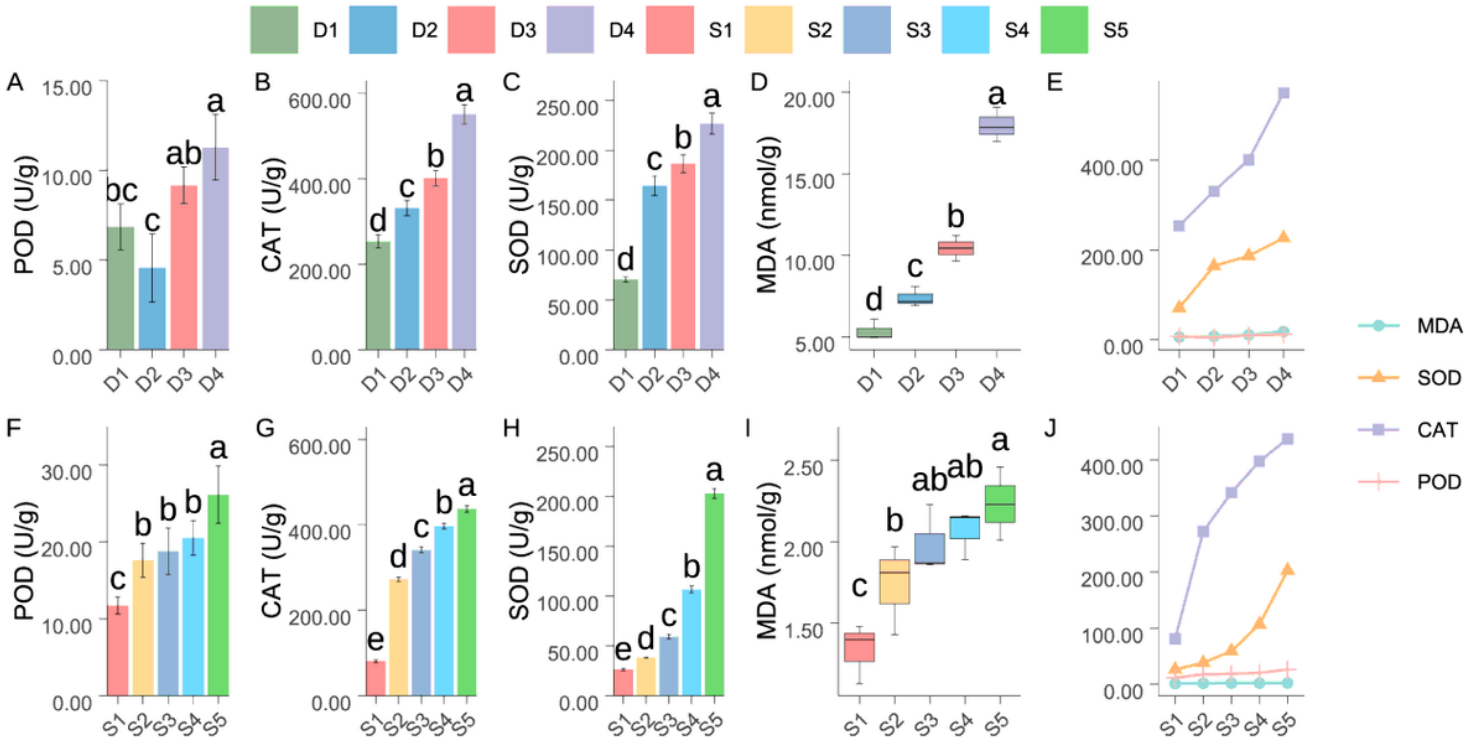

Figure 2

Effects of drought and salt stress on antioxidant enzyme activity and lipid peroxidation. The panels A to C show the activities of POD, CAT, and SOD in *S. salsa* under drought stress for different treatments (D1, D2, D3, and D4). Panels F to H show the activities of POD, CAT, and SOD under salt stress for different treatments (S1, S2, S3, S4, and S5). Panels D and I show MDA content in *S. salsa* under drought and salt stress respectively. Panels E and J show the trend changes in MDA content and the activities of POD, CAT, and SOD in different treatments under drought and salt stress conditions. Different colors represent different indicators, and the points on the line graph for each treatment are the average of three biological repeats under that treatment.

Note: The vertical error bars in the graphs show the average variation within each group and it was calculated as the mean (±standard deviation), reflecting the variation within the treatment groups. Different letters above the bars or boxes indicate statistically significant differences between groups ($P<0.05$).

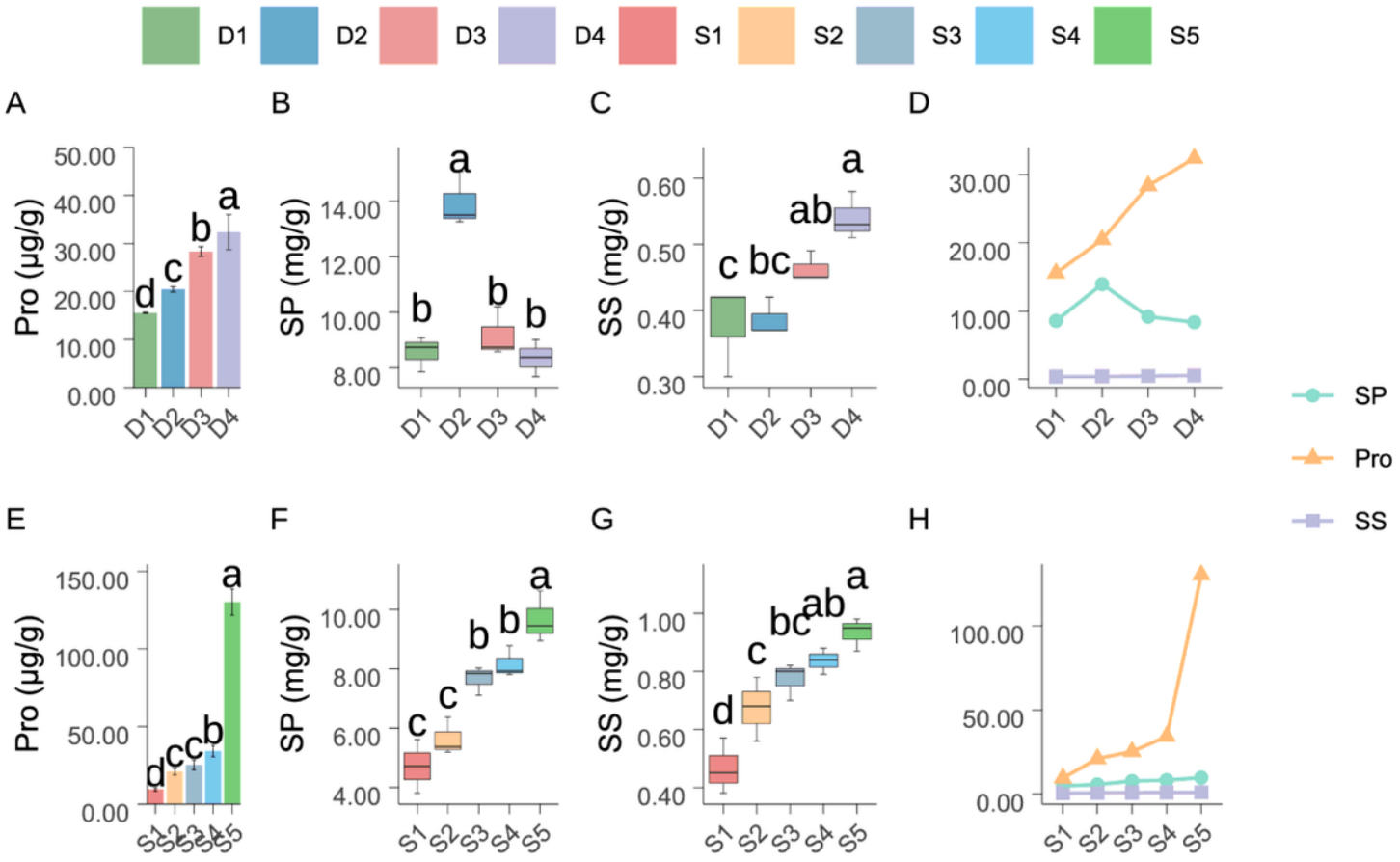

Figure 3

Effects of drought and salt stress on osmotic adjustment substances. The sentence delineates the composition of three osmotic adjustment substances - Proline (Pro), Soluble Proteins (SP), and Soluble Sugars (SS) - within S. salsa, under varying treatment conditions subjected to drought and salt stress. Panels A to C show osmotic adjustment substances under drought stress, while panels E to G show them under salt stress. Panels D and H show the trend changes in osmotic adjustment substances under different treatments, with different colors representing different indicators. The points in the line graph represent the average of three biological repeats under each treatment.

Note: The vertical error bars in the graphs show the average variation within each group and it was calculated as the mean (±standard deviation), reflecting the variation within the treatment groups. Different letters above the bars or boxes indicate statistically significant differences between groups ($P<0.05$).

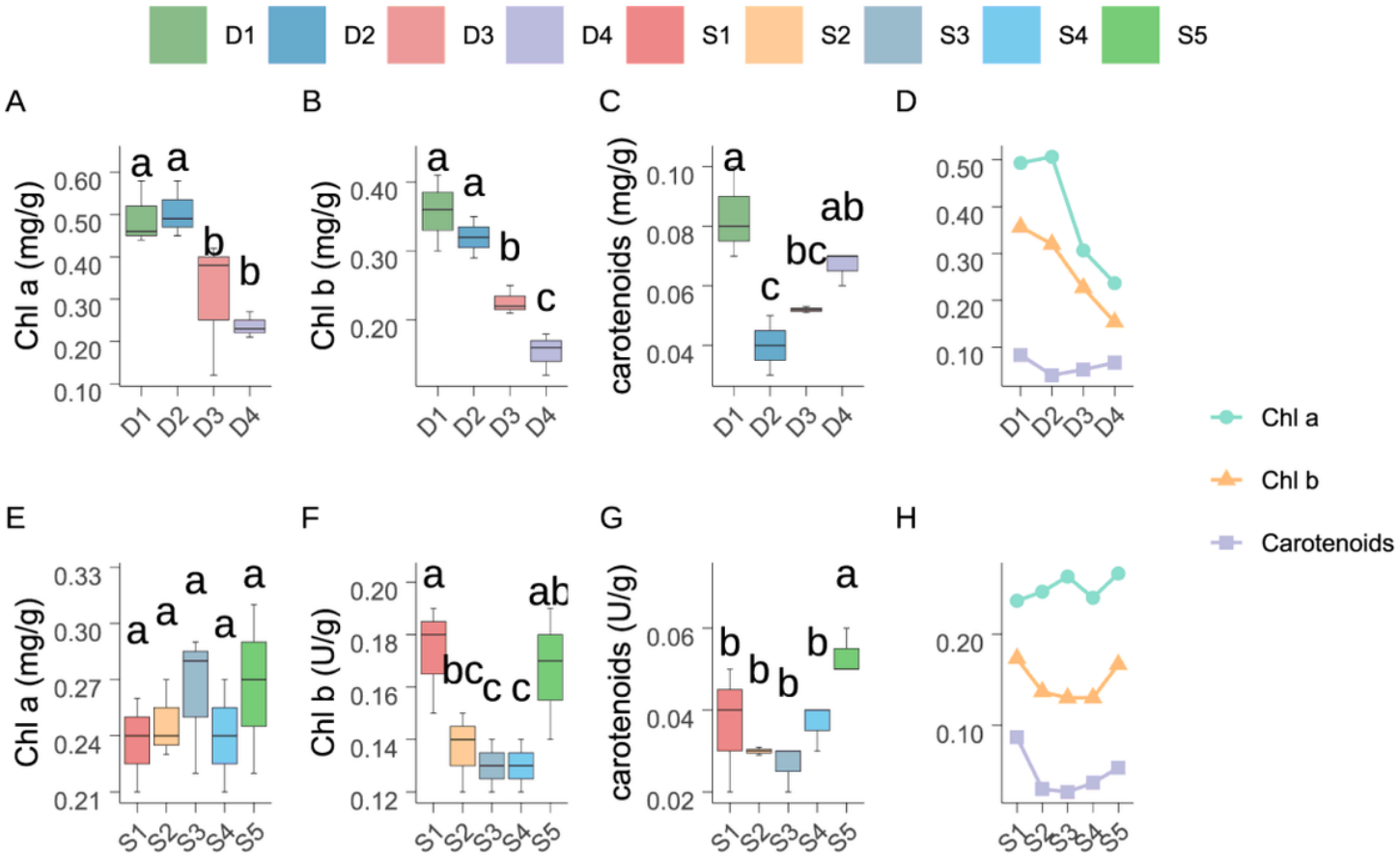

Figure 4

Effects of drought and salt stress on photosynthetic pigments. It describes a scientific experiment where the content of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids in *S. salsa* are measured under different treatment conditions, including drought stress (D1, D2, D3, and D4) and salt stress (S1, S2, S3, S4, and S5). Panels A to C show the results under drought stress, while panels E to G show the results under salt stress. Panels D and H show the trend changes in the three photosynthetic pigments under these different treatments, with different colors representing different indicators. The points on the line graph represent the average of three biological repeats under each treatment.

Note: The vertical error bars in the graphs show the average variation within each group and it was calculated as the mean (±standard deviation), reflecting the variation within the treatment groups. Different letters above the boxes indicate statistically significant differences between groups ($P<0.05$).

Supplementary Files

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- SupplementaryInformationSI.docx