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Effects of alkaline salt stress on growth, physiological properties and medicinal components of clonal *Glechoma longituba* (Nakai) Kupr.

Donghai Wang[1†], Fangshuai Song[1†], Yitong Zhou[2], Tingting Zhong[2], Yuyan Zhang[2], Qiao Deng[2], Xinqi Wang[2], Siqi Wang[2], Daocai Wang[1,2], Xiqiang Zhu[1,2], Ning Jiang[1,2] and Xiaopeng Liu[1,2*]

Abstract

Background *Glechoma longituba*, recognized as a medicinal plant, provides valuable pharmaceutical raw materials for treating various diseases. Saline-alkali stress may effectively enhance the medicinal quality of *G. longituba* by promoting the synthesis of secondary metabolites. To investigate the changes in the primary medicinal components of *G. longituba* under saline-alkali stress and improve the quality of medicinal materials, Na_2CO_3 was applied to induce short-term stress under different conditions and the biomass, physiologically active substances and primary medicinal components of *G. longituba* were measured in this study.

Results Under alkaline salt stress, the activities of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX) were elevated in *G. longituba*, accompanied by increased accumulation of proline (Pro) and malondialdehyde (MDA). Furthermore, analysis of the medicinal constituents revealed that *G. longituba* produced the highest levels of soluble sugars, flavonoids, ursolic acid, and oleanolic acid under 0.6% Na_2CO_3 stress for 48 h, 0.2% Na_2CO_3 stress for 72 h, 0.4% Na_2CO_3 stress for 12 h, and 0.4% Na_2CO_3 stress for 8 h, respectively.

Conclusions Short-term Na_2CO_3 stress enhances the synthesis of medicinal components in *G. longituba*. By manipulating stress conditions, the production of various medicinal substances could be optimized. This approach may serve as a basis for the targeted cultivation of *G. longituba*, offering potential applications in the treatment of diverse diseases.

Keywords *Glechoma longituba*, Salt stress, Antioxidant, Active substance, Medicinal constituents

†Donghai Wang and Fangshuai Song contributed equally to this work and should be considered co-first authors.

*Correspondence:
Xiaopeng Liu
liuxp999@163.com
[1]Hubei Key Laboratory of Biologic Resources Protection and Utilization, Hubei Minzu University, Enshi 445000, P. R. China
[2]School of Biological Science and Technology, Hubei Minzu University, Enshi 445000, P. R. China

Introduction

The perennial herb *Glechoma longituba* (Nakai) Kupr., belonging to the Labiatae family, has a long history as a valued medicinal herb in China [1]. The chemical composition of *G. longituba* is complex, primarily consisting of terpenoids, flavonoids, alcohols, soluble sugars, volatile oils, alkaloids. Among these, soluble sugars and flavonoids are the main medicinal constituents [2–4]. Additionally, two terpenoids, ursolic acid and oleanolic acid, have garnered attention due to their high content and significant medicinal effects. These pharmacologically active components underscore the potential of *G. longituba* for treating various diseases.

Modern pharmacological studies have revealed that *G. longituba* exhibits several therapeutic effects, including hypoglycemic, hypolipidemic, litholytic [3], antitumor [5], and anti-inflammatory properties. In terms of cultivation techniques, *G. longituba* can be propagated through tissue culture to produce clonal plants. It is widely accepted in modern botany that clonal plants possess inherent advantage in stress resistance due to their clonal integration characteristics. This trait allows for improved nutrient utilization and survival rates by facilitating the transfer of resources through structures such as stolons, rhizomes, or horizontal roots [6–8]. However, it remains to be determined whether this clonal integration advantage also applies to *G. longituba* under saline stress.

Salinized soils typically contains high concentrations of sodium salts, including NaCl, Na_2SO_4, and Na_2CO_3 [9]. Elevated sodium levels lead to reduced water potential in the environment, restricting water uptake by plants [10]. As a result, plants experience water loss and osmotic stress [11]. To counteract excessive water loss, plants often close their stomata, which reduces carbon dioxide (CO_2) fixation and diminishes the activity of CO_2-fixing enzymes [12, 13]. This response, combined with osmotic stress that damages photosynthetic pigments and disrupts membrane pH and potential balance [14], severely compromises photosynthesis.

Furthermore, in response to stress, the oxygen in plants can be converted into highly reactive oxygen species(ROS), such as hydrogen peroxide(H_2O_2), hydroxyl radical (OH•), and superoxide radical (O^{2-}•) [15, 16]. Plants must maintain ROS levels within a normal range for healthy growth, excessive ROS can cause oxidative damage to macromolecules such as lipids, nucleic acids, proteins, and carbohydrates. This leads to redox imbalance and severe oxidative stress, ultimately resulting in the gradual death of plant cells [17, 18].

Fortunately, plants have evolved unique strategies to cope with environmental stressors. To mitigate increased water loss, they often utilize lignin deposition to reduce root hydraulic conductivity, regulate the activity of water channel proteins [19], and activate various transcription factors that control the biosynthesis of compatible osmolytes [20]. These osmolytes include charged metabolites, polyols, soluble sugars, and proline [21]. For instance, salt stress has been shown to increase the content of soluble sugars and proline in *Limonium sinense* [22] and *Medicago sativa* [23].

Moreover, plants scavenge excess reactive oxygen species (ROS) through a system of antioxidant enzymes, primarily including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and so on [18, 24, 25]. Secondary metabolites also play a direct or indirect role in plant defense under stress, typically comprising substances such as flavonoids and terpenoids [26]. For example, it has been reported that salt-stressed *Glycyrrhiza uralensis* activates numerous expressed genes to enhance the synthesis of flavonoids and terpenoids, thereby increasing its stress tolerance [27].

Interestingly, the increase in polysaccharides, flavonoids, and terpenoids in response to stress not only aids in plant defense but also provides various pharmacological benefits, including anti-inflammatory, antioxidant, and antitumor properties. In comparison to neutral salts, alkaline salts impose more comprehensive stress on plants due to ion toxicity and high pH level, leading to an intensified oxidative stress response [28], Consequently, the synthesis of medicinal components such as soluble sugars, flavonoids, and terpenoids is further enhanced.

G. longituba, known for its diverse pharmacological properties, is a vital component of traditional Chinese patent medicines and possesses significant market potential. Currently, the primary method for obtaining *G. longituba* herbs is through field cultivation. However, this approach faces considerable limitations due to climatic challenges such as waterlogging, drought, and continuous rain, which severely affect both the yield and quality of the herbs by complicating the harvesting process [29]. Moreover, the cultivation of *G. longituba* competes with food crops for land, exacerbating global food scarcity issues [30]. Conventional field methods also struggle with problems such as yield decline and quality deterioration from continuous cultivation [31], as well as pesticide residues and excessive heavy metal content [32], These factors make it difficult to achieve high-quality, high-yield production of the herbs.

To address these challenges, proposals for factory production of *G. longituba* have emerged, however current research in this area remains insufficient. Our preliminary studies suggest that salt stress may enhance the content of medicinal components in *G. longituba*. Nevertheless, prolonged exposure to stress can reduce the plant's biomass, resulting in a lower overall yield of medicinal constituents despite an increased proportion

of these components. An effective strategy to improve the quality of *G. longituba* medicinal herbs involves using tissue culture combined with liquid culture techniques. This approach allows the plant to grow to sufficient biomass before applying short-term alkaline salt stress to enhance the content of medicinal components.

In this study, four different concentrations of Na_2CO_3 (0.2%, 0.4%, 0.6%, and 0.8%) were used to subject mature *G. longituba* to short-term stress treatments. We investigated the dynamic changes in biomass, antioxidant enzyme activities, osmotic substances, and medicinal component contents at various time intervals(4, 8, 12, 24, 48, 72, and 96 h). The objective was to elucidate the mechanism by which Na_2CO_3 stress enhances the medicinal constituents of *G. longituba* and to identify the optimal stress concentration and duration for targeted cultivation aimed at enhancing specific medicinal components.

Materials and methods

Plant material, growth conditions, and salt treatments

Cloned plants of *G. longituba* were cultured by tissue culture in our laboratory to make their roots up to 4 cm in length. The plants were transferred to a nutrient solution comprising 2.00 mmol•L^{-1} $Ca(NO_3)_2$, 4.00 mmol•L^{-1} KNO_3, 0.67 mmol•L^{-1} $NH_4H_2PO_4$, 1.00 mmol•L^{-1} $MgSO_4$, 24.99 μmol•L^{-1} $MnSO_4$, 25.07 μmol•L^{-1} H_3BO_3, 7.48 μmol•L^{-1} $ZnSO_4$, 1.25 μmol•L^{-1} KI, 0.26 μmol•L^{-1} Na_2MoO_4, 0.04 μmol•L^{-1} $CuSO_4$, 0.03 μmol•L^{-1} $CoCl_2$, and 72.6 μmol•L^{-1} Fe-EDTA for continued growth. After 45 days, the plants were transferred to a nutrient solution supplemented with 0 (control), 0.2%, 0.4%, 0.6%, and 0.8% (g/100 mL) Na_2CO_3 solutions, with three replicates at each concentration level. The growth conditions were observed at 4, 8, 12, 24, 48, 72, and 96 h of stress, respectively, and fresh stem and leaf tissues of *G. longituba* were collected and stored in a refrigerator at -80 °C for subsequent measurements of biomass, physiologically active substances, and medicinal substances. Greenhouse conditions were as follows: 16 h light/8 h dark (500 μmol•m^{-2}•s^{-1} of light intensity), at 24±2 °C and 60±5% relative humidity.

Chemicals and reagents

Na_2CO_3 was purchased from Wuhan Yitai Science and Technology Co. Ltd. in China. Anthrone was purchased from China National Pharmaceutical Group Chemical Reagent Co. Ltd. in China. Concentrated sulfuric acid was purchased from Zhongtian Chemical Co. Ltd. in Wuhan City, China. $NaNO_2$, NaOH, $Al(NO_3)_3$, and other chemicals were purchased from Fuchen Chemical Reagent Factory in Tianjin, China. Methanol was purchased from National Pharmaceutical Group Chemical Reagent Co. Ltd. in China. Bicinchoninic acid (BCA) protein, H_2O_2, MDA, Pro, SOD, POD, APX, and CAT detection kitswere purchased from Jiancheng Technology Co.Ltd. in Nanjing City, China.

Determination of biomass and relative water content

The stems and leaves of *G. longituba* were collected as samples at each stress time and the fresh weight (FW) of the samples was recorded. The samples were submerged in distilled water and allowed to stand for 15 h at 5 °C in the dark, then the samples were removed, patted surface dry, and full turgor weight (TW) was recorded [33]. Finally, the samples were dried in an oven at 80 ℃ until constant weight, and their dry weight (DW) was recorded and used as dry biomass [34]. Relative water content (RWC) was calculated using the following equation:

$$RWC = [(FW - DW)/(TW - DW)] \times 100\%$$

Determination of physiologically active substances

Fresh leaves of *G. longituba* at each stress time were taken and ground on ice by adding PBS buffer at a ratio of 1:9 (g/mL), and the supernatant was taken after centrifugation (685 g, 10 min) to determine the activities of antioxidant enzymes CAT, SOD, POD, and APX, as well as the contents of H_2O_2, proline, and MDA, respectively. The assays were analyzed according to the method provided by the detection kits manufacturer.

Determination of soluble sugar content

The stems and leaves of *G. longituba* at each stress time were taken and fixed in an oven at 105 °C for 15 min to inactivate the enzyme rapidly [35], to avoid changes in the medicinal components due to the catalytic reaction of the enzyme. Then the stem and leaves were dried at 80 °C, crushed, and the powder was mixed with distilled water according to the material-liquid ratio of 1:15 (g/mL), and extracted in a water bath at 80 °C for 3 h. After the extraction, centrifugation (1370 g, 15 min) was performed to take the supernatant, and the soluble sugars in the supernatant were precipitated using ethanol and then dissolved again in distilled water, the content of soluble sugars was determined by the sulfuric acid-anthracenone method [36].

Determination of total flavonoids content

The stems and leaves of *G. longituba* at each stress time were taken and fixed in an oven at 105 °C for 15 min and then placed under 80 °C for drying, crushed, and the powder was mixed with 95% ethanol according to the material-liquid ratio of 1:15 (g/mL) and extracted in a water bath for 2 h at 80 °C, after the extraction, the supernatant was centrifuged (1790 g, 20 min), and the

flavonoid content of the supernatant was determined by spectrophotometric method [37].

Determination of ursolic acid and oleanolic acid content

The stems and leaves of *G. longituba* at each stress time were taken and fixed in an oven at 105 °C for 15 min, then placed at 80 °C for drying, and pulverized after complete drying. Anappropriate amount of dried powder of *G. longituba* was extracted by Soxhlet to obtain the sample solution to be tested, and the content of ursolic acid and oleanolic acid was determined using High-Performance Liquid Chromatography (HPLC). Chromatographic conditions: C_{18} column (4.6 mm×250 mm, 5 μm), methanol-0.1% aqueous phosphoric acid (85:15) as mobile phase, flow rate 1.0 mL/min, detection wavelength 210 nm, column temperature 30 ℃, injection volume 20 μL.

Data analysis

All experimental data were statistically analyzed using SPSS 22.0 software. The experiment was repeated three times. The data were expressed as mean±standard error (SE), which were analyzed by one-way analysis of variance. Tukey method was used to compare the multiplicity between groups ($P=0.05$). All statistical charts were completed by Origin 8.0 software.

Results

The growth state of *G. longituba*

The growth of each group during the defferent stress period is illustrated in Fig. 1. Within 0 to 8 h, the growth status of *G. longituba* was almost unaffected. When the stress time reached 24 h, the high Na_2CO_3 concentration groups first showed leaf yellowing and water loss, and the chlorophyll in *G. longituba* began to decrease and the growth rate slowed down. With the further extension of the stress time, the leaf surface of the low Na_2CO_3 concentration groups gradually began to yellow and lose water, while the high Na_2CO_3 concentration groups appeared to dry up or even die. The degrees of dehydration of plants deteriorated with increasing stress concentration and time, ultimately leading to the death of the plants. By 96 h, The 0.4%, 0.6% and 0.8% Na_2CO_3-treated groups were almost dead.

Effect of salt stress on biomass

The effects of varying concentrations of Na_2CO_3 on the fresh weight, dry weight, and relative water content of *G. longituba* were analyzed at different stress durations, as presented in Table 1. The results indicated a significant decreasing trend in both fresh weight and relative water content. In contrast, dry weight exhibited an initial increase followed by a subsequent decline. Specifically, the water loss phenomenon of *G. longituba* already appeared in different degrees from the beginning of the stress, and became more and more obvious with the increase of the Na_2CO_3 concentration and the duration of the stress. Particularly, it is noteworthy that plants treated with 0.4% Na_2CO_3 for up to 4 h showed a 31.67% ($P<0.05$) increase in dry weight, despite a 19.18% and 3.27% decrease in fresh weight and relative water content, respectively, compared with the control group, suggesting that this stress condition is favorable for the accumulation of biomass.

Alterations of H_2O_2 content

H_2O_2 is an important type of ROS produced by plants in response to a counter-environment, and the changes in H_2O_2 content induced by Na_2CO_3 stress are shown in Fig. 2. Within 0 to 24 h, the H_2O_2 content of the treatment groups was lower than that of the control group. Notably, at the 12-hour mark, the H_2O_2 content of treatment groups decreased to the lowest value, which was reduced by 59.57–66.03% compared with the control, but there was no significant difference between the treatment groups ($P>0.05$). From 12 to 48 h, the H_2O_2 content of the treated groups increased significantly, reaching nearly maximum level at 48 h. However, with the exception of the plants treated with 0.2% Na_2CO_3, the H_2O_2 levels in the other groups returned to their initial levels. Subsequently, the H_2O_2 content under a further 0.2% Na_2CO_3 treatment reached a maximum value at 96 h, which was 46.52% higher than the control.

Oxidative damage to *G. longituba*

MDA is an important indicator of the degree of lipid peroxidation damage and can indirectly reflect the ability of plants to resist stress. The MDA content of each group during alkaline salt stress is shown in Fig. 3. Between 4 and 48 h, the MDA content increased gradually, and higher concentration of Na_2CO_3 resulted in greater MDA levels in the plants, with the 0.8% Na_2CO_3-treated group exhibiting the highest MDA content at 48 h, which was 281.44% higher than that of the control group. This indicates that lipid peroxidation damage was the greatest under this stress condition. After 48 h, the 0.6% and 0.8% Na_2CO_3-treated groups showed extensively mortality, while the MDA levels in plants treated with 0.2% and 0.4% Na_2CO_3 began to decrease transiently, returning to initial levels by 72 h. Notably, the MDA content of the 0.2% Na_2CO_3-treated group peaked at 96 h, which was 247.12% higher than that of the control group.

Antioxidant enzyme activities of *G. longituba* under Na_2CO_3 stress

The activities of the four major antioxidant enzymes in *G. longituba* are illustrated in Fig. 4. For CAT activity, all treatment groups exhibited an initial increase followed

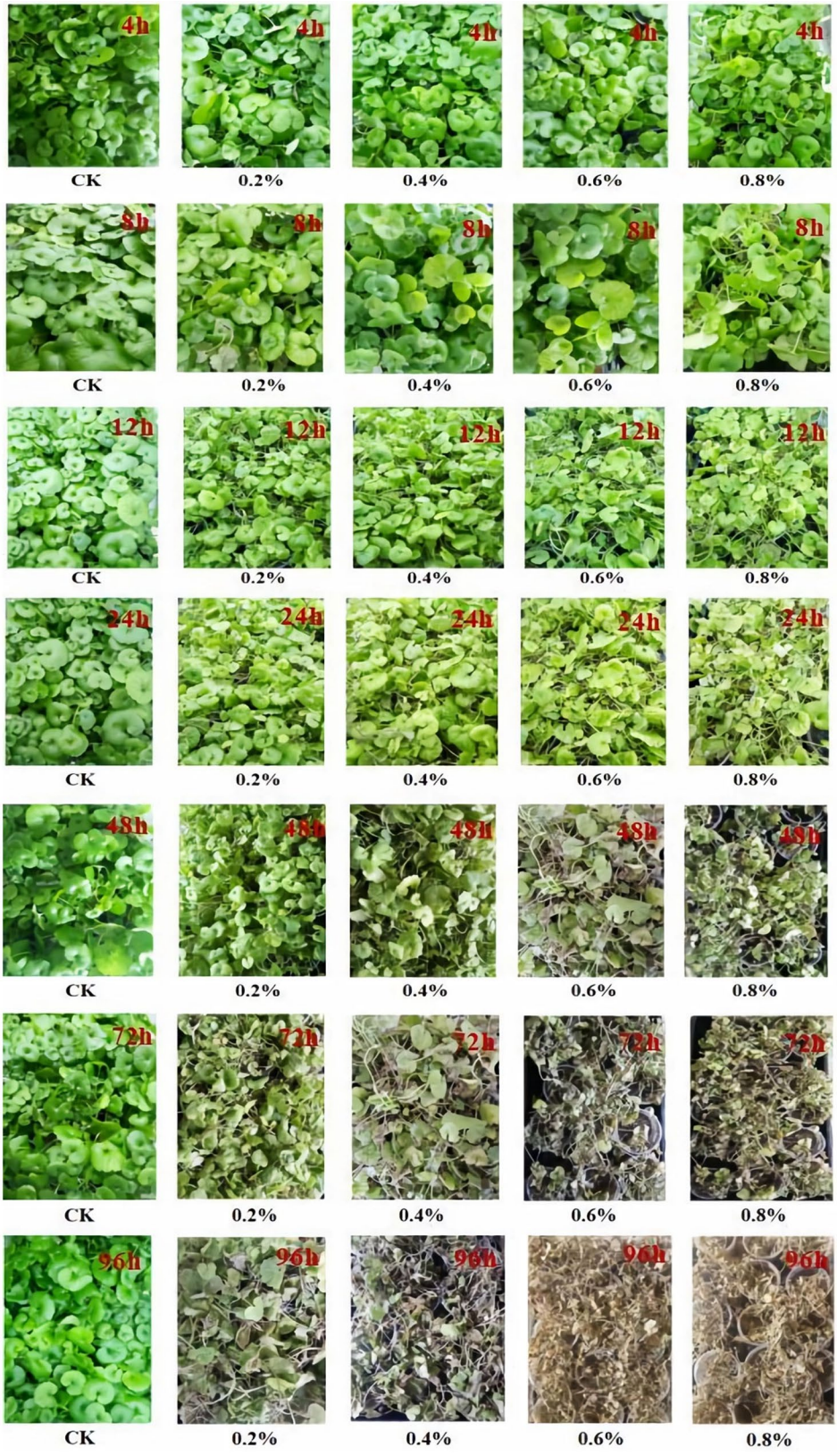

Fig. 1 Effects of concentrations and durations of Na_2CO_3stress on the growth status of *G. longituba*

Table 1 Effect of concentrations and durations of Na_2CO_3 stress on fresh weight (FW), dry weight (DW), and relative water content (RWC) of *G. longituba*($\bar{x} \pm SE$)

The concentration of Na_2CO_3(m/v)						
Time/h	Weight/g	0	0.2%	0.4%	0.6%	0.8%
	FW/g	23.93 ± 0.51^{a}	20.10 ± 0.37^{b}	19.36 ± 0.61^{b}	15.25 ± 0.48^{c}	14.08 ± 0.67^{c}
4	DW/g	1.20 ± 0.02^{cd}	1.38 ± 0.03^{b}	1.58 ± 0.05^{a}	1.33 ± 0.04^{bc}	1.12 ± 0.05^{d}
	RWC/%	94.97 ± 0.01^{a}	93.12 ± 0.01^{b}	91.86 ± 0.01^{d}	91.30 ± 0.01^{e}	92.02 ± 0.01^{c}
	FW/g	24.04 ± 0.11^{a}	17.91 ± 0.83^{b}	17.12 ± 0.37^{b}	13.11 ± 0.72^{c}	11.93 ± 0.42^{c}
8	DW/g	1.21 ± 0.01^{c}	1.54 ± 0.07^{a}	1.41 ± 0.03^{b}	1.11 ± 0.06^{cd}	1.03 ± 0.04^{d}
	RWC/%	94.96 ± 0.01^{a}	91.38 ± 0.02^{e}	91.78 ± 0.01^{b}	91.53 ± 0.01^{c}	91.40 ± 0.01^{d}
	FW/g	24.09 ± 0.11^{a}	14.09 ± 0.54^{b}	13.03 ± 0.52^{bc}	12.52 ± 0.30^{c}	11.95 ± 0.30^{c}
12	DW/g	1.21 ± 0.01^{a}	1.17 ± 0.05^{a}	1.10 ± 0.04^{a}	1.09 ± 0.03^{a}	1.14 ± 0.03^{a}
	RWC/%	94.98 ± 0.11^{a}	91.68 ± 0.02^{b}	91.53 ± 0.01^{c}	91.32 ± 0.01^{d}	90.46 ± 0.02^{e}
	FW/g	23.84 ± 0.43^{a}	15.04 ± 0.37^{b}	12.86 ± 0.27^{c}	10.16 ± 0.05^{d}	8.85 ± 0.47^{e}
24	DW/g	1.20 ± 0.02^{a}	1.27 ± 0.05^{a}	1.26 ± 0.03^{a}	0.98 ± 0.00^{b}	0.89 ± 0.05^{c}
	RWC/%	94.98 ± 0.01^{a}	91.54 ± 0.11^{b}	90.20 ± 0.01^{d}	90.32 ± 0.02^{c}	89.94 ± 0.02^{e}
	FW/g	24.08 ± 0.16^{a}	12.81 ± 0.42^{b}	10.96 ± 0.26^{c}	10.00 ± 0.64^{c}	7.92 ± 0.48^{d}
48	DW/g	1.21 ± 0.02^{ab}	1.27 ± 0.12^{a}	1.09 ± 0.03^{ab}	1.02 ± 0.06^{bc}	0.87 ± 0.05^{c}
	RWC/%	94.99 ± 0.00^{a}	90.11 ± 0.88^{b}	90.02 ± 0.01^{b}	89.74 ± 0.02^{b}	89.06 ± 0.01^{b}
	FW/g	24.03 ± 0.15^{a}	9.98 ± 0.16^{b}	7.92 ± 0.21^{c}	NA	NA
72	DW/g	1.21 ± 0.02^{a}	1.22 ± 0.02^{a}	0.83 ± 0.01^{b}		
	RWC/%	94.98 ± 0.01^{a}	87.81 ± 0.02^{c}	89.47 ± 0.35^{b}		
	FW/g	24.09 ± 0.13^{a}	12.81 ± 0.42^{b}	NA		
96	DW/g	1.21 ± 0.01^{a}	1.27 ± 0.12^{a}			
	RWC/%	94.98 ± 0.00^{a}	85.17 ± 0.46^{b}			

Different letters within a column indicate significant differences; NA indicates no analysis

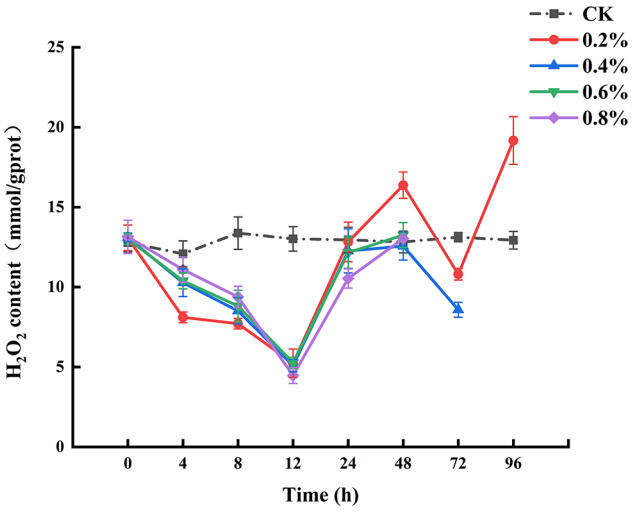

Fig. 2 Effects of concentrations and durations of Na_2CO_3 stress on H_2O_2 content in *G. longituba* (mean $\pm \bar{x} \pm SE$ SE)

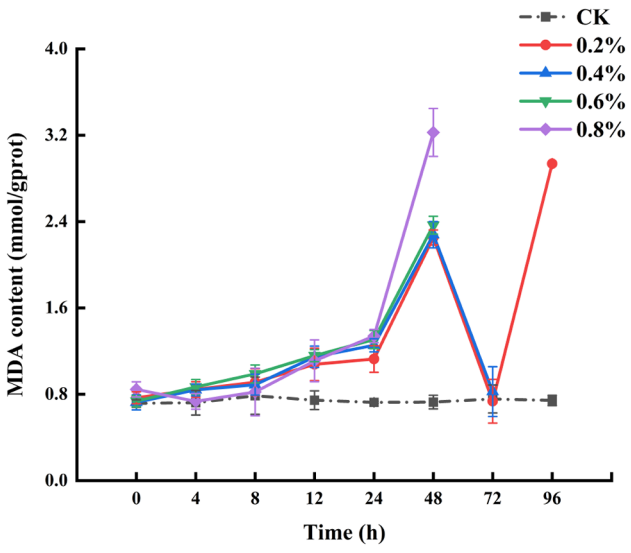

Fig. 3 Effects of concentrations and durations of Na_2CO_3 stress on MDA content in *G. longituba* (mean $\pm \bar{x} \pm SE$ SE)

by a decline, with levels consistently higher than those of the control group, and all of them reached their peak at 12 h. Notably, the CAT activity in the 0.6% Na_2CO_3-treated group was significantly higher than in the other groups ($P<0.05$), being 958.72% higher than that of the control group (Fig. 4a).

In terms of SOD activity, all treatment groups exhibited higher levels than the control group, The 0.4% Na_2CO_3-treated group showed the highest SOD activity at 8 h, which was 178.01% higher than that the control group (Fig. 4b). This indicates that *G. longituba* maximally mobilized the SOD to scavenge the reactive oxygen species during this time.

For POD activity, the duration of stress that effectively increased levels was between 4 and 24 h. The of All treatment groups reached their maximum POD activity at

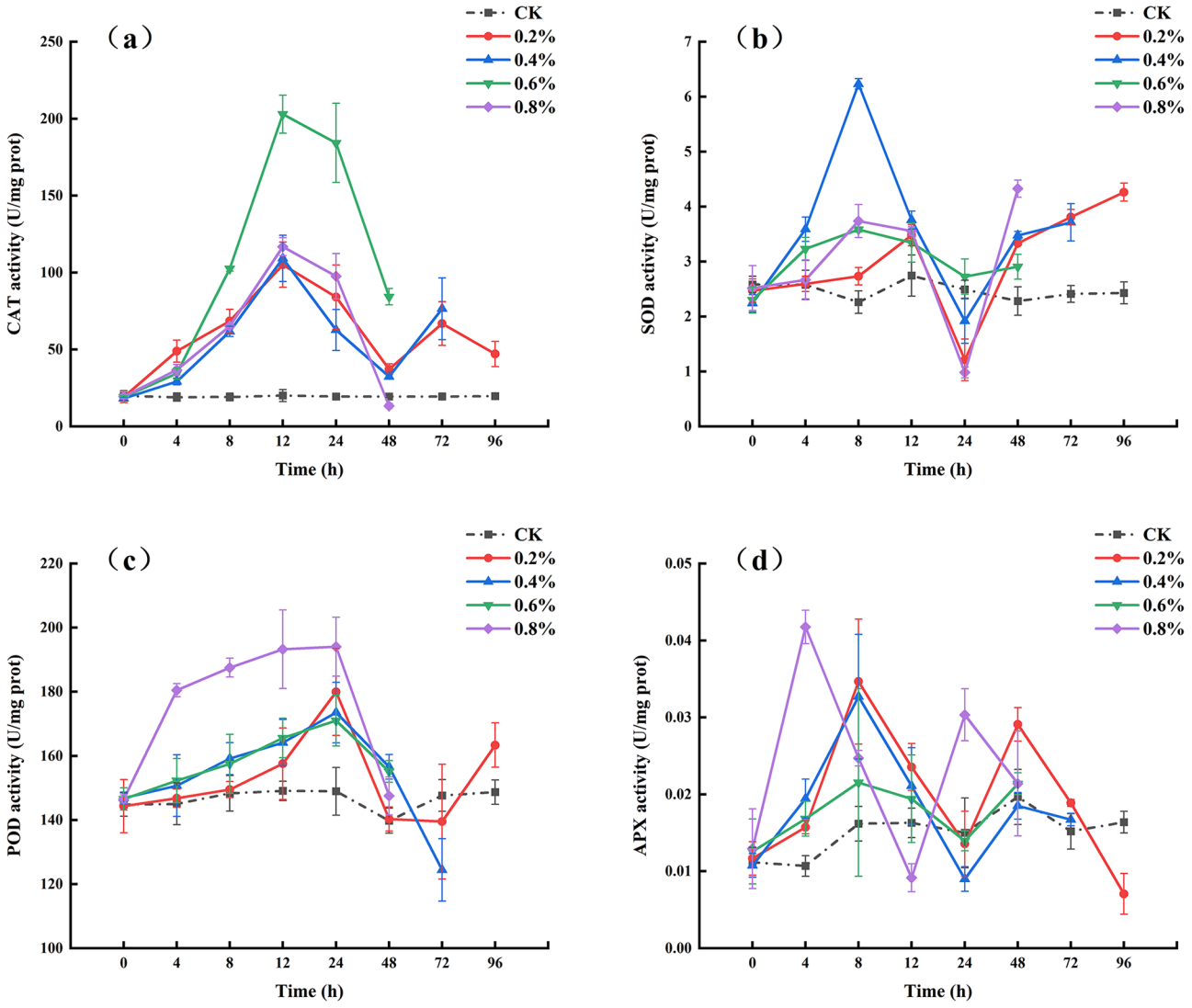

Fig. 4 Effects of concentrations and durations of Na_2CO_3 stress on antioxidant enzyme activities in *G. longituba* (mean $\pm \bar{X} \pm SE$ SE)

24 h with the 0.8% Na_2CO_3-treated group showing an increase of 32.40% compared to the control group, making it the highest among all the groups (Fig. 4c).

Regarding APX activity, fluctuations were observed across the treatment groups. The 0.8% Na_2CO_3-treated plants exhibited the highest APX activity at 4 h, which was 290.30% greater than that of the control group(Fig. 4d).

In summary, Na_2CO_3 treatment enhanced the activities of the four major antioxidant enzymes in *G. longituba* to varying degrees. This increase reflects the activation of oxidative stress responses in the plant.

Accumulation of proline and soluble sugar

Proline and soluble sugars are essential osmotic substances in plants. The effects of Na_2CO_3 stress on proline content in *G. longituba* are illustrated in Fig. 5a. From the early stages of stress, proline levels in all treatment groups gradually increased, surpassing those in the control group. A significant increase in proline content ($P<0.05$) was observed after 24 h of stress. The 0.6% and 0.8% Na_2CO_3-treated groups reached their maximum proline content at 48 h, with increases of 255.80% and 375.55%, respectively, compared to untreated plants. Notably, after 72 h, the proline content in the 0.2% and 0.4% Na_2CO_3-treated groups was significantly higher than that in the control and other treatment groups. The 0.2% Na_2CO_3-treated group exhibited the highest proline content at 96 h, which was 16.88 times greater than that of the control group.

Additionally, Na_2CO_3 treatment also enhanced the soluble sugar content in *G. longituba*, as shown in Fig. 5b. Between 4 and 48 h, all treatment groups demonstrated an increasing trend in soluble sugar content, peaking at

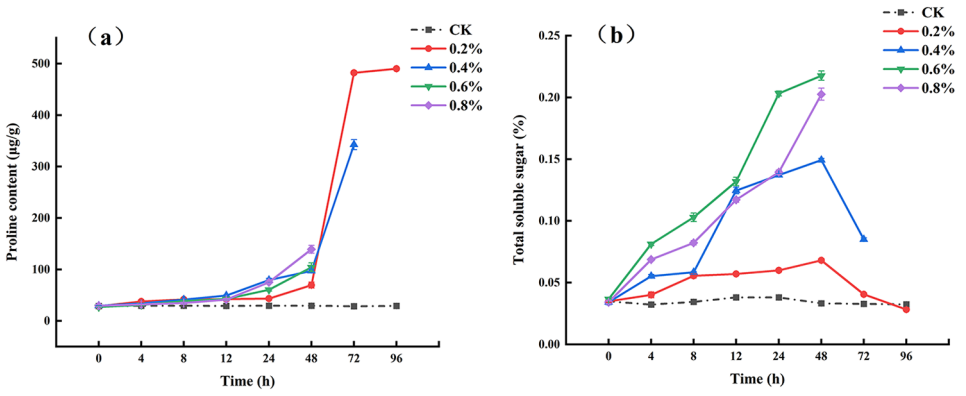

Fig. 5 Effects of concentrations and durations of Na_2CO_3 stress on accumulation of Pro and soluble sugar in *G. longituba* (mean $\pm\bar{X} \pm SE$ SE)

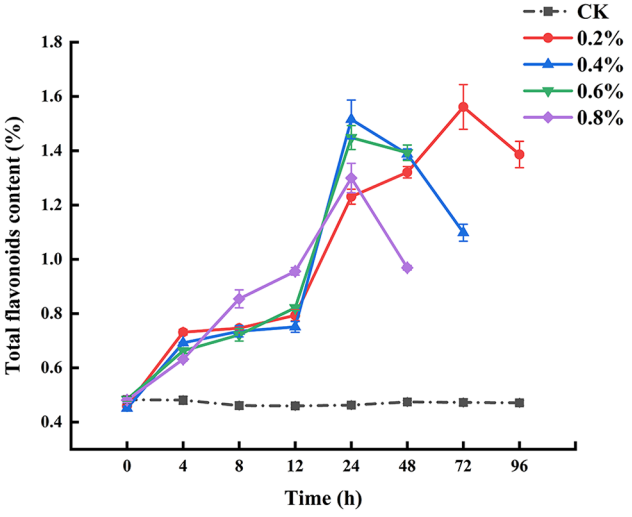

Fig. 6 Effects of concentrations and durations of Na_2CO_3 stress on total flavonoids content in *G. longituba* (mean $\pm\bar{X} \pm SE$ SE)

48 h. The highest soluble sugar content was recorded in the 0.6% Na_2CO_3-treated group, followed closely by the 0.8% Na_2CO_3-treated group. Compared to the control group, the soluble sugar contents in these two groups increased by 494.32% and 492.42%, respectively. These findings indicate that alkaline salt treatment promotes the production of osmotic substances in *G. longituba*, thereby enhancing the plant's resistance capabilities.

Changes in total flavonoids content

Flavonoids are a significant class of secondary metabolites in plants. High levels of flavonoids enhance plants' tolerance to abiotic stress conditions. The effects of different treatment conditions on the total flavonoids content in *G. longituba* are illustrated in Fig. 6. All treatment groups exhibited a significant increase in total flavonoids content between 4 and 24 h, with most groups reaching their peak levels at 24 h. Notably, the 0.2% Na_2CO_3-treated group demonstrated a sustained increasing trend that continued until 72 h. This group ultimately achieved a 2.3-fold increase in total flavonoids content compared to the control group, representing the highest level among all treatment groups.

Changes in the content of ursolic acid and oleanolic acid

Ursolic acid and oleanolic acid are the primary terpenoids found in *G. longituba*. The HPLC chromatograms of ursolic acid and oleanolic acid and the changes in their contents under Na_2CO_3 treatment are shown in Fig. 7. The results indicate that the content of ursolic acid in all treatment groups initially increased before subsequently decreasing, with the maximum concentration observed at a stress duration of 12 h, significantly exceeding that of the control group. Among the treatment groups, the 0.4% Na_2CO_3-treated group exhibited the highest ursolic acid content, with an increase of 365.75% compared to the control (Fig. 7b). In contrast, the content of oleanolic acid remained consistently higher in all treatment groups relative to the control. The highest level of oleanolic acid was recorded in plants treated with 0.4% Na_2CO_3 for 8 h, reflecting an increase of 158.85% compared to the control group (Fig. 7c). These findings suggest that saline stress is advantageous for the accumulation of terpenoids in *G. longituba*, with 0.4% identified as the optimal treatment concentration.

Discussion

Numerous studies have investigated the effects of salt stress on plant secondary metabolites. For instance, Ma et al. [38]explored the relationship between carbon and nitrogen metabolism and the synthesis of flavonoids and saponins in *Glycyrrhiza uralensis* seedlings treated with 75 mM NaCl for 45 days. Similarly, Zhang et al. [39] found through transcriptomic analysis that the expression levels of flavonoid and rutin synthesis genes were up-regulated in *Dendrobium officinale* treated with 250 mM NaCl for 14 days. However, these studies primarily focused on the physiological and biochemical responses of plants to stress, as well as the patterns of secondary

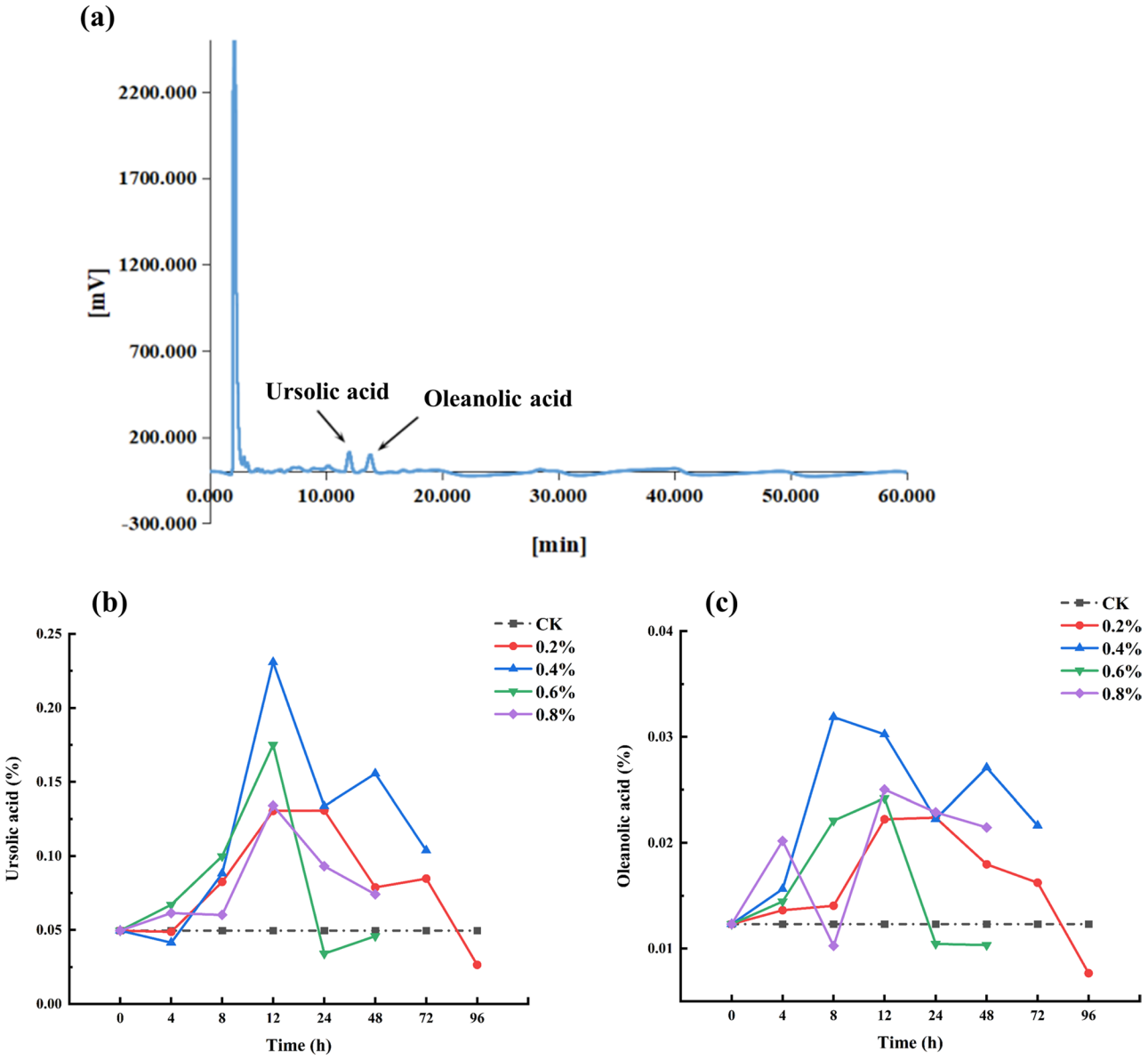

Fig. 7 Effects of concentrations and durations of Na_2CO_3 stress on ursolic acid and oleanolic acid content in *G. longituba* (mean $\pm\bar{x} \pm SE$ SE)

metabolites synthesis, often overlooked the impact on herb quality. Additionally, the long-term stress treatments described in these reports can significantly reduce biomass, hindering the cultivation of medicinal materials.

In this study, to address this issue, *G. longituba* was cultured to an adequate biomass via liquid culture before applied to short-term Na_2CO_3 stress, which may effectively mitigate the reduction in biomass. The optimal concentration and duration of stress were determined through preliminary experiments. In these pre-experiment, *G. longituba* was cultured in Na_2CO_3 solutions at concentrations of 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 1.2%, 1.4%, and1.6%. Then the growth status of *G. longituba* at 4, 8, 12, 24, 36, 48, 60, 72, 84, and 96 h were observed and the key medicinal components such as soluble sugars and flavonoids were measured. Notably concentrations exceeding 0.8% resulted in the death of *G. longituba* within 24 h, with no significant increase in the aforementioned metabolites. Additionally, assay results at 36, 60, and 84 h showed no significant changes compared to earlier measurements.

Based on these findings, the experiment was designed as follows: Na_2CO_3 concentrations of 0.2%, 0.4%, 0.6%, and 0.8% were selected, with stress durations of 4, 8, 12, 24, 48, 72, and 96 h. Biomass, water content, soluble sugars, flavonoids, ursolic acid and oleanolic acid were measured, as these indices are critical indicators of herb quality. Additionally, oxidative stress, antioxidant enzyme

activities, and osmotic substances—factors that influence the synthesis of secondary metabolites—were assessed to enhance our understanding of how stress affects medicinal constituents. Specifically, the correlations among these traits were analyzed(Fig. 8).

Inhibition of plant growth by stress

In saline and alkaline environments, plant growth is primarily inhibited by osmotic and ionic stress [40]. High concentrations of Na^+ reduce the water potential in the surrounding environment, causing plants to lose water. This water loss leads to the closure of stomata, which subsequently decreases CO_2 fixation and directly slows photosynthesis [41]. Additionally, Na^+ competes with essential nutrients such as K^+, Ca^{2+}, and Mg^{2+} for uptake, resulting in reduced chlorophyll production [42]. Furthermore, elevated Na^+ levels diminish the activity of various ion-dependent enzymes [43]. These Na^+-induced ionic stresses are significant mechanisms contributing to decreased plant biomass.

In our study, alkaline salt stress inhibited the growth of *G. longituba*, consistent with findings from previous research on *Tropaeolum majus L* [44]. , *Lonicera japonica* Thunb [45]., and *Festuca arundinacea* Schreb [46]. under salinity stress. However, a significant increase in dry weight at 4 h in the 0.4% Na_2CO_3-treated group was observed in this study. This suggests that mild stress may activate beneficial stress responses that promote biomass accumulation in plants. Although we noted a reduction in relative water content, it is posited that a moderate decrease in the relative water content of medicinal plants could enhance their quality without compromising active ingredient levels. This is particularly relevant, as the browning reaction induced by necessary post-harvest drying could diminish medicinal value [47].

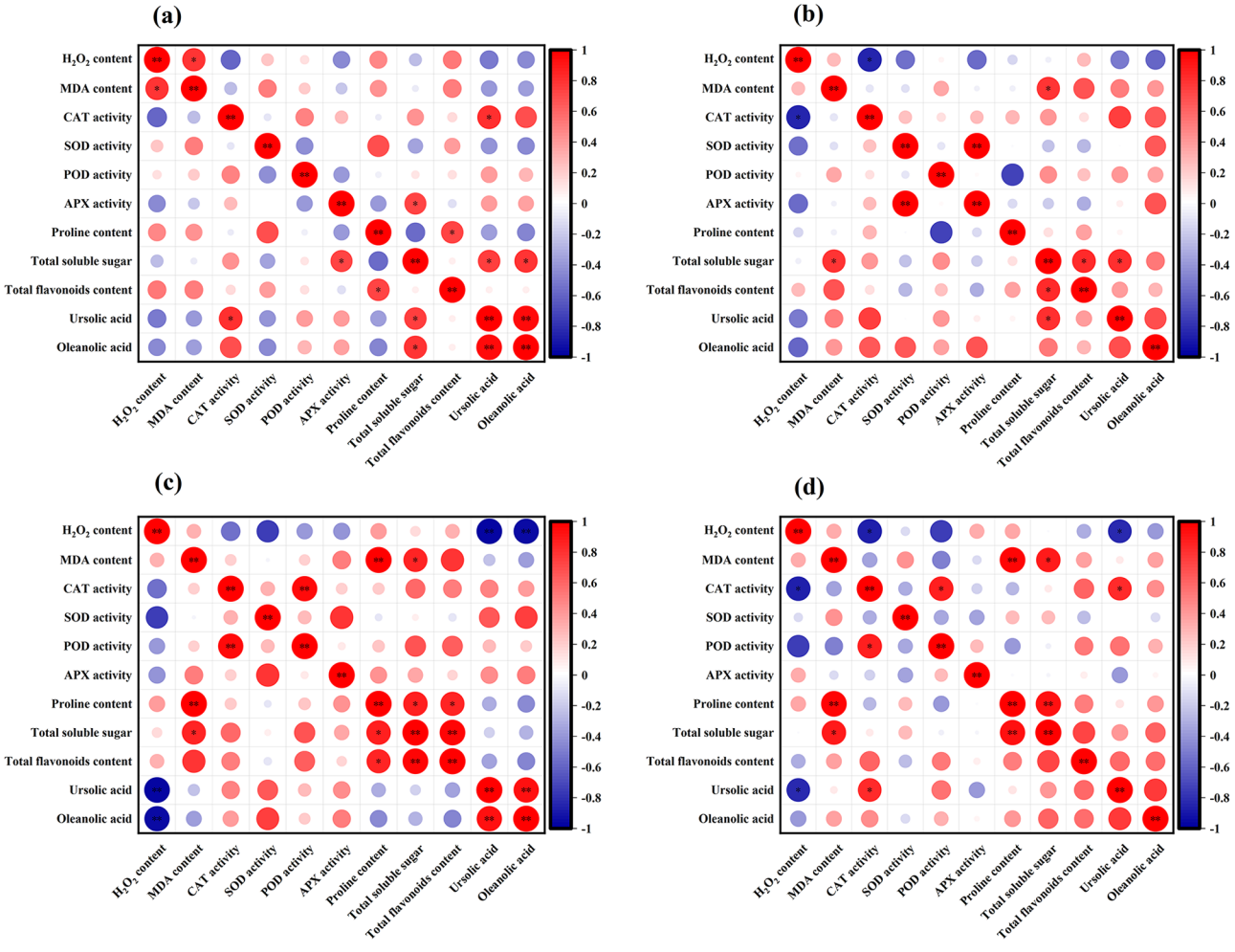

Fig. 8 Correlation analysis of physiological and biochemical properties of *G. longituba* under the stress of 0.2% (**a**), 0.4% (**b**), 0.6% (**c**), and 0.8% (**d**) alkali salt concentrations, respectively. * indicates significance at a 5% level, ** shows significance at a 1% level, and color depth denotes correlation coefficient. The strength of the correlation is indicated by the size of the circle

Oxidative stress induced by alkaline salt treatment

In response to salt stress, reactive oxygen species (ROS) play a crucial role as signaling molecules in plants. Maintaining ROS levels within a specific range is essential for normal plant growth [48]. In saline environments, excessive ROS can oxidatively damage proteins, lipids, and nucleic acids, ultimately leading to cell death [49]. To mitigate this damage, plants employ an effective antioxidant enzyme system to scavenge ROS [50]. Superoxide dismutase (SOD) catalyzes the conversion of superoxide radicals to H_2O_2, which is subsequently decomposed to non-toxic water and oxygen by enzymes such as catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) [51], this process may represent a potential mechanism for modulating H_2O_2 levels. H_2O_2 acts as an intermediate product in the ROS scavenging pathway and is often used as a reference for measuring ROS content.

In our study, H_2O_2 levels remained low across all treatment groups from 0 to 24 h, while the activities of CAT, APX, and POD significantly increased during this period. These findings indicate that *G. longituba* utilizes a ROS scavenging mechanism involving the activation of these three antioxidant enzymes in response to saline and alkaline stress, which aligns with aligns with the results reported by Pan et al. [52]. However, we observed a gradual increase in malondialdehyde (MDA) levels from 0 to 24 h. Elevated MDA levels, a final product of lipid peroxidation, suggest considerable damage to cellular membranes [53]. Despite the increase in SOD activity during this timeframe, it was insufficient to convert all superoxide radicals into H_2O_2. Consequently, the remaining superoxide radicals could still cause oxidative damage to cell and organelle membranes, a phenomenon documented in studies involving salt stress in the *Rosaceae* family [54].

As the duration of stress extended to 48 h, CAT and POD activities declined, coinciding with a notable increase in H_2O_2 and MDA levels, consistent with expected trends. The inhibition of antioxidant enzymes is likely due to high concentrations of Na^+, leading to a deficiency of K^+ and Ca^{2+}. This deficiency interferes with various enzymatic processes that require K^+ and Ca^{2+}, resulting in cytoplasmic ionotoxicity and reduced enzyme activity [55]. However, the high activity of SOD at 72 h caused H_2O_2 and MDA levels to decrease, suggesting that this clonal plant exhibits greater salinity tolerance.

Accumulation of osmotic substances and medicinal value of soluble sugars

The rapid accumulation of cytoplasmic osmoregulators is an effective strategy for saline adaptation in halophytic plants [56]. Under saline stress, compatible osmolytes, which are low molecular weight compounds, accumulate. These include charged metabolites, polyols, soluble sugars, ions, and free proline [57, 58]. The synthesis of these osmotic substances synthesis may be linked to the abscisic acid (ABA) signaling network [59]. Specifically, the small peptide CLE25 is induced and translocated to the leaves to regulate ABA synthesis in response to water deficit, ABA, in turn, inactivates the inhibitory protein phosphatase 2 C (PP2C), leading to phosphorylation of the subtype III SnRK2s [60]. This pathway regulates various downstream processes, including ion transport, stomatal closure, and the biosynthesis of compatible osmolytes [61].

In our study, the stress increased the levels of proline and soluble sugars in *G. longituba*, which aligns with findings from previous studies on *Tagetes minuta* [62], *Juglans microcarpa* [63], *Cannabis sativa* [64]. We hypothesize that stress acts as an exogenous signal that activates the ABA pathway, resulting in the increased accumulation of proline and soluble sugars. Our results indicate a positive correlation between the contents of proline and soluble sugars and the levels of malondialdehyde (MDA). This suggests that MDA may function as an endogenous signal that activates the ABA pathway. thereby promoting the production of proline and soluble sugars in *G. longituba*. Although research on the activation signals of the ABA pathway is limited, our findings contribute to the understanding of this signaling mechanism.

In the field of pharmacology, soluble sugars are a key medicinal component of plants, demonstrating the ability to enhance blood circulation [65] and regulate human immunity [66]. Additionally, their significant hypoglycemic effects make soluble sugars a promising area of research for diabetes treatment [67]. Our study found that the soluble sugar content in *G. longituba* was highest under 0.6% Na_2CO_3 stress for 48 h. Therefore, *G. longituba* grown under these conditions could serve as a valuable raw material for the development of diabetes-specific drugs.

Synthesis of flavonoids and its application in the field of medicine

Flavonoids are a class of compounds characterized by the linkage of two phenolic hydroxyl benzene rings through a central chain of three carbon atoms. They are significant secondary metabolites in plants and serve as important antioxidants [68–70]. Flavonoids scavenge ROS by utilizing their hydroxyl groups to release single electrons from free radicals and by enhancing the expression of antioxidant genes [71–73]. The synthesis of flavonoids depends on the phenylpropanoid and polyketide pathways, where p-coumaroyl-CoA and malonyl-CoA are catalyzed by chalcone synthase (CHS) to produce ketones, which act as intermediates for further flavonoid synthesis [74].

In our study, Na_2CO_3 stress significantly increased flavonoid biosynthesis in *G. longituba*, consistent with previous studies on *Sophora alopecuroides* [75], *Solanum nigrum* [76], and *Glycyrrhiza Uralensis* [77]. This increase in flavonoids is hypothesized to result from stress-induced activation of the phenylpropanoid and polyketide pathways, suggesting a potential mechanism for enhancing flavonoids synthesis in *G. longituba*. However, from a bioenergetic perspective, stress can be energetically costly, and flavonoid biosynthesis requires substantial energy [78]. Our observations indicated that flavonoid content was positively correlated with the levels of proline and soluble sugars. The substantial accumulation of proline and soluble sugars may serve as substrates for the tricarboxylic acid cycle [79], providing additional energy support for flavonoid synthesis.

In the medical field, flavonoids are recognized as dietary compounds with pharmacological properties, including antioxidant, anti-inflammatory, anti-tumor, and blood circulation-promoting effects. They have been extensively utilized in the development of potent drugs for treating cardiovascular diseases and cancer [80]. Furthermore, ongoing pharmacological research is exploring the potential of flavonoids in treating neurological disorders, such as Parkinson's and Alzheimer's diseases [81]. Notably, the flavonoid content of *G. longituba* was found to reach its maximum at 0.2% Na_2CO_3 stress for 72 h, this condition renders *G. longituba* a promising source of medicinal herbs for the treatment of cardiovascular diseases, cancer, and neurological disorders.

Accumulation and medicinal potential of ursolic acid and oleanolic acid

Terpenoids are another important class of secondary metabolites found in *G. longituba*, with ursolic acid and oleanolic acid being the most abundant compounds. These terpenoids function as direct or indirect defense substances in response to adverse environmental conditions. Their biosynthesis begins with the formation of terpene skeletons, which are subsequently transformed into various types of terpenoids. The formation of these skeleton depends on either the mevalonate (MVA) pathway in the cytosol or the 2-c-methyl-d-erythritol-4-phosphate (MEP) pathway in the plastids [82]. Additionally, the production pathways of these terpenoids can be influenced by exogenous signals, including both biotic and abiotic stresses.

In our study, the levels of ursolic acid and oleanolic acid in *G. longituba* increased significantly following stress exposure. This finding is consistent with previous research on the medicinal plant *Salvia mirzayanii* [83], which demonstrated that salt stress similarly elevated terpenoid levels. We hypothesize that Na_2CO_3 treatment serves as an exogenous signal that activates the MEV and MEP pathways, thereby promoting terpene production in *G. longituba*.

Correlation analysis revealed that the levels of ursolic acid and oleanolic acid were negatively correlated with hydrogen peroxide (H_2O_2) level and positively correlated with catalase (CAT) activity. This suggests that the overproduction of reactive oxygen species (ROS) adversely affects the MVA and MEP pathways. Such findings support the theory that an imbalance in ROS disrupts cellular metabolism [18]. Notably, the biosynthesis of terpenoids, including ursolic acid and oleanolic acid, was primarily sustained by increased CAT activity, which helped maintain ROS at relatively low levels.

From a pharmacological perspective, ursolic acid is known to induce apoptosis in cancer cells and enhance the activity of osteoblasts and osteoclasts, making it a potent agent for cancer treatment and osteoporosis prevention [84]. The highest concentration of ursolic acid was achieved when *G. longituba* was treated with 0.4% Na_2CO_3 for 12 h. These results suggest that *G. longituba* cultivated under this method may have enhanced potential for cancer treatment and osteoporosis prevention.

Similarly, oleanolic acid is recognized for its significant anti-inflammatory and hypoglycemic properties, making it valuable in managing vascular inflammation and diabetes mellitus [85]. The maximum yield of oleanolic acid was observed after 8 h of stress treatment with 0.4% Na_2CO_3. This indicates that *G. longituba* grown under these stress conditions might offer considerable therapeutic potential for managing vascular inflammation and diabetes.

Conclusion

In the clonal plant *G. longituba*, the stress effect of Na_2CO_3 caused water loss and inhibited the growth of the plant, but mild stress treatment favored the accumulation of biological yield. In addition, Na_2CO_3 stress increased the activities of antioxidant enzymes SOD, CAT, APX, and POD. These enzymes serve as primary reactive oxygen species (ROS) scavengers and exhibit a synergistic relationship. Furthermore, stress conditions facilitated the accumulation of proline, soluble sugars, flavonoids, ursolic acid, and oleanolic acid. Proline and soluble sugars function as osmotic agents, regulating cellular osmotic pressure to mitigate water. Most importantly, soluble sugars, flavonoids, ursolic acid, and oleanolic acid are major medicinal constituents of the plant that could be used in the treatment of a variety of diseases such as diabetes, cardiovascular diseases, neurological diseases, inflammation, and cancer. Our results have identified the optimal stress conditions under which these substances could be produced in large quantities, and if it is desired to use *G. longituba* for the treatment of specific diseases, then modification of the cultivation to

obtain the appropriate medicinal constituents based on the results of the study might be sufficient for therapeutic purposes.

Abbreviations

ROS	Reactive oxygen species
CAT	Catalase
SOD	Superoxide dismutase
POD	Peroxidase
APX	Ascorbate peroxidase
Pro	Proline
MDA	Malondialdehyde
FW	Fresh weight
DW	Dry weight
RWC	Relative water content

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Author contributions
Conceptualization, X.L., N.J. and X.Z.; methodology, X.L., N.J., X.Z., F.S. and D-H.W.; formal analysis, X.L., N.J., X.Z., F.S. and D-C.W.; investigation, D-H.W., F.S., Y.Z., T.Z., Y.Z., Q.D., X.W. and S.W.; data curation, X.L., N.J., D-H.W., F.S. and X.Z.; writing—original draft preparation, D-H.W. and F.S.; writing—review and editing, X.L, N.J. and D-H.W. All authors have read and agreed to the published version of the manuscript.

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Data availability
The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Ethical consideration
The trials conducted in this study did not violate any legislation.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Yuan Z-L, Chen X-B, Cai J, Huang L-Y, Guo S-H, Ouyang X-L. Polyphenols and Organic acids from Glechoma Longituba. Chem Nat Compd. 2023;59(3):562–4.
2. Jin L, Liu L, Guo Q. Phosphorus and iron in soil play dominating roles in regulating bioactive compounds of Glechoma longituba (Nakai) Kupr. Sci Hort. 2019;256.
3. Liang Q, Li X, Zhou W, Su Y, He S, Cheng S, et al. An explanation of the underlying mechanisms for the in vitro and in vivo antiurolithic activity of Glechoma Longituba. Oxid Med Cell Longev. 2016;2016:3134919.
4. Zhu Y-D, Zou J, Zhao W-M. Two new monoterpenoid glycosides from Glechoma Longituba. J Asian Nat Prod Res. 2008;10(2):199–204.
5. Ouyang XL, Qin F, Huang RZ, Liang D, Wang CG, Wang HS, et al. NF-kappaB inhibitory and cytotoxic activities of hexacyclic triterpene acid constituents from Glechoma Longituba. Phytomedicine. 2019;63:153037.
6. Liu F, Liu J, Dong M. Ecological consequences of clonal integration in plants. Front Plant Sci. 2016;7.
7. Clair SS, Zhang W, Yang G, Sun J, Chen J, Zhang Y. Clonal integration enhances the performance of a Clonal Plant Species under soil alkalinity stress. PLoS ONE. 2015;10(3).
8. Sun H, Zheng C, Chen T, Postma JA, Gao Y. Motherly care: how Leymus chinensis ramets support their offspring exposed to saline-alkali and clipping stresses. Sci Total Environ. 2021;801.
9. Zhang B, Chen X, Lu X, Shu N, Wang X, Yang X et al. Transcriptome analysis of Gossypium hirsutum L. reveals different mechanisms among NaCl, NaOH and Na2CO3 stress tolerance. Sci Rep. 2018;8(1).
10. Chandrasekaran M, Chanratana M, Kim K, Seshadri S, Sa T. Impact of Arbuscular Mycorrhizal Fungi on Photosynthesis, Water Status, and Gas Exchange of plants under Salt Stress–A Meta-analysis. Front Plant Sci. 2019;10.
11. Basu S, Kumari S, Kumar A, Shahid R, Kumar S, Kumar G. Nitro-oxidative stress induces the formation of roots' cortical aerenchyma in rice under osmotic stress. Physiol Plant. 2021;172(2):963–75.
12. Bai Q, Niu Z, Chen Q, Gao C, Zhu M, Bai J, et al. The C2H2-type zinc finger transcription factor OSIC1 positively regulates stomatal closure under osmotic stress in poplar. Plant Biotechnol J. 2023;21(5):943–60.
13. Yang J, Zhao S, Zhao B, Li C. Overexpression of TaLEA3 induces rapid stomatal closure under drought stress in Phellodendron amurense Rupr. Plant Sci. 2018;277:100–9.
14. Bose J, Munns R, Shabala S, Gilliham M, Pogson B, Tyerman SD. Chloroplast function and ion regulation in plants growing on saline soils: lessons from halophytes. J Exp Bot. 2017;68(12):3129–43.
15. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol. 2020;21(7):363–83.
16. Waszczak C, Carmody M, Kangasjarvi J. Reactive oxygen species in Plant Signaling. Annu Rev Plant Biol. 2018;69:209–36.
17. Sahu B, Sahu AK, Thomas V, Naithani SC. Reactive oxygen species, lipid peroxidation, protein oxidation and antioxidative enzymes in dehydrating Karanj (Pongamia pinnata) seeds during storage. South Afr J Bot. 2017;112:383–90.
18. Richards A, Chaurasia S, Guido LF. Antioxidant activity and reactive oxygen species (ROS) scavenging mechanism of Eriodictyon californium, an Edible Herb of North America. J Chem. 2022;2022:1–8.
19. Cesarino I. Structural features and regulation of lignin deposited upon biotic and abiotic stresses. Curr Opin Biotechnol. 2019;56:209–14.
20. Zhang D, Tong J, He X, Xu Z, Xu L, Wei P et al. A novel soybean intrinsic protein gene, GmTIP2;3, involved in responding to osmotic stress. Front Plant Sci. 2016;6.
21. Isayenkov S, Hilo A, Rizzo P, Tandron Moya YA, Rolletschek H, Borisjuk L et al. Adaptation strategies of Halophytic Barley Hordeum marinum ssp. marinum to high salinity and osmotic stress. Int J Mol Sci. 2020;21(23).
22. Calone R, Mircea D-M, González-Orenga S, Boscaiu M, Zuzunaga-Rosas J, Barbanti L et al. Effect of Recurrent Salt and Drought stress treatments on the endangered Halophyte Limonium Angustebracteatum Erben. Plants. 2023;12(1).
23. Bian W, Bao G, Qian H, Song Z, Qi Z, Zhang M et al. Physiological response characteristics in Medicago sativa under freeze-thaw and deicing salt stress. Water Air Soil Pollut. 2018;229(6).
24. Malecka A, Konkolewska A, Hanc A, Baralkiewicz D, Ciszewska L, Ratajczak E et al. Insight into the phytoremediation capability of Brassica juncea (v. Malopolska): Metal Accumulation and antioxidant enzyme activity. Int J Mol Sci. 2019;20(18).
25. Lukić N, Kukavica B, Davidović-Plavšić B, Hasanagić D, Walter J. Plant stress memory is linked to high levels of anti-oxidative enzymes over several weeks. Environ Exp Bot. 2020;178.
26. Agati G, Brunetti C, Fini A, Gori A, Guidi L, Landi M et al. Are flavonoids effective antioxidants in plants? Twenty years of our investigation. Antioxidants. 2020;9(11).
27. Wang C, Chen L, Cai Z, Chen C, Liu Z, Liu S et al. Metabolite Profiling and Transcriptome Analysis explains difference in Accumulation of Bioactive constituents in Licorice (Glycyrrhiza uralensis) under salt stress. Front Plant Sci. 2021;12.
28. Hu X, Wang D, Ren S, Feng S, Zhang H, Zhang J et al. Inhibition of root growth by alkaline salts due to disturbed ion transport and accumulation in Leymus Chinensis. Environ Exp Bot. 2022;200.
29. Wang A, Tao H, Ding G, Zhang B, Huang J, Wu Q. Global cropland exposure to extreme compound drought heatwave events under future climate change. Weather Clim Extremes. 2023;40.

30. Wang C-J, Wan J-Z. Assessing the habitat suitability of 10 serious weed species in global croplands. Global Ecol Conserv. 2020;23.
31. Deng X, Yuan X, Chen L, Chen Y, Rong X, Zeng Q et al. Field-scale remediation of cadmium-contaminated farmland soil by Cichorium intybus L.: planting density, repeated harvests, and safe use of its Cd-enriched biomass for protein feed. Ind Crops Prod. 2022;188.
32. Yuan X, Zhou J, Peng C, Qiao J, Xia S, Fan Y et al. Effects of Varied Forms of Arsenic Stress on Seedling Growth and Arsenic Distribution in Honeysuckle Plants. Processes. 2023;11(7).
33. Zygielbaum AI, Gitelson AA, Arkebauer TJ, Rundquist DC. Non-destructive detection of water stress and estimation of relative water content in maize. Geophys Res Lett. 2009;36(12).
34. Aroca R, Xie L, Lehvävirta S, Timonen S, Kasurinen J, Niemikapee J et al. Species-specific synergistic effects of two plant growth—promoting microbes on green roof plant biomass and photosynthetic efficiency. PLoS ONE. 2018;13(12).
35. Chen X, Jiang X, Zhao S, Liang H, Zhang X, Xu X, et al. The effects of fixation methods on the composition and activity of Sea Buckthorn (Hippophae rhamnoides L.) Leaf Tea. J Food Qual. 2022;2022:1–10.
36. Zhou R-R, Huang J-H, He D, Yi Z-Y, Zhao D, Liu Z et al. Green and efficient extraction of Polysaccharide and Ginsenoside from American Ginseng (Panax quinquefolius L.) by deep eutectic solvent extraction and aqueous two-phase system. Molecules. 2022;27(10).
37. Alide T, Wangila P, Kiprop A. Effect of cooking temperature and time on total phenolic content, total flavonoid content and total in vitro antioxidant activity of garlic. BMC Res Notes. 2020;13(1).
38. Ma X, Yu X, Cui G, Guo Z, Lang D, Zhang X. Methyl jasmonate mitigates osmotic stress by regulating carbon and nitrogen metabolism of Glycyrrhiza uralensis seedlings subjected to salt stress. Acta Physiol Plant. 2023;45(8).
39. Zhang M, Yu Z, Zeng D, Si C, Zhao C, Wang H et al. Transcriptome and metabolome reveal salt-stress responses of Leaf tissues from Dendrobium officinale. Biomolecules. 2021;11(5).
40. Chérel I, Gaillard I. The Complex Fine-tuning of K + fluxes in plants in relation to osmotic and ionic abiotic stresses. Int J Mol Sci. 2019;20(3).
41. Liang H, Shi Q, Li X, Gao P, Feng D, Zhang X et al. Synergistic effects of carbon cycle metabolism and photosynthesis in Chinese cabbage under salt stress. Hortic Plant J. 2022;10(2).
42. Ullah A, Tariq A, Sardans J, Peñuelas J, Zeng F, Graciano C et al. Alhagi Sparsifolia acclimatizes to saline stress by regulating its osmotic, antioxidant, and nitrogen assimilation potential. BMC Plant Biol. 2022;22(1).
43. Fan G, Wang L, Deng M, Zhao Z, Dong Y, Zhang X et al. Changes in transcript related to osmosis and Intracellular Ion Homeostasis in Paulownia tomentosa under salt stress. Front Plant Sci. 2016;7.
44. Barbosa LB, da Silva TI, Dias MG, Pereira ED, Cruz RRP, Silva JS, et al. Application of phytohormones reduces damage caused by salt stress in Tropaeolum majus. South Afr J Bot. 2024;166:69–78.
45. He W, Yan K, Zhang Y, Bian L, Mei H, Han G. Contrasting photosynthesis, photoinhibition and oxidative damage in honeysuckle (Lonicera japonica Thunb.) Under iso-osmotic salt and drought stresses. Environ Exp Bot. 2021;182.
46. Kaplan M, Baser M, Kale H, Irik HA, Ulger I, Unlukara A. Change in yield and chemical composition of tall fescue (Festuca arundinacea schreb.) plants under salt stress. Turkish J Field Crops. 2017;22(2).
47. Liang Z, Tong L, Yin S, Liu C, Wang L. Bidirectional hot air drying: an effective inhibitor of the browning of biomass similar to thick-layered honeysuckle. Drying Technol. 2020;40(1):116–26.
48. Hasanuzzaman M, Bhuyan MHMB, Parvin K, Bhuiyan TF, Anee TI, Nahar K et al. Regulation of ROS metabolism in plants under environmental stress: a review of recent experimental evidence. Int J Mol Sci. 2020;21(22).
49. Ye C, Zheng S, Jiang D, Lu J, Huang Z, Liu Z et al. Initiation and execution of programmed cell death and regulation of reactive oxygen species in plants. Int J Mol Sci. 2021;22(23).
50. Li J, Yu B, Ma C, Li H, Jiang D, Nan J et al. Functional characterization of Sugar Beet M14 antioxidant enzymes in Plant Salt stress tolerance. Antioxidants. 2022;12(1).
51. Hasanuzzaman M, Bhuyan MHM, Zulfiqar F, Raza A, Mohsin S, Mahmud J et al. Reactive oxygen species and antioxidant defense in plants under Abiotic stress: revisiting the crucial role of a Universal Defense Regulator. Antioxidants. 2020;9(8).
52. Pan C, Lu H, Liu J, Yu J, Wang Q, Li J et al. SODs involved in the hormone mediated regulation of H2O2 content in Kandelia obovata root tissues under cadmium stress. Environ Pollut. 2020;256.
53. Akbari B, Najafi F, Bahmaei M, Mahmoodi NM, Sherman JH. Modeling and optimization of malondialdehyde (MDA) absorbance behavior through response surface methodology (RSM) and artificial intelligence network (AIN): an endeavor to estimate lipid peroxidation by determination of MDA. J Chemom. 2023;37(4).
54. Wang D, Gao Y, Sun S, Lu X, Li Q, Li L et al. Effects of Salt stress on the antioxidant activity and Malondialdehyde, solution protein, Proline, and chlorophyll contents of three Malus Species. Life. 2022;12(11).
55. Thabet SG, Alqudah AM. New genetic insights into improving barley cope with salt stress via regulating mineral accumulation, cellular ion homeostasis, and membrane trafficking. Environ Exp Bot. 2023;208.
56. Alhaithloul HA, Soliman MH, Ameta KL, El-Esawi MA, Elkelish A. Changes in Ecophysiology, Osmolytes, and secondary metabolites of the Medicinal Plants of Mentha Piperita and Catharanthus roseus Subjected to Drought and heat stress. Biomolecules. 2019;10(1).
57. Chen J-f, Liu Y, Zhang T-y, Zhou Z-f, Huang J-y, Zhou T et al. Integrated physiological and transcriptional dissection reveals the core genes involving nutrient transport and osmoregulatory substance biosynthesis in allohexaploid wheat seedlings under salt stress. BMC Plant Biol. 2022;22(1).
58. Yang Y, Yao Y, Li J, Zhang J, Zhang X, Hu L et al. Trehalose alleviated salt stress in Tomato by regulating ROS metabolism, photosynthesis, Osmolyte Synthesis, and Trehalose Metabolic pathways. Front Plant Sci. 2022;13.
59. Park C, Lim CW, Lee SC. The Pepper CaOSR1 protein regulates the osmotic stress response via Abscisic Acid Signaling. Front Plant Sci. 2016;7.
60. Saini LK, Singh N, Pandey GK. Plant Protein Phosphatase 2 C: Critical Negative Regulator of ABA Signaling. Protein Phosphatases and Stress Management in Plants2020. pp. 83–102.
61. Hewage KAH, Yang JF, Wang D, Hao GF, Yang GF, Zhu JK. Chemical Manipulation of Abscisic Acid Signaling: a New Approach to Abiotic and biotic stress management in Agriculture. Adv Sci. 2020;7(18).
62. Moghaddam M, Farhadi N, Panjtandoust M, Ghanati F. Seed germination, antioxidant enzymes activity and proline content in medicinal plant Tagetes minuta under salinity stress. Plant Biosystems - Int J Dealing all Aspects Plant Biology. 2019;154(6):835–42.
63. Ji X, Tang J, Zhang J. Effects of Salt stress on the morphology, growth and physiological parameters of Juglansmicrocarpa L. Seedlings. Plants. 2022;11:18.
64. Hu H, Liu H, Du G, Fei Y, Deng G, Yang Y, et al. Fiber and seed type of hemp (Cannabis sativa L.) responded differently to salt-alkali stress in seedling growth and physiological indices. Ind Crops Prod. 2019;129:624–30.
65. Jenis J, Kim JY, Uddin Z, Song YH, Lee H-H, Park KH. Phytochemical profile and angiotensin I converting enzyme (ACE) inhibitory activity of Limonium Michelsonii Lincz. J Nat Med. 2017;71(4):650–8.
66. Zhou X, Zeng M, Huang F, Qin G, Song Z, Liu F. The potential role of plant secondary metabolites on antifungal and immunomodulatory effect. Appl Microbiol Biotechnol. 2023;107(14):4471–92.
67. Wang H, Li H, Hou Y, Zhang P, Tan M. Plant polysaccharides: sources, structures, and antidiabetic effects. Curr Opin Food Sci. 2023;51.
68. Lei L, Liu Y, Wang X, Jiao R, Ma KY, Li YM, et al. Plasma cholesterol-lowering activity of Gingerol- and shogaol-enriched extract is mediated by increasing sterol excretion. J Agric Food Chem. 2014;62(43):10515–21.
69. Zhang Z, Yang L, Hou J, Tian S, Liu Y. Molecular mechanisms underlying the anticancer activities of licorice flavonoids. J Ethnopharmacol. 2021;267.
70. Raffa D, Maggio B, Raimondi MV, Plescia F, Daidone G. Recent discoveries of anticancer flavonoids. Eur J Med Chem. 2017;142:213–28.
71. Wu J, Lv S, Zhao L, Gao T, Yu C, Hu J et al. Advances in the study of the function and mechanism of the action of flavonoids in plants under environmental stresses. Planta. 2023;257(6).
72. Sharma P, Kumar V, Khosla R, Guleria P. Exogenous naringenin improved digestible protein accumulation and altered morphology via VrPIN and auxin redistribution in Vigna radiata. 3 Biotech. 2020;10(10).
73. Ye JB, Chen QW, Tao TT, Xu F, Cheng SY. Promotive effects of genistein on photosynthesis, flavonoid biosynthesis and antioxidant enzyme activities in ginkgo biloba. Int J Agric Biology. 2016;18(5):917–22.
74. Shomali A, Das S, Arif N, Sarraf M, Zahra N, Yadav V et al. Diverse physiological roles of flavonoids in Plant Environmental stress responses and tolerance. Plants. 2022;11(22).
75. Ma T-L, Li W-J, Hong Y-S, Zhou Y-M, Tian L, Zhang X-G et al. TMT based proteomic profiling of Sophora alopecuroides leaves reveal flavonoid biosynthesis processes in response to salt stress. J Proteom. 2022;253.
76. Ben Abdallah S, Aung B, Amyot L, Lalin I, Lachâal M, Karray-Bouraoui N et al. Salt stress (NaCl) affects plant growth and branch pathways of carotenoid

and flavonoid biosyntheses in Solanum nigrum. Acta Physiol Plant. 2016;38(3).
77. Tong X, Cao A, Wang F, Chen X, Xie S, Shen H et al. Calcium-dependent protein kinase genes in Glycyrrhiza Uralensis Appear to be involved in promoting the biosynthesis of glycyrrhizic acid and flavonoids under salt stress. Molecules. 2019;24(9).
78. Chevilly S, Dolz-Edo L, Morcillo L, Vilagrosa A, López-Nicolás JM, Yenush L et al. Identification of distinctive physiological and molecular responses to salt stress among tolerant and sensitive cultivars of broccoli (Brassica oleracea var. Italica). BMC Plant Biol. 2021;21(1).
79. Zhang Y, Fernie AR. On the role of the tricarboxylic acid cycle in plant productivity. J Integr Plant Biol. 2018;60(12):1199–216.
80. Mitra S, Lami MS, Uddin TM, Das R, Islam F, Anjum J et al. Prospective multifunctional roles and pharmacological potential of dietary flavonoid narirutin. Biomed Pharmacother. 2022;150.
81. Melrose J. The potential of flavonoids and Flavonoid metabolites in the Treatment of Neurodegenerative Pathology in disorders of Cognitive decline. Antioxidants. 2023;12(3).
82. Wang J, Wang H, Fu Y, Huang T, Liu Y, Wang X. Genetic variance and transcriptional regulation modulate terpenoid biosynthesis in trichomes of Nicotiana tabacum under drought. Ind Crops Prod. 2021;167.
83. Valifard M, Mohsenzadeh S, Kholdebarin B, Rowshan V, Niazi A, Moghadam A. Effect of salt stress on terpenoid biosynthesis in Salvia mirzayanii: from gene to metabolite. J Hortic Sci Biotechnol. 2018;94(3):389–99.
84. Woźniak Ł, Skąpska S, Marszałek K. Ursolic Acid—A pentacyclic triterpenoid with a wide spectrum of pharmacological activities. Molecules. 2015;20(11):20614–41.
85. Wasim M, Bergonzi MC. Unlocking the potential of oleanolic acid: integrating pharmacological insights and advancements in Delivery systems. Pharmaceutics. 2024;16(6).

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