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RESEARCH ARTICLE

$NaHCO_3$ stress and hemp (*Cannabis sativa* L.) adaptation: Growth physiology-metabolism synergy in saline-alkaline environments

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ABSTRACT

Salt and alkaline stress pose major challenges to global agriculture, negatively affecting crop production. It is essential to understand how hemp (*Cannabis sativa* L.) adapt to these stresses in order to improve cultivation in affected regions. This study investigated the adaptive responses of hemp to sodium bicarbonate ($NaHCO_3$) stress by evaluating growth, photosynthetic performance, physiological characteristics, nutrient distribution, and secondary metabolite accumulation under different $NaHCO_3$ concentrations (0, 100, 200, and 300 mM). Low $NaHCO_3$ concentration (100 mM) had nonsignificant effect on hemp growth ($p > 0.05$) and even increased root dry weight by 32.31%. However, higher concentrations (200, 300 mM) significantly inhibited growth, reducing plant height by 20.70%-28.87%, stem diameter by 30.40%-52.48%, root length by 23.08%-33.25%, and leaf dry weight by up to 81.60%. Mild stress enhanced photosynthetic performance, with increases in SPAD (6.02%), net photosynthetic rate (20.64%), stomatal conductance (26.94%), transpiration rate (18.99%), and intercellular CO_2 concentration (9.61%), whereas severe stress markedly suppressed these parameters. The $NaHCO_3$ stress promoted Na^+ accumulation, particularly in roots (up to 630.91%), while reducing the uptake of K^+, Ca^{2+}, Mg^{2+}, N, and P. Moderate stress enhanced antioxidant defense and stimulated secondary metabolism, resulting in increases of 86.75% in total flavonoids, 145.92% in total phenolics, and 60.63% in cannabidiol, whereas these metabolites declined under severe stress. These findings could help to optimize hemp cultivation to improve salinity tolerance and contribute to the understanding of salinity stress responses in other important economic crops.

Key words: Antioxidant enzymes, *Cannabis sativa*, mineral elements, $NaHCO_3$ stress, nutrient uptake, secondary metabolites.

INTRODUCTION

Salinity stress is a major environmental challenge for global agriculture, severely affecting plant growth, physiological function, and productivity. Salt accumulation in arable lands causes significant abiotic stress, resulting in a 10% loss in global arable land area and jeopardizing food production and agricultural sustainability (Saleem et al., 2025). Land salinization is a significant global environmental problem. Approximately 1 billion hectares of the global land surface is currently salt-affected, representing about 7% of the Earth's land surface (Hopmans et al., 2021). China has about 3.6 billion hectares of saline land, accounting for 4.88% of its available land area (Hu et al., 2018). The ongoing impact of climate change and poor land management on land salinization highlights the importance of research on plant responses and adaptation to salinity stress. Typically, soils with high NaCl or NaCl and Na_2SO_4 are classified as saline, while those with elevated $NaHCO_3$ and Na_2CO_3 are classified alkaline (Anderson and Fidel, 2025).

Soil alkalinity, which is more detrimental to plant growth than salinity, is widespread worldwide. Salinity induces osmotic and ionic stress in plants; osmotic stress directly inhibits water uptake, cell expansion, and subsequent plant development (Ye et al., 2025). Alkaline salt stress from compounds such as sodium bicarbonate ($NaHCO_3$) results in ionic toxicity, osmotic stress, and problems associated with high pH. Elevated pH decreases the availability of metal ions (Fe^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}), causing physiological and metabolic disorders that inhibit plant growth (Wang et al., 2022b). Salt stress significantly affected leaf chlorophyll content in hemp (*Cannabis sativa* L.), reduced the efficiency of photosystem II (PSII) and simultaneously increased the accumulation of flavonoids (Kasprowiak et al., 2025). In addition, excessive accumulation of reactive oxygen species (ROS) accumulation under alkaline stress causes oxidative stress, leading to malondialdehyde (MDA) accumulation, membrane damage, degradation of photosynthetic pigments, and impaired photosynthetic performance (Liu et al., 2021).

Hemp, a versatile cash crop, is important in the fiber, food, and biomass energy industries and has significant medical potential due to bioactive compounds such as cannabidiol (CBD) (Britch et al., 2021; Han et al., 2024; Chaisan et al., 2025). Identification of morphological traits affecting high seed yield potential from new hemp germplasm collected in Thailand (Chaisan et al., 2025). The CBD, an important non-psychotropic component, treats conditions such as epilepsy, Alzheimer's disease, anxiety, pain, and inflammation (Poleg et al., 2019; Shomali et al., 2022). Although hemp tolerates various environmental stresses well, few studies have investigated its specific responses and adaptations to salinity stress. Current literature suggests that hemp can adapt to saline and alkaline environments by modulating physiological and biochemical pathways and activating defense mechanisms (Cao et al., 2021). However, while these studies primarily focus on salt stress, research on alkaline salt stress (e.g., $NaHCO_3$) remains scarce.

Therefore, this study aims to fill this gap by systematically analyzing the effects of $NaHCO_3$ stress on the growth, physiology, and secondary metabolites of hemp to better understand its response mechanisms to alkaline salt stress. This study investigated the adaptive mechanisms of hemp to alkaline salt stress by evaluating changes in growth, physiological regulation, elemental uptake, and secondary metabolites at different $NaHCO_3$ concentrations. This research improves our understanding of hemp's biological responses to salt and alkaline stress, they provide a scientific basis for future efforts to enhance its tolerance using approaches such as molecular breeding, genetic engineering or metabolic engineering, and also offer guidance for crop management and variety improvement.

MATERIALS AND METHODS

Plant material and experimental design

The plant material for hemp (*Cannabis sativa* L.) No.5 used in this experiment was obtained from the Daqing Branch of the Heilongjiang Academy of Sciences, Harbin, Heilongjiang China. First, the industrial hemp seeds were surface-sterilized (Huang et al., 2019) and then sown into an autoclaved substrate to establish seedlings.

Once the seedlings had grown, they were transplanted into a 3:1 (v/v) mixture of vermiculite and soil for further cultivation. Plants were grown in a growth chamber under controlled conditions: 25 ± 1.3 °C, 16:8 h light/dark cycle, white light intensity of approximately 250 µmol quanta m^{-2} s^{-1}, and 40 ± 4% humidity. After 1 wk, seedlings with uneven growth were removed, leaving three healthy seedlings per pot (diameter 16 cm, height 17 cm). Alkaline stress treatments started when the seedlings reached the three-leaf stage.

The experiment was divided into four groups: A control and three treatment groups exposed to 100 (S1), 200 (S2), and 300 mM (S3) $NaHCO_3$ solutions, respectively. Each treatment group received 250 mL their respective $NaHCO_3$ solution every 3 d, for a total of six applications. The control group received the same amount of water. Each treatment had three replicates. At the end of the treatment (3 wk), roots, stems, and leaves were rapidly harvested, snap frozen in liquid nitrogen, and stored at -80 °C for further analysis.

Determination of seedling growth

Plant height, root length, and fresh weight were measured using a balance, followed by oven-drying: First at 105 °C for 10 min, then at 80 °C until a constant weight was reached, to determine biomass.

Physiological and biochemical indices

Malondialdehyde (MDA) content was measured using the thiobarbituric acid method (Wang et al., 2019), soluble protein was measured using the Khao Maas Brilliant Blue method (Bradford, 1976), and superoxide dismutase (SOD) and peroxidase (POD) activities were evaluated according to the method described by Tian et al. (2022).

SPAD, photosynthetic, and fluorescence parameters

The SPAD values were measured using a chlorophyll analyzer (TYS-A, Shandong Jinke Lida Instrument, Jining, Shandong, China). Net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured using a portable photosynthesis system (LI-6400XT, LI-COR Biosciences, Lincoln, Nebraska, USA). Measurements were conducted on the youngest fully expanded leaves between 09:00 and 11:00 h on sunny days under a photosynthetic photon flux density of 1000 μmol m^{-2} s^{-1}. Chlorophyll fluorescence parameters were subsequently determined using a portable chlorophyll fluorometer according to the manufacturer's instructions.

Determination of secondary metabolites

Total phenolic content was determined using the Folin-Ciocalteu method (Jin et al., 2016), and flavonoid content was determined using the aluminum chloride colorimetric method (Ibrahim et al., 2010). Cannabidiol (CBD) content was quantified by HPLC after ultrasound-assisted extraction (Agarwal et al., 2018). The same batch of hemp leaves was used for each determination; the experiment was repeated three times, and the average value was calculated.

Ion content determination

Nitrogen content in plant roots, stems, and leaves was measured using a Kjeldahl nitrogen analyzer (KDN-19K, Shanghai Xianjian Instrument, Shanghai, China). An inductively coupled plasma mass spectrometry (ICP-MS) (7850, Agilent Technologies, Santa Clara, California, USA) was used for further analysis.

Statistical analysis

Data are presented as the mean ± SD of three independent experiments. ANOVA was performed using SPSS 21.0 (IBM, Armonk, New York, USA), with Duncan's multiple range tests at $P < 0.05$ (or 0.01) significance. Plots were generated using Origin 8.0 software (OriginLab, Northampton, Massachusetts, USA).

RESULTS

Effects of $NaHCO_3$ stress on growth indicators of hemp

This study measured the effects of four different $NaHCO_3$ concentrations on hemp plant height, stem thickness, root length, and leaf number under alkaline salt stress. As shown in Figure 1, hemp growth parameters—plant height, stem thickness, root length, and leaf number—tended to decrease with increasing soil salinity. Treatment with 100 mM $NaHCO_3$ did not significantly alter these growth parameters compared to the control. However, concentrations higher than 100 mM inhibited growth more significantly. Treatments with 200 to 300 mM $NaHCO_3$ resulted in reductions in plant height (20.70% to 28.87%), stem thickness (30.40% to 52.48%), and root length (23.08% to 33.25%) compared to the control.

Effects of $NaHCO_3$ stress on biomass and relative water content of different organs of hemp

The $NaHCO_3$ stress significantly reduced DM weight of roots, stems, and leaves, as well as the relative water content of leaves (Figure 2). At 300 mM, the greatest decreases were observed: Stems and leaves dry weights decreased by 56.83% and 81.60% respectively, roots by 64.46%, and leaf water content by 35.46%. In contrast, at 100 mM, root dry weight increased by 32.31% compared to the control. This suggests that at lower concentrations, plants may adapt by increasing root vigor to take up more water and nutrients, thereby slightly increasing root biomass.

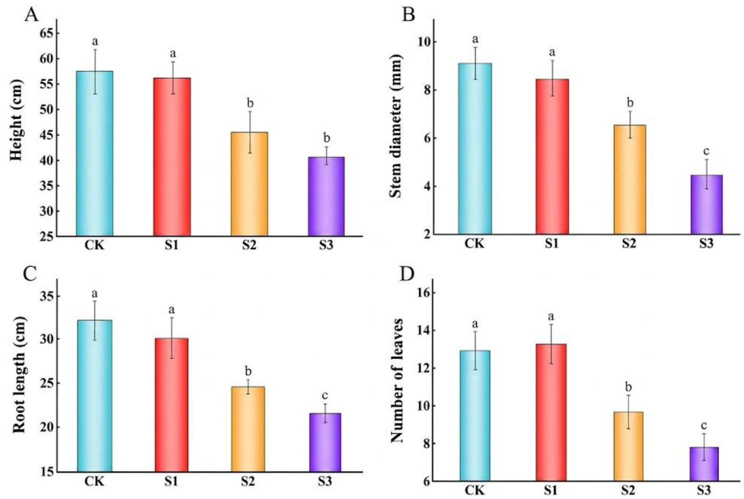

Figure 1. Effect of different $NaHCO_3$ concentrations (S1: 100 mM, S2: 200 mM, S3: 300 mM) on the growth indicators of hemp. Different letters indicate significant differences between treatments (P < 0.05). A: Plant height; B: Stem diameter; C: Root length; D: Number of leaves.

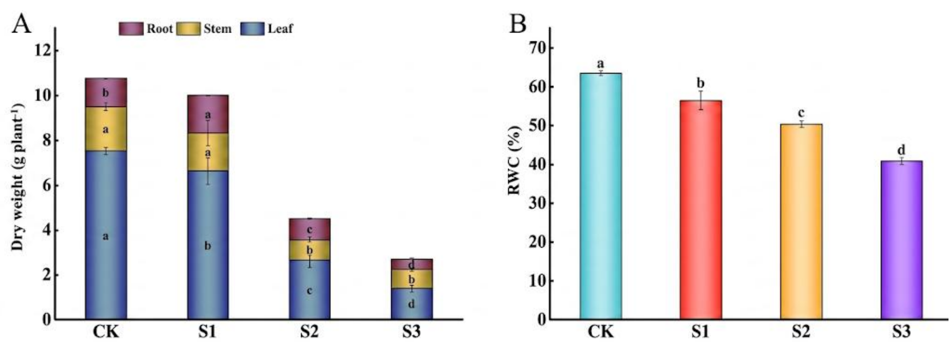

Figure 2. Effect of different alkaline salt concentrations on the biomass (A) and relative water content (RWC) (B) of hemp leaves. Different letters indicate significant differences between treatments (P < 0.05). S1: 100 mM $NaHCO_3$; S2: 200 mM $NaHCO_3$; S3: 300 mM $NaHCO_3$.

Effects of $NaHCO_3$ stress on photosynthetic and fluorescence indexes of hemp

With increased $NaHCO_3$ concentration, the photosynthetic indices first increase and then decreased (Figure 3). Under mild salinity stress, SPAD, photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) increased by 6.02%, 20.64%, 18.99%, 26.94%, and 9.61%, respectively. This increase may be attributed to $NaHCO_3$ acting as an inorganic C source, increasing CO_2 availability, causing stomata to widen and absorb more CO_2, thus enhancing photosynthesis.

Effects of $NaHCO_3$ stress on physiological indicators of hemp

With increased $NaHCO_3$ concentration, MDA content increased progressively, while the soluble protein content, SOD, and POD activity first increased and then decreased (Figure 4). At 100 mM, hemp MDA content increased by 20.18%, soluble protein by 14.85%, but SOD activity did not show any significant change compared to the control. At 200 mM $NaHCO_3$, MDA content increased to 81.68%, and SOD and POD activities peaked, significantly higher than in the control. This suggests that hemp maximized its antioxidant system to combat reactive oxygen species (ROS). At 300 mM $NaHCO_3$, SOD and POD activities decreased significantly by 12.44% and 22.47%, respectively. This reduction highlights the cell membrane damage caused by $NaHCO_3$ stress, which worsened as the stress increased.

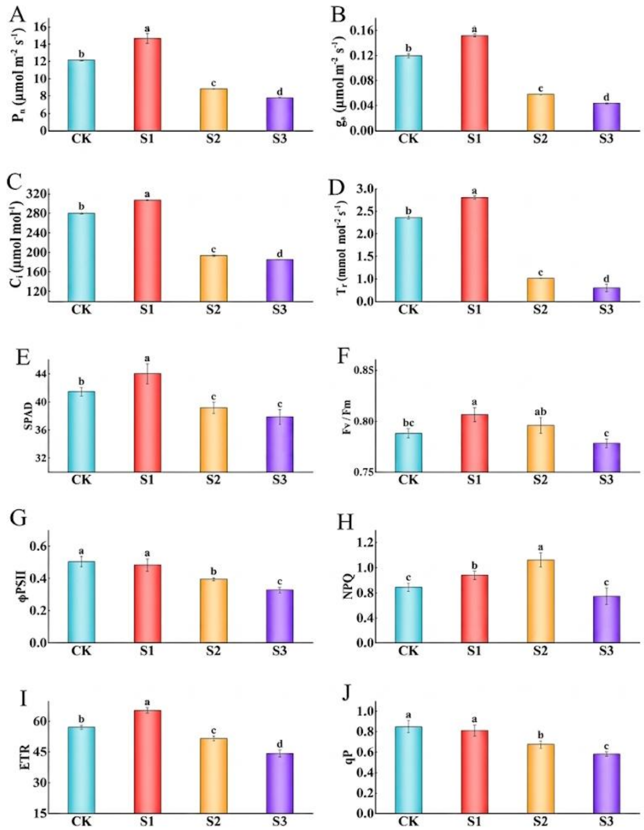

Figure 3. Effect of different alkaline salt concentrations on the photosynthesis and fluorescence indicators of hemp. Different letters indicate significant differences between treatments ($P < 0.05$). A: Photosynthetic rate (P_n); B: Stomatal conductance (g_s); C: Intercellular CO_2 concentration (C_i); D: Transpiration rate (T_r); E: Chlorophyll content (SPAD); F: F_v/F_m; G: Photosystem II (PSII); H: Non-photochemical quenching (NPQ); I: Electron transport rate (ETR); J: Photochemical quenching coefficient (qP). S1: 100 mM $NaHCO_3$; S2: 200 mM $NaHCO_3$; S3: 300 mM $NaHCO_3$.

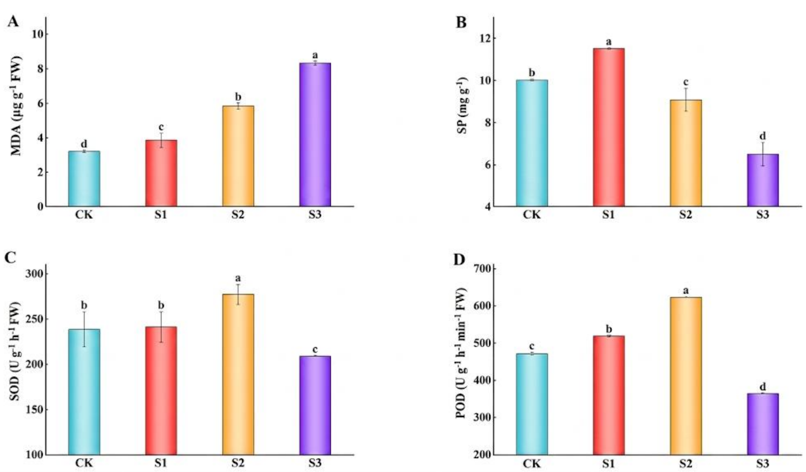

Figure 4. Effect of different alkaline salt concentrations on physiological indicators of hemp. Different letters indicate significant differences between treatments ($P < 0.05$). A: Malondialdehyde (MDA); B: Soluble protein (SP); C: Superoxide dismutase (SOD); D: Peroxidase (POD). S1: 100 mM $NaHCO_3$; S2: 200 mM $NaHCO_3$; S3: 300 mM $NaHCO_3$.

Na^+ contents and partitioning ratios

With increased $NaHCO_3$ concentration, Na^+ content in plant roots, stems, and leaves increased significantly (Figure 5B). At higher concentrations, root Na^+ content increased by up to 630.91% over the control, whereas leaf Na^+ content showed a more modest increase of 36.63% to 134.34%. This suggests that the roots absorbed substantial amounts of Na^+ under stress, resulting in a marked increase in root system Na^+ levels. With increasing $NaHCO_3$ concentrations, the allocation rate of Na^+ increased in roots (Figure 5A), while it decreased in stems and leaves. The $NaHCO_3$ stress increased Na^+ content and altered its distribution in roots, stems, and leaves. Roots, which showed the greatest increase, were the primary accumulation sites for Na^+. Although the Na^+ content of stem was generally lower, it also increased under stress. The Na^+ translocation to stems and leaves occurred as concentrations increased, with the smallest increase in leaves, probably as a protective measure to protect vital photosynthetic organs.

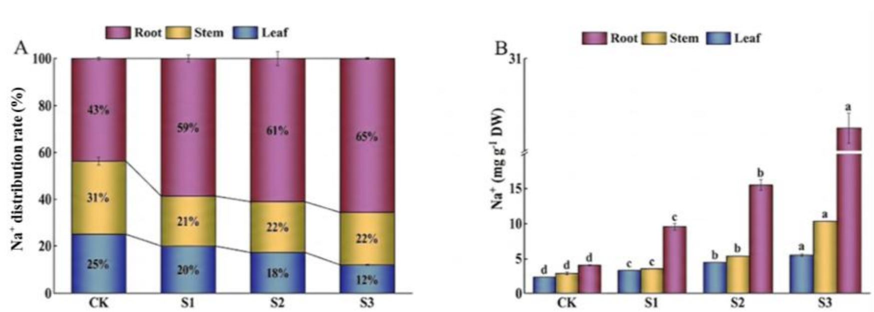

Figure 5. Effect of different alkaline salt concentrations on the Na^+ distribution rate (A) and content (B) in roots, stems, and leaves of hemp. Different letters indicate significant differences between treatments ($P < 0.05$). S1: 100 mM $NaHCO_3$; S2: 200 mM $NaHCO_3$; S3: 300 mM $NaHCO_3$.

K^+ contents and partitioning ratios

With increased $NaHCO_3$ concentration, root K^+ content first increased and then decreased, with a significant decrease of 40.55% at 200 mM (Figure 6B). At 300 mM, stem and leaf K^+ content decreased by 54.83% and 82.68%, respectively. Under mild stress, the increase in root K^+ content probably represents an adaptive mechanism to maintain osmotic pressure in root cells. Conversely, under high stress, the significant decrease in root K^+ content was due to inhibited K^+ uptake and transport. Stress reduces K^+ activity in the soil solution, with Na^+ competing for uptake sites in the roots, thereby reducing the K^+ uptake capacity of the root system.

Under $NaHCO_3$ stress, more K^+ is allocated to the roots than to stems and leaves (Figure 6A), resulting in reduced K^+ in aboveground parts and affecting their growth and physiological functions. Increased K^+ allocation to roots under stress supports ionic homeostasis, antioxidant defense, and cell membrane functions, which rely on sufficient K^+. At 300 mM $NaHCO_3$, root K^+ partitioning was significantly reduced, probably due to increased carbonate ions forming insoluble potassium carbonate, and high Na^+ levels competitively inhibiting K^+ uptake.

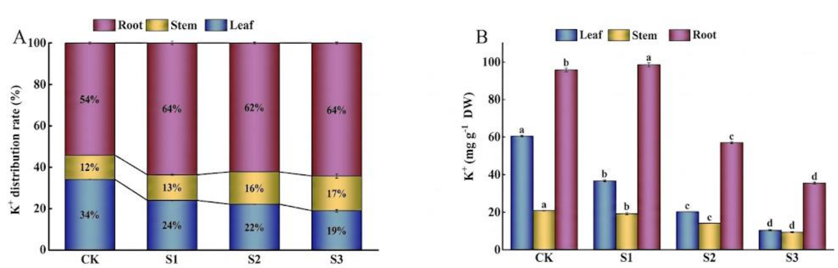

Figure 6. Effect of different alkaline salt concentrations on the K^+ distribution rate (A) and content (B) in roots, stems, and leaves of hemp. Different letters indicate significant differences between treatments ($P < 0.05$). S1: 100 mM $NaHCO_3$; S2: 200 mM $NaHCO_3$; S3: 300 mM $NaHCO_3$.

Ca^{2+}, Mg^{2+} contents and partitioning ratios

With $NaHCO_3$ stress concentration increased, Ca and Mg contents decreased in all plant organs (Figures 7A and 7C). Specifically, Ca^{2+} content in roots decreased by 49.84%-80.18%, and Mg^{2+} by 15.03%-81.09%, indicating a significant inhibition of root uptake capacity for these ions. The Ca^{2+} content in stems decreased by 11.11%-35.28% and Mg^{2+} by 22.00%-53.74%, reflecting different effects on ion accumulation in stems. The Mg^{2+} content in leaves decreased significantly when $NaHCO_3$ concentration reached 200 mM, with the greatest reduction at 300 mM (73.62%). This probably had a significant effect on chlorophyll synthesis and photosynthesis.

The $NaHCO_3$ stress caused a redistribution of Ca^{2+} and Mg^{2+} between plant organs (Figures 7B and 7D), with decreasing partitioning rates in roots and increasing rates in stems and leaves. This shift may represent an adaptive strategy to mitigate adverse effects on roots and maintain physiological functions of stems and leaves.

Overall, $NaHCO_3$ stress disrupted the Ca and Mg balance in plants, reducing ion levels in different organs and altering ion partitioning. This response is likely to serve as a protective mechanism to adapt to saline and alkaline conditions.

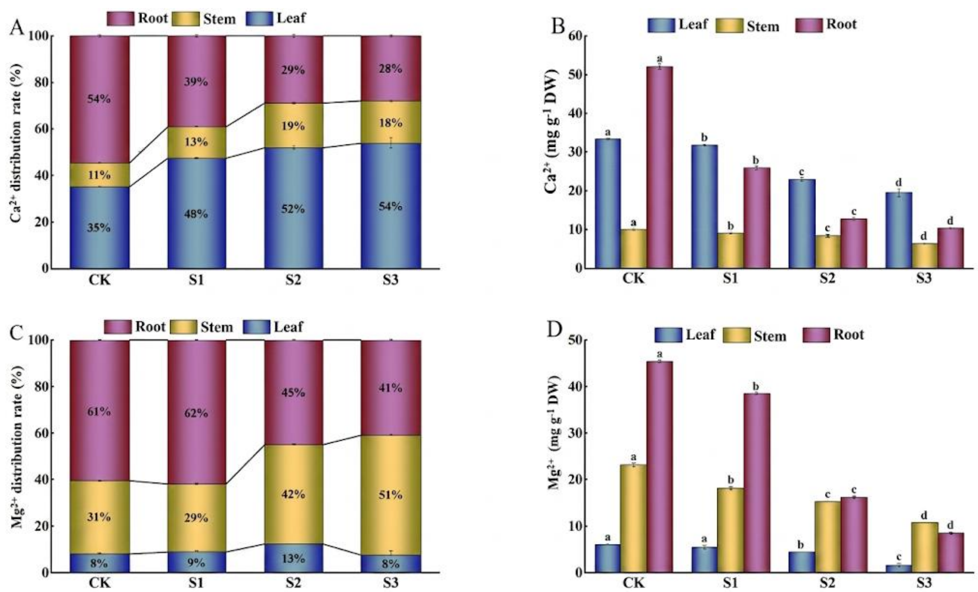

Figure 7. Effect of different alkaline salt concentrations on Ca^{2+} distribution rate (A) and content (B) and Mg^{2+} distribution rate (C) and content (D) in roots, stems, and leaves of hemp. Different letters indicate significant differences between treatments (*P* < 0.05). S1: 100 mM $NaHCO_3$; S2: 200 mM $NaHCO_3$; S3: 300 mM $NaHCO_3$.

N contents and partitioning ratios

Nitrogen content in plant roots decreased with increasing $NaHCO_3$ concentration, decreasing by 51.22% at the highest concentration (Figure 8A). This suggests that $NaHCO_3$ stress affected the N uptake capacity of the roots. Stem N content also varied, decreasing by approximately 43.99% under 300 mM $NaHCO_3$ treatment. This was probably due to reduced root uptake capacity, which reduced N transport to aboveground parts. Leaf N content decreased significantly by 29.82% and 47.35% in the 200 and 300 mM $NaHCO_3$ treatments, respectively. Given the role of N in chlorophyll synthesis, its reduction may hinder photosynthesis.

The $NaHCO_3$ stress also significantly reduced N partitioning rate in the roots, which decreased to about 18% at the highest concentration (Figure 8B). This indicates a reduced capacity for N uptake and accumulation in the root system. With increasing $NaHCO_3$ stress, stem and leaf N partition partitioning rates increased, reaching about 20% and 34% increases, respectively, under the 300 mM treatment.

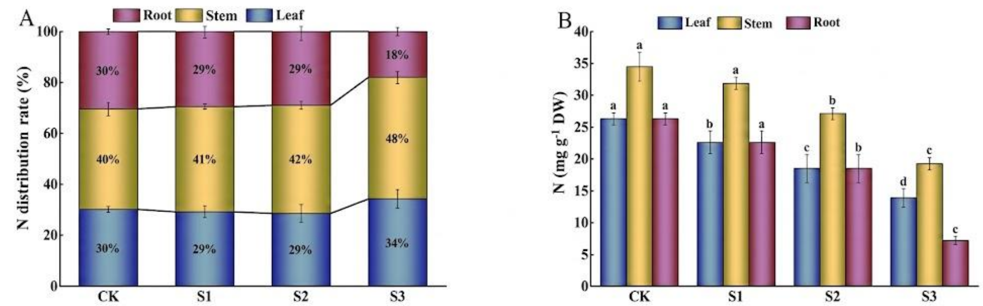

Figure 8. Effect of different alkaline salt concentrations on the N distribution rate (A) and content (B) in roots, stems, and leaves of hemp. Different letters indicate significant differences between treatments ($P < 0.05$). S1: 100 mM $NaHCO_3$; S2: 200 mM $NaHCO_3$; S3: 300 mM $NaHCO_3$.

P contents and partitioning ratios

As the $NaHCO_3$ concentration increased, P content of the plant roots decreased significantly, with the highest concentration reducing the root P content by 75.84% (Figure 9A). This suggests that $NaHCO_3$ stress impaired P uptake capacity of the roots. At the same time, P content in stems and leaves decreased by about 32.63%-44.93% and 32.50%-38.16% under 200 and 300 mM $NaHCO_3$ treatments, respectively.

The $NaHCO_3$ stress also significantly affected the distribution of P, reducing the root partitioning rate to about 26% at the highest concentration (Figure 9B). Conversely, P partitioning into stems and leaves increased by about 19% and 55% respectively, under the 300 mM $NaHCO_3$ treatment, suggesting a strategic redistribution to maintain aboveground physiological functions.

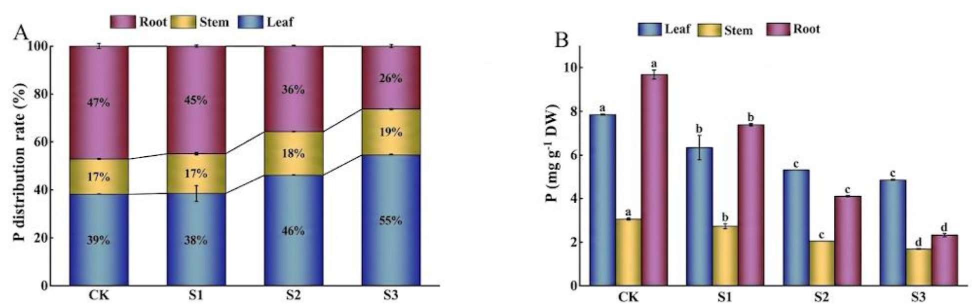

Figure 9. Effect of different alkaline salt concentrations on the P distribution rate (A) and content (B) in roots, stems, and leaves of hemp. Different letters indicate significant differences between treatments ($P < 0.05$). S1: 100 mM $NaHCO_3$; S2: 200 mM $NaHCO_3$; S3: 300 mM $NaHCO_3$.

Total flavonoid content

Total flavonoids, a class of secondary metabolites with diverse biological activities, are widely distributed in plants and are essential for growth, development, and stress resistance. With increasing $NaHCO_3$ concentration, total flavonoid content of hemp first increased and then decreased (Figure 10A), with significant peaking at concentrations of 100 and 200 mM, with increases of 47.20% and 86.75%, respectively. This indicates that moderate salinity stress can significantly activate plant defense mechanisms. As important antioxidants, flavonoids scavenge excess ROS produced under stress, thereby protecting cells from oxidative damage. When the $NaHCO_3$ concentration reached 300 mM, total flavonoid content of hemp began to decrease by 13.49%, indicating that stress beyond a certain threshold can harm the plant. High concentrations of $NaHCO_3$can seriously affect plant physiological functions, such as reducing photosynthetic efficiency, damaging cell membranes, and causing ionic imbalances, thereby inhibiting secondary metabolic pathways and reducing flavonoid synthesis.

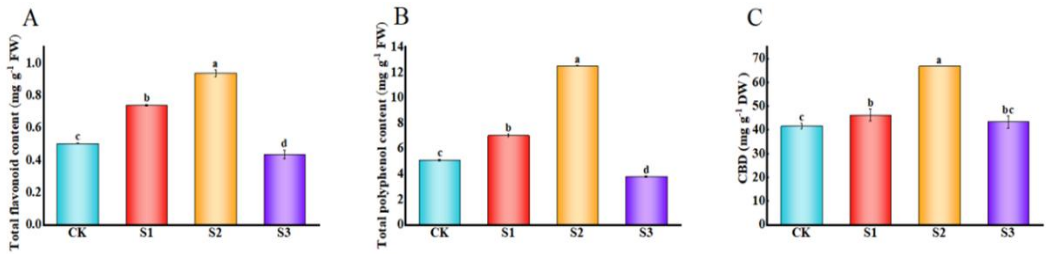

Figure 10. Effect of $NaHCO_3$ stress on the content of secondary metabolites in hemp. Different letters indicate significant differences between treatments ($P < 0.05$). A: Total flavonoids; B: Total phenols; C: Cannabidiol (CBD). S1: 100 mM $NaHCO_3$; S2: 200 mM $NaHCO_3$; S3: 300 mM $NaHCO_3$.

Total phenol content

As $NaHCO_3$ concentration increased, total phenolic content of hemp initially increased and then decreased (Figure 10B). At 100 mM $NaHCO_3$, the total phenol content of hemp increased by 38.00% compared to the control; at 200 mM, it increased by 145.92%. This increase is attributed to moderate salinity stress, which activates the plant's defense mechanisms, thus stimulating the synthesis and accumulation of phenolic compounds. Phenolic compounds, essential antioxidants, scavenge ROS and protect plant cells from oxidative damage. When 300 mM $NaHCO_3$ was reached, the total phenolic content in hemp began to decline, falling by 25.40% compared to the control. The decline is attributed to excessive salt and alkali stress, which exceeded hemp's tolerance and affected physiological functions such as photosynthesis, cell membrane integrity, and ionic balance. These impairments inhibited enzymes involved in synthesis of phenolic compounds, thereby reducing the total phenolic content.

Cannabidiol content

As the $NaHCO_3$ stress concentration increased, cannabidiol (CBD) content in hemp first increased, and then decreased (Figure 10C). At 100 mM $NaHCO_3$, CBD content in hemp increased by 11.02% over the control, suggesting that mild stress promotes CBD synthesis. At 200 mM, CBD content peaked at 60.63% above control. This significant increase may be due to the fact that moderate stress activates the hemp's defense mechanisms, thereby enhancing the CBD biosynthetic pathway. When the $NaHCO_3$ concentration reached 300 mM, the increase in CBD content decreased to 4.13%, indicating that the plant's adaptive response of the plant was approaching its limit. Under such intense stress, energy may be diverted from CBD synthesis to essential survival functions such as cell repair and maintenance of homeostasis.

DISCUSSION

Effects of $NaHCO_3$ stress on growth indicators

The effects of $NaHCO_3$ stress on hemp growth were characterized by significant reductions in plant height, stem thickness, root length, and leaf number with increasing $NaHCO_3$ concentration. Similar growth inhibition under salinity stress has been observed in wheat and soybean, attributed to effects on cell division and elongation, and reduced water and nutrient uptake by the roots (Wei et al., 2025; Fang et al., 2025). Interestingly, at 100 mM $NaHCO_3$, hemp's growth metrics were similar to the control, with even an increase in root dry weight, possibly due to physiological adaptations such as increased water and nutrient uptake efficiency due to increased root surface area.

Effects of $NaHCO_3$ stress on photosynthesis and physiological indicators

Preliminary experimental results suggest that under mild salt stress conditions, the use of $NaHCO_3$ as an additional source of inorganic C can temporarily increase photosynthetic efficiency. This may be due to the increased concentration of inorganic C available to the leaves from the CO_2 released from $NaHCO_3$ dissociation, providing a more abundant substrate for photosynthesis. Consequently, the net photosynthetic rate can increase even when stomatal conductance is not significantly elevated or is slightly reduced (Wu, 2021). However, as stress levels increased, particularly above 200 mM, there was a significant decrease in photosynthesis-related indices. This decrease can be attributed to ionic toxicity and osmotic stress from high $NaHCO_3$ concentrations, which affect the photosynthetic apparatus and cause stomatal closure (Liu et al., 2022). Furthermore, variations in physiological indices such as malondialdehyde (MDA) content and antioxidant enzyme activities indicate the extent of plant cell membrane damage and the plant's ability to neutralize reactive oxygen species (ROS) (Ma et al., 2021; Yuan et al., 2024). At lower $NaHCO_3$ concentrations, plants likely activate protein synthesis and antioxidant systems to counteract ROS induced by mild stress. However, at higher $NaHCO_3$ concentrations, there was a marked decrease in superoxide dismutase (SOD) and peroxidase (POD) activities, possibly due to alkaline conditions exceeding the plant's regulatory capacity, impairing enzyme function and reducing its ability to neutralize ROS (Choudhury et al., 2017). This suggests that hemp can cope with stress by increasing soluble protein content and enhancing antioxidant enzyme activities at lower concentrations. However, beyond critical concentration, these defense mechanisms failed, reducing the plant's resistance and leading to increased cell damage.

Effect of $NaHCO_3$ stress on the changes of elemental uptake and partitioning in hemp

Increasing $NaHCO_3$ concentrations were found to significantly increase Na^+ content in plants, mainly in the roots. This is consistent with the plant strategy to mitigate leaf toxicity by enhancing Na^+ accumulation in roots under salinity stress (Liu and Saneoka, 2019). At the same time, the levels of K^+, Ca^{2+}, Mg^{2+}, N, and P decreased to varying degrees. Notably, the reduction in K^+ and Ca^{2+} may be due to the competitive uptake of Na^+, which disrupts water relations and ionic balance, thereby hindering growth (Wang et al., 2022a). The $NaHCO_3$ stress impeded K^+ transport from roots to stems and leaves, reducing its availability aboveground. The plant may prioritize K^+ allocation to essential organs such as roots over stems and leaves to maintain basic physiological functions. In addition, $NaHCO_3$ stress decreased root concentrations of Ca^{2+} and Mg^{2+}, while their relative accumulation in stems and leaves may be due to an ionic imbalance in the root system that inhibits Ca^{2+} and Mg^{2+} uptake (Wei et al., 2021). Reduced Mg^{2+} content impaired leaf photosynthesis, while altered Ca^{2+} levels could affect cell wall structure and signaling (Ashrafi et al., 2018; Yin et al., 2019). Decreased N and P levels indicate reduced root uptake and translocation, with a notable decrease in N levels of roots and leaves, which may be a major factor in growth inhibition. Overall, $NaHCO_3$ stress disrupts ionic balance and nutrient supply, affecting the uptake, transport, and partitioning of key elements and ultimately inhibiting growth. However, plants maintain essential physiological functions under stress by adjusting elemental partitioning through adaptive mechanisms, thereby minimizing damage.

Regulation of hemp's secondary metabolites by $NaHCO_3$ stress

Sodium bicarbonate stress affects secondary metabolites of hemp, triggering complex physiological and biochemical responses. These include changes in total flavonoids, phenols, and cannabidiol (CBD), reflecting an adaptive dual effect on the environment. Flavonoids, vital secondary metabolites in plants, play a crucial role in resisting stress injury (Shomali et al., 2022). Phenolics, important secondary metabolites in plants, are regulated by various signaling pathways (Shah and Smith, 2020). The CBD, unique to industrial cannabis flowers and leaves, exhibits anti-inflammatory, antioxidant, antimicrobial, and neuroprotective properties (Capano et al., 2020; Tahir et al., 2021). Moderate $NaHCO_3$ stress increased the accumulation of flavonoids, phenols, and CBD, suggesting that mild to moderate stress enhances plant resilience to environmental challenges by activating defense mechanisms, such as the synthesis of antioxidants to scavenge excess ROS. However, when $NaHCO_3$ stress exceeds the plant's tolerance, the synthesis of these compounds decreases, probably due to impaired metabolic pathways and altered energy priorities. This results in a significant decrease in flavonoids, phenolics, and CBD under high stress, highlighting the negative response of the plant to intense stress. Therefore, control of $NaHCO_3$ concentration is crucial for optimizing these bioactive compounds in hemp, thereby enhancing plant stress tolerance in both practical and research settings. This has significant implications for the agriculture and cultivation of medicinal plants.

CONCLUSIONS

In this study, we systematically analyzed the effects of $NaHCO_3$ stress on the growth, physiology, and secondary metabolites of hemp. We observed that hemp adapted to low $NaHCO_3$ concentrations (100 mM); however, higher concentrations significantly impaired its growth and physiological functions. The response of hemp to

$NaHCO_3$ stress included regulation of photosynthetic efficiency, antioxidant enzyme activities, and ion (Na^+ and K^+) uptake and transport. It also dynamically adjusted levels of secondary metabolites, including flavonoids, phenolics, and cannabidiol. These findings provide an important theoretical basis for breeding salinity-tolerant hemp varieties and guide the optimization of cultivation practices in saline environments. Future research should explore the molecular mechanisms of hemp salinity tolerance, improve field trials, and disseminate findings to promote hemp production in saline environments.

Author contribution

Conceptualization: Y.Z. Methodology: Y.Y. Software: Y.Y. Validation: Z.L. Formal analysis: H.W. Investigation: Y.Y. Resources: Y.Z. Data curation: Z.L. Writing-original draft: Y.Y. Writing-review & editing: Y.Z. Visualization: Z.L. Supervision: Y.Z. Project administration: Y.Z. Funding acquisition: Y.Z. All co-authors reviewed the final version and approved the manuscript before submission.

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References

Agarwal, C., Máthé, K., Hofmann, T., Csóka, L. 2018. Ultrasound-assisted extraction of cannabinoids from *Cannabis sativa* L. optimized by response surface methodology. Journal of Food Science 83(3):700-710. doi:10.1111/1750-3841.14075.

Anderson, A., Fidel, R. (eds.) 2025. Introduction to Soil Science. 2nd ed. Iowa State University, Ames, Iowa, USA.

Ashrafi, E., Razmjoo, J., Zahedi, M. 2018. Effect of salt stress on growth and ion accumulation of alfalfa (*Medicago sativa* L.) cultivars. Journal of Plant Nutrition 41(7):818-831. doi:10.1080/01904167.2018.1426017.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1-2):248-254.

Britch, S.C., Babalonis, S., Walsh, S.L. 2021. Cannabidiol: Pharmacology and therapeutic targets. Psychopharmacology 238(1):9-28. doi:10.1007/s00213-020-05712-8.

Cao, K., Sun, Y., Han, C., Zhang, X., Zhao, Y., Jiang, Y., et al. 2021. The transcriptome of saline-alkaline resistant industrial hemp (*Cannabis sativa* L.) exposed to $NaHCO_3$ stress. Industrial Crops and Products 170:113766. doi:10.1016/j.indcrop.2021.113766.

Capano, A., Weaver, R., Burkman, E. 2020. Evaluation of the effects of CBD hemp extract on opioid use and quality of life indicators in chronic pain patients: A prospective cohort study. Postgraduate Medicine 132(1):56-61. doi:10.1080/00325481.2019.1685298.

Chaisan, T., Thobunluepop, P., Thongthip, N., Rakpenthai, A., Puangsin, B., Samipak, S., et al. 2025. Identification of morphological traits affecting high seed yield potential from new hemp germplasm collected in Thailand. Chilean Journal of Agricultural Research 85:88-97. doi:10.4067/S0718-58392025000100088.

Choudhury, F., Rivero, R., Blumwald, E., Mittler, R. 2017. Reactive oxygen species, abiotic stress and stress combination. Plant Journal 90(5):856-867. doi:10.1111/tpj.13299.

Fang, X.M., Zhang, J., Fan, C.Y., Liu, B.H., Kong, F.J., Li, H.Y. 2025. Molecular regulatory network of soybean responses to abiotic stress. Plant, Cell & Environment 49:4100-4115. doi:10.1111/pce.15600.

Han, K.V., Wang, J.Y., Wang, P.Y., Peng, Y.C.H. 2024. Therapeutic potential of cannabidiol (CBD) in anxiety disorders: A systematic review and meta-analysis. Psychiatry Research 339:116049. doi:10.1016/j.psychres.2024.116049.

Hopmans, J.W., Qureshi, A.S., Kisekka, I., Munns, R., Grattan, S.R., Rengasamy, P., et al. 2021. Critical knowledge gaps and research priorities in global soil salinity. p. 1-191. In Sparks, D.L. (eds.) Advances in Agronomy. Academic Press, New York, USA. doi:10.1016/bs.agron.2021.03.001.

Hu, H.R., Liu, H., Liu, F.H. 2018. Seed germination of hemp (*Cannabis sativa* L.) cultivars responds differently to the stress of salt type and concentration. Industrial Crops and Products 123:254-261. doi:10.1016/j.indcrop.2018.06.089.

Huang, Y.M., Chen, H.Q., Reinfelder, J.R., Liang X.Y., Sun, C.J., Liu, C.P., et al. 2019. A transcriptomic (RNA-seq) analysis of genes responsive to both cadmium and arsenic stress in rice root. Science of Total Environment 666:445-460. doi:10.1016/j.scitotenv.2019.02.281.

Ibrahim, M.H., Jaafar, H.Z., Rahmat, A., Rahman, Z.A. 2010. The relationship between phenolics and flavonoids production with total non structural carbohydrate and photosynthetic rate in *Labisia pumila* Benth. under high CO_2 and nitrogen fertilization. Molecules 16(1):162-174. doi:10.3390/molecules16010162.

Jin, L., Li, X.B., Tian, D.Q., Fang, X.P., Yu, Y.M., Zhu, H.Q., et al. 2016. Antioxidant properties and color parameters of herbal teas in China. Industrial Crops & Products 87:198-209. doi:10.1016/j.indcrop.2016.04.044.

Kasprowiak, F., Wilmowicz, E., Kućko, A. 2025. Jasmonate-mediated mitigation of salinity stress during germination and early vegetative development in hemp. Plants 14(18):2864. doi:10.3390/plants14182864.

Liu, D., Ma, Y., Rui, M.M., Lv, X.C., Chen, R.J., Chen, X.Y., et al. 2022. Is high pH the key factor of alkali stress on plant growth and physiology? A case study with wheat (*Triticum aestivum* L.) seedlings. Agronomy 12(8):1820. doi:10.3390/agronomy12081820.

Liu, L.Y., Saneoka, H. 2019. Effects of $NaHCO_3$ acclimation on rye (*Secale cereale*) growth under sodic-alkaline stress. Plants 8(9):314. doi:10.3390/plants8090314.

Liu, J.J., Zhang, D.Z., Yuan, Y.H., Chen, P.L., Zhang, P.P., Jin, F., et al. 2021. A promising crop for cadmium-contamination remediation: Broomcorn millet. Ecotoxicology and Environmental Safety 224:112669. doi:10.1016/j.ecoenv.2021.112669.

Ma, Q., Wu, C., Liang, S., Yuan, Y., Liu, C., Liu, J., et al. 2021. The alkali tolerance of broomcorn millet (*Panicum miliaceum* L.) at the germination and seedling stage: The case of 296 broomcorn millet genotypes. Frontiers in Plant Science 12:711429. doi:10.3389/fpls.2021.711429.

Poleg, S., Golubchik, P., Offen, D., Weizman, A. 2019. Cannabidiol as a suggested candidate for treatment of autism spectrum disorder. Progress in Neuro-psychopharmacology & Biological Psychiatry 89:90-96. doi:10.1016/j.pnpbp.2018.08.030.

Saleem, M.A., Khan, A., Tu, J.J., Huang, W.K., Liu, Y., Feng, N.J., et al. 2025. Salinity stress in rice: Multilayered approaches for sustainable tolerance. International Journal of Molecular Sciences 26(13):6025. doi:10.3390/ijms26136025.

Shah, A., Smith, D.L. 2020. Flavonoids in agriculture: Chemistry and roles in, biotic and abiotic stress responses, and microbial associations. Agronomy 10(8):1209. doi:10.3390/agronomy10081209.

Shomali, A., Das, S., Arif, N., Sarraf, M., Zahra, N., Yadav, V., et al. 2022. Diverse physiological roles of flavonoids in plant environmental stress responses and tolerance. Plants 11(22):3158. doi:10.3390/plants11223158.

Tahir, M.N., Shahbazi, F., Rondeau-Gagné, S., Trant, J.F. 2021. The biosynthesis of the cannabinoids. Journal of Cannabis Research 3:1-12. doi:10.1186/s42238-021-00062-4.

Tian, T., Wang, J.G., Wang, H.J., Cui, J., Shi, X.Y., Song, J.H., et al. 2022 Nitrogen application alleviates salt stress by enhancing osmotic balance, ROS scavenging, and photosynthesis of rapeseed seedlings (*Brassica napus*). Plant Signaling & Behavior 17(1):2081419. doi:10.1080/15592324.2022.2081419.

Wang, Y.L., Gao, S.S., He, X.Y., Li, Y., Li, P.Y., Zhang, Y., et al. 2019. Growth, secondary metabolites and enzyme activity responses of two edible fern species to drought stress and rehydration in Northeast China. Agronomy 9(3):137. doi:10.3390/agronomy9030137.

Wang, C.F., Han, G.L., Qiao, Z.Q., Li, Y.X., Yang, Z.R., Wang, B.S. 2022a. Root Na^+ Content negatively correlated to salt tolerance determines the salt tolerance of *Brassica napus* L. inbred seedlings. Plants 11(7):906. doi:10.3390/plants11070906.

Wang, J.Y., Li, Q., Zhang, M., Wang, Y.C. 2022b. The high pH value of alkaline salt destroys the root membrane permeability of *Reaumuria trigyna* and leads to its serious physiological decline. Journal of Plant Research 135(6):785-798. doi:10.1007/s10265-022-01410-y.

Wei, T.J., Li, G., Wang, M.M., Jin, Y.Y., Zhang, G.H., Liu, M., et al. 2021. Physiological and transcriptomic analyses reveal novel insights into the cultivar-specific response to alkaline stress in alfalfa (*Medicago sativa* L.) Ecotoxicology and Environmental Safety 228:113017. doi:10.1016/j.ecoenv.2021.113017.

Wei, L., Ren, X.M., Qin, L.M., Zhang, R., Cui, M.H., Xia, G.M., et al. 2025. TaWRKY55-TaPLATZ2 module negatively regulate saline-alkali stress tolerance in wheat. Journal of Integrative Plant Biology 67(1):19-34. doi:10.1111/jipb.13793.

Wu, Y.Y. 2021. Is bicarbonate directly used as substrate to participate in photosynthetic oxygen evolution. Acta Geochimica 40(4):650-658. doi:10.1007/s11631-021-00484-0.

Ye, Y.S., Wang, H.Y., Zhang, P.P., Zhang, Y.H. 2025. Adaptation strategies for hemp in alkaline salt environments: Fertilizer management for nutrient uptake and optimizing growth. Agriculture 15(2):125. doi:10.3390/agriculture15020125.

Yin, Z.P., Zhang, H., Zhao, Q., Yoo, M.J., Zhu, N., Yu, J.L., et al. 2019. Physiological and comparative proteomic analyses of saline-alkali $NaHCO_3$-responses in leaves of halophyte *Puccinellia tenuiflora*. Plant and Soil 437(1-2):137-158. doi:10.1007/s11104-019-03955-9.

Yuan, H.P., Si, H., Ye, Y.S., Ji, Q.Y., Wang, H.Y., Zhang, Y.H. 2024. Arbuscular mycorrhizal fungi-mediated modulation of physiological, biochemical, and secondary metabolite responses in hemp (*Cannabis sativa* L.) under salt and drought stress. Journal of Fungi 10(4):283. doi:10.3390/jof10040283.