Mechanistic study on integrated water and fertilizer management to alleviate Na $^{+}$ toxicity and enhance salt tolerance and yield of pakchoi under salt stress (#120973)

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Mechanistic study on integrated water and fertilizer management to alleviate Na $^{+}$ toxicity and enhance salt tolerance and yield of pakchoi under salt stress

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Planting salt-tolerant Pakchoi is a promising strategy for utilizing coastal saline soils, yet supporting agronomic technologies remain underdeveloped. This study investigates the mechanisms by which coordinated water and fertilizer regulation mitigates Na^+ toxicity under salt stress and enhances salt tolerance and yield in Pakchoi, aiming to provide a scientific basis for optimizing agronomic management of salt-tolerant Pakchoi. A pot experiment using a split-plot design was conducted with three irrigation methods (conventional, drip, and mulched drip) and three fertilizer types (conventional, mixed, and controlled-release). The MP treatment (mulched drip irrigation + mixed fertilizer) significantly reduced soil EC in the upper layer and increased K^+ and Ca^{2+} content while lowering Na^+, thereby increasing the K^+/Na^+ ratio and decreasing the Na^+/Ca^{2+} ratio. MP also reduced MDA and electrolyte leakage and enhanced activities of SOD, CAT, POD, and APX enzymes. Leaf water potential improved to –0.60 MPa, and total soluble organic osmolytes accumulation were minimized. MP-treated plants showed superior gas exchange (Pn = 14.8 μmol CO_2·m^{-2}·s^{-1}, Gs = 0.622 mol H_2O·m^{-2}·s^{-1}, Tr = 5.28 mmol H_2O·m^{-2}·s^{-1}) with efficient CO_2 assimilation (C_i = 251 μmol·mol^{-1}) and high chlorophyll content (total = 2.68 mg·g^{-1}). Yield performance improved markedly, with 39% and 42% higher fresh and dry weight than FI. MP treatment offers a highly effective water-fertilizer strategy for Pakchoi in saline regions.

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Abstract

Planting salt-tolerant Pakchoi is a promising strategy for utilizing coastal saline soils, yet supporting agronomic technologies remain underdeveloped. This study investigates the mechanisms by which coordinated water and fertilizer regulation mitigates Na^+ toxicity under salt stress and enhances salt tolerance and yield in Pakchoi, aiming to provide a scientific basis for optimizing agronomic management of salt-tolerant Pakchoi. A pot experiment using a split-plot design was conducted with three irrigation methods (conventional, drip, and mulched drip) and three fertilizer types (conventional, mixed, and controlled-release). The MP treatment (mulched drip irrigation + mixed fertilizer) significantly reduced soil EC in the upper layer and increased K^+ and Ca^{2+} content while lowering Na^+, thereby increasing the K^+/Na^+ ratio and decreasing the Na^+/Ca^{2+} ratio. MP also reduced MDA and electrolyte leakage and enhanced activities of SOD, CAT, POD, and APX enzymes. Leaf water potential improved to –0.60 MPa, and total soluble organic osmolytes accumulation were minimized. MP-treated plants showed superior gas exchange (*Pn* = 14.8 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, *Gs* = 0.622 mol $H_2O \cdot m^{-2} \cdot s^{-1}$, *Tr* = 5.28 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) with efficient CO_2 assimilation (C_i = 251 μmol·mol^{-1}) and high chlorophyll content (total = 2.68 mg·g^{-1}). Yield performance improved markedly, with 39% and 42% higher fresh and dry weight than FI. MP treatment offers a highly effective water-fertilizer strategy for Pakchoi in saline regions.

Keywords: Pakchoi, Salt stress, Integrated water and fertilizer management, Ion homeostasis and Na^+ toxicity, Photosynthetic performance and ROS defense

INTRODUCTION

Pakchoi (*Brassica rapa* L. ssp. *chinensis*), belonging to the Brassicaceae family, is native to

China and is commonly known as Chinese cabbage. It is an important leafy vegetable crop in China and has been extensively cultivated in recent years in countries such as Europe, America, Japan, and Southeast Asia, gradually becoming a globally recognized vegetable (*Yu et al., 2022*). However, soil salinization has become a major limiting factor for greenhouse vegetable production (*Xu et al., 2018*) and a significant agricultural issue worldwide (*Liang et al., 2018*). Currently, there are approximately 950 million hectares of saline-alkali land globally, with China having around 100 million hectares of saline soil (*Li et al., 2022a*). In the coastal areas of Guangdong, there are nearly 200,000 hectares of saline-alkali land, with Zhanjiang accounting for half of this area (*Luo, 2012; Wang et al., 2018*). Moreover, it is estimated that the degree of soil salinization on approximately 20% of agricultural land worldwide is continuously worsening, with more than 50% of arable land expected to become saline by 2050 (*Li et al., 2022b*).

Coastal soil salinization typically occurs due to rapid soil moisture evaporation, leading to the gradual accumulation of sodium chloride (NaCl) in the soil (*Khalifa et al., 2016*). Under NaCl stress, plant growth and development slow down, metabolic capacity is inhibited, and severe symptoms of wilting may occur, eventually leading to plant death (*Parida and Das, 2005*). Proper irrigation and fertilization practices are essential for managing salinity while ensuring water and nutrient supply to plants. Inappropriate fertilization and irrigation can result in the accumulation of salt in the root zone, leading to Na^+ toxicity in crops (*Lima et al., 2020*). This can damage cell membrane structures and functions (*Liu et al., 2017*), causing disruption in physiological metabolism and water imbalance, which adversely affect crop growth and yield reduction (*Piao et al., 2020*). Therefore, rational fertilization and irrigation are crucial measures to reduce soil salinity and increase crop yield (*Lima et al., 2020*). Studies have shown that film mulched trickle irrigation can effectively prevent rapid water evaporation, reduce the aggregation of salt ions in the root zone, and alleviate plant salt stress (*Lima et al., 2020*). Pakchoi, belonging to the leafy vegetable category, is a nitrogen-loving crop, and nitrogen fertilizer application plays a decisive role in its yield (*Xiong et al., 2018*). Controlled-release nitrogen fertilizer is particularly beneficial for crops as it releases nutrients slowly, reducing soil salt accumulation. It also facilitates nitrogen absorption by crops, contributing to proper physiological metabolic functions in Pakchoi (*Zhang et al., 2016*). Selective ion uptake through the plant root system, such as K^+ and Ca^{2+}, helps inhibit Na^+ entry and promote Na^+ excretion, thereby maintaining higher K^+/Na^+ and Na^+/Ca^{2+} ratios, reducing or avoiding the harmful effects of Na^+ ions (*Zhang et al., 2018*).

Studies have demonstrated that adopting appropriate water and fertilizer management strategies in saline-alkali soils can effectively control salinity and enhance crop yield (*Lima et al., 2019*). However, current research on Pakchoi cultivation in coastal saline areas has primarily focused on the selection and breeding of salt-tolerant varieties, with limited attention paid to corresponding water and nitrogen management practices. Therefore, the main objective of this study is to investigate and evaluate the effects of different fertilization regimes (e.g., conventional and controlled-release fertilizers) and irrigation methods (e.g., surface irrigation, drip irrigation, and plastic-film mulched drip irrigation) under salt stress conditions (0.15% NaCl). The evaluation encompasses changes in soil electrical conductivity (EC), Na^+, K^+, and Ca^{2+} concentrations and their ratios in Pakchoi, plant nitrogen and phosphorus contents, leaf water potential, organic osmolyte contents, reactive oxygen species (ROS) and malondialdehyde (MDA) levels, electrolyte leakage rate, antioxidant enzyme activities, chlorophyll content, photosynthetic parameters, as well as plant height and yield. This study aims to explore how different water and fertilizer management models alleviate Na^+ toxicity under salinity stress and enhance the growth of Pakchoi, ultimately providing scientific support for the optimized management of water and fertilizer in coastal saline-alkali land to achieve high-efficiency production.

MATERIALS AND METHODS

Experimental Materials

Test Soil: Soil samples were obtained from the cultivation layer (0-20cm) of Guangdong Ocean University's breeding base (N21°8′31″, E110°18′23″). The soil characteristics were as follows: pH 6.45, EC 670 μS/cm, salinity 0.01%, bulk density 1.20 g/cm³, organic matter content 14.25 g/kg, available nitrogen 65.57 mg/kg, available phosphorus 10.81 mg/kg, available potassium 58.37 mg/kg, water-soluble calcium 3.43 mg/kg, and water-soluble magnesium 2.28 mg/kg. After air-drying, the soil samples were crushed and sieved through a 2.00 mm mesh, and 5g of NaCl was added and mixed uniformly. The soil was then placed in pots (inner diameter 21 cm, height 20 cm, bottom without holes), with each pot containing 3.8 kg of soil. After adjustment, the measured soil salinity was 0.15%.

Test Crop: Improved Pakchoi (*Brassica rapa* L. ssp. *chinensis*) seeds of the "Jinpin 28" variety were purchased from Fujian Jinpin Agricultural Science and Technology Co., Ltd. This variety is known to thrive in soils with a total salt content of up to 0.3%. Its root system primarily extends within the top 10cm of the soil. The growth stages include seedling stage (20 days), rosette stage (20 days), flowering stage (15 days), and fruiting stage (15 days). Due to its classification as a leafy vegetable, it is typically harvested during the later stages of the rosette period. The seeds were germinated in a cultivation box until they had two leaves and one heart. Uniformly growing seedlings were selected and transplanted into experimental pots.

Test Fertilizers: Fertilizers were provided by Environmental Friendly Fertilizer Engineering Technology Research Center in Guangdong. The fertilizers included compound controlled-release fertilizer ($N:P_2O_5:K_2O$ = 14:14:14, effective for 1-2 months), conventional urea (N ≥ 46.0%), monoammonium phosphate ($N:P_2O_5:K_2O$ = 12:61:0), and potassium chloride (K_2O ≥ 60%).

Experimental Design

The experiment was conducted in a glass greenhouse at the Guangdong Ocean University Agricultural Biotechnology Research Institute from October 23, 2021, to December 29, 2021. A two-factor split-plot design was employed. The main factor consisted of different irrigation methods: conventional irrigation (I), trickle irrigation (T), and film mulched trickle irrigation (P). The sub-factor involved the application of different fertilizers: conventional fertilizer (F), controlled-release fertilizer (C), and mixed fertilizer (M) with F and C. Additionally, a control treatment (CK) was established, which did not include NaCl and utilized conventional fertilizer and irrigation. In total, there were ten treatments (as shown in Table 1), each with five replicate pots, and each pot contained three Pakchoi plants.

Experimental methods

Fertilization Method: Nitrogen, phosphorus, and potassium fertilizers were uniformly mixed with the soil as base fertilizer, with each treatment receiving 0.2g N/pot, 0.2g P_2O_5/pot, and 0.2g K_2O/pot. In the mixed fertilizer (M) treatment, it was a blend of 50% of the total NPK nutrients from the conventional fertilizer (F) and 50% from the controlled-release fertilizer (C).

Irrigation Method: In the irrigation treatment, a graduated cylinder was used to uniformly and quantitatively water the surface of the potted plants. For the trickle irrigation treatment, water was measured using a graduated cylinder and added to the drip emitter, with a constant dripping rate of 30 ml/h for targeted irrigation. In the film mulched trickle irrigation treatment, black plastic film was used to cover the soil surface in the pots, and holes were made in the plastic film corresponding to the drip emitter outlets for localized drip irrigation. Prior to the experiment, the field capacity of the base soil was determined (26%), and each pot was supplied with an equal amount of water equivalent to 80% of the field capacity (790 ml). Subsequently, water was added daily between 6:00 PM and 7:00 PM to maintain the soil moisture content at 80% of field capacity.

Collection of Soil and Plant Samples: The experiment involved transplanting Pakchoi seedlings on October 23, 2021. Soil samples were collected for the first time on November 13, 2021, during the seedling stage. Soil samples were obtained from the middle of each pot, collecting soil from both the upper layer (0-10 cm, root zone) and the lower layer (10-20 cm). One soil core was collected from each pot, and this process was repeated five times for each treatment (5 pots per treatment). The samples were sieved through a 2 mm mesh to remove stones and plant roots and were air-dried for subsequent analysis. The same procedure was repeated on December 4, 2021, during the later stage of the rosette stage of Pakchoi. Plant samples were harvested, and plants were washed with deionized water, blotted dry with absorbent paper to determine fresh weight. A small amount of fresh leaves was taken for physiological index measurement. The remaining plant samples were then oven-dried at 75°C to a constant weight, followed by grinding for further analysis.

Soil and Plant Sample Measurements

Soil electrical conductivity (EC) was determined using a soil salinity meter (Germany, STEP PNT300 model) with a 1:1 soil-to-water ratio. Plant height was measured using a ruler from the ground to the tip of the uppermost leaf along the main stem axis on days 7 (early seedling stage), 17 (late seedling stage), 27 (early rosette stage), and 37 (late rosette stage) after transplanting.

On the harvest day, functional leaves were sampled for physicochemical analysis. Leaf water potential was measured using the pressure chamber method with a PMS Model 1000 instrument (USA), with fresh samples collected and measured in the early morning (*Turner, 1988*).

Chlorophyll a, chlorophyll b, and total chlorophyll contents were extracted with 80% acetone and calculated based on absorbance values measured at 663 nm and 645 nm using a spectrophotometer (*Lichtenthaler and Wellburn, 1983*). On clear, windless days between 9:00 and 11:00 a.m., fully expanded functional leaves from the middle part of pakchoi plants were selected for measurement. A portable photosynthesis system (LI-6400XT, LI-COR Inc., USA) was used to determine key photosynthetic parameters. The chamber light intensity was set at 1200 μmol·m⁻²·s⁻¹, with an ambient CO_2 concentration of approximately 400 μmol·mol⁻¹, and relative humidity maintained between 50% and 60%. The following photosynthetic parameters were recorded: Net photosynthetic rate (*Pn*), transpiration rate (*Tr*), stomatal conductance (*Gs*), intercellular CO_2 concentration (C_i).

The measured values were computed and interpreted based on the following definitions and equations:

Net Photosynthetic Rate (*Pn*, μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), represents the net assimilation of CO_2 by the leaf per unit area per unit time.

$$Pn = A = (C_a - C_i) \times g_c$$

Where:

C_a = Ambient CO_2 concentration in the leaf chamber ($\mu mol \cdot mol^{-1}$)

C_i = Intercellular CO_2 concentration ($\mu mol \cdot mol^{-1}$)

g_c = CO_2 conductance ($mol \cdot m^{-2} \cdot s^{-1}$)

(Note: In practice, the instrument typically calculates *A* (i.e., *Pn*) directly based on the difference in CO_2 concentration and the airflow rate.)

Transpiration Rate (*Tr*, mmol $H_2O \cdot m^{-2} \cdot s^{-1}$), reflects the amount of water vapor lost through stomata per unit area per unit time.

$$Tr = g_w \times (W_i - W_a)$$

Where:

g_w = Water vapor conductance (mol $H_2O \cdot m^{-2} \cdot s^{-1}$)

W_i = Water vapor concentration inside the leaf chamber ($mmol \cdot mol^{-1}$)

W_a = Ambient water vapor concentration ($mmol \cdot mol^{-1}$)

Stomatal Conductance (*Gs*, mol $H_2O \cdot m^{-2} \cdot s^{-1}$), indicates the permeability of stomata to water vapor diffusion per unit area per unit time.

$$Gs = \frac{Tr}{VPD}$$

Where:

Tr = Transpiration rate (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)

VPD = Vapor Pressure Deficit between the leaf and chamber air (kPa)

Intercellular CO_2 Concentration (C_i, $\mu mol \cdot mol^{-1}$), reflects the concentration of CO_2 in the intercellular spaces of the mesophyll, indicating the CO_2 assimilation capacity.

$$C_i = C_a - \left(\frac{Pn}{g_s/1.6}\right)$$

Where:

C_a = Ambient CO_2 concentration (μmol·mol^{-1})
Pn = Net photosynthetic rate (μmol CO_2·m^{-2}·s^{-1})
g_s = Stomatal conductance (mol H_2O·m^{-2}·s^{-1})
(Note: Division by 1.6 accounts for the fact that stomatal conductance to water vapor is approximately 1.6 times that to CO_2.)

Contents of sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) in plant tissues were determined after dry ashing and dissolution in 1% HCl using a Shimadzu AA-7000 atomic absorption spectrophotometer (Japan) (*Isaac and Johnson, 1985*). Total nitrogen (N) was determined using the Kjeldahl digestion method, and total phosphorus (P) was determined using the molybdenum blue colorimetric method with absorbance measured at 880 nm (*Bremner, 1965*).

Proline content was measured via the acid ninhydrin colorimetric method, with absorbance recorded at 520 nm (*Bates et al., 1973*). Soluble sugars, glucose, fructose, and sucrose were quantified using the anthrone method: soluble sugars and fructose were measured at 620 nm, glucose was quantified using a glucose oxidase enzymatic reaction, and sucrose was hydrolyzed into monosaccharides before colorimetric measurement (*Yemm and Willis, 1954*). Soluble protein was measured using the Coomassie Brilliant Blue G-250 binding assay at 595 nm (*Bradford, 1976*).

For reactive oxygen species (ROS)-related indicators, superoxide anion (O_2^-) was measured using the nitro blue tetrazolium (NBT) reduction method, while hydrogen peroxide (H_2O_2) was quantified using an iodometric assay with absorbance at 390 nm. Electrolyte leakage was calculated from the electrical conductivity before and after leaf tissue incubation using a conductivity meter. Malondialdehyde (MDA) content was determined via the thiobarbituric acid (TBA) method, and absorbance was read at 532 nm and 600 nm to calculate the differential values (*Heath and Packer, 1968*).

Antioxidant enzyme activities were measured as follows: superoxide dismutase (SOD) activity based on the inhibition of NBT photoreduction; peroxidase (POD) activity using the guaiacol-H_2O_2 reaction; catalase (CAT) activity via the decomposition rate of H_2O_2 measured as the decline in absorbance; and ascorbate peroxidase (APX) activity based on the oxidation of ascorbic acid, with continuous measurement at 290 nm to track the decline in absorbance over time (*Aebi, 1984*).

Data Processing

Data analysis was conducted using SPSS 22.0 software.

RESULTS AND ANALYSIS

Effect of Water and Fertilizer Management on Soil Electrical Conductivity

As shown in Table 2, different irrigation methods (I, T, P) had a significant impact on both upper and lower layer soil electrical conductivity (EC), while fertilization methods (F, M, C) had no significant effect. During the Pakchoi seedling and rosette stages, the upper layer soil EC of the T (FT, MT, CT) and P (FP, MP, CP) treatments was significantly lower than that of the I (FI, MI, CI) treatment, whereas the lower layer soil EC of the T and P treatments was higher than that of the I treatment. The upper and lower layer soil EC of CK control treatment was significantly lower than the other treatments. The upper layer soil EC showed a decreasing trend with the extension of the Pakchoi growth period, while there was no clear trend observed in the lower layer soil EC.

Effects of Water and Fertilizer Management on Leaf Water Potential in Pakchoi

As shown in Figure 1, the CK treatment exhibited the highest leaf water potential (–0.40 MPa), which was significantly greater than that observed in all other treatments (significance level: a). This indicates that, in the absence of salt stress, bok choy maintained optimal water status, with no signs of water potential decline or associated physiological stress. In contrast, the FI treatment demonstrated the lowest leaf water potential (–1.53 MPa), revealing that traditional irrigation combined with conventional fertilizer was ineffective in sustaining plant water status under saline conditions, leading to severe water stress in the plants.

Overall, the sequence of leaf water potential values across treatments followed the order: FI < CI < MI < FT < CT < MT < FP < CP < MP < CK. This gradient distribution underscores the substantial influence of water and fertilizer management strategies on the regulation of leaf water potential in Pakchoi. Notably, treatments MP (–0.60 MPa) and CP (–0.73 MPa) maintained relatively high water potential values, indicating that plastic mulching drip irrigation combined with mixed or controlled-release fertilizers can effectively preserve plant water status and alleviate salt-induced water deficits. In contrast, the lower water potentials recorded in CI (–1.35 MPa), MI (–1.22 MPa), and FT (–1.08 MPa) suggest that under traditional irrigation or with the use of single fertilizer types, bok choy plants struggle to maintain adequate hydration levels.

It is particularly noteworthy that although controlled-release fertilizers (CI, CT, CP) are designed to release nutrients gradually and stabilize plant water status, their effectiveness under saline conditions varied considerably depending on the irrigation method. For instance, the CI treatment exhibited a relatively low water potential (–1.35 MPa), whereas the CP treatment achieved a significantly higher value (–0.73 MPa), indicating that the regulatory efficacy of controlled-release fertilizers is contingent upon a compatible and efficient water delivery system.

Effects of Water and Fertilizer Management on the Contents and Ratios of Na+, K^+, and Ca^{2+} in Pakchoi

This experiment investigated the effects of different water and fertilizer management strategies on the K^+, Na^+, and Ca^{2+} contents in Pakchoi, as well as the ratios of K^+/Na^+ and Na^+/Ca^{2+}. As shown in Table 3, significant differences were observed in these indicators among the treatments, highlighting the significant impact of water and fertilizer management on ion absorption and ion balance in Pakchoi.

Firstly, there were significant differences in K^+ content among the treatments. The K^+ content in CK (3.89%), MP (3.28%), MT (4.06%), and CP (3.73%) treatments was higher compared to other treatments. These treatments accumulated significantly more K^+ than FI (1.32%) and FT (2.36%), suggesting that optimized water and fertilizer management can effectively promote K^+

absorption and accumulation, enhancing the plant's water-salt balance ability. The FI treatment had the lowest K^+ content, reflecting the inhibitory effect of traditional water and fertilizer management on K^+ absorption.

Similarly, the trend for Na^+ content followed that of K^+. The Na^+ content in MP (3.5%) and CP (6.39%) was significantly lower than that in the FI treatment (9.22%). This indicates that optimized water and fertilizer management strategies can effectively mitigate salt stress, reduce Na^+ accumulation, and improve the ion balance in Pakchoi. In contrast, the higher Na^+ content in the FI treatment suggests excessive Na^+ accumulation, which likely leads to increased salt stress on the plant.

In terms of Ca^{2+} content, CK treatment had the highest Ca^{2+} content (0.98%), significantly higher than the other treatments. MP and CP treatments also had relatively high Ca^{2+} content (0.91% and 0.83%, respectively), indicating that these optimized water and fertilizer management practices can effectively promote Ca^{2+} absorption and accumulation, which is crucial for enhancing plant cell wall stability and stress resistance. On the other hand, FI and MI treatments had lower Ca^{2+} content, suggesting that these management strategies may lead to insufficient calcium absorption.

Regarding ion ratios, the K^+/Na^+ ratio was highest in the MP treatment (0.94), while it was lowest in the FI treatment (0.14). Treatments like MP and CP effectively increased the utilization of K^+ and reduced Na^+ accumulation, thereby enhancing the plant's resistance to salt stress. The low K^+/Na^+ ratio in the FI treatment indicates a stronger salt stress response in Pakchoi and poorer ion balance under traditional irrigation and fertilization methods.

The Na^+/Ca^{2+} ratio also showed significant differences. The Na^+/Ca^{2+} ratio was highest in the FI treatment (22.55), indicating that under salt stress, traditional water and fertilizer management leads to excessive Na^+ accumulation, disrupting the balance of calcium. The Na^+/Ca^{2+} ratio was significantly lower in the MP and CP treatments, suggesting that these treatments effectively improve the negative impact of salt stress on the plant's ion balance.

In conclusion, optimized water and fertilizer management strategies, such as the MP and CP treatments, can significantly increase K^+ accumulation, reduce Na^+ accumulation, and improve Ca^{2+} absorption, maintaining ion balance in the plant and effectively alleviating salt stress, thus improving Pakchoi's salt tolerance. In contrast, traditional water and fertilizer management strategies, such as the FI treatment, lead to higher Na^+ accumulation and lower K^+ content, increasing the risk of salt stress.

Effects of Water and Fertilizer Management on Nitrogen and Phosphorus Content in Pakchoi Plants

As shown in Figure 2, the nitrogen (N) content in the CK treatment was significantly higher than in all other treatments (2.84%), indicating that salt stress substantially inhibited nitrogen uptake in bok choy. The N content was generally low under salt stress conditions, particularly in the FI (0.762%), FT (0.723%), and CI (0.690%) treatments, reflecting severe suppression of nitrogen accumulation in these groups. In contrast, treatments such as MP (1.29%), MT (1.19%), and MI (0.89%) exhibited relatively higher N levels, suggesting that the combination of plastic mulching drip irrigation and mixed fertilizer effectively mitigated salt damage and enhanced nitrogen uptake.

A similar trend was observed for phosphorus (P) content. The CK group exhibited a markedly higher P content (0.268%) compared to all other treatments. The lowest P level was recorded in the FI treatment (0.0567%), and generally low P levels were also observed in traditional irrigation (FI) and controlled-release fertilizer (CI) treatments, with values of 0.0567% and 0.0866%, respectively. Conversely, relatively higher P contents were found in the MP (0.111%), CP (0.109%), and MI (0.109%) treatments, indicating that the combination of plastic mulching drip irrigation with mixed or controlled-release fertilizers can significantly improve phosphorus uptake.

Overall, the data demonstrate that salt stress significantly suppresses nitrogen and phosphorus absorption in bok choy, especially under traditional irrigation and the use of single fertilizer types. Among all the treatments, the combination of plastic mulching drip irrigation and mixed fertilizer (MP) showed the most pronounced effect in alleviating salt stress and enhancing nutrient accumulation.

Effects of Water and Fertilizer Management on Organic Solute Accumulation in Pakchoi Plants

This study further evaluated the accumulation of various organic solutes in Pakchoi under different water and fertilizer management regimes, focusing on six physiological indicators: proline, soluble protein, soluble sugar, glucose, fructose, and sucrose (Figure 3). These solutes are critical for osmoregulation and stress adaptation under saline conditions. The significant differences among treatments and the distribution of values across groups highlight the substantial impact of water-fertilizer strategies on the osmotic adjustment capacity and metabolic activity of Pakchoi.

Proline content (Figure 3a) was highest in the FI treatment (333.68 μg/g FW), significantly exceeding all other treatments, while the CK group exhibited the lowest concentration (76.43 μg/g FW). This finding indicates that salt stress strongly induces proline biosynthesis in Pakchoi, facilitating osmotic regulation and protecting cells from dehydration. Notably, treatments such as MP and CP displayed markedly lower proline levels compared to FI, FT, and CI, suggesting that plastic mulching drip irrigation combined with optimized fertilization alleviated stress intensity and reduced the need for excessive accumulation of protective solutes.

In terms of soluble protein content (Figure 3b), the FI and CI treatments showed the highest levels (28.78 and 28.91 mg/g FW, respectively), with values significantly greater than those recorded under MP, FP, CP, and CK treatments. As soluble proteins contribute to osmotic balance and stress response, their elevated levels in high-salinity treatments reflect the intensity of cellular metabolic response under salt stress. Conversely, the CK group had the lowest protein content (9.59 mg/g FW), consistent with its unstressed condition and lack of induced protein expression. MP treatment also exhibited relatively low protein accumulation (13.53 mg/g FW), further confirming its efficacy in mitigating environmental stress.

Soluble sugars (Figure 3c), another key class of osmotic regulators, also played an essential role in stress response. The FI and CI treatments had soluble sugar concentrations exceeding 48 mg/g FW, significantly higher than that of the CK group (23.91 mg/g FW). This suggests that under severe salt stress, Pakchoi accumulates sugars to maintain cellular osmotic potential. Meanwhile, MP and CP treatments showed reduced sugar content (25.26 and 26.04 mg/g FW, respectively), indicating that these practices contributed to alleviating salt-induced osmotic stress.
Glucose (Figure 3d) and fructose (Figure 3e) levels followed a similar trend. The highest glucose content was observed in the FT treatment (25.82 μmol/g FW), while MP and CK exhibited the lowest levels (13.54 and 10.22 μmol/g FW, respectively). Elevated glucose levels in FI, CI, and MI suggest that salt stress activates carbohydrate metabolism pathways, leading to enhanced glucose synthesis. Similarly, fructose concentrations were significantly higher in FI and CI (14.15 and 11.51 mg/g FW, respectively), with CK once again showing the lowest accumulation (4.76 mg/g FW). These results mirror the proline pattern, highlighting the role of sugar metabolism in maintaining cellular water balance under saline conditions.
Sucrose content (Figure 3f) further substantiated the above findings. Treatments FI, CI, FT, MI, and CT recorded significantly higher levels (16–17.5 mg/g FW), whereas MP and CK presented the lowest values (8.15 and 5.08 mg/g FW, respectively). These differences indicate that plants under lower or no stress conditions exhibit less demand for sucrose-mediated osmotic adjustment.
Overall, the FI and CI treatments consistently exhibited the highest concentrations of osmotic regulators, implying that traditional irrigation combined with conventional or controlled-release fertilizers resulted in severe salt stress in Pakchoi. In contrast, MP and CP treatments showed lower accumulation across multiple parameters, demonstrating that plastic mulching drip irrigation in conjunction with optimized fertilization effectively reduced stress intensity and minimized the plant's reliance on osmoregulatory solutes. The CK group, with the lowest values across all indicators, further validated that salt stress is the primary driver of organic solute accumulation in Pakchoi.

Effects of Water and Fertilizer Management on ROS, MDA Content, and Electrolyte Leakage in Pakchoi Plants

This experiment further analyzed the degree of oxidative damage and membrane stability in Pakchoi under salt stress, by measuring four key physiological indicators: superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) content, electrolyte leakage, and malondialdehyde (MDA) content (Figure 4). These indicators comprehensively reflect the accumulation of reactive oxygen species (ROS) and lipid peroxidation of membranes under stress. The results reveal significant differences across treatments, highlighting the substantial impact of water and fertilizer management strategies on alleviating salt stress and enhancing cellular protection mechanisms.
Firstly, the O_2^- content was highest in the FI treatment (1.97 μmol/g FW), significantly higher than in the FP, MP, CP, and CK treatments, with the CK group showing the lowest value (0.45 μmol/g FW). This indicates a gradient shift from high salt stress to no stress, as shown in Figure 4a. Similarly, the O_2^- content in CI, MI, and FT treatments was relatively high, suggesting a pronounced accumulation of ROS under traditional irrigation or single fertilizer regimes. In

contrast, the MP and CP treatments exhibited a notable decrease in O_2^- content, indicating that plastic mulching drip irrigation combined with optimized fertilization can effectively suppress ROS generation and reduce oxidative damage.

The trend in H_2O_2 content further supports these findings (Figure 4b). FI treatment exhibited the highest H_2O_2 level (3.31 μmol/g FW), followed by CI (2.70 μmol/g FW) and MI (2.58 μmol/g FW), with MP (1.22 μmol/g FW) and CK (0.85 μmol/g FW) showing the lowest levels, with significant differences. The clear suppression of H_2O_2 accumulation in the MP and CP treatments highlights their important role in mitigating salt-induced ROS accumulation.

Electrolyte leakage, which serves as an important indicator of cell membrane integrity, also followed the same trend as the oxidative stress markers (Figure 4c). The electrolyte leakage rate in the FI treatment was significantly highest (31.77%), while the CK group exhibited the lowest rate (10.88%), indicating enhanced membrane damage and leakage under salt stress. The MP treatment had a leakage rate of 13.28%, slightly higher than CK but significantly lower than in the high-stress treatments (FI, CI, FT), demonstrating the protective effect of this treatment on the cell membrane. The CP treatment also exhibited a relatively low leakage rate (14.05%), further suggesting that optimized water and fertilizer management can significantly reduce membrane damage under salt stress.

MDA content, a key indicator of lipid peroxidation, showed similar trends (Figure 4d). The MDA content was highest in the FI treatment (3.61 nmol/g FW), and lowest in the CK treatment (0.82 nmol/g FW). The CI treatment also exhibited high levels of MDA (3.36 nmol/g FW), indicating severe membrane lipid peroxidation under these conditions. In contrast, the MDA content in MP and CP treatments was significantly lower (1.13 and 1.44 nmol/g FW, respectively), further confirming the effectiveness of optimized water and fertilizer management strategies in reducing membrane oxidative damage and enhancing Pakchoi's stress tolerance.

In summary, FI and CI treatments exhibited significantly higher values across all oxidative stress-related indicators, reflecting the inability of traditional irrigation combined with conventional or controlled-release fertilizers to effectively protect cells under salt stress, leading to increased oxidative damage. In contrast, the MP and CP treatments demonstrated significantly lower values in all indicators, indicating their superior ability to reduce ROS accumulation and mitigate membrane system damage, making them the most effective management strategies for alleviating salt stress. The CK group consistently exhibited the lowest values in all indicators, reinforcing the notion that salt stress is the primary driver of oxidative damage and cell membrane injury.

Effects of Water and Fertilizer Management on Antioxidant Enzyme Activities in Pakchoi

This experiment also evaluated the effects of different water and fertilizer management strategies on the antioxidant enzyme activities in Pakchoi, specifically analyzing the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) (Figure 5). The results revealed significant differences in enzyme activities across treatments, highlighting the impact of water and fertilizer management on Pakchoi's response to oxidative stress.

SOD activity, a key enzyme in the elimination of superoxide anion radicals, was highest in the MP treatment (158.19 U/g FW), followed by CP (150.85 U/g FW), both significantly higher than the other treatments. The lowest SOD activity was observed in the FI treatment (54.66 U/g FW), significantly lower than the MP and CP treatments. This trend suggests that under optimized water and fertilizer management conditions (MP and CP treatments), Pakchoi's oxidative stress response is significantly enhanced, while the response under traditional management practices (FI treatment) is weaker (Figure 5a).

Similarly, POD activity was highest in the MP treatment (113.91 U/g FW), followed by CP (111.46 U/g FW), both significantly higher than the other treatments. The lowest POD activity was observed in the FI treatment (36.28 U/g FW), which aligns with its lower oxidative stress response (Figure 5b).

CAT activity was highest in the MP treatment (20.33 U/g FW), with the CP treatment showing the second-highest level (19.50 U/g FW). The lowest CAT activity was found in the FI treatment (6.51 U/g FW). This indicates that optimized water and fertilizer management (such as MP and CP treatments) significantly enhances Pakchoi's ability to detoxify hydrogen peroxide (H_2O_2), thus providing better protection against oxidative damage (Figure 5c).

APX activity, an enzyme responsible for reducing H_2O_2 to water, was highest in the MP treatment (6.01 U/g FW), followed by CP (5.68 U/g FW). The FI treatment exhibited the lowest APX activity (1.31 U/g FW), suggesting that under traditional management practices, the activity of ascorbate peroxidase is low, leading to weaker H_2O_2 detoxification capacity (Figure 5d).

In summary, these results conclude that water and fertilizer management strategies, particularly MP and CP treatments, significantly enhance Pakchoi's antioxidant defense system, improving its resistance to oxidative damage induced by salt stress. Traditional treatments (FI and CI) consistently showed lower enzyme activities, indicating a weaker response to oxidative stress.

Effects of Water and Fertilizer Management on Chlorophyll Content in Pakchoi

This study further analyzed the effects of different water and fertilizer management practices on the chlorophyll a (Chl-a), chlorophyll b (Chl-b), and total chlorophyll (Chl-a + Chl-b) contents in Pakchoi leaves. The results revealed significant differences among treatments across all three indicators, indicating that water and fertilizer management plays a critical role in regulating the photosynthetic potential and physiological activity of Pakchoi (Figure 6).

In terms of Chl-a content, the CK treatment exhibited the highest value (1.612), significantly exceeding all other treatments. This was followed by MP (1.512) and FP (1.44), suggesting that both the absence of salt stress and optimized water and fertilizer management (as in MP treatment) promote the synthesis of photosynthetic pigments. In contrast, CI (1.11) and FI (1.228) treatments showed the lowest Chl-a contents, indicating that traditional irrigation combined with conventional fertilizers may suppress chlorophyll synthesis, likely due to salt stress and suboptimal nutrient availability.

The trend in Chl-b content mirrored that of Chl-a. The CK group again had the highest value (0.552), with MP (0.522) and FP (0.508) following closely behind, all significantly higher than FI (0.414) and CI (0.368). This further confirms that optimized water and fertilizer management effectively enhances chlorophyll accumulation. Intermediate Chl-b values in CT and MI treatments suggest that partially improved management measures (e.g., controlled-release fertilizers) offer moderate stress alleviation benefits.

For total chlorophyll content (Chl-a + Chl-b), CK treatment recorded the highest level (2.16), followed by MP (2.03), both significantly greater than all other treatments. This indicates that optimized water and fertilizer strategies or non-saline environments significantly boost photosynthetic capacity in Pakchoi. In contrast, CI had the lowest total chlorophyll content (1.48), followed by FI (1.64) and CT (1.63), suggesting that traditional management approaches impair chlorophyll biosynthesis, which could potentially hinder plant growth and yield formation.

The overall trends highlight the effectiveness of optimized water and fertilizer regimes—especially the MP treatment (plastic mulching drip irrigation combined with mixed fertilizers)—in significantly increasing chlorophyll content in Pakchoi. Its chlorophyll levels were second only to the non-saline CK treatment and markedly superior to those under traditional irrigation (FI) and single-fertilizer treatments (CI). These findings are consistent with earlier results showing the MP treatment's capacity to alleviate salt stress and enhance stress resilience, further confirming the central role of water and fertilizer management in modulating the physiological metabolism of Pakchoi.

Effects of Water and Fertilizer Management on Photosynthetic Parameters of Pakchoi

This study systematically evaluated the regulatory effects of different water and fertilizer management strategies on key photosynthetic parameters in Pakchoi, including net photosynthetic rate (*Pn*), transpiration rate (*Tr*), stomatal conductance (*Gs*), and intercellular CO_2 concentration (C_i). The experimental data revealed significant differences across all four parameters among treatments, indicating that water and fertilizer management significantly affects the gas exchange capacity and photosynthetic efficiency of Pakchoi (Figure 7).

Firstly, regarding *Pn* (Figure 7a), the CK treatment recorded the highest value (15.2 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), significantly surpassing all other treatments, followed by MP (14.8) and FP (14.4). The CI treatment exhibited the lowest value (11.6), suggesting that under non-saline conditions (CK) and optimized water-fertilizer strategies (e.g., MP and FP), photosynthetic efficiency in Pakchoi is markedly enhanced. Conversely, the low photosynthetic rates observed in CI and FI treatments indicate that traditional irrigation combined with conventional fertilizers under high salinity conditions significantly suppresses photosynthesis.

Tr showed a similar trend to that of *Pn* (Figure 7b). The MP treatment exhibited the highest transpiration rate (5.28 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$), which was not significantly different from CK (5.2) but was significantly higher than CI (3.68) and FI (4.14). This suggests that optimized water and fertilizer management enhances water uptake and transpiration, which helps regulate leaf temperature and maintain photosynthetic balance. Lower *Tr* values may reflect stomatal closure under salt stress, limiting CO_2 intake and thereby inhibiting photosynthesis.

For *Gs* (Figure 7c), the CK treatment again showed the highest value (0.68 mol $H_2O \cdot m^{-2} \cdot s^{-1}$), followed by MP (0.622), while CI had the lowest (0.458). This pattern is highly consistent with the trends observed in *Pn* and *Tr*. MP and FP treatments also demonstrated notable increases in *Gs*, supporting their role in promoting stomatal opening and enhancing photosynthetic capacity. In contrast, treatments such as CI and CT exhibited lower *Gs*, which limited CO_2 exchange efficiency, indicating restricted photosynthetic support.

C_i was highest in the CI treatment (306 μmol·mol^{-1}), significantly greater than in other treatments, while CK had the lowest C_i (245) (Figure 7d). This parameter reflects the balance between stomatal aperture and CO_2 assimilation efficiency. The high C_i in CI, combined with its low *Pn* and *Gs*, suggests that despite CO_2 accumulation, the carbon assimilation capacity is poor. In contrast, CK and MP treatments had the lowest C_i but the highest *Pn*, indicating higher CO_2 utilization efficiency and stronger photosynthetic activity.

Overall, the MP treatment demonstrated the most favorable photosynthetic performance under salt stress, characterized by high *Pn*, *Tr*, and *Gs* values and low C_i. This indicates superior gas exchange capacity and CO_2 assimilation efficiency, making it an effective strategy for optimizing photosynthetic function. Although CK performed best overall, it served as a non-saline control, further validating the suppressive effects of salt stress on photosynthesis. The CI, FI, and CT treatments consistently showed lower performance across all parameters, highlighting the limitations of traditional water and fertilizer management in mitigating salt-induced photosynthetic inhibition.

Effects of Water and Fertilizer Management on Pakchoi Plant Height

As shown in Figure 2, with the growth period of Pakchoi extending, the plant height of Pakchoi in all treatments gradually increased. Significant differences in plant height among treatments began to appear in the later stages of Pakchoi seedlings. The CK control treatment had significantly taller Pakchoi plants compared to the salt-stressed treatments. The M treatment had significantly taller Pakchoi plants compared to the C and F treatments. Among the salt-stressed treatments, MP treatment had the tallest plants, significantly taller than the other treatments. MT treatment was significantly taller than MI treatment, while MI was slightly taller than the C (CI, CT, CP) and F (FI, FT, FP) treatments. There were no significant differences between the C and F treatments.

Effects of Water and Fertilizer Management on Pakchoi Biomass

As shown in Table 4, the CK control treatment had the highest fresh and dry weights of Pakchoi, significantly greater than the salt-stressed treatments. Among the salt-stressed treatments, the MP treatment had the highest fresh weight, significantly greater than the other treatments, and its dry weight was significantly greater than that of the F and C treatments. The fresh weight and dry weight of the MP treatment were 15% and 3% higher than those of MT, 19% and 7% higher than those of MI, 21% and 12% higher than those of CP, 23% and 9% higher than those of CT, 22% and 20% higher than those of CI, 24% and 18% higher than those of FP, and 26% and 20% higher than those of FT, and 39% and 42% higher than those of FI.

DISCUSSION

In this study, we systematically evaluated the effects of various water and fertilizer management strategies on the growth, physiological, and biochemical responses of Pakchoi under salt stress. The results revealed that optimized treatments—particularly MP (plastic film mulched drip irrigation combined with mixed fertilizer)—significantly alleviated salt-induced damage, enhanced photosynthetic capacity, maintained ionic balance, reduced oxidative injury, and increased biomass yield. By integrating findings across 11 different physiological and morphological dimensions (2.1–2.11), this study provides robust empirical support for crop salt tolerance strategies.

Phenotypic Performance: Significant Improvements in Growth, Biomass, and Water Status

Under salt stress, the MP treatment showed the most outstanding growth performance, with plant height and both fresh and dry biomass significantly exceeding all other salt-treated groups and even approaching levels observed in the non-saline control (CK) (Figure 8, Table 4). Similar trends have been documented in other studies. For instance, Chen et al. (2016) demonstrated that drip irrigation combined with slow-release fertilizers significantly improved the height and yield of bell pepper under salinity stress (*Chen et al., 2016*). Notably, our study observed these benefits even under moderate salinity levels (0.3% NaCl), suggesting stronger adaptation capacity through MP application.

Leaf water potential data further indicated that MP treatment effectively mitigated salt-induced dehydration stress (–0.60 MPa), second only to the CK group (–0.40 MPa), whereas the lowest value occurred in the FI treatment (–1.53 MPa), suggesting poor water balance under conventional irrigation (Figure 1). This aligns with findings by Zhang et al. (2020), who emphasized that drip irrigation can improve rhizosphere soil moisture distribution and enhance plant water status (*Zhang et al., 2020*).

Moreover, soil electrical conductivity (EC) results showed that MP effectively reduced EC accumulation in the upper soil layer while promoting downward salt migration, thus easing rhizosphere salt stress (Table 2). This corresponds with observations by Xie et al. (2021), who reported that plastic film mulching with drip irrigation successfully prevented surface salt accumulation in tomato cultivation (*Xie et al., 2021*).

Physiological Mechanisms: Synergistic Enhancements in Ion Homeostasis, Photosynthetic Performance, and ROS Defense

This study clearly demonstrated that MP treatment facilitated preferential ion uptake. Specifically, MP significantly increased the accumulation of K^+ and Ca^{2+} (3.28% and 0.91%, respectively) while reducing Na^+ (3.5%), thereby elevating the K^+/Na^+ ratio to 0.94 and decreasing the Na^+/Ca^{2+} ratio to 3.84 (Table 3). These values indicate improved membrane selectivity and electrochemical balance under MP, contributing to better cellular stability during salt stress. This supports the conclusions of Maathuis and Amtmann (1999), who highlighted the crucial role of a high K^+/Na^+ ratio in conferring salt tolerance in plants (*Maathuis and Amtmann, 1999*).

In terms of photosynthetic capacity, MP achieved values close to CK for net photosynthetic rate (*Pn*: 14.8 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), stomatal conductance (*Gs*: 0.622 mol $H_2O \cdot m^{-2} \cdot s^{-1}$), and transpiration rate (*Tr*: 5.28 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$), reflecting efficient CO_2 assimilation and stomatal regulation. Moreover, intercellular CO_2 concentration (C_i) was relatively low (251 μmol·mol⁻¹), indicative of high carbon assimilation efficiency (Figure 7). These observations are consistent with Chaves et al. (*2009*), who suggested that appropriate water and nutrient strategies can alleviate stress-induced inhibition of the photosynthetic apparatus (*Chaves et al., 2009*).

Regarding antioxidant defenses, the MP treatment significantly enhanced the activities of four key enzymes: SOD, POD, CAT, and APX. Notably, SOD and CAT activities reached 158.19 and 20.33 U/g FW, respectively—far exceeding those observed under FI (Figure 5). Simultaneously, MP sharply reduced ROS levels (O_2^- and H_2O_2), electrolyte leakage, and MDA content (Figure 4). These trends suggest that MP not only limits ROS generation but also enhances the plant's detoxification capacity, effectively mitigating lipid peroxidation.

These results align with the findings of Munns and Tester (*2008*), who emphasized that salt-induced oxidative damage can be minimized via efficient nutrient and water delivery systems, particularly under integrated water-fertilizer regimes (*Munns and Tester, 2008*).

Innovation: Systemic Mechanistic Framework for Water-Fertilizer Coordinatio

The major innovation of this study lies in its factorial design, combining irrigation modes (I, T, P) and fertilizer types (F, M, C) into nine distinct treatments. This allowed comprehensive analysis of phenotypic, physiological, metabolic, and photosynthetic traits in response to salt stress and led to the construction of an integrated model describing water regulation–ion homeostasis–ROS defense–photosynthetic optimization (Figure 9).

While most previous studies have focused on singular mechanisms such as irrigation or fertilization alone, our approach provides a multi-level, systems-based framework for understanding plant resilience under abiotic stress. As Zhu (*2016*) emphasized, salt tolerance mechanisms should be explored across various physiological layers. Our sequential investigation from water potential to ion transport, ROS detoxification, and biomass production embodies such a model-based paradigm (*Zhu, 2016*).

Furthermore, osmoregulatory compounds such as proline, soluble sugars, and fructose were significantly reduced under MP and CP treatments (Figure 3), suggesting that under reduced stress conditions, plants no longer require excessive energy expenditure for osmoprotection. This reflects higher energy-use efficiency and physiological stability and aligns with the "low-cost stress tolerance" model proposed by Ashraf and Foolad (*2007*).

Prospects

This study demonstrates that coordinated management of irrigation and fertilization under saline conditions can effectively alleviate osmotic stress, ion toxicity, and oxidative damage in Pakchoi, thereby improving photosynthetic efficiency and biomass accumulation. Future research should apply multi-omics approaches—transcriptomics, proteomics, and metabolomics—to dissect how MP treatment modulates signaling pathways for water sensing, selective K^+/Na^+ transport, ROS scavenging, and ABA-dependent responses at the molecular level, thereby shifting from physiological to mechanistic understanding (*Zhang et al., 2022*). Moreover, genotypic screening of salt-sensitive Pakchoi varieties under MP conditions will help establish a "Genotype–

Regulation–Stress" triadic response model, providing precise guidance for crop management in coastal saline lands.

To enhance practical applicability, future studies should scale up from controlled conditions to field trials across varying salinity gradients, water qualities, and climate zones to assess the robustness and scalability of the MP strategy. Additionally, integrating remote sensing, sensor data, and machine learning can facilitate intelligent water-fertilizer regulation models, creating a closed-loop system that links soil moisture/salinity, plant physiology, and management commands for smart agriculture in saline regions (*Wang et al., 2021*). Finally, future research must address combined abiotic stress scenarios (e.g., salinity-drought, salinity-heat), to test the broader resilience of MP treatment and expand its application range, ensuring sustainable leafy vegetable production under climate change conditions.

CONCLUSION

This study systematically evaluated the effects of different water and fertilizer management strategies on the growth performance, physiological-ecological traits, and antioxidant mechanisms of Pakchoi under salt stress conditions (0.3% NaCl), with a focus on the significant advantages of the MP treatment (mulched drip irrigation combined with mixed fertilizer). The main findings are summarized as follows:

(1) Water-fertilizer regimes significantly regulate soil salinity distribution and ionic balance.

Different irrigation methods caused distinct vertical patterns in soil electrical conductivity (EC). Treatments T (drip irrigation) and P (mulched drip irrigation) effectively reduced salt accumulation in the upper 0–20 cm soil layer while promoting downward salt migration, thus optimizing the rhizosphere salinity profile. Within plant tissues, the MP treatment markedly increased K^+ (3.28%) and Ca^{2+} (0.91%) concentrations while reducing Na^+ (3.5%), resulting in a higher K^+/Na^+ ratio (0.94) and lower Na^+/Ca^{2+} ratio (3.84). These changes reflect enhanced selective ion uptake and Na^+ exclusion capacity, contributing to stabilized cellular osmotic potential and membrane electrochemical gradients, and thus improved salt tolerance.

(2) MP treatment alleviates oxidative stress and enhances membrane stability.

Under MP management, *Pakchoi* leaves exhibited significantly lower levels of malondialdehyde (MDA) and electrolyte leakage, indicating reduced membrane lipid peroxidation and enhanced cell membrane stability. Concurrently, activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) were substantially elevated, forming an efficient reactive oxygen species (ROS) scavenging system. These responses were significantly superior to those observed under conventional treatments such as FI and CI, providing effective protection for cellular structures.

(3) Water status and osmotic adjustment capacity were significantly improved.

Leaf water potential under MP treatment (–0.60 MPa) was markedly higher than in other salt-stressed groups and second only to the non-saline control (CK, –0.40 MPa), suggesting that mulched drip irrigation effectively mitigated cellular dehydration. The total content of osmolytes such as proline, soluble sugars, and MDA was minimized, indicating that plants under MP management did not require high-energy-demanding osmotic regulation strategies to maintain turgor, thereby reflecting enhanced energy use efficiency and stress resilience.

(4) Photosynthetic performance and gas exchange efficiency, along with pigment accumulation, were significantly enhanced.

MP treatment resulted in the highest gas exchange parameters among all salt-stressed groups, with net photosynthetic rate (*Pn*), stomatal conductance (*Gs*), and transpiration rate (*Tr*) reaching 14.8 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, 0.622 mol $H_2O \cdot m^{-2} \cdot s^{-1}$, and 5.28 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$, respectively. The intercellular CO_2 concentration (C_i) was the lowest at 251 $\mu mol \cdot mol^{-1}$, suggesting greater CO_2 assimilation efficiency and enhanced carbon fixation potential. Moreover, MP-treated plants showed significantly increased levels of chlorophyll a, chlorophyll b, and total chlorophyll (1.97, 0.71, and 2.68 $mg \cdot g^{-1}$, respectively), approaching the values observed in the CK group. These results indicate that MP effectively preserves the structural and functional integrity of photosystem II (PSII), thereby improving light harvesting and energy conversion. The concurrent enhancement in pigment content and gas exchange parameters strongly supports the superior photosynthetic capacity and biomass accumulation observed under MP.

(5) MP treatment exhibited the most favorable performance in yield formation.

Under salt stress, plants treated with M fertilizers showed significantly higher plant height and biomass than those under C and F treatments. MP-treated plants had the greatest fresh and dry weights, exceeding MT by 15% and 3%, MI by 19% and 7%, CP by 21% and 12%, CT by 23% and 9%, CI by 22% and 20%, FP by 24% and 18%, FT by 26% and 20%, and FI by 39% and 42%, respectively. This confirms the strong yield potential and physiological resilience of MP under saline conditions. These advantages highlight the MP strategy as a superior integrated water-fertilizer approach for enhancing crop vigor, nutrient uptake, and biomass productivity.

In summary, the MP treatment effectively mitigated salt-induced stress in *Pakchoi* by synergistically optimizing soil salinity profiles, improving selective ion absorption, enhancing antioxidant defenses, and boosting photosynthetic performance and water status. These comprehensive improvements support MP as the most promising water and fertilizer management strategy for high-efficiency cultivation of *Pakchoi* in coastal saline-alkali soils. We recommend the promotion of MP application in relevant agro-ecological zones, integrated with salt-tolerant cultivar selection and intelligent fertigation technologies to advance sustainable, efficient, and resilient protected vegetable production systems.

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Competing Interests

The authors declare that they have no competing interests.

Data Availability

All data related to this paper can be requested from the corresponding author upon reasonable request.

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Figures Captions

Figure 1: The leaf water potential of Pakchoi under different irrigation and fertilization modes

Note: Lowercase letters indicate significant differences in leaf water potential among treatments at the 5% level ($P < 0.05$, DMRT).

Figure 2: Effects of different irrigation and fertilization modes on nitrogen and phosphorus content in Pakchoi plants

Note: Capital letters above the bars indicate significant differences in nitrogen content among treatments at the 5% level. Lowercase letters indicate significant differences in phosphorus content among treatments at the 5% level ($P < 0.05$, DMRT).

Figure 3: The organic solute accumulation in Pakchoi under different irrigation and fertilization modes: (a)Proline

content, (b)Soluble protein content, (c)Soluble sugar content, (d)Glucose content, (e)Fructose content, (f)Sucrose content.

Note: Lowercase letters indicate significant differences in organic solute accumulationl among treatments at the 5% level ($P < 0.05$, DMRT).

Figure 4: The ROS, MDA contents, and electrolyte leakage in Pakchoi under different irrigation and fertilization modes: (a) O_2^- content, (b) H_2O_2 content, (c) Electrolyte leakage, (d) MDA content.

Note: Lowercase letters indicate significant differences in ROS, MDA content, and electrolyte leakage among treatments at the 5% level ($P < 0.05$, DMRT).

Figure 5: The antioxidant enzyme activities in Pakchoi under different water and fertilizer management modes: (a)SOD activitie, (b)POD activitie, (c)CAT activitie, (d)APX activitie.

Note: Lowercase letters indicate significant differences in antioxidant enzyme activities among treatments at the 5% level ($P < 0.05$, DMRT).

Figure 6: The chlorophyll content of Pakchoi under different water and fertilizer management regimes

Note: Different capital letters above the bars indicate significant differences in total chlorophyll content among treatments at the 5% level. Different lowercase letters indicate significant differences in chlorophyll a or b content among treatments at the 5% level ($P < 0.05$, DMRT).

Figure 7: The photosynthetic parameters of Pakchoi under different water and fertilizer management regimes: (a)Pn, (b)Tr, (c)Gs, (d)Ci

Note: Different lowercase letters indicate significant differences in photosynthetic parameters among treatments at the 5% level ($P < 0.05$, DMRT).

Figure 8: The plant height of Pakchoi under different irrigation and fertilization modes

Figure 9: Integrated Mechanistic Model of Water Regulation, Ion Homeostasis, ROS Defense, and Photosynthetic Optimization in Pakchoi under Salt Stress

Table Captions

Table 1. The design of experiment

Table 2. The soil EC under different irrigation and fertilization modes[1)]

1) Number in the table is mean±standard deviation. Different letters in a column indicate significant difference among treatments at the 5% level ($P<0.05$, DMRT).

Table 3. The Na+, K+, Ca2+contents of single plant and their ratio under different irrigation and fertilization modes[1)]

1) Number in the table is mean±standard deviation.Different letters in a column indicate significant difference among treatments at the 5% level ($P<0.05$, DMRT).

Table 4. The biomass of Pakchoi under different irrigation and fertilization modes[1)]

1) Number in the table is mean±standard deviation. Different letters in a column indicate significant difference among treatments at the 5% level ($P<0.05$, DMRT).

Table 1(on next page)

The design of experiment

Table 1. The design of experiment

Treatments	Irrigation methods	Fertilizer types	With or without NaCl
FI	Conventional irrigation	Conventional fertilizer	With
FT	Trickle irrigation	Conventional fertilizer	With
FP	Film mulched trickle irrigation	Conventional fertilizer	With
MI	Conventional irrigation	Mixed fertilizer	With
MT	Trickle irrigation	Mixed fertilizer	With
MP	Film mulched trickle irrigation	Mixed fertilizer	With
CI	Conventional irrigation	Controlled-release fertilizer	With
CT	Trickle irrigation	Controlled-release fertilizer	With
CP	Film mulched trickle irrigation	Controlled-release fertilizer	With
CK	Conventional irrigation	Conventional fertilizer	Without

Table 2

(on next page)

The soil EC under different irrigation and fertilization modes [1)]

1) Number in the table is mean±standard deviation. Different letters in a column indicate significant difference among treatments at the 5% level ($P<0.05$, DMRT) .

Table 2. The soil EC under different irrigation and fertilization modes[1)]

Treatments	EC in upper soil layer ($us\cdot cm^{-1}$)			EC in lower soil layer ($us\cdot cm^{-1}$)	
	Seedling stage	Rosette stage		Seedling stage	Rosette stage
FI	1711±155 ab	1578±92 a		1628±254 c	1690±63 e
FT	1332±99 bc	1168±63 cd		2295±286 ab	2842±124 ab
FP	1364±149 bc	1125±85 d		2385±359 ab	2285±296 cd
MI	1721±202 a	1556±98 a		2044±185 bc	1927±228 de
MT	1297±162 bc	1182±87 bcd		2599±261 a	3165±385 a
MP	1322±70 bc	1265±87 bc		2487±242 ab	2454±118 c
CI	1792±216 a	1577±142 a		1907±375 bc	2071±136 d
CT	1458±157 abc	1290±65 bc		2219±242 ab	2585±145 bc
CP	1309±102 bc	1208±70 bcd		2334±213 ab	2323±76 c
CK	598±50 d	319±61 e		850±61 d	784±20 f

1) Number in the table is mean±standard deviation. Different letters in a column indicate significant difference among treatments at the 5% level ($P<0.05$, DMRT).

Table 3 (on next page)

The Na^{+}, K^{+}, Ca^{2+} contents of single plant and their ratio under different irrigation and fertilization modes [1)]

1) Number in the table is mean±standard deviation.Different letters in a column indicate significant difference among treatments at the 5% level ($P<0.05$, DMRT) .

Tab. 3 The Na^+, K^+, Ca^{2+}contents of single plant and their ratio under different irrigation and fertilization modes[1)]

Treatments	K^+ (%)	Ca^{2+} (%)	Na^+ (%)	K^+/Na^+	Na^+/Ca^{2+}
FI	1.32±0.14 g	0.41±0.03 g	9.22±0.29 a	0.14±0.01 g	22.64±2.13 a
FT	2.36±0.28 de	0.67±0.02 e	7.08±0.43 b	0.33±0.05 e	10.53±0.59 cd
FP	1.78±0.14 f	0.65±0.02 e	4.9±0.15 d	0.36±0.03 e	7.6±0.22 e
MI	2.13±0.28 e	0.38±0.02 g	6.62±0.42 bc	0.32±0.04 e	17.28±1.70 b
MT	4.06±0.43 a	0.83±0.02 c	5.07±0.35 d	0.81±0.09 b	6.15±0.47 f
MP	3.28±0.43 bc	0.91±0.02 b	3.5±0.56 e	0.96±0.16 b	3.84±0.61 g
CI	1.39±0.15 g	0.56±0.02 f	6.06±0.41 c	0.23±0.04 f	10.82±0.90 cd
CT	2.83±0.29 cd	0.71±0.02 d	6.67±0.56 bc	0.42±0.06 d	9.43±0.84 d
CP	3.73±0.86 ab	0.83±0.02 c	6.39±0.41 bc	0.59±0.12 c	7.72±0.70 e
CK	3.89±0.42 ab	0.98±0.03 a	1.12±0.28 f	3.61±0.86 a	1.15±0.28 h

1) Number in the table is mean±standard deviation.Different letters in a column indicate significant difference among treatments at the 5% level ($P<0.05$, DMRT).

Table 4(on next page)

The biomass of Pakchoi under different irrigation and fertilization modes [1)]

1) Number in the table is mean±standard deviation. Different letters in a column indicate significant difference among treatments at the 5% level (P<0.05 , DMRT) .

Table 4. The biomass of Pakchoi under different irrigation and fertilization modes[1)]

Treatments	Fresh matter (g)	Dry matter (g)
FI	32.47±1.89 e	2.09±0.18 g
FT	35.86±1.19 d	2.46±0.14 f
FP	36.54±1.45 cd	2.5±0.15 ef
MI	38.12±0.81 c	2.77±0.11 bcd
MT	39.39±1.23 c	2.88±0.07 bc
MP	45.26±2.04 b	2.96±0.12 b
CI	37.19±0.71 c	2.47±0.11 f
CT	36.75±1.12 cd	2.71±0.07 cde
CP	37.47±1.63 c	2.64±0.12d ef
CK	49.38±0.46 a	3.46±0.06 a

1) Number in the table is mean±standard deviation. Different letters in a column indicate significant difference among treatments at the 5% level (P<0.05, DMRT).

Figure 1

The l eaf w ater p otential of Pakchoi under d ifferent irrigation and fertilization modes

Note : Lowercase letters indicate significant differences in l eaf w ater p otential among treatments at the 5% level ($P < 0.05$, DMRT).

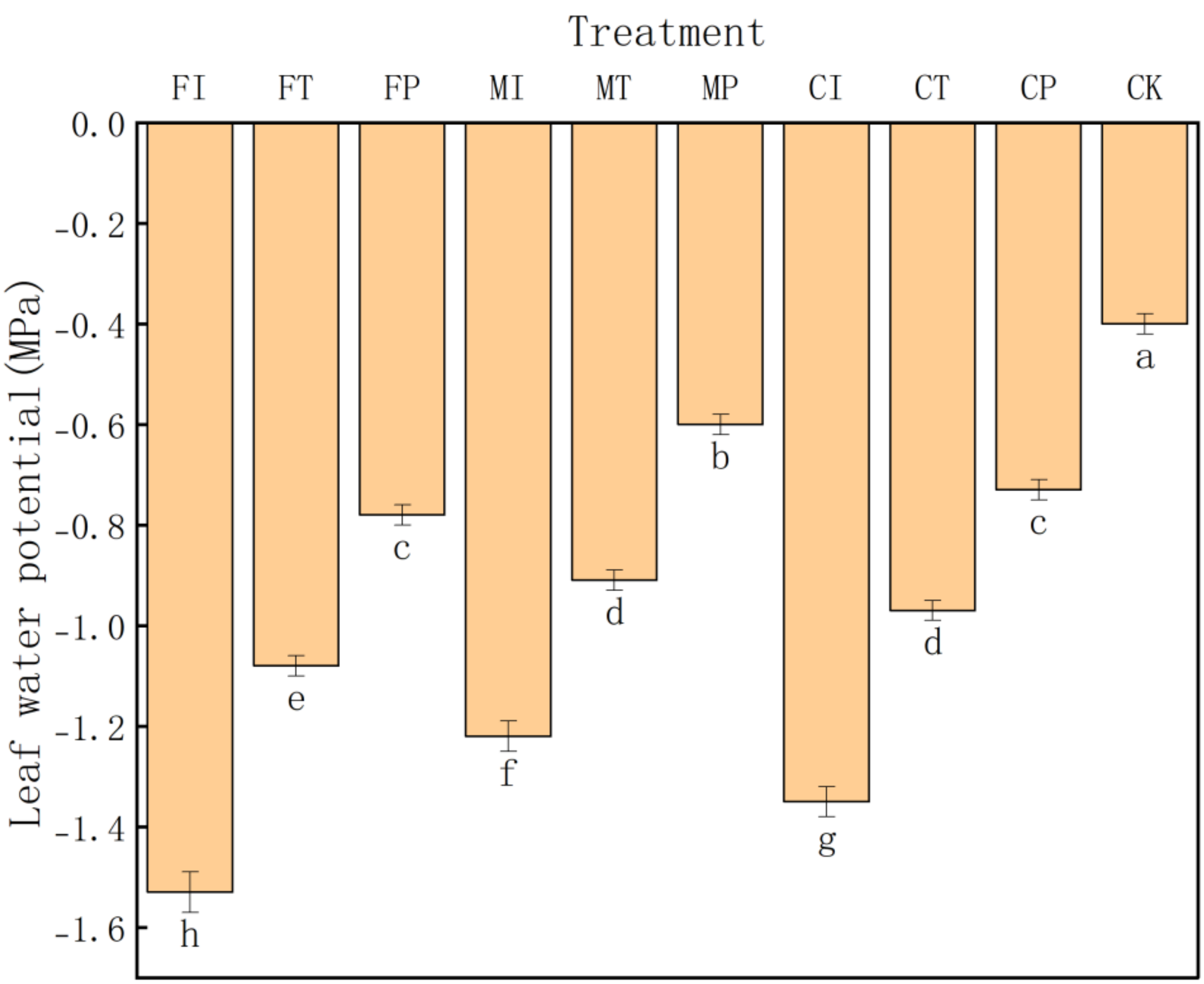


Figure 2

Effects of d ifferent irrigation and fertilization modes on n itrogen and p hosphorus c ontent in Pakchoi p lants

Note: Capital letters above the bars indicate significant differences in nitrogen content among treatments at the 5% level. Lowercase letters indicate significant differences in phosphorus content among treatments at the 5% level ($P < 0.05$, DMRT).

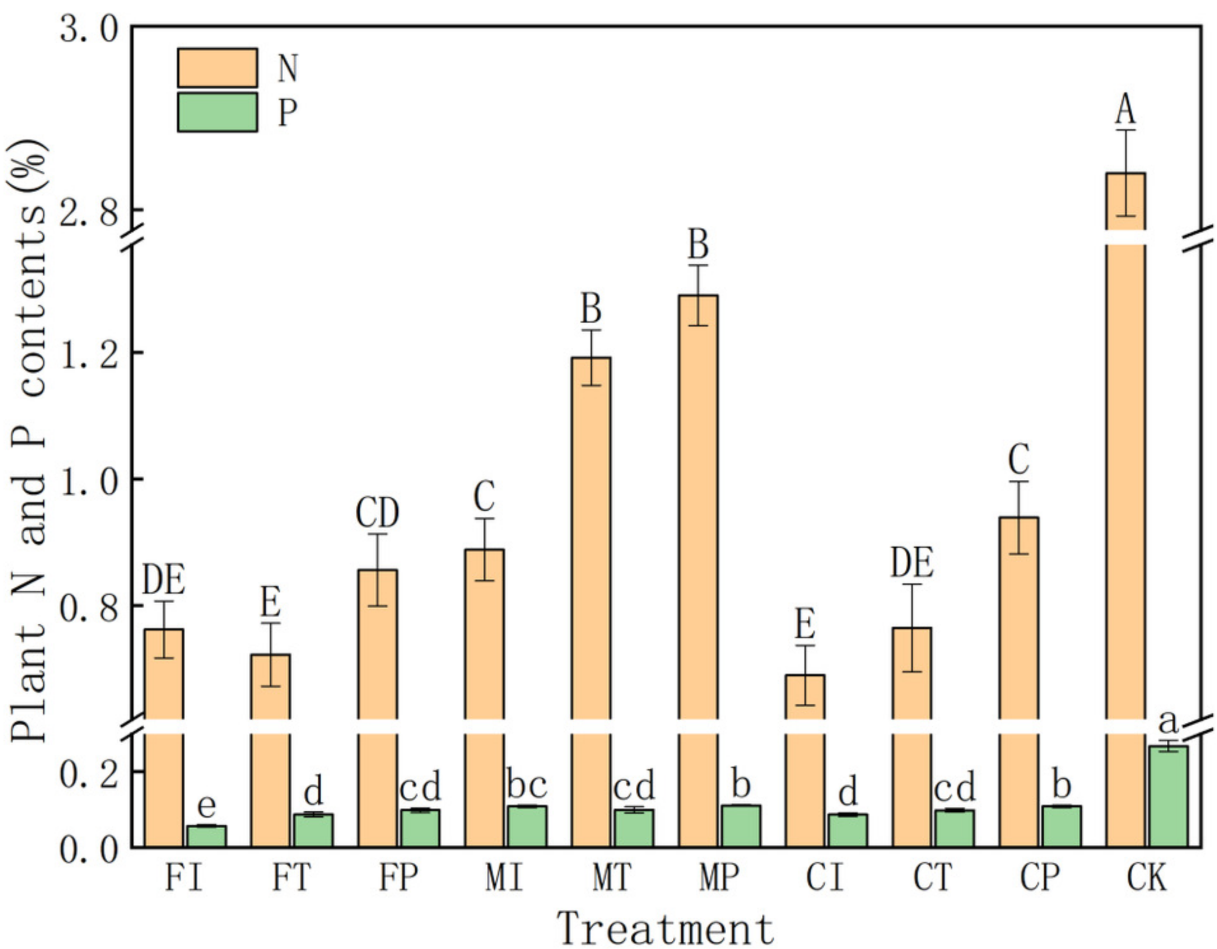
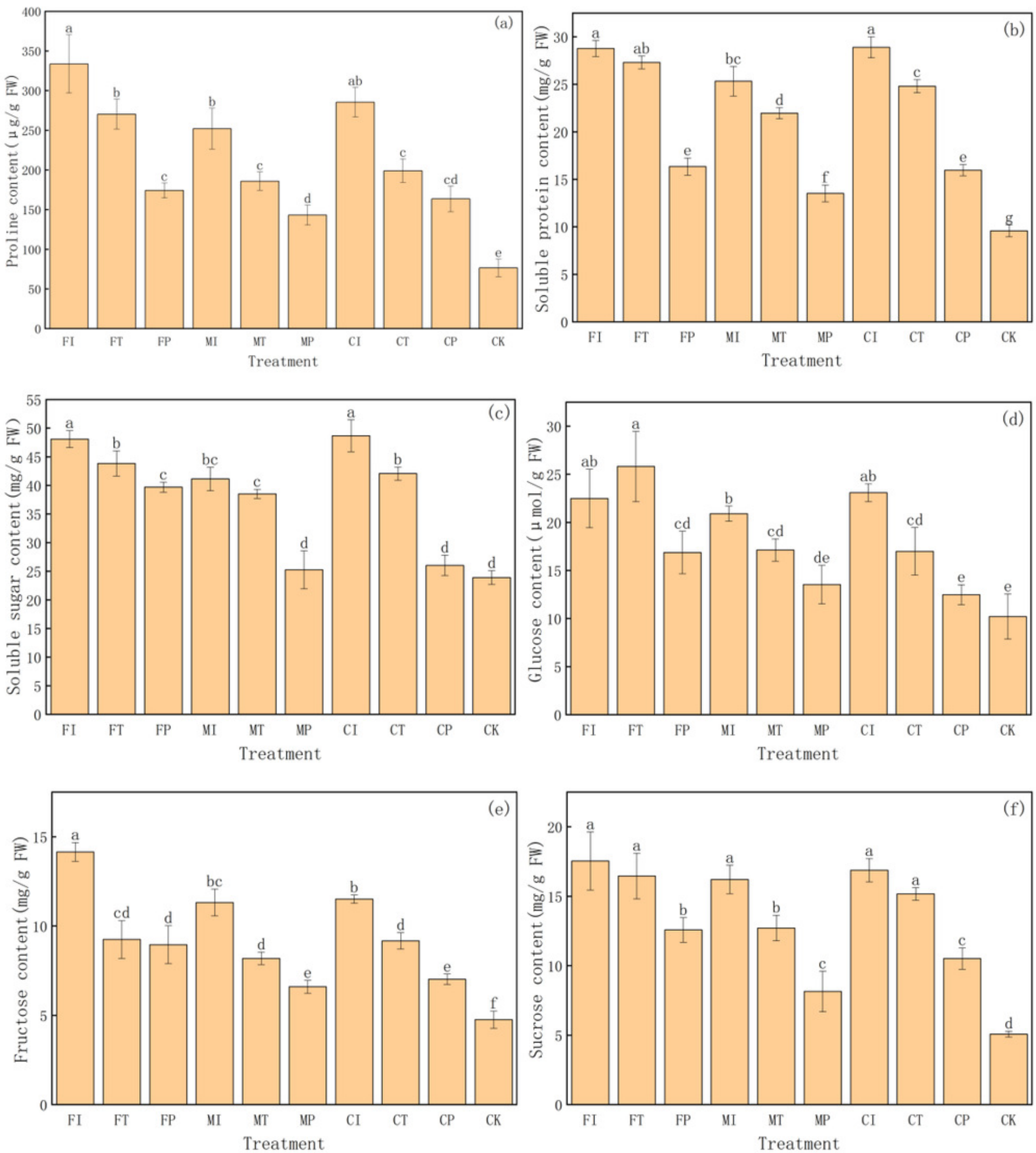


Figure 3

The organic solute accumulation in Pakchoi under d ifferent irrigation and fertilization modes : (a)Proline content, (b)Soluble protein content, (c)Soluble sugar content, (d)Glucose content, (e)Fructose content, (f)Sucrose content

Note : Lowercase letters indicate significant differences in organic solute accumulation l among treatments at the 5% level ($P < 0.05$, DMRT).



(a)
Proline content(μg/g FW)
Treatment
FI FT FP MI MT MP CI CT CP CK
(b)
Soluble protein content(mg/g FW)
Treatment
(c)
Soluble sugar content(mg/g FW)
Treatment
(d)
Glucose content(μmol/g FW)
Treatment
(e)
Fructose content(mg/g FW)
Treatment
(f)
Sucrose content(mg/g FW)
Treatment

Figure 4

The ROS, MDA contents, and electrolyte leakage in Pakchoi under d ifferent irrigation and fertilization modes : (a) O_2^- content, (b) H_2O_2 content, (c) Electrolyte leakage, (d) MDA content.

Note: Lowercase letters indicate significant differences in ROS, MDA content, and electrolyte leakage among treatments at the 5% level ($P < 0.05$, DMRT).

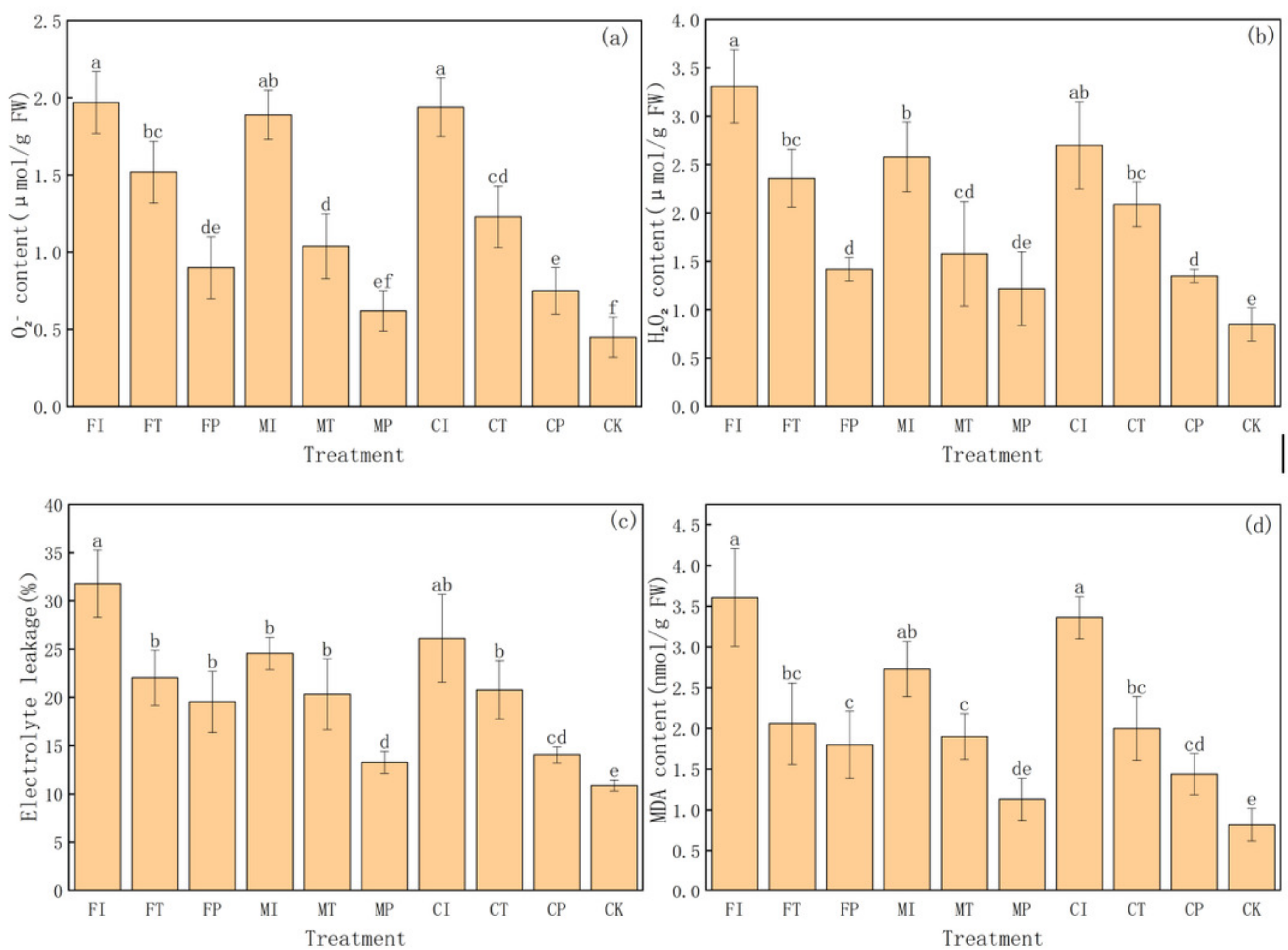


Figure 5

The antioxidant enzyme activities in Pakchoi under different water and fertilizer management modes : (a)SOD activitie, (b)POD activitie, (c)CAT activitie, (d)APX activitie.

Note: Lowercase letters indicate significant differences in antioxidant enzyme activities among treatments at the 5% level (P < 0.05, DMRT).

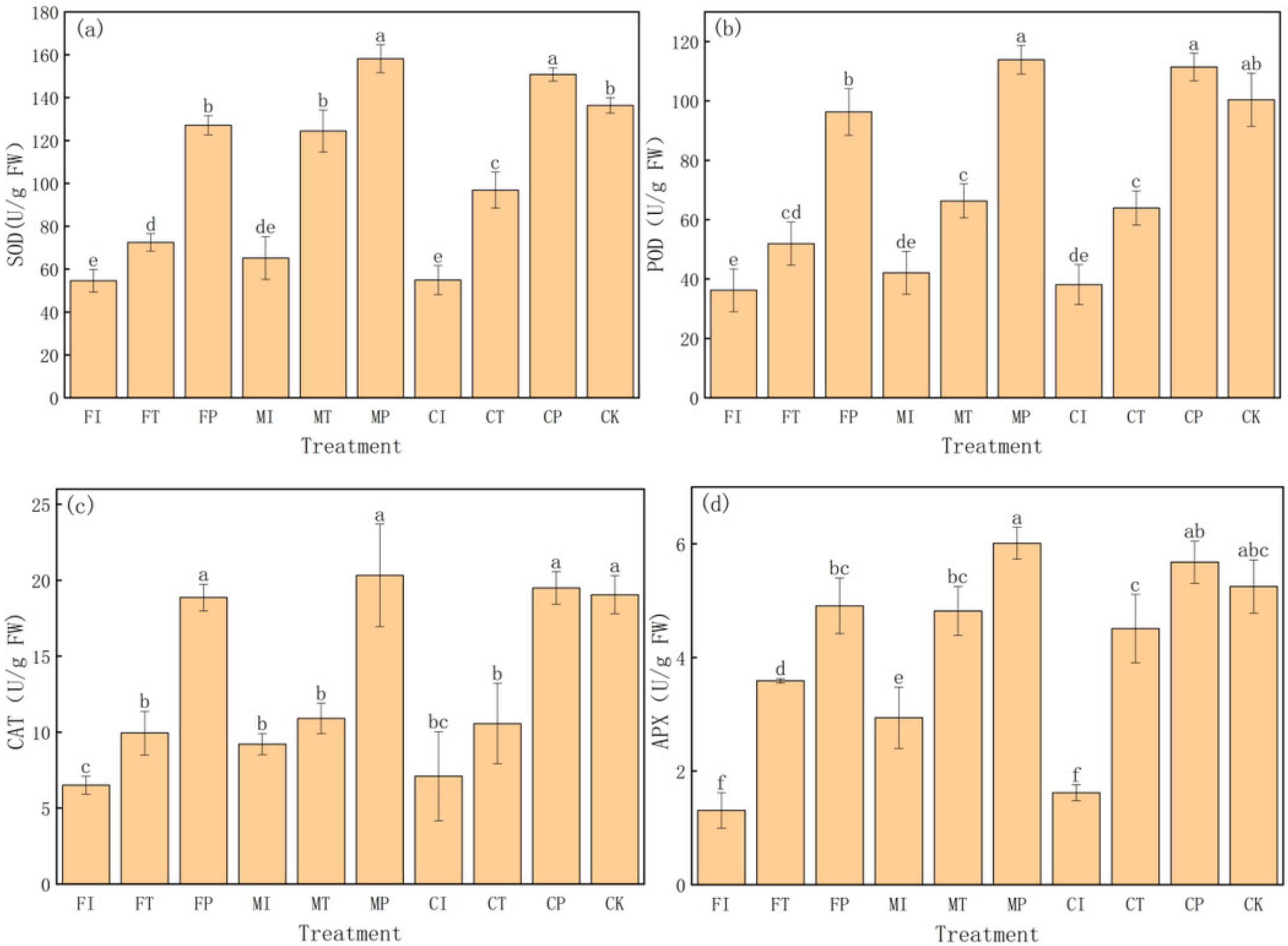


Figure 6

The chlorophyll content of Pakchoi under different water and fertilizer management regimes

Note: Different c apital letters above the bars indicate significant differences in total chlorophyll content among treatments at the 5% level . D ifferent lowercase letters indicate significant differences in chlorophyll a or b content among treatments at the 5% level (P < 0.05, DMRT).

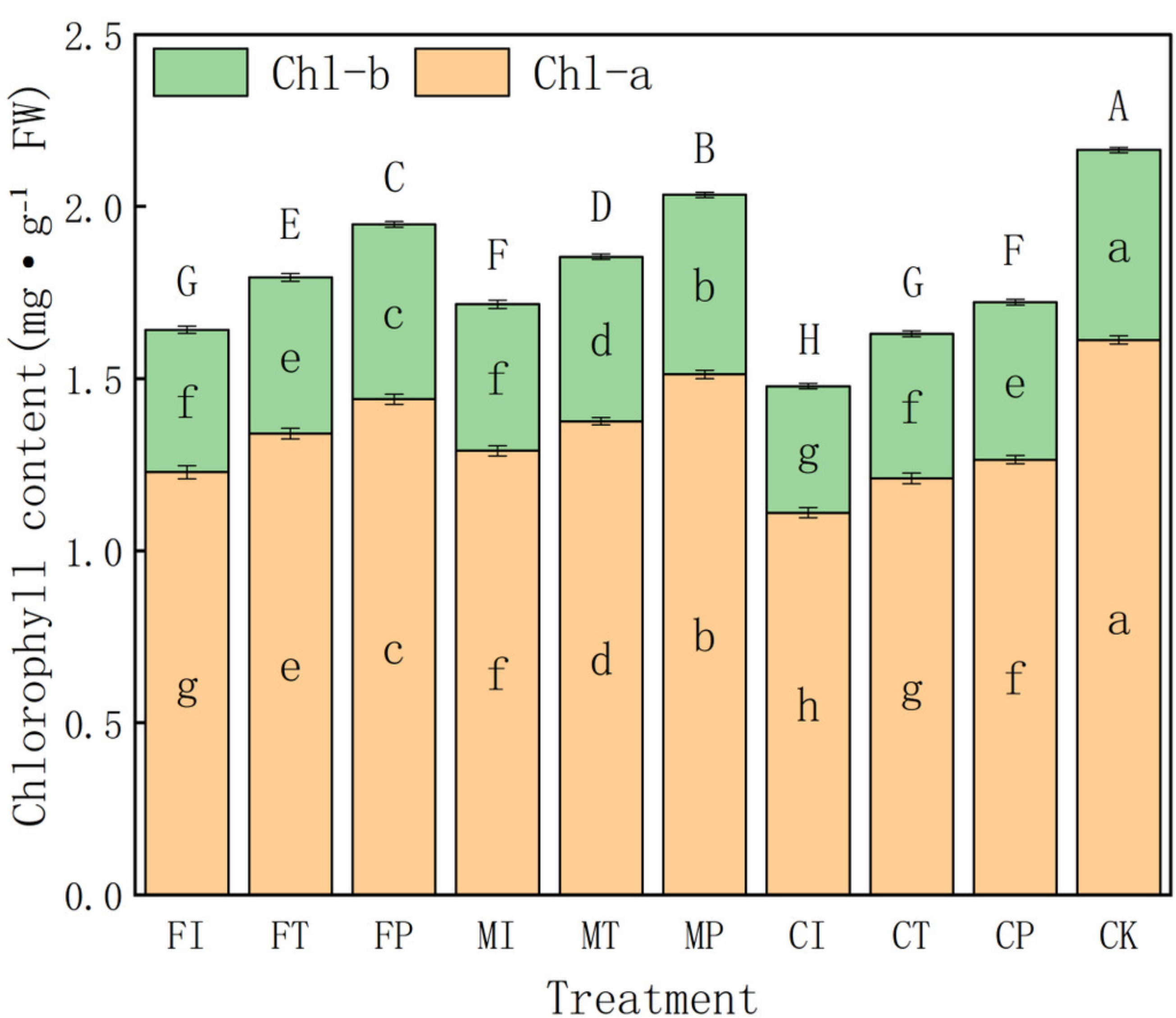


Figure 7

The photosynthetic parameters of Pakchoi under different water and fertilizer management regimes: (a) P_n , (b) T_r , (c) G_s , (d) $C\,i$

Note: D ifferent lowercase letters indicate significant differences in photosynthetic parameters among treatments at the 5% level (P < 0.05, DMRT).

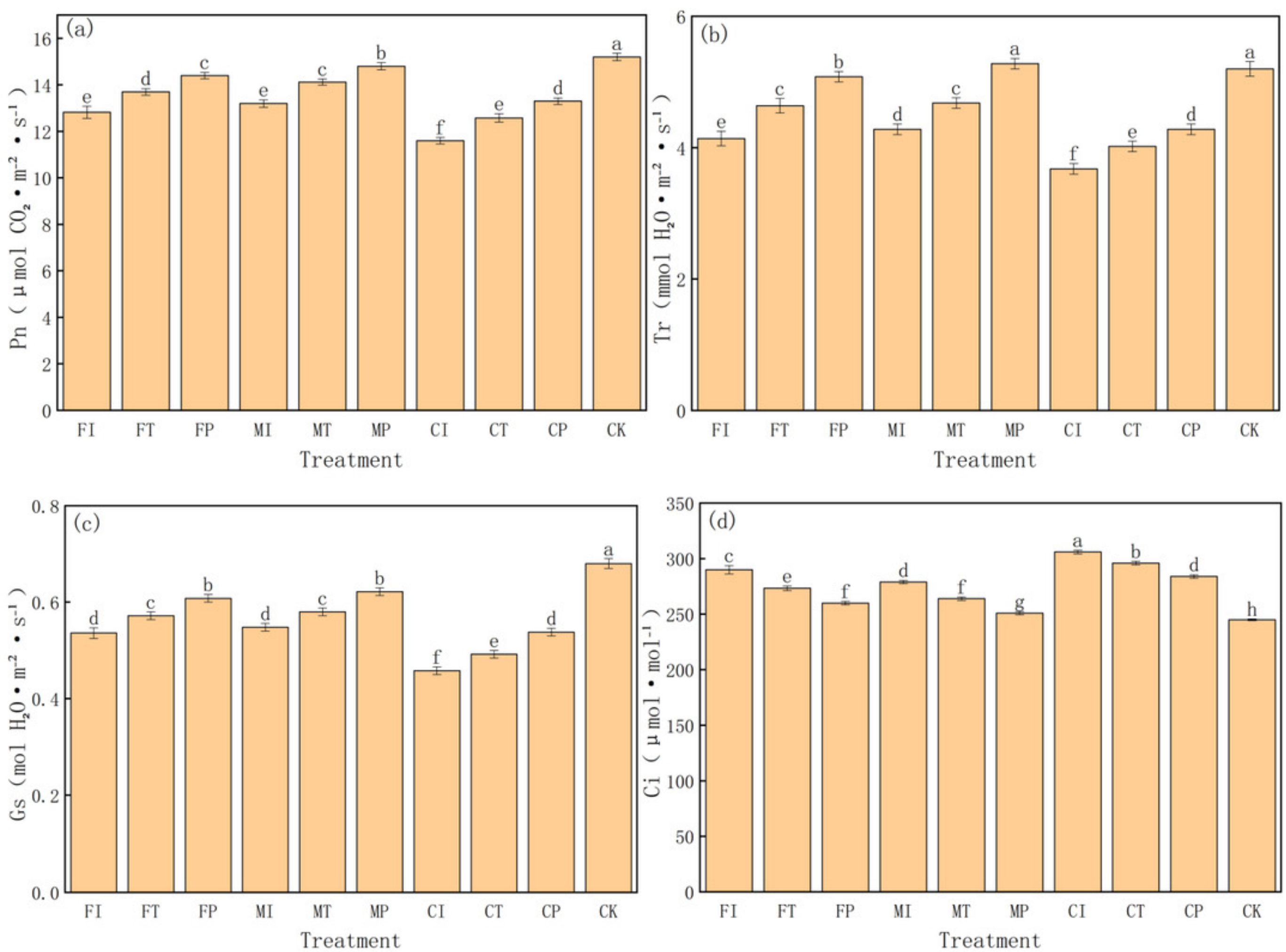


Figure 8

The plant height of Pakchoi under different irrigation and fertilization modes

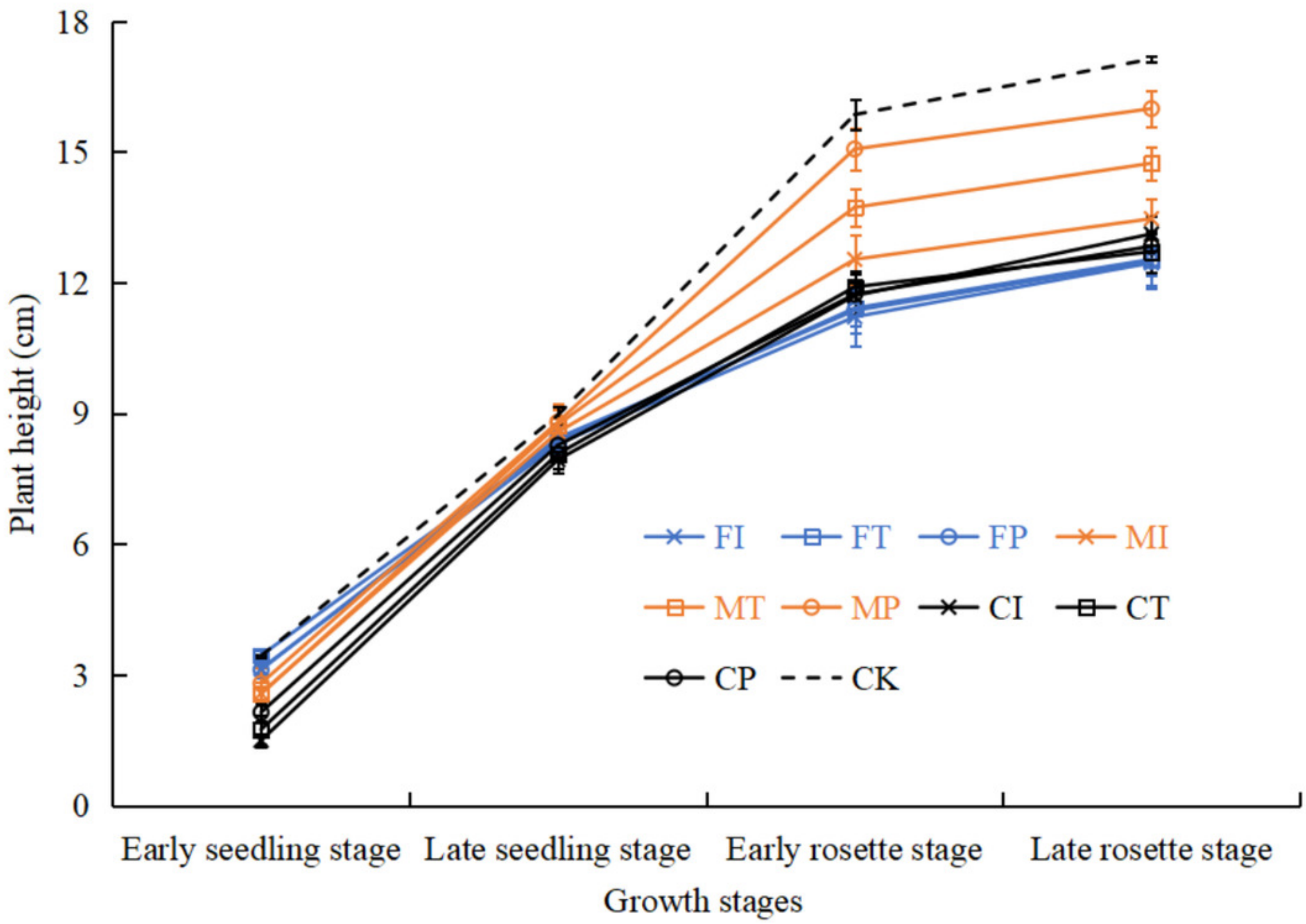
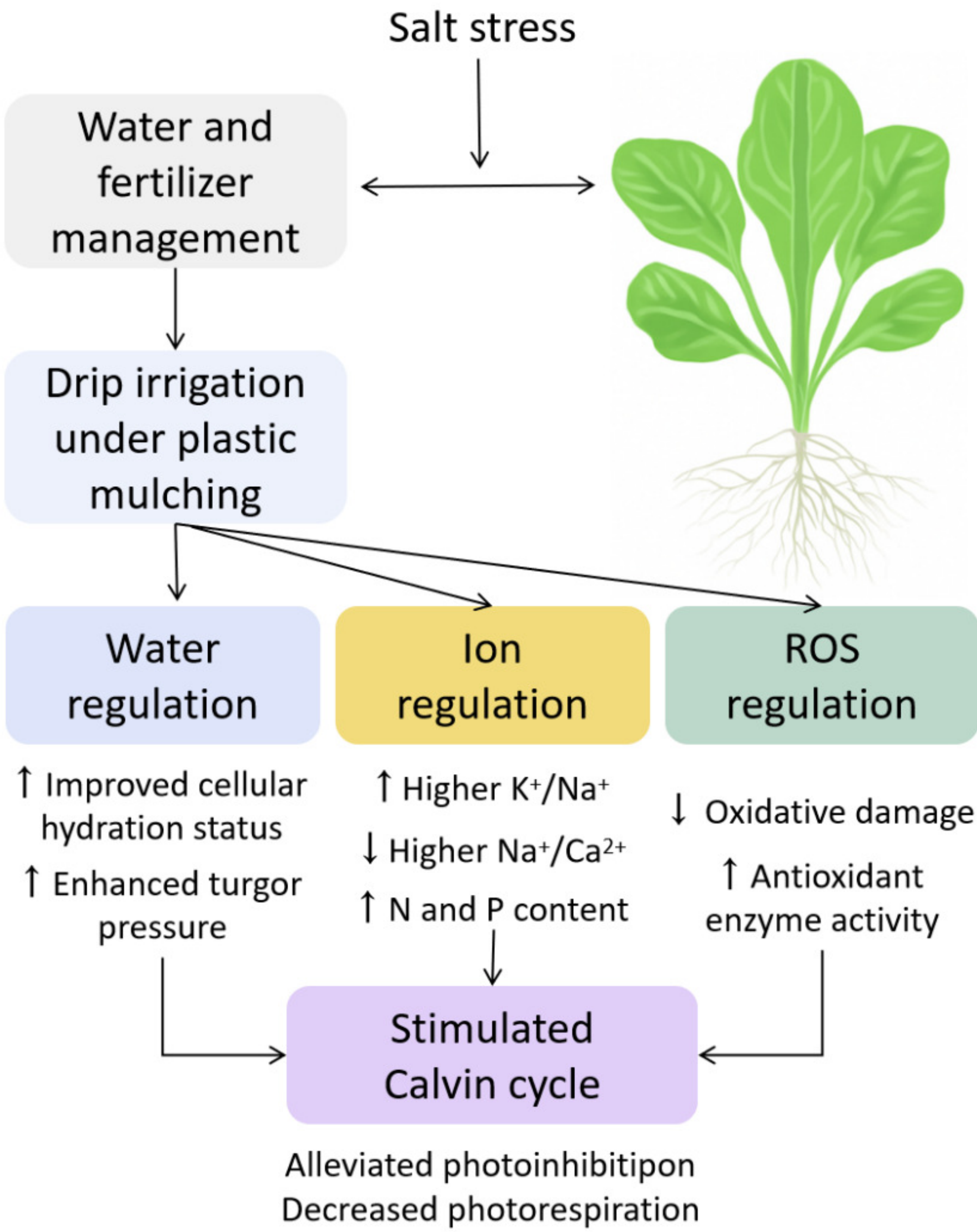


Figure 9

Integrated Mechanistic Model of Water Regulation, Ion Homeostasis, ROS Defense, and Photosynthetic Optimization in Pakchoi under Salt Stress


Salt stress
Water and fertilizer management
Drip irrigation under plastic mulching
Water regulation
Ion regulation
ROS regulation
↑ Improved cellular hydration status
↑ Enhanced turgor pressure
↑ Higher K+/Na+
↓ Higher Na+/Ca2+
↑ N and P content
↓ Oxidative damage
↑ Antioxidant enzyme activity
Stimulated Calvin cycle
Alleviated photoinhibitipon
Decreased photorespiration