agronomy

MDPI

Article

Prohexadione Calcium and Gibberellin Improve Osmoregulation, Antioxidant Response and Ion Homeostasis to Alleviate NaCl Stress in Rice Seedlings

Meiling Liu [1,2], Naijie Feng [1,3,*], Dianfeng Zheng [1,3,*] and Rongjun Zhang [1]

[1] College of Coastal Agriculture Sciences, Guangdong Ocean University, Zhanjiang 524088, China; lml19960416@126.com (M.L.); 18846192767@163.com (R.Z.)
[2] College of Agronomy, Shenyang Agricultural University, Shenyang 110866, China
[3] Shenzhen Research Institute of Guangdong Ocean University, Zhanjiang 518108, China
* Correspondence: fengnj@gdou.edu.cn (N.F.); byndzdf@126.com (D.Z.)

Abstract: Prohexadione calcium (EA) and gibberellin (GA) are two different types of plant growth regulators that have different effects on the regulation of plant development. The objective of this study was to evaluate the impacts of EA and GA on rice plant growth, development and morph-physiological traits in two rice varieties: 'Huang Huazhan' and 'Guang Hong 3'. At the three-leaf seedling stage, the plants were treated with 50 mM NaCl 24 h after foliar application of EA (100 mg·L^{-1}) and GA (1 mg·L^{-1}). Data on morphological indexes, osmotic regulators and antioxidant activities were compared with the treatment of EA and GA on the 4th, 7th, 10th and 13th days after NaCl stress. Our data analysis showed that NaCl stress inhibited the leaf area growth of rice seedlings, altered the microstructure and disrupted the antioxidant system, ion uptake and transport balance. The significant increase in malondialdehyde (MDA) content and superoxide anion production rate ($O_2^{\cdot-}$) indicated that NaCl stress caused a severe oxidative stress response to rice seedlings. Treatment with EA and GA activated the antioxidant system under NaCl stress, significantly elevated superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities and suppressed the increase in MDA content and the $O_2^{\cdot-}$ production rate. Under NaCl stress, EA and GA treatments improved the osmoregulatory balance, significantly increased soluble protein and proline contents and maintained lower Na^+/K^+ levels. EA and GA treatments significantly increased the K^+ and Ca^{2+} contents, thereby maintaining ionic balance, which was favorable for maintaining the growth of rice seedlings. In this study, moth plant growth regulators maintained the growth and development of rice seedlings under NaCl stress by inducing an increase in osmoregulation and antioxidant levels, reducing the degree of membrane damage and regulating the selective uptake of ions by rice seedlings. Current findings also clarified that foliar application of EA was more effective than GA in three-leaf seedlings by enhancing the morph-physiological and antioxidant parameters under NaCl stress.

Keywords: NaCl stress; rice seedlings; prohexadione calcium; gibberellin; physiological metabolism

check for updates

Citation: Liu, M.; Feng, N.; Zheng, D.; Zhang, R. Prohexadione Calcium and Gibberellin Improve Osmoregulation, Antioxidant Response and Ion Homeostasis to Alleviate NaCl Stress in Rice Seedlings. *Agronomy* **2024**, *14*, 1318. https://doi.org/10.3390/agronomy14061318

Academic Editor: Federico Vita

Received: 17 May 2024
Revised: 4 June 2024
Accepted: 10 June 2024
Published: 18 June 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Abiotic stresses are the leading factors limiting crop grain yield [1]. Soil salinity poses a significant threat to plant growth and development, and the main component of soil salinization is NaCl [2]. The effects of salt stress on crops are apparently at morphological, physiological, molecular and genetic levels [3]. Plants are first affected by osmotic stress under salt stress, and when salt stress reaches a certain level, ionic stress occurs, and both osmotic stress and ionic stress are the main causes of oxidative damage [4]. The most toxic constituents of salinity are Na^+ ions, which are responsible for toxicity, induce osmotic potential and generate oxidative stress [5,6]. Salt stress leads to enhanced intracellular ROS synthesis and the disruption of ion uptake and transport balance, resulting in oxidative

damage to cell membranes and protein synthesis [7,8]. Hydrogen peroxide, superoxide radicals and hydroxyl radicals are the main ROS [9]. Plants defense systems have the ability to resist abiotic stresses, such as through the synthesis of osmoregulatory substances and the enhancement of antioxidant systems [10]. The accumulation of osmoregulatory substances like proline contributes to cell regulation, ROS detoxification and maintenance of cell membrane integrity [11]. The main antioxidant enzymes that maintain cellular oxidative homeostasis are superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) [12].

Rice (*Oryza sativa* L.) is one of the major food crops for more than half of the global population and is often susceptible to soil salinity [13]. The inhibitory effects of salt stress on rice growth include a reduction in leaf area, affecting the intracellular oxidative balance and ionic osmotic balance [14]. The extent of the effect of salt stress on rice growth depends on the type and duration of salt stress [1]. The rice seedling period is one of the most critical stages during the entire reproductive period and is sensitive to salt-stressed environments [10]. Previous studies claimed that salt stress inhibits the growth of rice seedlings and disrupts lipid membrane integrity [15]. Therefore, the establishment of better salt tolerance at the seedling stage helps to maintain better growth and development at later stages [13].

Plant growth regulators are widely used in regulating plant growth and development. Prohexadione calcium is a class of delayed plant growth regulators that inhibit the synthesis of gibberellins in the GA_1–GA_{20} range in plants [16] and reduce plant internode length [17]. Prohexadione calcium application reduced plant height and collapse index in rice [18] and maize [19]. Gibberellins promote plant growth mainly by affecting cell division and longitudinal length [20]. Exogenous gibberellins promote germination and enhance seedling resistance in sweet sorghum [21,22]. In this experiment, the effects of prohexadione calcium and gibberellin foliar application on morphogenesis, antioxidant system, osmoregulation and ion homeostasis of rice seedlings under NaCl stress were comparatively studied. Exploring the regulatory mechanisms of prohexadione calcium and gibberellin on rice seedlings under NaCl stress will provide a theoretical basis for further promotion of the stress resistance of rice plant growth regulators.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

In the present study, two rice varieties i.e., Guang Hong 3 (No. 3) and Huang Huazhan (HHZ), were used as the plant materials, and the co-tested growth regulators were prohexadione calcium (EA) and gibberellin (GA). The seeds and growth regulators were provided by the chemical control group of the College of Coastal Agriculture, Guangdong Ocean University.

A pot experiment was conducted in the greenhouse at Guangdong Ocean University from November 2021 to October 2022 under the day and night temperatures of 25/20 ± 2 °C. Healthy and uniform seeds were selected and sterilized with 3% hydrogen peroxide for 15 min, then thoroughly rinsed with distilled water. Sterilized rice seeds need to undergo a soaking and germination process in the dark at 28 ± 2 °C before they can be sown. Uniform-sized seeds of both rice varieties were sown in non-porous plastic pots (upper diameter/lower diameter/height = 15 cm:11.5 cm:14.7 cm) with brick red soil and vermiculite (3:1) as a substrate. When rice seedlings grew three leaves and one heart, 100 mg·L^{-1} prohexadione calcium and 1 mg·L^{-1} gibberellin were applied to leaves as foliar spray until the leaf surface was moist. The treatment was carried out 24 h after the conditioner treatment with NaCl solution (50 mM) (*w*/*w*) [6]. Six treatments were set up for each rice variety: (1) CK—water treatment, (2) EA—100 mg·L^{-1} prohexadione calcium foliar spray, (3) GA—1 mg·L^{-1} gibberellin foliar spray, (4) N—50 mM NaCl treatment, (5) EAN—100 mg·L^{-1} prohexadione calcium foliar spray + 50 mM NaCl treatment, (6) GAN—1 mg·L^{-1} gibberellin foliar spray + 50 mM NaCl treatment.

2.2. Determination of Morphological Indicators and Microstructure

Rice seedlings of uniform growth and size were selected on the 4th, 7th, 10th and 13th days after NaCl stress, respectively, and leaf area was determined using a Yaxin leaf area meter (Yaxin-1241: Beijing Ya Xin Li Yi Technology Co., Ltd., Beijing, China).

Furthermore, rice leaves were cut into small 1 cm sections and fixed with FAA fixative (formalin/glacial acetic acid/70% ethanol = 1:1:18) poured onto a slide containing the leaves for 24 h. The leaves were washed three times with 70% ethanol for 1 h each time. The sample was dehydrated with ethanol, made transparent with pure alcohol and xylene and then put into a mixture of half xylene and half paraffin wax for 15 min so that the paraffin wax penetrated into the tissue to reach a suitable saturation level for embedding. Finally, paraffin sections were stained with 1% Fancy Red and 1% Solid Green, dehydrated with ethanol, infiltrated with xylene and covered with neutral resin. Images were observed using light microscopy (ContourGT-InMotion: Bruker Nano Surface Instrumentation Division, Berlin, Germany) [23].

2.3. Measurement of Physiological Indicators

Superoxide anion production rate ($O_2^{\cdot-}$) and malondialdehyde (MDA) were measured according to the protocol described by Wang et al. [24].

Superoxide dismutase (SOD) activity, peroxidase (POD) activity and catalase (CAT) activity were determined according to the method of Forghani et al. [22].

Soluble protein content was determined using the Komas Brilliant Blue staining method [25]. The proline content was measured according to the protocol described by Bates et al. [26].

We used a grinder to grind the dried leaves into a powder, and after treatments such as ablation and ashing, the Na^+, K^+ and Ca^{2+} contents were determined using a flame photometer [12].

2.4. Statistical Analysis

Statistical analyses were conducted using SPSS (24) software (IBM, Chicago, IL, USA). All treatment and control data were analyzed by one-way analysis of variance (ANOVA) with Duncan's multiple range test at the $p < 0.05$ significance level. The results were expressed as the mean ± SE of the three replicates. The figures were drawn using OriginPro 9.1 software (OriginLab, Northampton, MA, USA).

3. Results

3.1. Changes in Leaf Area and Leaf Microstructure of Rice Seedlings

NaCl stress inhibited the growth and development of rice seedling leaf areas (Figure 1). The effect of prohexadione calcium treatment in alleviating the inhibition of leaf growth by NaCl stress was observed to be better than that of the gibberellin treatment. NaCl stress reduced the leaf area of Guang Hong 3 and Huang Huazhan on the 13th day of NaCl treatment. However, the EAN and GAN treatments of Guang Hong 3 showed a significant increase in the leaf area by 28.99% and 9.71% on the 13th day compared to N treatment, and the EAN and GAN treatments of Huang Huazhan showed a significant increase by 25.45% and 17.66% on the 13th day when compared to N treatment (Figure 1A,B). The cavities in the leaves of Guang Hong 3 and Huang Huazhan treated with prohexadione calcium and gibberellin were larger under NaCl stress (Figure 1G,I,K,M). Vascular bundles of rice seedlings under NaCl stress were smaller and there were fewer chloroplasts (Figure 1D,F), traits which were alleviated by prohexadione calcium and gibberellin treatments under NaCl stress (Figure 1H,J,L,N).

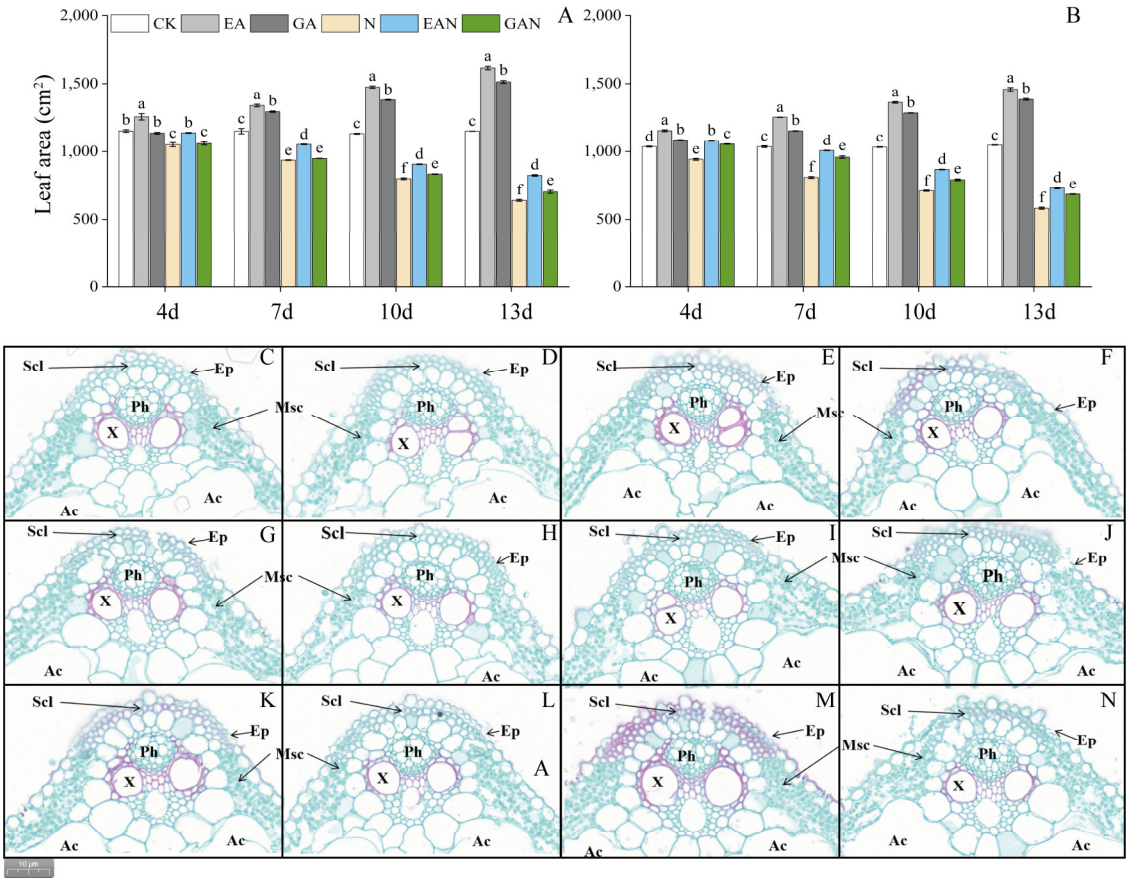

Figure 1. Effect of prohexadione calcium and gibberellin on leaf area and leaf microstructure of rice seedlings under NaCl stress. (**A**,**C**,**D**,**G**,**H**,**K**,**L**) for Guang Hong 3 and (**B**,**E**,**F**,**I**,**J**,**M**,**N**) for Huang Huazhan. CK: water treatment; EA: prohexadione calcium; GA: gibberellin; N: NaCl treatment; EAN: prohexadione calcium + NaCl treatment; GAN: gibberellin + NaCl treatment. (**C**,**E**) are two control treatments of rice; (**G**,**I**) are prohexadione calcium foliar spray treatments; (**K**,**M**) are gibberellin foliar spray treatments; (**D**,**F**) are NaCl treatments; (**H**,**J**) are 'prohexadione calcium + NaCl' treatments; (**L**,**N**) are 'gibberellin + NaCl' treatments. Ac: cavity, Msc: chloroplast, Ph: bast, X: xylem, Scl: thick-walled tissue, Ep: epidermis. Different letters (a–f) indicate significant differences between different treatments according to ANOVA (Duncan, $p < 0.05$).

3.2. Changes in Membrane Damage and Antioxidant Enzyme Activity of Rice Seedlings

NaCl stress increased the generation of intracellular reactive oxygen species (ROS), caused more cell membrane damage and increased antioxidant levels (Figure 2). The prohexadione calcium treatment considerably enhanced the activity of antioxidant enzymes and reduced membrane damage compared to gibberellin treatment under NaCl stress. The MDA content and $O_2^{\cdot-}$ production rate in the leaves of Guang Hong 3 and Huang Huazhan reached a maximum on the 13th day and were significantly lower in the EAN and GAN treatments compared to the N treatment (Figure 2A–D). The N treatment of Guang Hong 3 and Huang Huazhan showed an increasing trend with time for SOD and POD activities, while CAT activity started to decrease on the 10th day. The EAN treatment enhanced the antioxidant enzyme activities better than the GAN treatment, reaching a maximum on the 13th day. There were corresponding percent increases of 9.82% and 5.67% in SOD activity, 9.94 and 3.76% in POD activity and 44.92% and 38.66% in CAT activity for EAN and GAN treatments, respectively, compared to the N treatment for Guang Hong 3 (Figure 2E,G,I), and increases of 10.17% and 8.26% in SOD activity, 5.62% and 3.88% in POD activity and 57.16% and 45.63% in CAT activity for Huang Huazhan, reaching significant differences (Figure 2F,H,J).

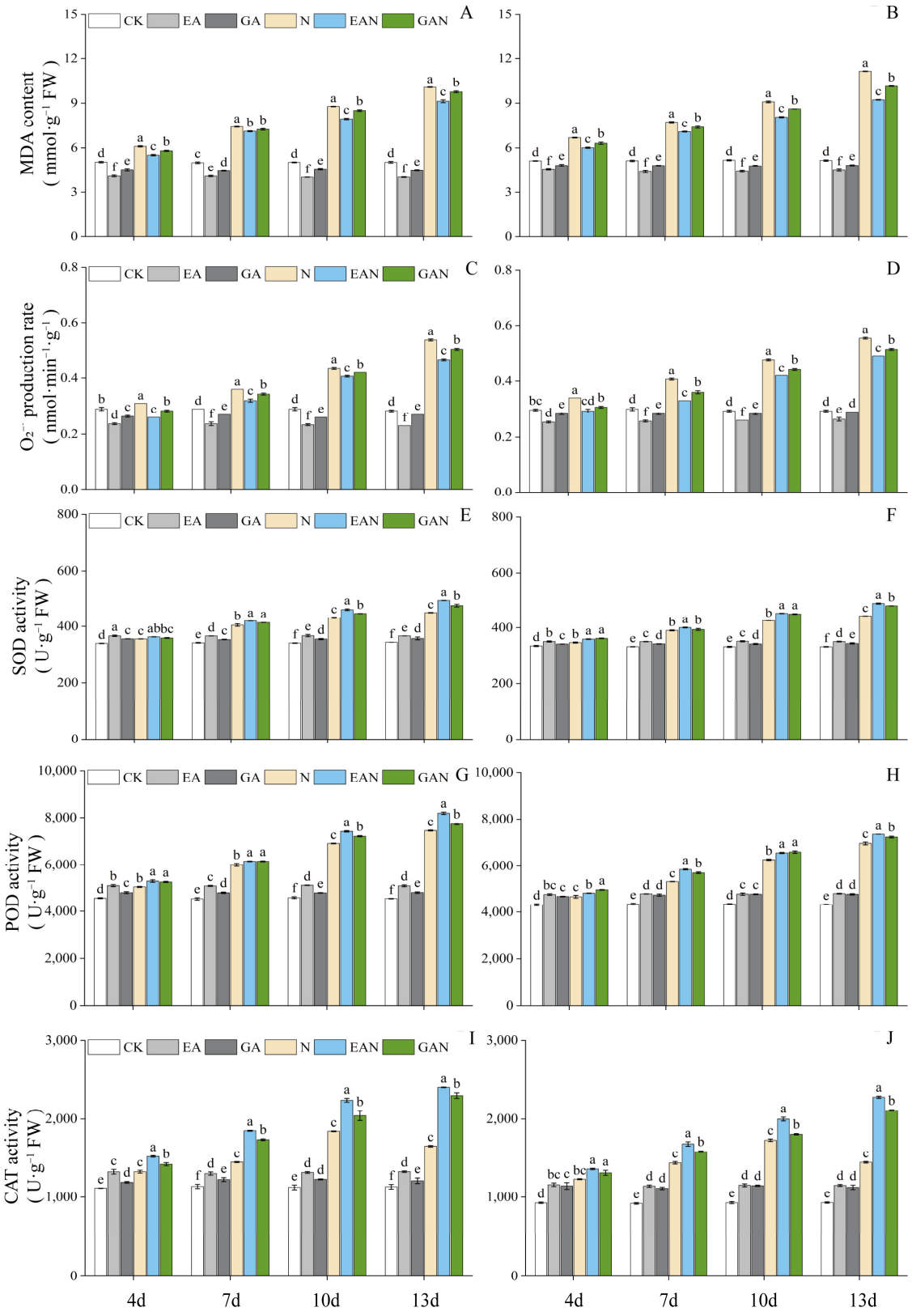

Figure 2. Effect of prohexadione calcium and gibberellin on membrane damage and antioxidant level of rice seedlings under NaCl stress. (**A**,**C**,**E**,**G**,**I**) are Guang Hong 3; (**B**,**D**,**F**,**H**,**J**) are Huang Huazhan. CK: water treatment; EA: prohexadione calcium; GA: gibberellin; N: NaCl treatment; EAN: prohexadione calcium + NaCl treatment; GAN: gibberellin + NaCl treatment. Different letters (a–f) indicate significant differences between different treatments according to ANOVA (Duncan, $p < 0.05$).

3.3. Changes in the Content of Osmoregulatory Substances in Rice Seedling Leaves

The results of the present study showed that prolonged effects of NaCl stress increased the content of osmoregulatory substances in the leaves of rice seedlings (Figure 3). The soluble protein and proline contents of Guang Hong 3 were higher than those of Huang Huazhan under NaCl stress. The foliar treatment of prohexadione calcium and gibberellin differentially regulated the soluble protein and proline contents under NaCl stress. However, the impact of prohexadione calcium was found to be better than gibberellin. The soluble protein content of Guang Hong 3 and Huang Huazhan reached the maximum on the 13th day of NaCl stress. There were significant increases of 22.56% and 10.96% in soluble protein content on the 13th day for the EAN and GAN treatments of Guang Hong 3, respectively, compared to N treatment (Figure 3A), which reached a significant difference of 14.77% and 12.24% for Huang Huazhan (Figure 3B). There were significant increases of 23.96–27.81% for EAN treatment and 10.50–19.97% for GAN treatment from the 7th to 13th day, compared to N treatment for Guang Hong 3 (Figure 3C), and 18.84–34.69% for EAN treatment and 9.96–19.75% for GAN treatment from the 7th to 13th day compared to N treatment for Huang Huazhan (Figure 3D).

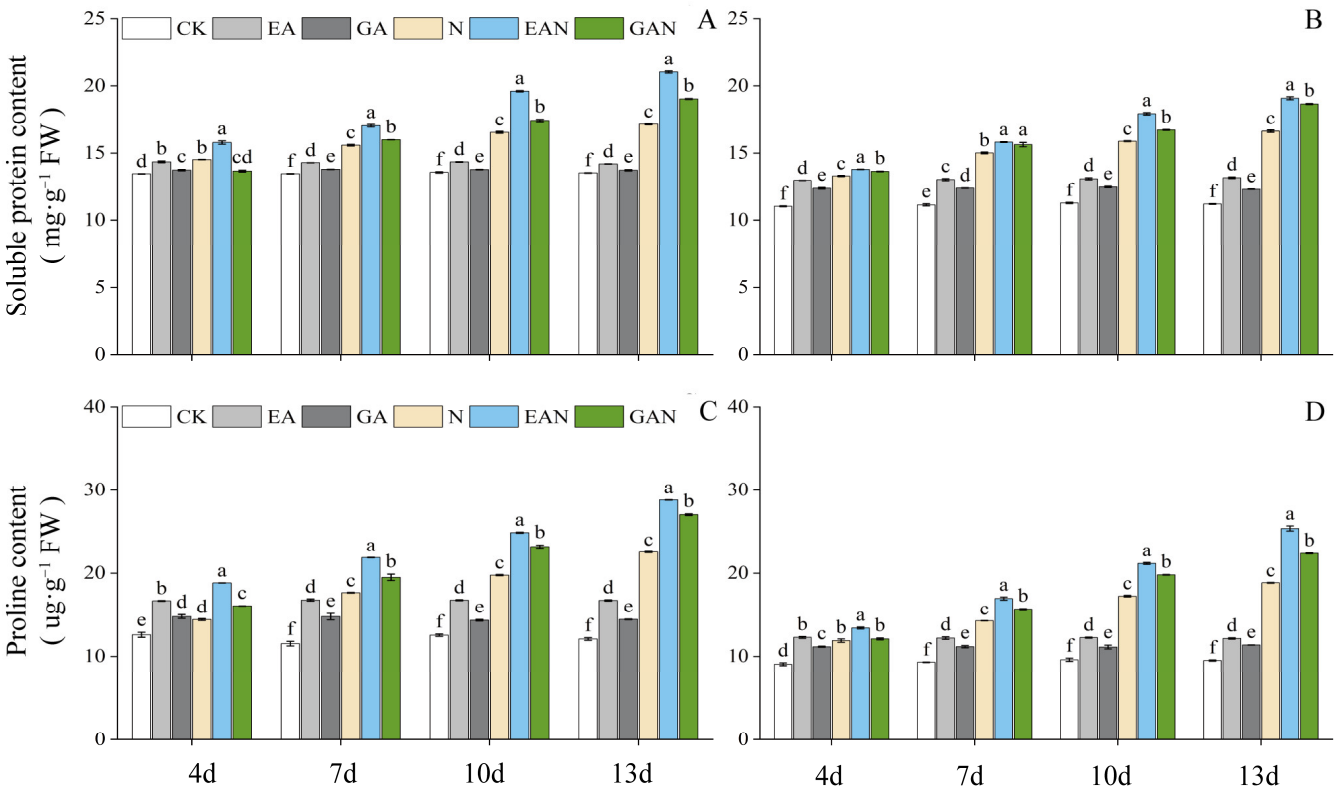

Figure 3. Effect of prohexadione calcium and gibberellin on osmoregulatory substances in rice seedlings under NaCl stress. (**A**,**C**) are Guang Hong 3; (**B**,**D**) are Huang Huazhan. CK: water treatment; EA: prohexadione calcium; GA: gibberellin; N: NaCl treatment; EAN: prohexadione calcium + NaCl treatment; GAN: gibberellin + NaCl treatment. Different letters (a–f) indicate significant differences between different treatments according to ANOVA (Duncan, $p < 0.05$).

3.4. Changes in Ion Content of Rice Seedling Leaves

NaCl stress significantly elevated Na^+ content and significantly decreased K^+ and Ca^{2+} content in rice seedlings (Figure 4). The mitigating effect of prohexadione calcium treatment on ion toxicity under NaCl stress was better than that of the gibberellin treatment. There were significant decreases of 52.22% and 33.13% in Na^+ content and 58.01% and 39.16% in Na^+/K^+ content for EAN and GAN treatments, respectively, while there were significant increases of 13.38% and 9.80% in K^+ content and 25.28% and 15.61% in Ca^{2+} content for

EAN and GAN treatments, respectively, for Guang Hong 3 compared to the N treatment (Figure 4). Compared to the N treatment, there were significant decreases of 41.58% and 31.05% in Na^+ content and 58.43% and 37.09% in Na^+/K^+ content and significant increases of 41.58% and 9.13% in K^+ content and 11.49% and 2.69% in Ca^{2+} content in EAN and GAN treatments for Huang Huazhan (Figure 4).

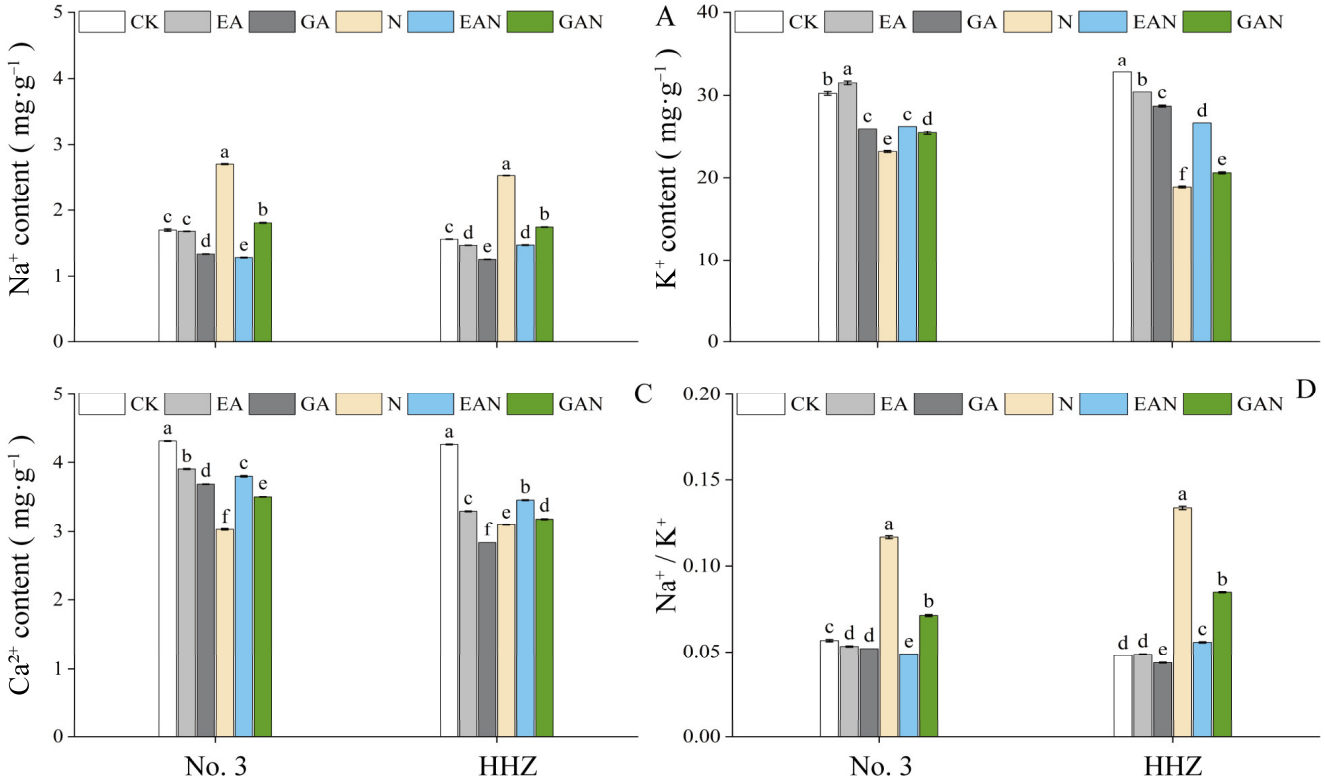

Figure 4. Effect of prohexadione calcium and gibberellin on ion content of rice seedlings under NaCl stress. (**A**,**C**) are Guang Hong 3; (**B**,**D**) are Huang Huazhan. CK: water treatment; EA: prohexadione calcium; GA: gibberellin; N: NaCl treatment; EAN: prohexadione calcium + NaCl treatment; GAN: gibberellin + NaCl treatment. Different letters (a–f) indicate significant differences between different treatments according to ANOVA (Duncan, $p < 0.05$).

3.5. Principal Component Analysis

In this study, the correlations between 12 indicators in Guang Hong 3 and Huang Huazhan were analyzed using principal component analysis (PCA) (Figure 5). The total variation of 12 traits in Guang Hong 3 was 92.7% (Figure 5A) and 89.5% in Huang Huazhan (Figure 5B). The EAN and GAN treatments of Guang Hong 3 and gibberellin were significantly different from the N treatment, respectively. SOD, POD, CAT, $O_2^{\cdot-}$, MDA, SP and pro of Guang Hong 3 and Huang Huazhan were distributed in the positive direction of the PC1 axis, with the same direction and small angle for each index, showing a positive correlation (Figure 5). Na^+ and Na^+/K^+ were distributed in the positive direction of the PC1 axis and the negative direction of the PC2 axis, with the same direction and positive correlation for both indicators, and in the opposite direction and negative correlation for Ca^{2+} and K^+. LA was closer to K^+ with the same direction and positive correlation.

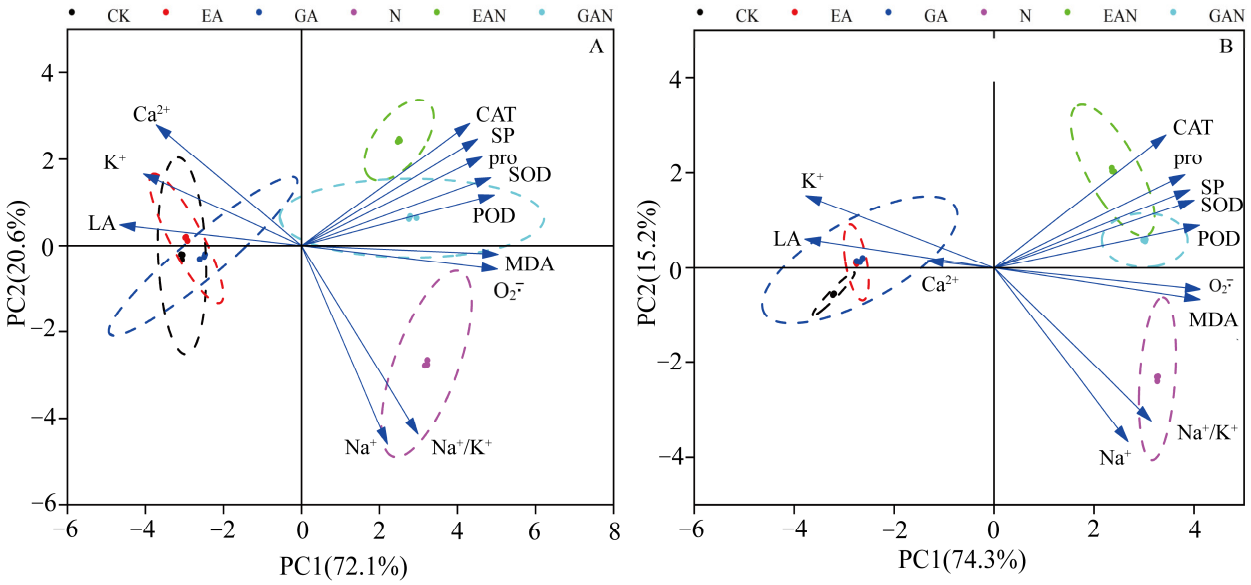

Figure 5. Principal component analysis of rice seedling determination indexes. (**A**) is Guang Hong 3 and (**B**) is Huang Huazhan. CK: water treatment; EA: prohexadione calcium; GA: gibberellin; N: NaCl treatment; EAN: prohexadione calcium + NaCl treatment; GAN: gibberellin + NaCl treatment. LA: leaf area; MDA: malondialdehyde; $O_2^{\cdot-}$: superoxide anion; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; SP: soluble protein; pro: proline.

4. Discussion

Salt stress negatively affects the growth and morphological characteristics of plants including their leaf area [14,27]. In the current study, NaCl stress caused a significant reduction in rice leaf area. Compared to gibberellin treatment, prohexadione calcium treatment was more effective in alleviating the leaf area of rice seedlings under NaCl stress (Figure 1A,B). The microstructure of rice seedling leaves showed that prohexadione calcium and gibberellin treatment alleviated the inhibitory effect of NaCl stress on the increase in leaf flesh cells and vascular bundles (Figure 1), which was beneficial to enhance the growth, water transport and other functions of rice seedlings under NaCl stress.

Salt stress disrupts cell membrane stability, leading to increased intracellular ROS levels in plants and worsened lipid peroxidation [7,28]. There are complex oxidative defense systems in plants, including antioxidant enzymes like SOD, POD and CAT [29], which can reduce the toxic effects of ROS on cells [30]. In this experiment, the rate of $O_2^{\cdot-}$ production and MDA content increased significantly with prolonged NaCl stress, which indicated that leaf cells undergo significant oxidative stress under NaCl stress (Figure 2A–D). This may be due to ionic toxicity and osmotic stress caused by NaCl stress. However, antioxidant enzyme activity trends were consistent with the degree of membrane damage and showed that elevated antioxidant enzyme activity was associated with cell membrane damage caused by NaCl stress (Figure 5). Enhanced antioxidant capacity can improve plant adaptation to oxidative stress [31], and plants with higher levels of antioxidants are more resistant to abiotic stresses [32]. SOD has a significant ability to scavenge superoxide radicals, and CAT catalyzes the conversion of H_2O_2 to H_2O and O_2 [33]. Compared to NaCl treatment, prohexadione calcium and gibberellin treatments significantly reduced ROS and MDA contents (Figure 2A–D), showing that prohexadione calcium and gibberellin treatments could improve the antioxidant damage capacity of rice seedlings. The different antioxidant enzymes (SOD, POD and CAT) had high synergistic activity for ROS clearance under NaCl stress, in which CAT activity started to decrease on the 10th day of NaCl stress, probably due to the rate of ROS production exceeding the CAT clearance capacity (Figure 2I,J). Overall, prohexadione calcium is more effective than gibberellin at regulating the antioxidant system. This was similar to previous results where

the antioxidant system was improved through calcium supplementation, which enhanced the detoxification of ROS [27].

Osmoregulatory substances stabilize plant cell structure and maintain the redox state under stressful conditions [34]. A significant mechanism of plant tolerance to salt stress is osmoregulation [34], and the accumulation of proline and soluble proteins under salt stress is an effective way to reduce osmotic stress [35]. Proline accumulation is higher in salt-tolerant varieties than in sensitive ones [36]. In this study, Guang Hong 3 accumulated more proline and soluble protein than the Huang Huazhan variety. It was previously claimed that high concentrations of proline enhance plant tolerance to NaCl stress by increasing the osmoregulatory capacity of plants and reducing cell damage by ROS [37,38]. Proline accumulation is a criterion for determining plant tolerance to salt stress [39]. In our experiments, we found that prohexadione calcium and gibberellin treatments significantly increased proline and soluble protein contents under NaCl stress (Figure 3), which were positively correlated with antioxidant enzyme activities (SOD, POD and CAT) and membrane damage (MDA and $O_2^{\cdot-}$) (Figure 5). This indicates that ROS accumulation leads to increased membrane damage and promotes proline accumulation to maintain the osmotic balance and cell membrane integrity of rice seedlings under NaCl stress. Prohexadione calcium treatment increased the content of osmoregulatory substances under NaCl stress to a higher extent than gibberellin treatment. It shows that prohexadione calcium is more effective than gibberellin treatment at increasing the content of osmoregulatory substances to improve osmoregulation, maintain cell swelling and enhance salt tolerance in rice seedlings under NaCl stress.

Over-uptake of Na^+ causes ion toxicity and severely inhibits plant growth [40], and Na^+/K^+ is an important indicator of salt tolerance in plants [7]. Under salt stress, over-accumulation of Na^+ leads to K^+ outflow, Ca on the cell membrane is replaced by Na and the Na^+/K^+ ratio decreases [41,42]. In the present study, Na^+ content was significantly increased, while K^+ and Ca^{2+} contents were significantly decreased under NaCl stress (Figure 4). The decrease in Ca^{2+} content may be caused by Na^+ displacement, and the K^+ outflow may be caused by relatively high ROS accumulation during NaCl stress. The level of Na^+ content in leaves correlates with salt stress tolerance [33]. The maintenance of a lower Na^+/K^+ ratio is a key indicator for assessing salt tolerance in plants, which prevents the destruction of various enzymes (e.g., antioxidant enzymes) [43]. Previous studies claimed that a relatively large leaf area helped to reduce the accumulation of Na^+ toxicity [44]. We found in our study that prohexadione calcium treatment was more effective than gibberellin treatment at alleviating increased Na^+ levels and significantly reducing Na^+/K^+ content under NaCl stress (Figure 4A,D), which resulted in increased K^+ uptake caused by prohexadione calcium and gibberellin, maintaining the integrity of the cell membrane under NaCl stress, but prohexadione calcium was more effective at regulating. The simultaneous prohexadione calcium and gibberellin treatments alleviated the limiting effect of NaCl stress on leaf area growth (Figure 1A,B), which may have contributed to the reduction in over-accumulation of Na^+ in the leaves. This is similar to previous studies that reduced the accumulation of Na^+ and alleviated ion toxicity in rice under salt stress using salicylic acid [32]. In summary, prohexadione calcium and gibberellin affected rice varieties with different salt tolerance to different extents. Prohexadione calcium had a significant effect on enhancing the growth of Huang Huazhan (salt-sensitive type) under salt stress. Prohexadione calcium and gibberellin had positive effects on the subsequent growth of rice by enhancing salt tolerance of other rice seedlings before transplanting. Therefore, improving the salt tolerance of rice is crucial for the growth and development of rice in coastal areas.

5. Conclusions

A significant effect of NaCl treatment on the morphogenesis, degree of membrane damage, antioxidant response, osmoregulation and ion homeostasis of rice seedlings was observed in this study. Prohexadione calcium and gibberellin treatments reduced the

growth-limiting effect of NaCl stress on rice seedlings, but the effects of application and the degree of alleviation of NaCl stress differed. The effect of prohexadione calcium treatment on leaf area changes and regulation of physiological metabolic processes under NaCl stress was superior compared to the gibberellin treatment. Prohexadione calcium treatment significantly increased the activity of antioxidant enzymes (SOD, POD and CAT) in rice seedlings under NaCl stress, reduced cell membrane damage, maintained osmoregulatory balance, promoted the uptake and translocation of K^+ and Ca^{2+}, reduced Na^+ uptake and protected cell membrane integrity (Figure 6). Regulation of overall physiological metabolic processes with prohexadione calcium was superior to that in the gibberellin treatment. The results provide a beneficial basis for the effects of different plant growth regulators on the growth of rice seedlings under NaCl stress. Furthermore, prohexadione calcium was more effective at improving the salt tolerance of rice seedlings. Our experiments will focus on further research and the completion of a comparative study of the different regulatory pathways of prohexadione calcium and gibberellin at the molecular level in rice seedlings under NaCl stress.

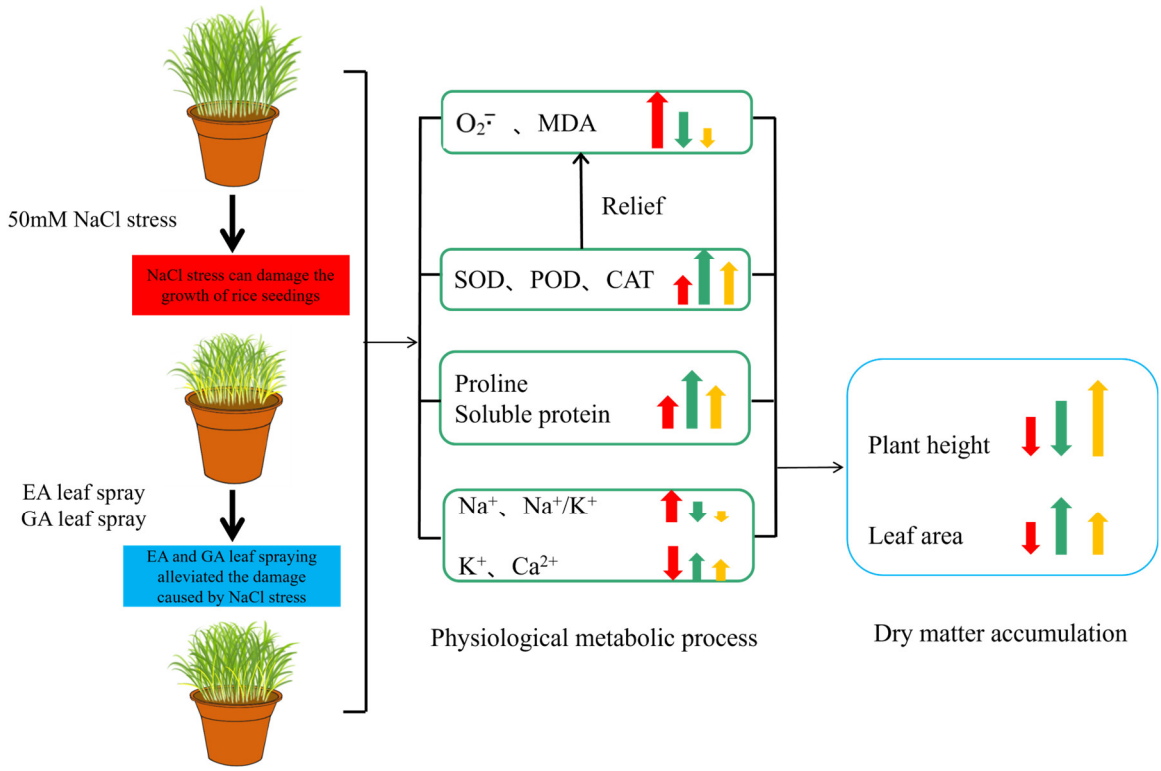

Figure 6. Effect of prohexadione calcium and gibberellin leaf spray on morphological establishment and physiological metabolic processes of rice seedlings under NaCl stress. The red arrows represent the effect of NaCl stress; the green arrows represent the effect of 'prohexadione calcium + NaCl' treatment; the yellow arrows represent the effect of 'gibberellin + NaCl' treatment. The size of each color arrow represents the effect of different treatments on the promotion or inhibition of different indexes of rice seedlings. EA: prohexadione calcium; GA: gibberellin; MDA: malondialdehyde; O_2^-: superoxide anion; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase.

Author Contributions: Conceptualization, N.F.; methodology and analysis, D.Z.; software, M.L.; validation, N.F. and D.Z.; formal analysis, M.L.; investigation, R.Z.; resources, R.Z.; data curation, M.L.; writing—original draft preparation, M.L.; writing—review and editing, N.F.; visualization, R.Z.; supervision, N.F. and D.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Special Project on Key Areas of General Universities of Guangdong Provincial Education Department (2021ZDZX4027), Guangdong General University Innovation Team Project (2021KCXTD011), Guangdong Ocean University Scientific Research Initiation Project (R20046; 060302052012) and Zhanjiang Municipal Bureau of Science and Technology (2022A01016).

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Hussain, S.; Zhang, J.; Zhong, C.; Zhu, L.; Cao, X.; Yu, S.; James, A.B.; Hu, J.; Jin, Q. Effects of salt stress on rice growth, development characteristics, and the regulating ways: A review. *J. Integr. Agric.* **2017**, *16*, 2357–2374. [CrossRef]
2. Sarkar, A.; Ghosh, P.; Pramanik, K.; Mitra, S.; Soren, T.; Pandey, S.; Mondal, M.; Maiti, T. A halotolerant *Enterobacter* sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. *Res. Microbiol.* **2018**, *169*, 20–32. [CrossRef] [PubMed]
3. Minh, L.T.; Khang, D.T.; Ha, P.T.T.; Tuyen, P.T.; Minh, T.N.; Quan, N.V.; Xuan, T.D. Effects of salinity stress on growth and phenolics of rice (*Oryza sativa* L.). *Int. Lett. Nat. Sci.* **2016**, *57*, 1–10. [CrossRef]
4. Liang, W.; Ma, X.; Wan, P.; Liu, L. Plant salt-tolerance mechanism: A review. *Biochem. Biophys. Res. Commun.* **2018**, *495*, 286–291. [CrossRef] [PubMed]
5. Yan, G.; Fan, X.; Peng, M.; Yin, C.; Xiao, Z.; Liang, Y. Silicon improves rice salinity resistance by alleviating ionic toxicity and osmotic constraint in an organ-specific pattern. *Front. Plant Sci.* **2020**, *11*, 260. [CrossRef] [PubMed]
6. Wei, L.; Zhao, H.; Wang, B.; Wu, X.; Lan, R.; Huang, X.; Chen, B.; Chen, G.; Jiang, C.; Wang, J.; et al. Exogenous melatonin improves the growth of rice seedlings by regulating redox balance and ion homeostasis under salt stress. *J. Plant Growth Regul.* **2022**, *41*, 2108–2121. [CrossRef]
7. Chunthaburee, S.; Dongsansuk, A.; Sanitchon, J.; Pattanagul, W.; Theerakulpisut, P. Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage. *Saudi J. Biol. Sci.* **2016**, *23*, 467–477. [CrossRef] [PubMed]
8. Farooq, M.; Asif, S.; Jang, Y.H.; Park, J.R.; Zhao, D.D.; Kim, E.G.; Kim, K.M. Effect of Different Salts on Nutrients Uptake, Gene Expression, Antioxidant, and Growth Pattern of Selected Rice Genotypes. *Front. Plant Sci.* **2022**, *13*, 895282. [CrossRef] [PubMed]
9. Kaur, N.; Dhawan, M.; Sharma, I.; Pati, P.K. Interdependency of reactive oxygen species generating and scavenging system in salt sensitive and salt tolerant cultivars of rice. *BMC Plant Biol.* **2016**, *16*, 131. [CrossRef]
10. Li, X.; Yu, B.; Cui, Y.; Yin, Y. Melatonin application confers enhanced salt tolerance by regulating Na+ and Cl− accumulation in rice. *Plant Growth Regul.* **2017**, *83*, 441–454. [CrossRef]
11. Agami, R.A. Applications of ascorbic acid or proline increase resistance to salt stress in barley seedlings. *Biol. Plant.* **2014**, *58*, 341–347. [CrossRef]
12. Mostofa, M.G.; Saegusa, D.; Fujita, M.; Tran, L.S. Hydrogen sulfide regulates salt tolerance in rice by maintaining Na+/K+ balance, mineral homeostasis and oxidative metabolism under excessive salt stress. *Front. Plant Sci.* **2015**, *6*, 1055. [CrossRef]
13. Krishnamurthy, S.L.; Pundir, P.; Warraich, A.S.; Rathor, S.; Lokeshkumar, B.M.; Singh, N.K.; Sharma, P.C. Introgressed saltol QTL lines improves the salinity tolerance in rice at seedling stage. *Front. Plant Sci.* **2020**, *11*, 833. [CrossRef]
14. Hussain, S.; Bai, Z.; Huang, J.; Cao, X.; Zhu, L.; Zhu, C.; Khaskheli, M.A.; Zhong, C.; Jin, Q.; Zhang, J. 1-Methylcyclopropene modulates physiological, biochemical, and antioxidant responses of rice to different salt stress levels. *Front. Plant Sci.* **2019**, *10*, 124. [CrossRef]
15. Rahman, A.; Nahar, K.; Hasanuzzaman, M.; Fujita, M. Calcium supplementation improves Na+/K+ ratio, antioxidant defense and glyoxalase systems in salt-stressed rice seedlings. *Front. Plant Sci.* **2016**, *7*, 609. [CrossRef] [PubMed]
16. Kashirskaya, N.; Kuzin, A.; Kochkina, A. Effect of prohexadione calcium on vegetative growth, yield and scab development on 'Sinap Orlovskii' apple trees. *E3S Web Conf. EDP Sci.* **2021**, *254*, 02011. [CrossRef]
17. Ozbay, N.; Susluoglu, Z. Assessment of growth regulator prohexadione calcium as priming agent for germination enhancement of pepper at low temperature. *JAPS J. Anim. Plant Sci.* **2016**, *26*, 1652–1658.
18. Na, C.I.; Hamayun, M.; Khan, A.L.; Kim, Y.H.; Choi, K.I.; Kang, S.M.; Kim, S.I.; Kim, J.T.; Won, J.G.; Lee, I.J. Influence of prohexadione-calcium, trinexapac-ethyl and hexaconazole on lodging characteristic and gibberellin biosynthesis of rice (*Oryza sativa* L.). *Afr. J. Biotechnol.* **2011**, *10*, 13097–13106.
19. Junior, J.F.C.C.; Souza, C.A.; Pinheiro, M.G.; Silva, E.R.; da Silva, A.L.P. Morpho-physiological responses of maize hybrids as a function prohexadione calcium doses applied in the vegetative phase. *Bulg. J. Agric. Sci.* **2021**, *27*, 167–178.
20. Žiauka, J.; Kuusienė, S. Different inhibitors of the gibberellin biosynthesis pathway elicit varied responses during in vitro culture of aspen (*Populus tremula* L.). *Plant Cell Tissue Organ Cult.* **2010**, *102*, 221–228. [CrossRef]
21. Zhu, G.; An, L.; Jiao, X.; Chen, X.; Zhou, G.; McLaughlin, N. Effects of gibberellic acid on water uptake and germination of sweet sorghum seeds under salinity stress. *Chil. J. Agric. Res.* **2019**, *79*, 415–424. [CrossRef]
22. Forghani, A.H.; Almodares, A.; Ehsanpour, A.A. The role of gibberellic acid and paclobutrazol on oxidative stress responses induced by in vitro salt stress in sweet sorghum. *Russ. J. Plant Physiol.* **2020**, *67*, 555–563. [CrossRef]

23. Ren, D.; Rao, Y.; Wu, L.; Xu, Q.; Li, Z.; Yu, H.; Zhang, Y.; Leng, Y.; Hu, J.; Zhu, L.; et al. The pleiotropic ABNORMAL FLOWER AND DWARF1 affects plant height, floral development and grain yield in rice. *J. Integr. Plant Biol.* **2016**, *58*, 529–539. [CrossRef]
24. Wang, S.; Zhou, H.; Feng, N.; Xiang, H.; Liu, Y.; Wang, F.; Li, W.; Feng, S.; Liu, M.; Zheng, D. Physiological response of soybean leaves to uniconazole under waterlogging stress at R1 stage. *J. Plant Physiol.* **2022**, *268*, 153579. [CrossRef] [PubMed]
25. Abdel Latef, A.A.H.; Chaoxing, H. Does inoculation with Glomus mosseae improve salt tolerance in pepper plants? *J. Plant Growth Regul.* **2014**, *33*, 644–653. [CrossRef]
26. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil.* **1973**, *39*, 205–207. [CrossRef]
27. Rahman, A.; Hossain, M.S.; Mahmud, J.A.; Nahar, K.; Hasanuzzaman, M.; Fujita, M. Manganese-induced salt stress tolerance in rice seedlings: Regulation of ion homeostasis, antioxidant defense and glyoxalase systems. *Physiol. Mol. Biol. Plants* **2016**, *22*, 291–306. [CrossRef] [PubMed]
28. Hoang, T.; Tran, T.; Nguyen, T.; Williams, B.; Wurm, P.; Bellairs, S.; Mundree, S. Improvement of salinity stress tolerance in rice: Challenges and opportunities. *Agronomy* **2016**, *6*, 54. [CrossRef]
29. Paul, S.; Roychoudhury, A.; Banerjee, A.; Chaudhuri, N.; Ghosh, P. Seed pre-treatment with spermidine alleviates oxidative damages to different extent in the salt (NaCl)-stressed seedlings of three indica rice cultivars with contrasting level of salt tolerance. *Plant Gene* **2017**, *11*, 112–123. [CrossRef]
30. AlTaey, D.K.A. Alleviation of Salinity Effects by Poultry Manure and Gibberellin Application on growth and Peroxidase activity in pepper. *Int. J. Environ. Agric. Biotechnol.* **2017**, *2*, 238861. [CrossRef]
31. Mostofa, M.G.; Fujita, M.; Tran, L.S.P. Nitric oxide mediates hydrogen peroxide-and salicylic acid-induced salt tolerance in rice (*Oryza sativa* L.) seedlings. *Plant Growth Regul.* **2015**, *77*, 265–277. [CrossRef]
32. Jini, D.; Joseph, B. Physiological mechanism of salicylic acid for alleviation of salt stress in rice. *Rice Sci.* **2017**, *24*, 97–108. [CrossRef]
33. Mekawy, A.M.M.; Abdelaziz, M.N.; Ueda, A. Apigenin pretreatment enhances growth and salinity tolerance of rice seedlings. *Plant Physiol. Biochem.* **2018**, *130*, 94–104. [CrossRef] [PubMed]
34. Farhangi-Abriz, S.; Torabian, S. Antioxidant enzyme and osmotic adjustment changes in bean seedlings as affected by biochar under salt stress. *Ecotoxicol. Environ. Saf.* **2017**, *137*, 64–70. [CrossRef] [PubMed]
35. Ahmad, P.; Abdel Latef, A.A.; Hashem, A.; Abd_Allah, E.F.; Gucel, S.; Tran, L.S.P. Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. *Front. Plant Sci.* **2016**, *7*, 347. [CrossRef] [PubMed]
36. Mansour, M.M.F.; Ali, E.F. Evaluation of proline functions in saline conditions. *Phytochemistry* **2017**, *140*, 52–68. [CrossRef] [PubMed]
37. Esan, A.M.; Olaiya, C.O. Effect of salicylic acid (SA) seeds soaking on the NaCl salt stress induced changes in soluble sugar and protein accumulation in organs of two genotypes of okra plants. *Afr. J. Plant Sci.* **2016**, *10*, 105–110. [CrossRef]
38. Hasanuzzaman, M.; Alam, M.M.; Rahman, A.; Hasanuzzaman, M.; Nahar, K.; Fujita, M. Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. *BioMed. Res. Int.* **2014**, *2014*, 757219. [CrossRef] [PubMed]
39. Hmidi, D.; Abdelly, C.; Athar, H.U.; Ashraf, M.; Messedi, D. Effect of salinity on osmotic adjustment, proline accumulation and possible role of ornithine-δ-aminotransferase in proline biosynthesis in Cakile maritima. *Physiol. Mol. Biol. Plants* **2018**, *24*, 1017–1033. [CrossRef]
40. Formentin, E.; Sudiro, C.; Perin, G.; Riccadonna, S.; Barizza, E.; Baldoni, E.; Lavezzo, E.; Stevanato, P.; Sacchi, G.A.; Fontana, P.; et al. Transcriptome and cell physiological analyses in different rice cultivars provide new insights into adaptive and salinity stress responses. *Front. Plant Sci.* **2018**, *9*, 204. [CrossRef]
41. Tahjib-Ul-Arif, M.; Sayed, M.A.; Islam, M.M.; Siddiqui, M.N.; Begum, S.N.; Hossain, M.A. Screening of rice landraces (*Oryza sativa* L.) for seedling stage salinity tolerance using morpho-physiological and molecular markers. *Acta Physiol. Plant.* **2018**, *40*, 70. [CrossRef]
42. Kibria, M.G.; Hossain, M.; Murata, Y.; Hoque, M.A. Antioxidant defense mechanisms of salinity tolerance in rice genotypes. *Rice Sci.* **2017**, *24*, 155–162. [CrossRef]
43. Wang, J.; Yuan, J.; Ren, Q.; Zhang, B.; Zhang, J.; Huang, R.; Wang, G.G. Arbuscular mycorrhizal fungi enhanced salt tolerance of Gleditsia sinensis by modulating antioxidant activity, ion balance and P/N ratio. *Plant Growth Regul.* **2022**, *97*, 33–49. [CrossRef]
44. Kakar, N.; Jumaa, S.H.; Redona, E.D.; Warburton, M.L.; Reddy, K.R. Evaluating rice for salinity using pot-culture provides a systematic tolerance assessment at the seedling stage. *Rice* **2019**, *12*, 57. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.