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Strigolactone Alleviates NaCl Stress by Regulating Antioxidant Capacity and Hormone Levels in Rice (*Oryza sativa* L.) Seedlings

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Abstract: Salt stress is a key environmental factor altering rice plant growth. Strigolactones (GR24) play a vital role in responding to various abiotic stresses and regulating plant growth. However, the regulatory mechanisms of SLs on rice seedlings under salt stress have not yet been clarified. A pot experiment was undertaken to evaluate the effects of GR24 soaking on the rice variety 'Huanghuazhan' (salt-sensitive) seedling growth, antioxidant metabolism, and endogenous hormones under NaCl stress. Results showed that NaCl stress significantly inhibited rice growth; disrupted antioxidant enzymes activity; and increased the content of soluble proteins (SPs), proline (Pro), malondialdehyde (MDA) and hydrogen–peroxide (H_2O_2). GR24 significantly improved photosynthetic pigments and antioxidant–enzyme activities, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate–peroxidase (APX); increased SP, ascorbic acid (AsA); and reduced glutathione (GSH) content and MDA, H_2O_2, and Pro content, resulting in the mitigation of oxidative injury caused by NaCl stress. Moreover, GR24 significantly increased the content of strigolactones (SLs), cytokinin (CTK), auxin (IAA), Gibberellin A3 (GA3), and IAA/ABA and CTK/ABA ratios and decreased the abscisic acid (ABA). Findings indicated that GR24 alleviated oxidative damage caused by NaCl stress by increasing photosynthetic and antioxidant capacity and maintaining the balance of endogenous hormones, thus improving the salt tolerance of rice seedlings.

Keywords: rice seedlings; strigolactone; NaCl stress; morphogenesis; antioxidant metabolism; hormone level

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1. Introduction

Soil salinization is of considerable consequence at the global level, exerting a profound impact on crop growth and productivity [1]. Approximately 20% of the global arable land and 33% of irrigated farmlands are subjected to soil salinity [2]. Salt stress constrains plant growth and crop production [3]. Excessive salt accumulation in the soil disrupts the ionic balance within plants, leading to ionic toxicity, high osmotic pressure, oxidative stress, etc., thereby retarding plant growth and development and reducing their capacity to absorb water and nutrients from the soil, ultimately resulting in plant mortality [4]. When the NaCl concentration of irrigation water exceeds 32.8 mM, it can reduce rice productivity [5]. Salt stress decreased the number of effective spikes and grains per spike, and resulted in a low rice grain yield [6]. Therefore, to enhance crop productivity in highly saline areas, it is necessary to effectively utilize salinized farmland and choose a suitable method for alleviating salt stress and enhance plant salt tolerance mechanisms.

Rice (*Oryza sativa* L.) is a vital cereal crop, and over half of the world's population relies on rice as a staple food [7]. However, rice is highly sensitive to salt stress during its seedling stage [8]. Enhancing rice seedling establishment in saline soils remains a significant challenge with severe consequences for sustainable agriculture [9]. Therefore, it is imperative to explore mitigating strategies to alleviate adverse influence of salt stress and enhance salt tolerance during the seedling stage.

To mitigate the toxic effects induced by salt stress, plants have evolved a variety of defense mechanisms [10], such as salt stress mitigated by maintaining a low Na^+/K^+ ratio [11] and increasing the content of osmoregulatory substances [12]. Plants usually scavenge ROS with the help of antioxidant enzymes and non-enzymatic systems to maintain redox homeostasis against oxidative damage caused by salt stress [13]. In addition, phytohormones play an important role in regulating plant growth and tolerance to abiotic stresses [14]. Mu et al. reported that exogenous brassinolide facilitated the protection of membrane lipid peroxidation and the regulation of the antioxidant defense system and ionic and endogenous hormone homeostasis and attenuates the damage of salt stress on rice seedlings [15]. The application of exogenous gibberellins enhanced the tolerance of wheat to ZnO by reducing the accumulation of reactive oxygen species and enhancing antioxidant enzyme activities [16]. Yi Feng et al. found that cytokinin increased salt tolerance in apple seedlings [17].

Strigolactone (SL), a novel phytohormone derived from carotenoids, plays an important role in the regulation of biochemical processes such as seed germination and morph-physiological characteristics [18]. Recent studies had highlighted the crucial role of SL in abiotic stress tolerance, particularly in plant responses to heavy metal stress, drought stress, cold stress, and salt stress [18–21]. Liu et al. demonstrated that GR24 enhanced the salt tolerance of tomato seedlings under salt stress by increasing photosynthetic pigment content, enhancing antioxidant capacity, and improving endogenous SL synthesis [22]. Lu et al. showed that GR24 could enhance the levels of soluble sugars and proteins, elevate the activity of antioxidant enzymes, and reduce the concentration of MDA in alfalfa leaves, thereby mitigating the adverse effects of salt stress in alfalfa [23]. The exogenous application of GR24 improved the photosynthetic pigment content under salt stress, thus validating the potential use of GR24 for mitigating salt stress in pepper [24]. Similarly exogenous GR24 reduced MDA and ROS and maintained the normal growth of rice seedlings under salt stress [25]. Oilseed rape plants treated with GR24 significantly increased in chlorophyll content and antioxidant enzyme activities under salt stress [26]. These findings suggested that SL would respond to salt stress by regulating morph-physiological traits.

To date, there has been no published research investigating the potential of GR24 seed-soaking to mitigate the adverse effects of salt stress on the growth of rice seedlings. The objective of this study was to examine the impact of GR24 seed-soaking on rice seedling morphology, photosynthetic pigments, the antioxidant defense system, and hormones levels under salt stress conditions. Furthermore, to investigate the mitigating effects and physiological mechanisms of exogenous GR24 on the growth and development of rice under salt stress, this study provides a theoretical basis for the application of GR24 in salinity-tolerant rice cultivation.

2. Materials and Methods

2.1. Test Materials

The test rice variety was Huanghuazhan, selected by the Rice Research Institute of Guangdong Academy of Agricultural Sciences. The test regulator was GR24 (a synthetic analog of SL), procured from Beijing Sorabo Technology Co. (Beijing, China) [27]. The cultivation substrate was composed of brick-red soil that had been air-dried and sieved and mixed with sand at a 3:1 ratio. The physical and chemical properties of the soil were as follows: pH—6.89; organic matter—4.11 g kg^{-1}; nitrogen—1.37 mg kg^{-1}; fast-acting phosphorus—2.66 mg kg^{-1}; fast-acting potassium—71.49 mg kg^{-1}. It was then packed into a nutrient bowl with an upper diameter of 210 mm, a diameter of 160 mm at the lower bottom, and a height of 180 mm, with a weight of 2.5 kg per pot.

2.2. Experimental Design

The experiment was conducted in 2023–2024 in a solar greenhouse at Guangdong Ocean University. The indoor humidity was 84% ± 5% and the average indoor temperature was 32 ± 2 °C during the daytime and 28 ± 2 °C during the nighttime. The experimental site was a solar greenhouse, so the light intensity varied with daylight. The optimal concentration of GR24 (1.2 μmol L^{-1}) screened in the pre-preparation experiment was used. The fully matured seeds were selected and sterilized with 3% hydrogen peroxide for 15 min and then rinsed repeatedly with water five times [28] for 15 min and then rinsed repeatedly with water a total of five times. The sterilized seeds were randomly divided into two portions and soaked in water and 1.2 μmol L^{-1} GR24 solution for 24 h in a dark incubator (Biochemical Incubator, Model: LRH-150, Manufacturer: Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai, China) at 30 °C, with the ratio of the total amount of seeds to the volume of the solution (w/v) 1:2. The germination of the soaked seeds was carried out in a dark incubator at 30 °C for 24 h. The soaked seeds were then incubated in a dark incubator at 30 °C. The seeds were germinated in a dark incubator at 30 °C for 24 h. Following the 24 h germination period, seeds exhibiting a consistent dewy white coloration were selected and distributed among nutrient pots, with 69 plants being evenly sown in each pot and spaced 0.5 cm apart. The seeds were covered with approximately 2 cm of soil.

One day before sowing, 1L of fertilizer water (urea: 0.146 g L^{-1}, KCl: 0.125 g L^{-1}, diamine phosphate: 0.2 g L^{-1}) or fertilizer + NaCl water (NaCl: 80 mmol L^{-1}, pH: 6.93, EC: 6.78 ms/cm) was poured into each pot. Subsequently, the 80 mmol L^{-1} NaCl solution and fresh water were irrigated at regular two-day intervals in a consistent and measured manner. The amount of watering was three times more than the amount of water held by the soil, and about 2/3 of the solution was run off, thus flushing out the accumulated NaCl to keep the NaCl concentration constant, and the control group was watered with an equal amount of fresh water.

The experiment was a completely randomized design and involved four treatments. Each treatment was replicated three times and sampled four times. The four treatments utilized in the experiment were as follows: (1) CK—water-soaked seed + water treatment; (2) G—1.2 μmol L^{-1} GR24-soaked seed + water treatment; (3) N—water-soaked seed + 80 mmol L^{-1} NaCl treatment; and (4) NG—1.2 μmol L^{-1} GR24-soaked seed + 80 mmol L^{-1} NaCl treatment. Samples were obtained at the one-leaf-one-heart stage (1.5), the two-leaf-one-heart stage (2.5), the three-leaf-one-heart stage (3.5), and the four-leaf-one-heart stage (4.5) to determine morphological and physiological indicators. During sampling, undamaged rice leaves from each treatment were cut and quickly frozen in liquid nitrogen. They were then stored in a refrigerator at −40 °C for the determination of various physiological indices.

2.3. Germination Characteristics

The seedling percentage was determined by calculating the ratio of the number of seedlings that emerged on the seventh day to the number of seeds that were sown.

$$\text{Seedling emergence rate} = (\text{The number of seedlings}/\text{The number of seeds sown}) \times 100$$

2.4. Determination of Growth Index of Rice Seedlings

The seedlings were rinsed with water. Dry the surface of seedlings with filter paper. Fifteen seedlings were randomly selected from each treatment. Plant height was measured with a straight edge tool, and stem base width was measured with vernier calipers. The above-ground and below-ground parts were separated and weighed for fresh weight. The rice seedlings were then dried to a constant weight and weighed for dry weight.

2.5. Determination of Root Morphology of Rice Seedlings

The root system of rice seedlings was scanned using a root scanner (Epson Perfection V800 Photo (Epson Indonesia Inc., Suwa, Nagano, Japan)) and the images were analyzed using the WinRHIZO root analysis system software (Regent Instruments, Québec City, QC, Canada) to analyze the images and measure the total root length, total root surface area, total root volume, and average root diameter.

2.6. Measurement of Photosynthetic Pigment Content

The contents of photosynthetic pigments were obtained with reference to the method proposed by Lichtenthaler [29]. We immersed 0.1 g of fresh rice leaf tissue in 10 mL of 95% ethanol and left it at 4 °C in dark conditions for 24 h until the leaves became colorless. Then, the absorbance at 665, 649, and 470 nm was measured using a spectrophotometer (UV-3600 Plus, Shimadzu, Kyoto, Japan). The photosynthetic pigment concentrations (mg L^{-1}) were calculated according to the following equations:

$$\text{Chlorophyll a (Chl a)} = 13.95\ \text{D}\ 665 - 6.88\ \text{D}\ 649$$

$$\text{Chlorophyll b (Chl b)} = 24.96\ \text{D}\ 649 - 7.32\ \text{D}\ 665$$

$$\text{Total chlorophyll content} = \text{Chl a} + \text{Chl b}$$

$$\text{Carotenoid (Car)} = (1000\ \text{D}\ 470 - 2.05\ \text{Chl a} - 111.48\ \text{Chl b})/245$$

2.7. Determination of Antioxidant Enzyme Activities

Superoxide dismutase (SOD) was determined by the nitrogen blue tetrazolium (NBT) method [30] and was detected using a full wavelength enzyme labeling instrument (Epoch, Bio Tek Instruments (Beijing) Co. (Bio Tek), Beijing, China). Peroxidase (POD) was determined by guaiacol method [31] and detected by OD at 470 nm using UV spectrophotometer (Thermo Scientific, Thermo Fisher (Shanghai) Instruments Co., Shanghai, China), with a change in OD of 0.01 per minute as 1 unit of enzyme activity (U). Catalase (CAT) was determined using the hydrogen peroxide catabolic reaction method [32]. Ascorbate peroxidase (APX) was measured using the amount of AsA oxidized per unit time [31].

2.8. Determination of Antioxidant Content

Ascorbic acid (AsA) content was determined by the method described by Kampfenke et al. [33] with minor modifications. Briefly, 0.5 g of sample was added with 10 mL of 5% TCA solution, ground and homogenized at 4 °C, and centrifuged at 10,000 r/min for 15 min, and the supernatant was used to determine the AsA content. Reduced glutathione (GSH) content was determined according to the method described by Tyburski and Tretyn [34] with slight modifications.

2.9. Determination of Membrane Damage Index

H_2O_2 was determined with reference to the method described by Bates et al. [35] with minor modifications. The determination of malondialdehyde (MDA) was conducted using the thiobarbituric acid method [36].

2.10. Determination of Osmoregulatory Substances

Proline content was determined with reference to the method described by Bates [35] et al. with minor modifications. Determination of soluble protein content was based on the Thomas Brilliant Blue G-250 staining method [35] with minor modifications.

2.11. Measurement of Endogenous Hormone Content

Endogenous hormones were determined at Shanghai Enzyme-Linked Biotechnology Co. (Shanghai, China). A double antibody one-step sandwich enzyme-linked immunosorbent assay (ELISA) kit was used. The linear regression coefficient of the standard was greater than or equal to 0.9900, and the lowest detection concentration was less than 1.0 pmol L^{-1}. It did not cross-react with other soluble structural analogs. The intra- and inter-plate coefficients of variation were less than 15%. The absorbance (OD) was measured at 450 nm with the enzyme marker, and the concentration of the samples was calculated.

2.12. Statistical Analysis

All data were processed using Microsoft Excel 2010, analysis of variance (ANOVA) using SPSS 20.0, and multiple comparisons using Duncan's test for multiple comparisons, and analyses were expressed as means and standard errors, with different lower-case letters indicating significant differences ($p < 0.05$). The graphs in this article were plotted using Origin 2021.

3. Results and Analysis

3.1. Effect of GR24 Seed-Soaking on Seedling Emergence Rate in Rice under NaCl Stress

NaCl stress significantly inhibited seedling emergence rate in rice seedlings. GR24 significantly reduced the inhibitory effect of NaCl stress on rice seedlings emergence rate (Table 1). Compared to CK, the NaCl treatment significantly reduced the seedling emergence by 23.74%, while the GR24 soaking treatment significantly enhanced the seedling emergence by 24.50%, under NaCl stress. The results indicate that the GR24 soaking treatment alleviated the inhibitory effect of NaCl stress.

Table 1. Effect of GR24 on seedling emergence rate under NaCl stress.

Treatment	Seedling Emergence Rate (%)
CK	95.65 ± 0.8367 ab
G	98.07 ± 0.4831 a
N	72.95 ± 0.4836 c
NG	90.82 ± 0.4831 b

Note: Values are expressed as the means ± SE of three replicates, and different letters after the data in the same column indicate significant differences between treatments ($p < 0.05$). CK: water-soaked seeds + water treatment; G: 1.2 μmol L^{-1} GR24-soaked seeds + water treatment; N: water-soaked seeds + 80 mmol L^{-1} NaCl treatment; NG: 1.2 μmol L^{-1} GR24-soaked seeds + 80 mmol L^{-1} NaCl treatment.

3.2. Effect of GR24 Soaking on Rice Seedling Growth under NaCl Stress

NaCl stress significantly inhibited the growth parameters of rice seedlings (Figures 1 and 2). The exogenous GR24 soaking treatment significantly increased the morphological indexes of rice seedlings under NaCl stress (Figure 1 and Table 2). Compared to CK, the NaCl treatment significantly reduced the plant height, root length, and stem base width on average by 16.54–40.07%, 10.08–25.61%, and 21.92–32.47%, respectively, in the 1.5 leaf stage to the 4.5 leaf stage in rice seedlings. Compared with CK, NaCl stress significantly reduced the above-ground fresh weight and the below-ground dry weight indexes, by 29.31% to 55.11% and 31.61% to 61.97%, respectively. The application of the GR24 soaking treatment markedly enhanced plant height, root length, stem basal width, fresh weight and dry weight on average by 7.32–41.64%, 5.5–27.09%, 15.38–32.06%, 18.39–40.21%, and 36.61–61.97%, respectively, in the 1.5 leaf stage to the 4.5 leaf stage in rice seedlings under NaCl stress. These results showed that GR24 seed soaking could effectively alleviate the inhibition of rice seedling growth by NaCl stress and improve the salt tolerance of rice seedlings.

Table 2. Effect of GR24 soaking on the growth of rice seedlings under NaCl stress.

Standard	Treatment	Stage			
		1.5th Leaf	2.5th Leaf	3.5th Leaf	4.5th Leaf
Plant height/cm	CK	14.23 ± 0.2 b	17.99 ± 0.18 b	19.23 ± 0.08 b	26.6 ± 0.14 b
	G	16.68 ± 0.12 a	20.13 ± 0.11 a	22.49 ± 0.21 a	27.84 ± 0.22 a
	N	8.53 ± 0.13 d	13.01 ± 0.16 d	15.7 ± 0.18 d	19.6 ± 0.2 d
	NG	12.08 ± 0.25 c	15.85 ± 0.19 c	16.85 ± 0.15 c	24.54 ± 0.16 c

Table 2. *Cont.*

Standard	Treatment	Stage			
		1.5th Leaf	2.5th Leaf	3.5th Leaf	4.5th Leaf
Stem width/mm	CK	0.91 ± 0.04 ab	1.35 ± 0.02 b	2.43 ± 0.04 b	3.45 ± 0.03 b
	G	0.98 ± 0.03 a	1.45 ± 0.03 a	3.06 ± 0.06 a	4.14 ± 0.07 a
	N	0.71 ± 0.04 c	1.03 ± 0.03 d	1.64 ± 0.03 d	2.6 ± 0.06 d
	NG	0.84 ± 0.02 b	1.26 ± 0.02 c	2.16 ± 0.03 c	3.0 ± 0.05 c
Shoot freshly weight/g	CK	0.0593 ± 0.0012 b	0.106 ± 0.0037 b	0.2108 ± 0.0044 b	0.3315 ± 0.007 b
	G	0.0734 ± 0.0012 a	0.1455 ± 0.0027 a	0.2483 ± 0.0031 a	0.3866 ± 0.0072 a
	N	0.0358 ± 0.0009 d	0.0725 ± 0.0012 d	0.1153 ± 0.003 d	0.1928 ± 0.0086 d
	NG	0.0488 ± 0.0004 c	0.0859 ± 0.0015 c	0.148 ± 0.0044 c	0.2513 ± 0.0055 c
Shoot dry weight/g	CK	0.0094 ± 0.0003 b	0.0235 ± 0.0005 b	0.0356 ± 0.0005 b	0.0709 ± 0.0018 b
	G	0.0102 ± 0.0001 a	0.0281 ± 0.0007 a	0.0444 ± 0.0006 a	0.0897 ± 0.002 a
	N	0.0049 ± 0.0001 d	0.0149 ± 0.0001 d	0.0226 ± 0.0006 d	0.0371 ± 0.0019 d
	NG	0.0072 ± 0.0001 c	0.0168 ± 0.0004 c	0.0267 ± 0.0004 c	0.0527 ± 0.0012 c
Root fresh weight/g	CK	0.0218 ± 0.0004 b	0.0983 ± 0.0032 b	0.1654 ± 0.0032 b	0.2314 ± 0.0098 b
	G	0.0254 ± 0.0005 a	0.1258 ± 0.0024 a	0.1906 ± 0.0051 a	0.2703 ± 0.0159 a
	N	0.0154 ± 0.0006 d	0.0521 ± 0.0026 d	0.0814 ± 0.0029 d	0.1039 ± 0.0031 d
	NG	0.0195 ± 0.0004 c	0.0628 ± 0.0009 c	0.1044 ± 0.0043 c	0.1519 ± 0.0037 c
Root dry weight/g	CK	0.0034 ± 0.0001 b	0.0093 ± 0.0002 b	0.0143 ± 0.0003 b	0.0278 ± 0.0005 b
	G	0.0039 ± 0.0001 a	0.0124 ± 0.0002 a	0.0168 ± 0.0004 a	0.0347 ± 0.0016 a
	N	0.0015 ± 0.0001 d	0.0048 ± 0.0001 d	0.006 ± 0.0001 d	0.0106 ± 0.0005 d
	NG	0.0019 ± 0.0001 c	0.0063 ± 0.0001 c	0.0079 ± 0.0003 c	0.0187 ± 0.0007 c

Note: Values are expressed as the means ± SE of three replicates, and different letters after the data in the same column indicate significant differences between treatments ($p < 0.05$). CK: water-soaked seeds + water treatment; G: 1.2 μmol L^{-1} GR24-soaked seeds + water treatment; N: water-soaked seeds + 80 mmol L^{-1} NaCl treatment; NG: 1.2 μmol L^{-1} GR24-soaked seeds + 80 mmol L^{-1} NaCl treatment.

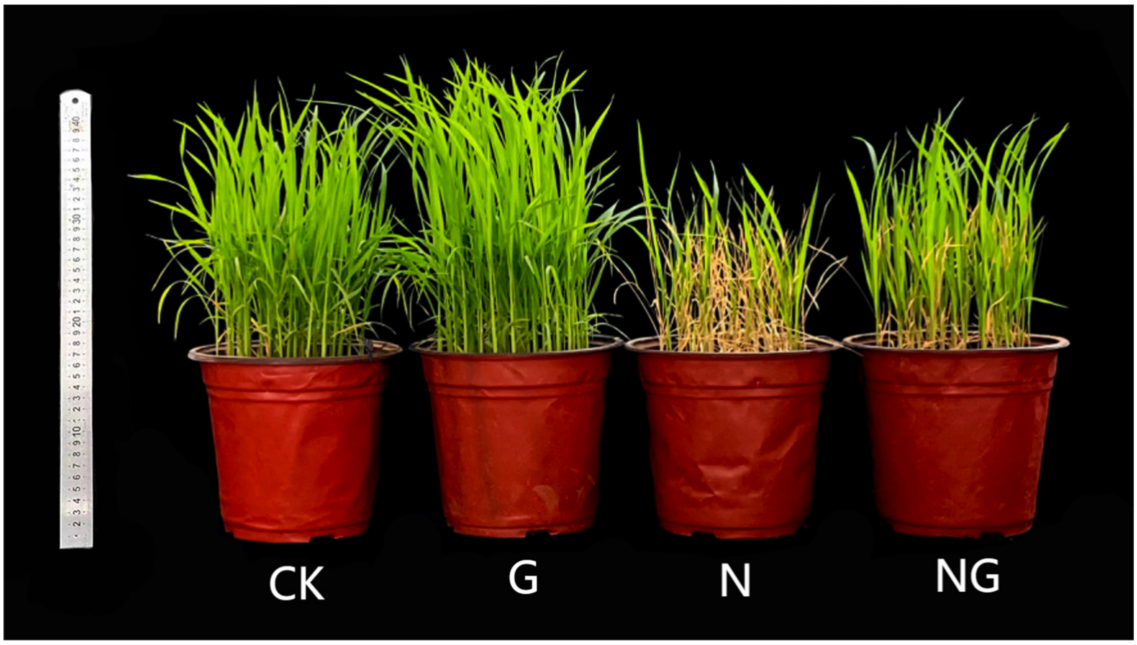

Figure 1. Effect of GR24 on seedlings of Huanghuazhan under NaCl stress (4.5 leaves). CK: water-soaked seeds + water treatment; G: 1.2 μmol L^{-1} GR24-soaked seeds + water treatment; N: water-soaked seeds + 80 mmol L^{-1} NaCl treatment; NG: 1.2 μmol L^{-1} GR24-soaked seeds + 80 mmol L^{-1} NaCl treatment.

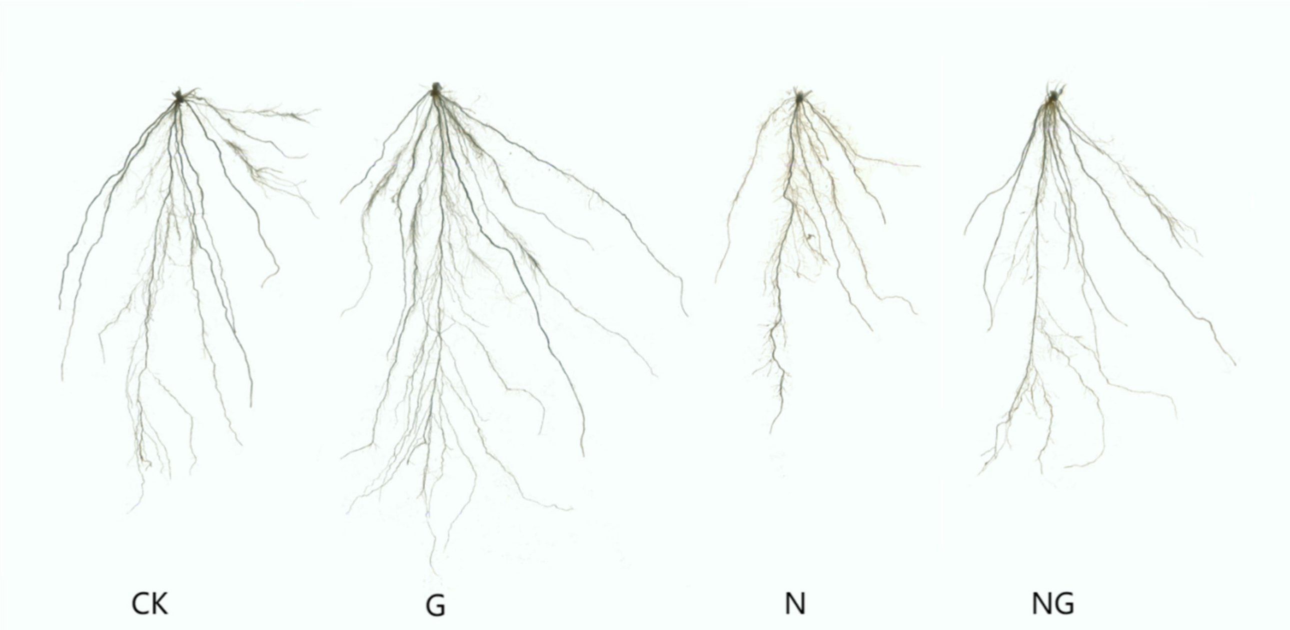

Figure 2. Effect of GR24 on root growth of rice seedlings under NaCl stress (3.5 leaf stage).

3.3. Effect of GR24 Soaking on Root Morphological Traits of Rice Seedlings under NaCl Stress

NaCl stress significantly affected the root morphology of rice seedlings (Table 3, Figure 2). Compared to CK, the NaCl treatment significantly reduced the total root length by 26.3–42.81%, the total root surface area by 37.75–50.97%, the total root volume by 38.76–52.42%, and the mean root diameter by 9.49–22.18%, respectively, in rice seedlings. Soaking with GR24 markedly enhanced the root morphological traits (Table 3, Figure 2). Compared to NaCl stress, the GR24 soaking treatment significantly improved the total root length by 12.28–33.25%, the total root surface area by 26.11–45.76%, the total root volume by 24.53–32.69%, and the mean root diameter by 7.96–18.22%, respectively, under NaCl stress in rice seedlings. These results suggested that the exogenous GR24 soaking treatment had a positive regulatory influence on root morphological traits under NaCl stress.

Table 3. Effect of GR24 soaking on root growth of rice seedlings under NaCl stress.

Standard	Treatment	Stage			
		1.5th Leaf	2.5th Leaf	3.5th Leaf	4.5th Leaf
Total root length/cm	CK	37.81 ± 1.1485 ab	141.01 ± 1.0612 b	227.09 ± 2.3753 b	367.18 ± 10.6145 b
	G	42.46 ± 2.9531 a	169.86 ± 4.5188 a	244.03 ± 6.6281 a	399.18 ± 2.5649 a
	N	25.56 ± 1.4765 c	103.92 ± 1.3854 d	142.97 ± 0.8088 d	209.98 ± 2.7684 d
	NG	33.91 ± 0.2505 b	116.69 ± 2.2825 c	163.5 ± 3.5481 c	279.79 ± 8.1806 c
Total root surface area/cm^2	CK	3.19 ± 0.1397 a	13.35 ± 0.208 b	20.51 ± 0.2558 b	34.53 ± 1.206 a
	G	3.28 ± 0.1754 a	16.03 ± 0.4771 a	21.45 ± 0.4202 a	36.18 ± 0.7503 a
	N	1.65 ± 0.087 c	8.31 ± 0.1429 d	11.18 ± 0.3127 d	16.93 ± 0.8313 c
	NG	2.4 ± 0.0291 b	10.48 ± 0.1152 c	14.26 ± 0.1372 c	23.88 ± 1.2475 b
Total root volume/cm^3	CK	0.03856 ± 0.0011 a	0.15354 ± 0.0038 b	0.36069 ± 0.0136 a	0.45062 ± 0.0141 b
	G	0.03838 ± 0.0013 a	0.19042 ± 0.0083 a	0.366 ± 0.006 a	0.52595 ± 0.0162 a
	N	0.02003 ± 0.0006 c	0.09403 ± 0.0033 d	0.1716 ± 0.0077 c	0.25028 ± 0.0115 d
	NG	0.02501 ± 0.0007 b	0.12476 ± 0.0032 c	0.22168 ± 0.0125 b	0.31168 ± 0.0059 c
Mean root diameter/mm	CK	0.294 ± 0.0024 a	0.294 ± 0.0033 a	0.317 ± 0.0046 a	0.333 ± 0.0023 a
	G	0.287 ± 0.0044 ab	0.306 ± 0.0023 a	0.325 ± 0.0037 a	0.339 ± 0.0021 a
	N	0.229 ± 0.009 c	0.266 ± 0.0049 a	0.272 ± 0.0009 b	0.293 ± 0.0039 c
	NG	0.271 ± 0.0036 b	0.293 ± 0.0089 b	0.28 ± 0.0085 b	0.316 ± 0.0059 b

Note: Values are expressed as the means ± SE of three replicates, and different letters after the data in the same column indicate significant differences between treatments ($p < 0.05$). CK: water-soaked seeds + water treatment; G: 1.2 μmol L^{-1} GR24-soaked seeds + water treatment; N: water-soaked seeds + 80 mmol L^{-1} NaCl treatment; NG: 1.2 μmol L^{-1} GR24-soaked seeds + 80 mmol L^{-1} NaCl treatment.

3.4. Effect of GR24 Soaking on the Photosynthetic Pigment Content of Rice Seedlings under NaCl Stress

The photosynthetic pigment content showed a decreasing and then increasing trend (Figure 3). The NaCl stress significantly reduced chlorophyll synthesis in the leaves of rice seedlings. Compared to CK, the NaCl treatment significantly reduced the chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents on average by 9.6–13.1%, 15.55–19.36%, 12.07–13.18%, and 19.81%, respectively, in the 1.5 leaf stage to the 3.5 leaf stage in rice seedlings. In contrast, compared to NaCl stress, the GR24 soaking treatment significantly increased photosynthetic pigment content in the 1.5 leaf stage to the 4.5 leaf stage in rice seedlings (Figure 3). The application of GR24 soaking treatment markedly enhanced the chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents by 12.32–6.10%, 21.32–13.26%, 14.45–7.24%, and 10.07–14.29%, respectively, for the 1.5 leaf stage to 3.5 leaf stage in rice seedlings under NaCl stress. Compared to NaCl stress, the application of the GR24 soaking treatment also increased the chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents at the 2.5 leaf and 4.5 leaf stages. The above findings suggeste that GR24 increased the content of photosynthetic pigments, indicating that it might be helpful to rice seedlings to adapt against NaCl stress.

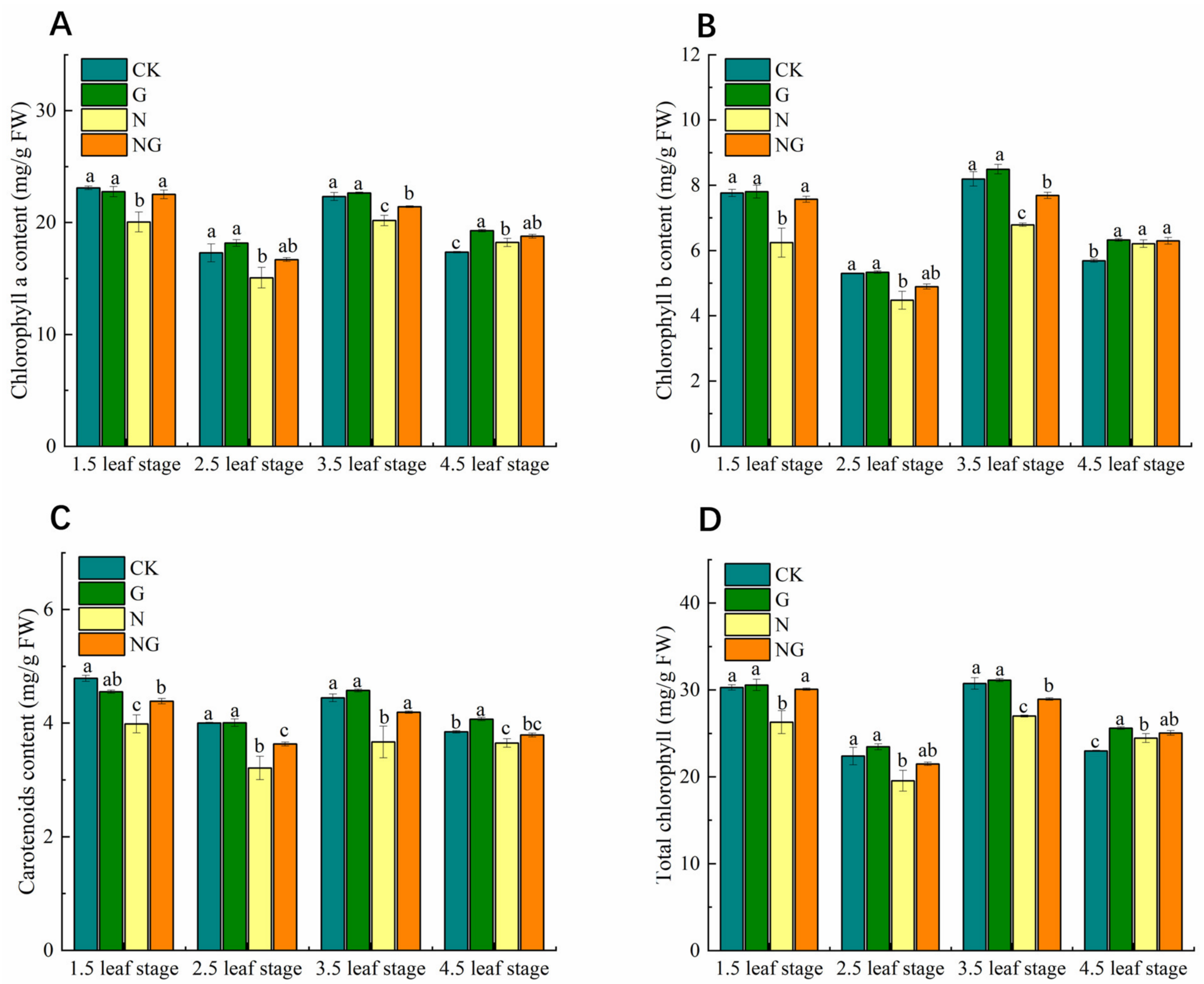

Figure 3. Effect of GR24 on photosynthetic pigment content of Huanghuazhan seedlings under NaCl stress. chlorophyll a content (**A**); chlorophyll b content (**B**); carotenoids content (**C**); total chlorophyll (**D**). CK: water-soaked seeds + water treatment; G: 1.2 μmol L^{-1} GR24-soaked seeds + water treatment; N: water-soaked seeds + 80 mmol L^{-1} NaCl treatment; NG: 1.2 μmol L^{-1} GR24-soaked seeds + 80 mmol L^{-1} NaCl treatment. Values are the means ± SE of three replicate samples. Different letters in the data column indicate significant differences ($p < 0.05$) according to Duncan's test.

3.5. Effect of GR24 Soaking on Antioxidant Enzyme Activities of Rice Seedling Leaves under NaCl Stress

As the NaCl stress increased, antioxidant enzyme activities significantly increased at the 1.5 leaf, 2.5 leaf, 3.5 leaf and 4.5 leaf stages after the NaCl stress treatment (Figure 4). Under NaCl stress, SOD, POD, and APX activities in the leaves of rice seedlings showed a trend of increasing and then decreasing, and CAT activity showed a trend of decreasing and then increasing. Compared to CK, the NaCl treatment significantly increased the SOD, POD, and CAT activities on average by 11.82–63.30% and 16.07–33.69%, respectively, for the 1.5 leaf stage to the 4.5 leaf stage in rice seedlings. Under NaCl stress treatment, the APX of the rice seedlings reached its maximum value at the 3.5 leaf stage, with a significant increase of 40.04%. The exogenous GR24 soaking treatment further increased antioxidant enzyme activity in the leaves of rice seedlings under NaCl stress (Figure 4). Compared to the NaCl stress treatment, under NaCl stress, the application of the GR24 soaking treatment enhanced SOD activity in the leaves of rice seedlings, and APX activity was significantly increased by 14.39% and 26.43% at the 1.5 leaf and 2.5 leaf stages, respectively. POD activity significantly increased by 33.66% at the 1.5 leaf stage (Figure 4B). At the 3.5 leaf and 4.5 leaf stages, the leaf CAT activity of rice seedlings was significantly increased by 13.51% and 19.95%, respectively. These results showed that exogenous GR24 application was able to increase the activity of antioxidant enzymes under salt stress, thus improving the antioxidant capacity of rice seedlings.

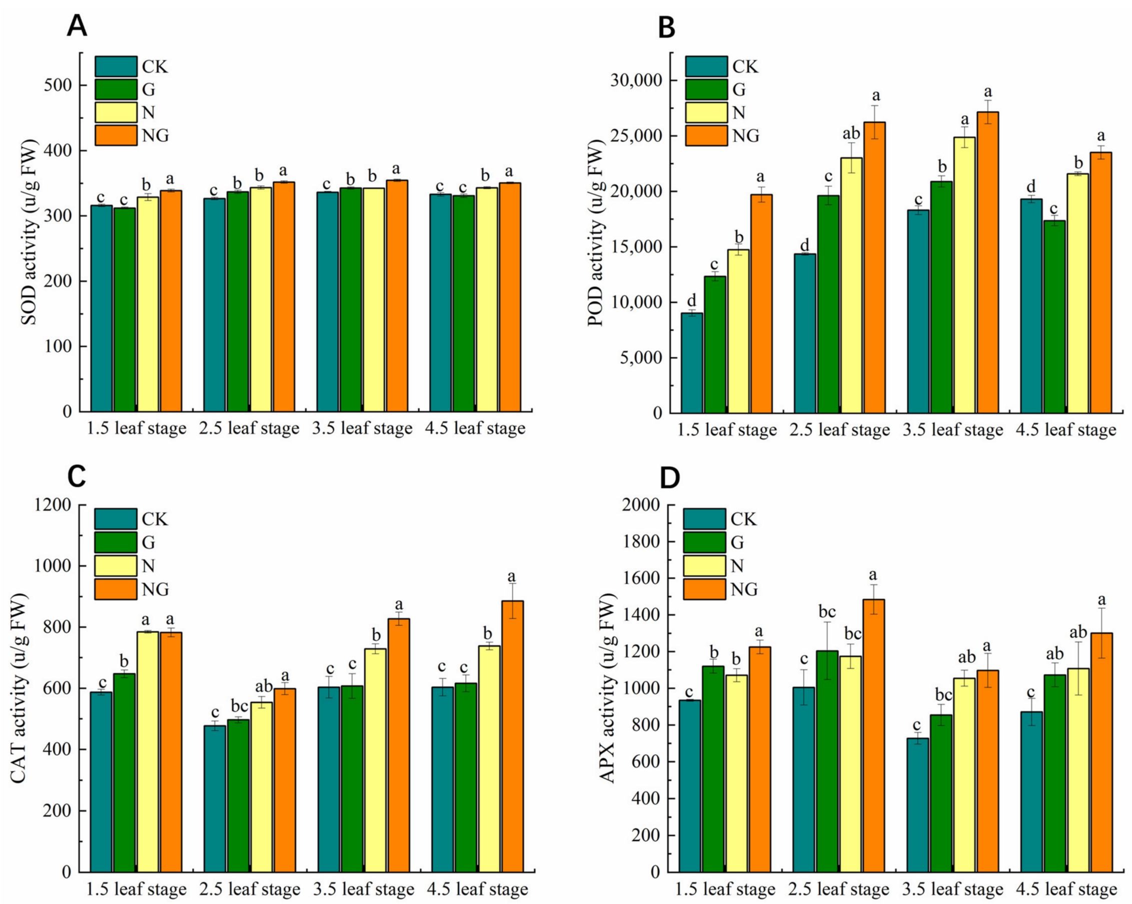

Figure 4. Effect of GR24 on antioxidant enzyme activities of Huanghuazhan seedlings under NaCl stress. (**A**) The activities of SOD. (**B**) The activities of POD. (**C**) The activities of CAT. (**D**) The activities of APX. CK: water-soaked seeds + water treatment; G: 1.2 μmol L^{-1} GR24-soaked seeds + water treatment; N: water-soaked seeds + 80 mmol L^{-1} NaCl treatment; NG: 1.2 μmol L^{-1} GR24-soaked seeds + 80 mmol L^{-1} NaCl treatment. Values are the means ± SE of three replicate samples. Different letters in the data column indicate significant differences ($p < 0.05$) according to Duncan's test.

3.6. Effect of GR24 Soaking on AsA and GSH Contents of Rice Seedling Leaves under NaCl Stress

Under NaCl stress, the levels of AsA and GSH were found to significantly decrease (Figure 5). Compared to CK, the NaCl treatment significantly reduced the AsA on average by 14.91%, 11.14%, and 16.66%, respectively, in the 2.5 leaf stage to 4.5 leaf stage in rice seedlings. Also, compared to CK, the NaCl treatment significantly reduced the GSH content on average by 31.11%, 34.52%, and 37.29%, respectively, in the 1.5, 2.5, and 4.5 leaf stages in rice seedlings. The application of exogenous GR24 led to an increase in AsA and GSH contents under NaCl stress (Figure 5). The application of the GR24 soaking treatment markedly enhanced AsA by 7.98%, 21.71%, 20.60%, and 17.09%, respectively, in the 1.5 leaf, 2.5 leaf, 3.5 leaf and to 4.5 leaf stages in rice seedlings under NaCl stress. Furthermore, compared to NaCl stress, the application of the GR24 soaking treatment markedly enhanced GSH content by 41.70% at the 4.5 leaf stage under NaCl stress. These results suggest that GR24 could increase the antioxidant content.

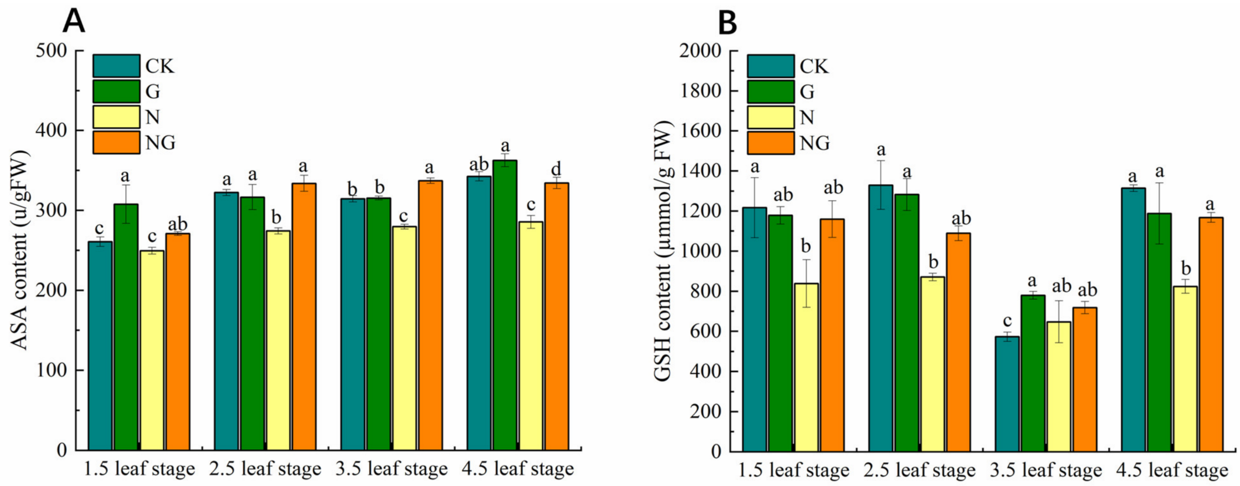

Figure 5. Effect of GR24 on ascorbic acid (**A**) and glutathione (**B**) of Huanghuazhan seedlings under NaCl stress. CK: water-soaked seeds + water treatment; G: 1.2 µmol L^{-1} GR24-soaked seeds + water treatment; N: water-soaked seeds + 80 mmol L^{-1} NaCl treatment; NG: 1.2 µmol L^{-1} GR24-soaked seeds + 80 mmol L^{-1} NaCl treatment. Values are the means ± SE of three replicate samples. Different letters in the data column indicate significant differences ($p < 0.05$) according to Duncan's test.

3.7. Effect of GR24 Soaking on the Degree of Leaf Membrane Damage in Rice Seedlings under NaCl Stress

Under NaCl stress, MDA and H_2O_2 contents showed increasing and decreasing trends compared to CK (Figure 6). Compared to CK, the NaCl treatment significantly increased MDA and H_2O_2 content on average by 35.43% and 17.18%, respectively, in the 1.5 leaf stage to 4.5 leaf stage in rice seedlings. Compared to NaCl stress, the application of GR24 soaking treatment markedly reduced MDA content by 23.10%, 7.56%, 5.60%, and 14.05%, respectively, for the 1.5 leaf, 2.5 leaf, 3.5 leaf, and 4.5 leaf stages in rice seedlings under NaCl stress (Figure 6A). Compared to NaCl stress, the application of the GR24 soaking treatment markedly reduced H_2O_2 content on average by 17.79%, 7.99%, and 14.42%, respectively, for the 1.5 leaf, 2.5 leaf, and 4.5 leaf stages in rice seedlings under NaCl stress. There was also a decrease in H_2O_2 content at the 3.5 leaf stage compared to NaCl stress (Figure 6B). These results indicated that GR24 soaking could alleviate ROS accumulation and maintain cell membrane stability in rice seedlings under NaCl stress.

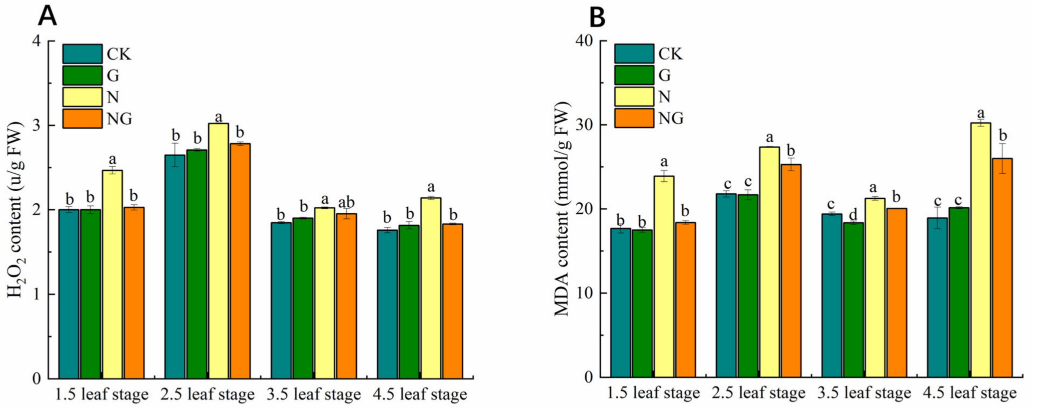

Figure 6. Effect of GR24 on H_2O_2 (**A**) and MDA (**B**) of Huanghuazhan seedlings under NaCl stress. CK: water-soaked seeds + water treatment; G: 1.2 μmol L^{-1} GR24-soaked seeds + water treatment; N: water-soaked seeds + 80 mmol L^{-1} NaCl treatment; NG: 1.2 μmol L^{-1} GR24-soaked seeds + 80 mmol L^{-1} NaCl treatment. Values are the means ± SE of three replicate samples. Different letters in the data column indicate significant differences ($p < 0.05$) according to Duncan's test.

3.8. Effects of GR24 Soaking on Osmoregulatory Substances in Rice Seedling Leaves under NaCl Stress

Soluble protein and proline contents were higher under NaCl stress compared CK. Among these, the soluble protein content initially showed an increasing trend, followed by a decrease, and then another increase, whereas the proline content continued to increase with the duration of salt stress (Figure 7). Compared to CK, the NaCl treatment significantly increased the soluble protein content on average by 10.95%, 1.92%, and 4.46%, respectively, for 1.5 leaf, 2.5 leaf, and 4.5 leaf stages in rice seedlings. Compared to CK, the NaCl treatment significantly increased the proline content on average by 37.47%, 63.81%, 127.05%, and 58.32%, respectively, for 1.5 leaf, 2.5 leaf, 3.5 leaf and 4.5 leaf stage in rice seedlings. The application of exogenous GR24 soaking treatments significantly increased the soluble protein content under NaCl stress but reduced the proline content (Figure 7). Application of the GR24 soaking treatment markedly enhanced soluble protein content at 2.5 and 3.5 leaf stages in rice seedlings under NaCl stress. Additionally, compared to NaCl stress, GR24 soaking caused a significant decrease in proline content at the 1.5 leaf, 2.5 leaf, and 4.5 leaf stages.

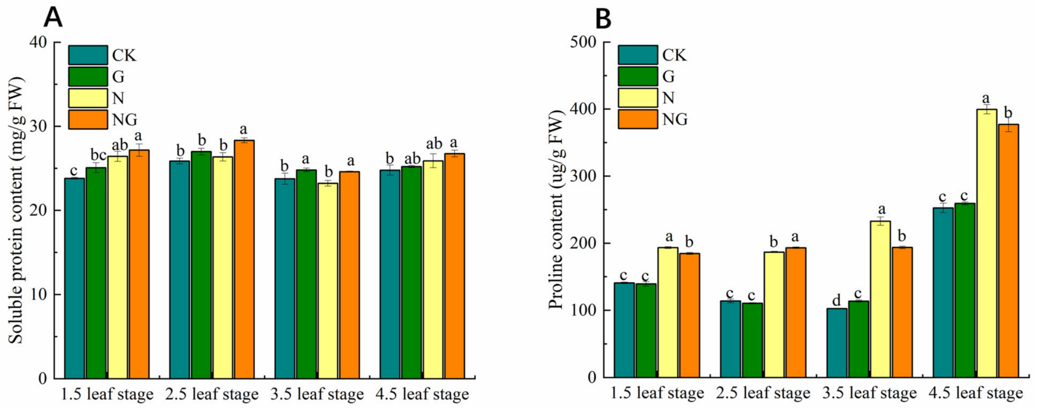

Figure 7. Effect of GR24 on soluble protein (**A**) and proline (**B**) content of Huanghuazhan seedlings under NaCl stress. CK: water-soaked seeds + water treatment, G: 1.2 μmol L^{-1} GR24-soaked seeds + water treatment, N: water-soaked seeds + 80 mmol L^{-1} NaCl treatment, NG: 1.2 μmol L^{-1} GR24-soaked seeds + 80 mmol L^{-1} NaCl treatment. Values are the means ± SE of three replicate samples. Different letters in the data column indicate significant differences ($p < 0.05$) according to Duncan's test.

3.9. Effect of GR24 Soaking on Leaf Hormone Content of Rice Seedlings under NaCl Stress

To determine whether GR24 also affects endogenous plant hormones in response to salt stress, changes in the content of five plant hormones, including SLs, IAA, ABA, CTK, and GA3, were determined in rice seedlings (Table 4). Leaf hormone contents were significantly different and showed large differences under different treatments. NaCl stress increased ABA content in leaves compared with CK; however, GR24 treatment significantly reversed the effect of NaCl (Table 4). Compared to CK, the NaCl treatment significantly reduced the content of CTK, IAA, and GA3 on average by 19.46%, 40.26%, and 22.74%, respectively, at 3.5 leaf stage of the rice seedlings. Compared with NaCl treatment, the application of the GR24 soaking treatment markedly enhanced SLs, CTK, IAA, and GA3 on average by 106.58%, 73.31%, 90.22%, and 52.29%, respectively, at the 3.5 leaf stage in rice seedlings under NaCl stress. The results showed that the exogenous GR24 soaking treatment could regulate the content of endogenous hormones in the leaves of rice seedlings, thus reducing the salinity toxicity of rice seedlings.

Table 4. Effect of GR24 on hormone content of Huanhuazhan seedlings under NaCl stress.

Treatment	SL (ng g^{-1})	ABA (ng g^{-1})	CTK (ng g^{-1})	IAA (ng g^{-1})	GA3 (ng g^{-1})	IAA/ABA	CTK/ABA
CK	1706.11 ± 33.7278 d	492.57 ± 12.6549 d	269.51 ± 3.5092 c	79.33 ± 1.2657 c	0.33 ± 0.0060 b	0.16 ± 0.0016 a	0.55 ± 0.0072 b
G	6652.79 ± 46.6260 a	582.3 ± 12.1714 c	419.13 ± 7.4722 a	85.16 ± 0.2175 b	0.33 ± 0.0064 b	0.15 ± 0.0027 b	0.72 ± 0.0025 a
N	2006.05 ± 32.5903 c	897.19 ± 2.3231 a	217.05 ± 3.5979 d	47.39 ± 0.7614 d	0.25 ± 0.0019 c	0.05 ± 0.007 d	0.24 ± 0.0036 d
NG	4144.17 ± 14.6975 b	748.19 ± 4.9391 b	376.16 ± 4.1620 b	90.15 ± 1.2119 a	0.38 ± 0.0037 a	0.12 ± 0.0013 c	0.5 ± 0.0025 c

Note: CK: water-soaked seeds + water treatment; G: 1.2 μmol L^{-1} GR24-soaked seeds + water treatment; N: water-soaked seeds + 80 mmol L^{-1} NaCl treatment; NG: 1.2 μmol L^{-1} GR24-soaked seeds + 80 mmol L^{-1} NaCl treatment. Values are the means ± SE of three replicate samples. Different letters in the data column indicate significant differences ($p < 0.05$) according to Duncan's test.

3.10. Effect of GR24 Dipping on the Hormone Balance of Rice Seedling Leaves under NaCl Stress

Compared to CK, the NaCl treatment significantly reduced the IAA/ABA and CTK/ABA on average by 67.22% and 55.82%, respectively, at the 3.5 leaf stage of the rice seedlings (Table 4). Compared to NaCl stress, the application of the GR24 soaking treatment markedly enhanced IAA/ABA and CTK/ABA on average by 128.11% and 107.82%, respectively, at the 3.5 leaf stage of rice seedlings under NaCl stress. These findings indicate that the application of GR24 has a regulatory effect on the hormonal balance of rice seedling leaves under stress conditions.

4. Discussion

Salt stress influences the physiological and biochemical functions of plants, consequently hindering their growth [37]. Variations in plant biomass represent a comprehensive response to stress from adverse conditions [38]. Numerous studies indicate that high salinity significantly hampers the growth and accumulation of biomass in various plants, including wheat [39], tomato [22], and oilseed rape [26]. In our study, the growth and development of rice seedlings were inhibited under NaCl stress (Figures 1 and 2, and Table 3), which significantly reduced fresh and dry weights (Table 2). Under NaCl stress, the greatest decreases in fresh and dry weights were 55.11% and 61.97%, respectively, indicating that the biomass accumulation of rice plants was severely inhibited under NaCl stress. Nevertheless, an exogenous GR24 seed soaking treatment was observed to mitigate the toxic effects of NaCl and markedly enhance the associated growth indices of rice seedlings subjected to salt stress (Figure 1). Our findings are in line with those reported by Liu et al. [22]. SLs interacted with IAA, resulting in improved growth and increased root branching [40].

There exists a direct correlation between the concentration of photosynthetic pigments and crop health, with their levels serving as one of the most critical indicators of a plant's capacity to photosynthesize under abiotic stress [41,42]. Under conditions of stress, the content of photosynthetic pigments in the leaves of plants decreases and the leaves turn yellow [43]. The present study also showed the same results. GR24 can regulate the binding of chlorophyll and membrane proteins in order to maintain chloroplast membrane stability and enhance photosynthetic efficiency [44,45]. Lu et al. [44] found that the application of exogenous GR24 could significantly increase the content of photosynthetic pigments. The increase in photosynthetic pigments was able to promote the utilization of light energy by plants and reduce the photosystem damage caused by excessive light energy and ROS. In this study, under NaCl stress, GR24 treatment significantly increased photosynthetic pigment content (Figure 3) and attenuated the yellowing of GR24-treated leaves (Figure 1). Under NaCl stress, the GR24 treatment significantly increased the content of photosynthetic pigments (Figure 3) and attenuated the yellowing phenomenon of GR24-treated leaves (Figure 1), and the content of photosynthetic pigments at the 1.5-leaf stage was close to the CK level. The results demonstrated that GR24 effectively mitigated chlorophyll degradation and enhanced PSII capacity in response to salt stress, which is in accordance with the findings of Danish et al. [24]. Song et al. [46] also found a significant decrease in the content of photosynthetic dyes under salt stress. Nevertheless, the exogenous application of GR24 led to a notable increase in the total content of photosynthesized pigments and the expression of genes involved in chlorophyll biosynthesis when compared to the effects of salt stress alone.

In plants undergoing typical growth processes, the production and removal of reactive oxygen species (ROS) are maintained in a state of equilibrium. Plants make more hydrogen peroxide under salt-stressed conditions [47]. Plants make more hydrogen peroxide when they are stressed by salt. This breaks down cell walls and makes cells die faster. This then damages other molecules, such as nucleic acids and proteins [48]. MDA is a byproduct of cell membrane damage. Its level can indicate how damaged a cell membrane is and how well a plant is able to resist damage [25]. In the present study, it was found that the H_2O_2 and MDA contents of rice seedlings were significantly increased under NaCl stress, whereas an overall average reduction of 23.10% and 17.79% in MDA and H_2O_2 content of rice seedling leaves under NaCl stress was found after the exogenous GR24 treatment (Figure 6), which is similar to the results of Shah et al. [49]. Plants balance the salt toxicity caused by ROS by increasing the synthesis rate of antioxidant enzymes and antioxidants [22]. SOD disproportionately converts superoxide to form H_2O_2, whereas CAT metabolizes H_2O_2 to produce H_2O and $O^{2\cdot-}$ [13]. The present study showed that SOD, POD, CAT, and APX activities increased in rice leaves under NaCl stress, and the application of exogenous GR24 further increased the activities of antioxidant enzymes in leaves under NaCl stress (Figure 4). This is similar to the findings of Ma [50] and Faisal et al. [27]. Ahsan et al. [51] found that GR24 helped plants against stress from salt by making their ROS scavenging abilities stronger. Zhang et al. [52] also found that GR24 improved the cold tolerance of rape seedlings by boosting antioxidants and reducing ROS. AsA and GSH are important antioxidants that participate in the GSH-AsA cycle to maintain the redox state of cells [53]. In this study, the AsA and GSH contents were significantly reduced in the leaves of rice seedlings under NaCl stress (Figure 5). However, GR24 reversed this effect. The contents of AsA and GSH in the leaves of rice seedlings under NaCl stress were significantly increased after the exogenous GR24 imbibition treatment (Figure 5), maintaining a higher antioxidant capacity in the leaves. This is similar to the findings of Li et al. [54]. Therefore, the results indicate that GR24 enhances the scavenging capacity for excessive ROS accumulation in rice leaves by up-regulating antioxidant enzyme activities, thereby maintaining cellular redox balances.

The synthesis and accumulation of osmoregulatory substances enables plants to improve osmoregulation in the presence of salt stress [12]. Soluble proteins constitute an essential component of cellular structure. It enhances the ability of cells to retain water, responds to external stressors, and plays a role in osmoregulation [55]. In this study, soluble proteins were significantly increased in seedlings treated with GR24 dipping under NaCl stress (Figure 7A), which was consistent with the results of Zhang et al. [52]. Proline has been shown to act as an osmoregulator under conditions of abiotic stress, thereby maintaining cellular homeostasis and preventing cellular dehydration. In addition, it has been demonstrated to stabilize protein structure and scavenge free radicals, which collectively affords protection to cells from damaging effects [56,57]. Nevertheless, the question of whether proline plays a role in osmoregulation remains a topic of debate among the scientific community. Ghosh et al. [58] proposed a positive linear relationship between proline content and the tolerance of plants to a range of abiotic stresses. Lacerda et al. [59] suggested that the accumulation of proline may not be a direct response to salt stress, but rather a plant response associated with salt damage. Furthermore, the reduction in proline synthesis may potentially enhance the growth of plants. In the present study, it was found that proline content increased under NaCl stress, but it was significantly decreased under NaCl stress after exogenous GR24 imbibition (Figure 7B). These results were consistent with those reported by Abdel et al. [60]. We hypothesized that this may be related to the improved growth of rice plants under NaCl stress after exogenous GR24 soaking (Tables 2 and 3, and Figure 1). Additionally, the gradual normalization of the osmotic pressure of the extracellular environment may be a contributing factor. Following the alleviation of salt stress by an exogenous application of GR24, the reduced plant demand for proline as an osmotic regulator may also play a role. This occurs as a result of increased antioxidant enzyme activity and antioxidant content, as well as a decrease in MDA and H_2O_2. Chen et al. [61] found that exogenous monocotyledonin lactone reduced the proline content of soybean seedlings under alkaline stress, and they hypothesized that the reason might be that the SL biosynthesis and signaling mechanisms of soybean seedlings under alkaline stress were not significantly related to proline regulation. Reduced proline content has also been shown in salinity tolerance studies in soybeans. Lv et al. [62] discovered that the tolerance of rice seedlings to salinity stress is not associated with an accumulation of proline.

The adaptation of plant life to adverse environmental conditions can be optimized via the regulation of alterations in plant hormones [14]. Nevertheless, the synthesis, catabolism, and stabilization of hormones in plants are disrupted when plants are subjected to adverse stress conditions [63]. ABA plays an important role in plant resilience, serving as a crucial accumulating agent under unfavorable conditions, such as those of drought, high salinity, and low temperature, thereby enhancing plant resistance [64]. Additionally, hormones such as IAA, CTK, and GA play a role in regulating plant stress tolerance [65,66]. SLs are essential hormones that regulate plant development and stress tolerance and interact with ABA, IAA, CTK, and GA3 [67]. this study, the content of SLs and ABA significantly increased under NaCl stress. The exogenous GR24 dipping treatment further significantly increased the SL content and decreased the ABA content in the leaves of rice seedlings under NaCl stress (Table 4). This finding is similar to that of Tang et al. [68]. Liu et al. [69] demonstrated that the exogenous application of GR24 was capable of inducing the production of elevated concentrations of SLs in plants. Ruiz-Lozano et al. [70] discovered a negative correlation between ABA and SLs. Under drought stress conditions, SLs produced by clumped mycorrhizae suppressed the accumulation of ABA. We hypothesized that the negative correlation between SL and ABA may be a strategy for plant adaptation to abiotic stress. In the present study, the exogenous GR24 imbibition treatment significantly increased the contents of GA, CTK, and IAA in the leaves of rice seedlings under NaCl stress and enhanced IAA/ABA and CTK/ABA (Table 4). These findings are consistent with those reported by Zhou et al. [71]. Alvi et al. found that exogenous GR24 reduced heat stress by decreasing ABA/GA ratio and increasing CTK content [72]. Min et al. found that exogenous GR24 treatment reduced IAA and zeatin (ZR) content in grape leaves and roots under drought stress [71]. Our results suggest that exogenous GR24 application induces changes in endogenous hormone levels, which maintains hormone homeostasis and consequently improves salt tolerance in rice seedlings.

5. Conclusions

The objective of this study was to elucidate the physiological mechanisms by which GR24 alleviates salt stress in rice seedlings (Figure 8). The results demonstrated that NaCl stress significantly inhibited the growth of rice seedlings, and GR24 alleviated salt stress in rice seedlings through multiple pathways. The application of GR24 led to improvements in the morphology of rice seedlings subjected to NaCl stress. Additionally, it enhanced the synthesis of photosynthetic pigments, increased the activities of antioxidant enzymes, elevated the levels of antioxidants AsA and GSH, augmented the content of leaf soluble proteins, and reduced the levels of proline. These changes resulted in a reduction in the production of MDA and H_2O_2, thereby mitigating the oxidative damage caused by NaCl stress to rice seedlings. Furthermore, the exogenous GR24 was observed to regulate the endogenous hormone balance of rice seedlings under NaCl stress, thereby reducing the damage of salt stress on rice seedlings and improving salt tolerance. These results offer a novel perspective on the biological functions of GR24 in plant growth and stress response. However, further investigation is required to elucidate the molecular mechanisms underlying the enhancement of salt tolerance in rice by GR24. In conclusion, this study has identified the physiological mechanisms by which GR24 alleviates salt stress in rice seedlings. The application of GR24 resulted in improvements in rice seedling morphology, photosynthetic pigment synthesis, antioxidant defense, and the regulation of endogenous hormone levels under conditions of NaCl stress. The application of GR24 resulted in a reduction in the damage caused by salt stress in rice seedlings, thereby improving salt tolerance. In sum, these findings offer new insights into the biological functions of GR24 in plant growth and stress response. Nevertheless, the physiological mechanism of GR24 and NaCl stress is intricate, and its molecular mechanism remains to be elucidated.

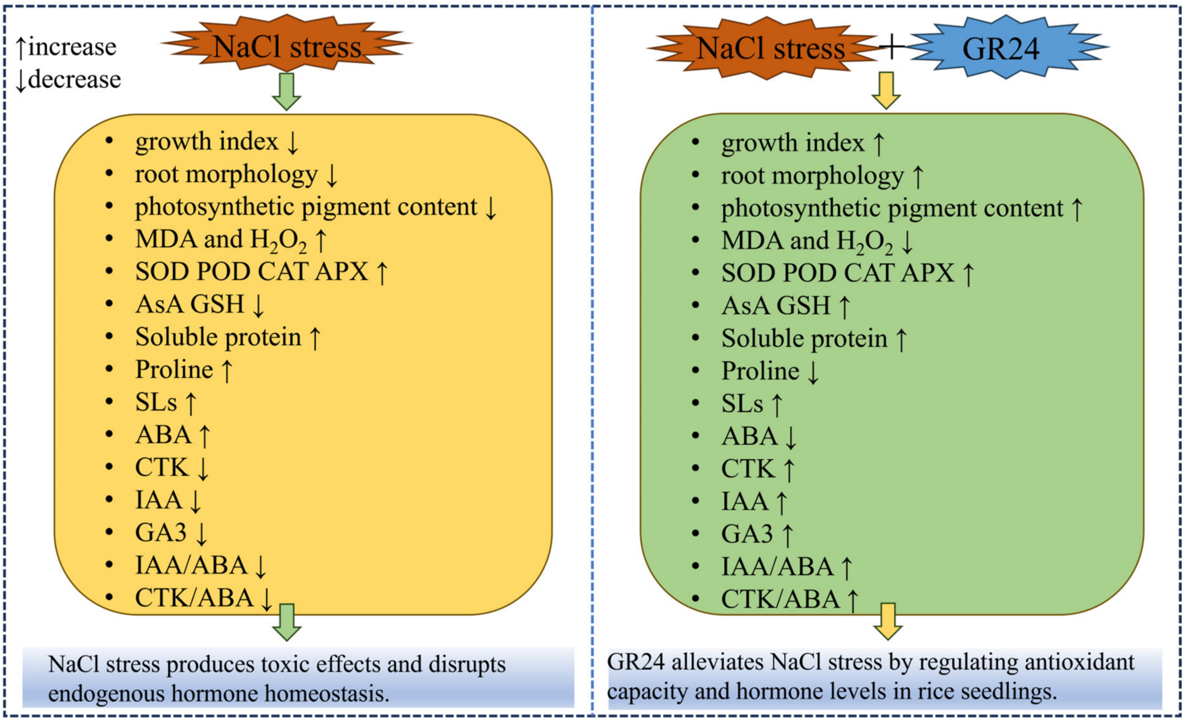

Figure 8. A working model of GR24 to alleviate NaCl stress by regulating the antioxidant capacity and hormone levels in rice seedlings. Enzymatic antioxidants and SL increased under both NaCl and NaCl + GR 24, but the increase was greater under NaCl + GR 24 than under NaCl stress.

Author Contributions: J.Z. was responsible for writing the original draft, investigation, data curation, and formal analysis. N.F. contributed to the methodology and conceptualization. D.Z. aided in the conceptualization, the acquisition of funding, administration, and the supervision of the project. A.K. contributed to the investigation and methodology. Y.D. helped with the investigation. Y.W. aided in the writing—review and editing. R.D. contributed to the investigation. J.W. helped with the investigation and formal analysis. J.X. contributed to the formal analysis. Z.S. contributed to the formal analysis. Q.Z. contributed to the formal analysis. M.W. contributed to the formal analysis. All authors contributed to the manuscript preparation, writing and revision. All authors have read and agreed to the published version of the manuscript.

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