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Regulation of seed soaking with indole-3-butyric acid potassium salt (IBA-K) on rapeseed (*Brassica napus* L.) seedlings under NaCl stress

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Abstract
The growth and yield of rapeseed are significantly hampered by salt stress. Indole-3-butyric Acid Potassium Salt (IBA-K) has been found to alleviate the impact of salt stress on plant growth. However, the regulatory effect of IBA-K dipping on salt-stressed rapeseed remains unclear. To explore the implications of IBA-K on the growth and development of rapeseed during the seedling stage, we conducted potting experiments using the Huayouza 62 variety. Five different concentrations of IBA-K for seed soaking (0, 10, 20, 40, 80 $mg{\cdot}L^{-1}$) were tested. The promotional impact of IBA-K on rapeseed demonstrated an initial increase followed by a decline, reaching a peak at 20 $mg{\cdot}L^{-1}$. Therefore, 20 $mg{\cdot}L^{-1}$ was determined as the optimal concentration for subsequent experiments. To further understand the mechanism of IBA-K's action on salt-stressed rapeseed seedlings, we utilized the moderately salt-resistant cabbage rapeseed variety Huayouza 158R and the highly salt-resistant Huayouza 62 as specimens. The investigation focused on their response and repair mechanisms under 150 $mmol{\cdot}L^{-1}$ NaCl stress. The findings demonstrated that compared with the sole NaCl stress, the 20 $mg{\cdot}L^{-1}$ IBA-K seed soaking treatment under salt stress significantly enhanced the plant height, stem diameter, and leaf area of both rapeseed varieties. It also led to greater biomass accumulation, increased chlorophyll content, and improved photosynthetic efficiency in rapeseed. Furthermore, this treatment bolstered the activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), while significantly reducing the levels of electrolyte leakage (EL) and malondialdehyde (MDA). Consequently, it alleviated the membrane lipid peroxidation damage induced by NaCl stress, enhanced the accumulation of soluble proteins, maintained cellular osmotic pressure, and effectively mitigated the adverse effects of NaCl stress on rapeseed.

Keywords Antioxidant, Osmoregulation, Potassium indolebutyrate, Rapeseed, Salt stress

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Background

Soil salinization has emerged as a pressing global ecological challenge [1]. In China, saline soils cover approximately 3.6 million hectares, representing 4.88% of the nation's usable land area [2]. This statistic highlights China's significant soil issues, which pose a considerable threat to regional agricultural productivity. Soil salinization directly impacts crop health and degrades land structure, it disrupts natural farming practices, leading to low and inconsistent yields of crops [3]. To address the constraints imposed by salt stress on both ecological development and crop production, we must implement effective strategies to enhance the management of saline soils and mitigate crop yield losses.

Rapeseed (*Brassica napus*), an annual herbaceous plant, ranks among the four primary oilseed crops in China, it serves as a crucial source of vegetable oil [4]. However, salt stress poses a significant challenge in rapeseed cultivation, impacting seed germination, and overall growth, and ultimately, reducing yield [5]. Research indicates that excessive sodium and chloride ions can lead to ionic toxicity in rapeseed; this toxicity diminishes soil osmotic potential and induces osmotic stress, causing dehydration of plant cells [6, 7]. Photosynthesis, essential for crop metabolism, suffers greatly under external environmental pressures. During salt stress, enzymes that facilitate carbohydrate metabolism become inhibited, and stomata may experience complete or partial closure. This restriction lowers the transpiration and photosynthesis rates, hampering gas exchange, which may lead to wilting or even leaf drop until the plant dies [8, 9]. Furthermore, salt stress induces oxidative stress, resulting in the accumulation of reactive oxygen species (ROS), such as superoxide($O_2\cdot^-$), hydroxyl radicals($\cdot OH$), and hydrogen peroxide(H_2O_2) [10, 11]. To cope with these challenges, rapeseed employs antioxidant enzyme systems and antioxidants to scavenge excessive ROS. This strategy maintains a dynamic balance between ROS production and removal, thereby mitigating oxidative stress within the cells of rapeseed [12, 13].

Salt stress impacts plants differently at various growth stages. Research highlights that seedling crops show heightened sensitivity to soil salinity, especially species with shallow roots, this stage often determines the extent of salt-related damage [14, 15]. Consequently, seedling growth serves as a vital indicator of salt stress effect on crops. Studies reveal that the establishment rate, final size, and yield of buckwheat [16], rice [17], and sunflower [18] correlate closely with their seedling salt tolerance. Thus, investigating the repercussions of salt stress during the seedling phase of rapeseed is crucial. Understanding these effects and developing adaptation strategies will enhance the salt tolerance of rapeseed during this critical stage, ultimately benefiting its growth and production.

To enhance crop resilience against salt stress, plant growth regulators, mineral elements, phytohormones, and antioxidants actively contribute [19]. Notably, plant growth regulators have demonstrated efficacy in mitigating various facets of plant growth under salt stress and augmenting salt tolerance. For instance, certain regulators improve the development of crops like wheat [20], cotton [21], and tomato [22] under saline conditions. IBA-K is a plant growth regulator with good stability, safety, and long-term and specific properties, which plays a role in breaking seed dormancy and promoting rooting and strengthening of roots. Historically, IBA-K has been utilized primarily for the propagation of tree and flower cuttings [23, 24]. However, recent studies focus on its application across diverse crops. Research indicates that IBA-K enhances maize root length and volume, thereby boosting overall crop quality [25]. Furthermore, it exerts significant regulatory effects on soybean leaves, roots, and physiological attributes, mitigating drought stress effects [26]. Zhou et al. [27] highlighted that the IBA-K application could lessen salt stress damage in rice. Thus, IBA-K presents considerable potential for alleviating abiotic stresses across various crops and is pivotal for cultivating saline-resistant crops.

Currently, the impact of IBA-K on rapeseed growth and salt resistance remains underexplored, and its mechanism of action needs to be clarified. Therefore, this study is designed to elucidate the effects of IBA-K on the growth and physiological traits of rapeseed seedlings facing NaCl stress. The goal is to offer novel insights for future research into IBA-K's potential to enhance the salt tolerance of rapeseed while also providing a theoretical foundation and practical guidance for cultivating salt-resistant rapeseed in saline soils.

Materials and methods

Experimental design

A randomized complete design was chosen for the experiment to ensure a comprehensive assessment of the treatments, conducted in the daylight linkage greenhouse of Binhai Agricultural College. The experiment was divided into two parts: (1) Concentration screening test: full-seeded, uniformly sized Huayouza 62 rapeseed seeds were manually selected, and the seeds were sterilized with 3% hydrogen peroxide solution for 10 min and then rinsed with ultrapure water for 4–5 times. The sterilized rapeseed seeds were soaked in deionized water and IBA-K concentrations of 0, 10, 20, 40, 80 $mg\cdot L^{-1}$ solution for 8 h (dark, 25±1℃), and then the seeds were sown in pots of caliber×bottom diameter×basin height=10 cm×7 cm×8.5 cm (without holes), Each pot contained 0.35 kg of a soil mix comprised of vermicompost and sand in a ratio of 1:3. (soil organic matter content: 46.38, total nitrogen content: 0.77%, total phosphorus content: 1.76%, total potassium content: 0.39%, and total nutrients: 2.92%). Ten seeds were sown at equal intervals, with subsequent thinning performed to maintain four seedlings per pot post-emergence. When two true leaves emerged, 50 ml of a modified 1/2 Hoagland (pH 6.5 ~ 6.9) nutrient solution was added to each pot. Under normal lighting conditions, the treatments were repeated three times each.

(2) Based on the concentration screening test, researchers selected an IBA-K concentration of 20 mg·L^{-1} for further experimentation. The experimental site and conditions mirrored those of the initial concentration screening test. Seeds of the medium salt-tolerant variety Huayouza 158R and the better salt-tolerant variety Huayouza 62 rapeseed, with plump and uniform grains, were carefully chosen. The seeds underwent sterilization using a 3% hydrogen peroxide solution for 10 min, followed by rinsing with ultrapure water for 4–5 cycles. The seeds were soaked in deionized water and 20 mg·L^{-1} of IBA-K solution in for 8 h under dark conditions at 25±1 °C. Subsequently, The seeds were dried using absorbent paper. Before sowing, the soil received salt treatment by dissolving a measured amount of NaCl in water, resulting in a solution NaCl concentration of 150 mmol·L^{-1} and mixing this solution thoroughly with the soil before sowing (Ensuring the pot size, soil volume, soil substrate, and sowing method were the same as in the concentration screening test). To prevent nutrient deficiency in the later stage, pour the modified 1/2 Hoagland nutrient solution (pH 6.5 ~ 6.9); once when the two true leaves grow, pour 50 ml per pot, A total of 8 treatments were established in the experiment, involving various seed soaking treatments and different NaCl treatments on Huayouza 158R and Huayouza 62 varieties. Under normal lighting conditions, the treatments were repeated three times each, the specific therapies implemented were:

1. CK (control, soaked seeds in deionized water).
2. I (20 mg·L^{-1}IBA-K soaked seeds).
3. S (150 mmol·L^{-1}NaCl stress + soaked seeds in deionized water).
4. SI (150 mmol·L^{-1} NaCl stress + 20 mg·L^{-1} IBA-K soaked seeds).

Measurement items and methodology

Sampling method

Samples were collected on the 14th, 19th, and 24th days after the initiation of the experiment to assess morphological, physiological, and biochemical parameters. Representative plants showing uniform growth under each treatment condition were chosen and documented during each sampling session. Fresh leaf samples were carefully selected, roots were washed, and excess moisture was removed using absorbent paper. Subsequently, the samples were flash-frozen in liquid nitrogen and subsequently preserved in a -80℃ ultra-low temperature freezer to ensure their integrity for further analysis.

Morphological parameters

The plant height, root length, and stem diameter were measured precisely using a straightedge and vernier calipers. The leaf area of each seedling was measured by Yanxin-1241 Leaf Area Meter. The above-ground and below-ground fresh weights were weighed, and then they were put into an envelope bag in a constant temperature oven at 120℃ to kill the green for 30 min and then dried at 80℃ to constant weight, and then weighed the above-ground and below-ground dry weights.

Determination of photosynthetic pigments and photosynthesis-related parameters

Representative seedlings were chosen for each group, and their green leaves were trimmed into segments and measured at 0.1 g before being placed into 10 ml test tubes. The chlorophyll pigments were extracted using anhydrous ethanol, and their absorbance values at 665 nm, 652 nm, 649 nm, and 470 nm were measured using a spectrophotometer. The concentrations of chlorophyll a (Chl a) and chlorophyll b (Chl b) were determined using specific formulas [28]. Use the following formula:

$$\mathrm{Chl\ a(mg.g^{-1})} = 13.95\mathrm{A}_{665} - 6.88\mathrm{A}_{649}.$$

$$\mathrm{Chl\ b(mg.g^{-1})} = 24.96\mathrm{A}_{649} - 7.32\mathrm{A}_{665}.$$

Photosynthesis parameters were measured between 9 a.m. and 11:30 a.m. using a LI-6800 portable photosynthesizer (USA, LI-COR, Inc.) to determine the net photosynthetic rate (*Pn*), transpiration rate (*Tr*), stomatal conductance (*Gs*), and intercellular CO_2 concentration (*Ci)* of the leaves. Measurement conditions were: light intensity of 1,000 mmol m^{-2} s^{-1}, CO_2 concentration of 400 μmol mol^{-1} in the leaf chamber, leaf temperature of 25℃, relative humidity of 60–70%, and an airflow rate of 500 mmol s^{-1}.

Determination of electrolyte leakage rate

It was determined according to the method of Dionisio and Tobita [29]. The leaves were diced and weighed 0.1 g in a test tube filled with 10 ml of deionized water and allowed to sit at room temperature (25 °C) for 24 h. The solution's conductivity was assessed with a conductivity meter (E1), and the samples were heated for 30 min in a controlled water bath at 100 °C, cooled, and then the solution's conductivity was measured again (E2). Compute the EL value using the equation below:

$$\mathrm{Electrolyte\ Leakage(EL)} = (\mathrm{E1}/\mathrm{E2}) \times 100\% \quad (1)$$

Determination of antioxidant enzyme activity

Preserved fresh leaves (0.5 g) were ground with liquid nitrogen and 10 ml of pre-cooled phosphate buffer (0.05 mol·L^{-1}; pH 7.8) was added in two batches, ground to a homogenate and poured into a centrifuge tube and centrifuged at 4 °C and 10,000 g for 20 min. The supernatant was used as the enzyme extract and the related enzyme activity was determined. SOD activity was assessed using the NBT photochemical reduction method [30]; POD and CAT activities were determined following the protocol by Klapheck et al. [31]and Ma et al. [32].

Assessment of membrane lipid peroxidation products

The thiobarbituric acid (TBA) [33] assay was employed to quantify malondialdehyde (MDA) levels. We collected and weighed 0.5 g samples of seedling root and leaf tissues. These samples were ground in liquid nitrogen to form a powder. Then, the powder with 10 ml of phosphate buffer (pH 7.8) and extracted using 0.6% TBA prepared in 10% trichloroacetic acid (TCA). The mixture underwent heating in boiling water at 100 °C for 15 min before rapidly cooling on ice. Following centrifugation at 7000 g for 20 min, we measured the absorbance of the supernatant at wavelengths of 450 nm, 532 nm, and 600 nm, respectively.

Determination of soluble protein content

The soluble protein content was quantified using the Thomas Brilliant Blue G-250 staining method [34]. We weighed 0.5 g of frozen leaf tissue and added 50 mmol·L^{-1} phosphate buffer (pH 7.8). Next, we homogenized the mixture and transferred it to a centrifuge tube. We centrifuged the sample for 20 min at 4℃ and 12,000 g. Afterward, we took 1 mL of the enzyme solution, combined it with 5 mL of Caulmers Brilliant Blue (G-250), mixed it thoroughly, and measured the absorbance at 595 nm.

Data processing

All data underwent one-way ANOVA analysis using SPSS 25.0. The Duncan test evaluated differences among treatments, with $P<0.05$ indicating statistically significant differences. The Origin 2019 software facilitated graphical representation. Three biological replicates per treatment.

Results and analysis

Selection of IBA-K concentration

Different concentrations of IBA-K at 0, 10, 20, 40, and 80 mg·L^{-1} had varying effects on the growth characteristics of Huayouza 62 cultivars (Table 1). In comparison to the 0 mg·L^{-1} IBA-K treatment, the highest plant height in rapeseed occurred at IBA-K concentrations of 20 and 80 mg.L^{-1}, with significant increases of 22.7% and 17.6%, respectively. As IBA-K concentration rose, root length, stem diameter, and below-ground dry weight initially increased, then decreased, peaking at 20 mg·L^{-1}, which exerted the most substantial growth promotion on rapeseed. Relative to the control, rapeseed exhibited significant improvements in root length, stem diameter, leaf area, and biomass of 9.3%, 10.4%, 57.7%, 49.6%, 49.3%, 54.6%, and 77.8% respectively (Table 1). Beyond 20 mg·L^{-1} concentration, the growth promotion effects of IBA-K soaking on rapeseed seedlings gradually diminished, especially at 80 mg.L^{-1}, where root length and stem thickness were suppressed, decreased by 6.4% and 6.3%, respectively, compared to the control. The shoot dry weight did not differ significantly from plants not treated with IBA-K. Therefore, a concentration of 20 mg·L^{-1} was selected as the optimal concentration of IBA-K solution for subsequent experiments.

According to Duncan's test, different lowercase letters indicate significance between treatments ($n=3$, $P<0.05$). Data represent means±SD, $n=3$. According to Duncan's test, different lowercase letters indicate significance between treatments ($P<0.05$).

Effect of IBA-K soaking on growth parameters of rapeseed seedlings under NaCl stress

NaCl stress significantly inhibited the growth parameters of rapeseed, such as plant height, stem diameter, and leaf area (Table 2 and Fig. 1). From days 14 to 24, the height of Huayouza 158R and Huayouza 62 decreased significantly by 36.59%, 32.60%, 35.17%, and 29.62%, 16.36%, 11.99%, respectively. Stem thickness showed an average decrease of 28.23% and 24.28%, respectively, under S treatment

Table 1 Effects of potassium indolebutyrate at different concentrations on the growth and development of rapeseed

Concentration (mg·L-1)	Plant height(cm)	Root length(cm)	Stem diameter(mm)	Leaf area(mm^2)	Shoot fresh weight(g)	Root fresh weight(g)	Shoot dry weight(g)	Root dry weight(g)
0	11.17 ± 0.17d	11.50 ± 0.25bc	1.60 ± 0.06bc	1940.0 ± 59.05c	0.98 ± 0.04c	0.05 ± 0.003c	0.08 ± 0.001d	0.006 ± 0.0000b
10	12.17 ± 0.09c	11.53 ± 0.17b	1.73 ± 0.03ab	2568.2 ± 72.64b	1.24 ± 0.04b	0.06 ± 0.004b	0.10 ± 0.001b	0.009 ± 0.0003a
20	13.70 ± 0.15a	11.57 ± 0.3a	1.77 ± 0.03a	3058.5 ± 113.48a	1.47 ± 0.07a	0.07 ± 0.004c	0.11 ± 0.002a	0.011 ± 0.0006a
40	11.47 ± 0.15d	11.13 ± 0.24bc	1.53 ± 0.03c	2034.8 ± 47.81c	1.03 ± 0.03c	0.05 ± 0.001c	0.08 ± 0.001c	0.007 ± 0.0006b
80	13.13 0.07b	10.77 ± 0.15c	1.50 ± 0.06c	2577.5 ± 44.62b	1.22 ± 0.05b	0.06 ± 0.000b	0.1 ± 0.003b	0.006 ± 0.0003b

Table 2 Effects of IBA-K soaking on growth parameters of rapeseed under salt stress

		Treatments	Plant height(cm)	Stem diameter(mm)	Leaf area(mm^2)
Huayouza 158R	14	CK	14.30 ± 0.38a	2.03 ± 0.07a	801.57 ± 27.08b
		I	15.00 ± 0.12a	2.07 ± 0.07a	1027.13 ± 9.39a
		S	9.07 ± 0.29c	1.40 ± 0.00b	145.17 ± 11.32c
		SI	10.20 ± 0.17b	1.53 ± 0.03b	176.17 ± 7.87c
	19	CK	19.63 ± 0.19b	2.30 ± 0.12b	1746.17 ± 48.77b
		I	22.13 ± 0.48a	2.53 ± 0.07a	2959.20 ± 69.91a
		S	13.23 ± 0.52c	1.57 ± 0.03d	630.00 ± 20.35d
		SI	14.13 ± 0.39c	1.87 ± 0.03c	842.00 ± 17.54c
	24	CK	22.20 ± 0.30b	2.77 ± 0.03b	7153.60 ± 122.1b
		I	24.17 ± 0.20a	3.00 ± 0.03a	8149.40 ± 227.46a
		S	15.67 ± 0.35d	2.17 ± 0.03d	2942.27 ± 71.06c
		SI	17.43 ± 0.35c	2.30 ± 0.06c	3320.87 ± 124.34c
Huayouza 62	14	CK	11.37 ± 0.23a	1.83 ± 0.03a	837.83 ± 15.39b
		I	11.73 ± 0.09a	1.87 ± 0.03a	1007.50 ± 50.07a
		S	8.00 ± 0.12b	1.33 ± 0.03c	184.47 ± 14.72d
		SI	8.30 ± 0.06b	1.53 ± 0.03b	334.77 ± 10.52c
	19	CK	14.47 ± 0.46b	2.23 ± 0.03a	2605.67 ± 59.07b
		I	17.37 ± 0.61a	2.40 ± 0.15a	2846.10 ± 30.73a
		S	12.10 ± 0.32bc	1.73 ± 0.03b	721.80 ± 6.24c
		SI	13.20 ± 0.21c	1.90 ± 0.06b	813.45 ± 48.35c
	24	CK	16.97 ± 0.15b	2.73 ± 0.03a	7945.00 ± 90.64b
		I	19.63 ± 0.20a	2.90 ± 0.06a	10090.57 ± 368.53a
		S	14.93 ± 0.22c	2.10 ± 0.06c	2479.17 ± 81.38d
		SI	16.60 ± 0.06b	2.43 ± 0.07b	3236.90 ± 53.30c

CK: Control, soaked seeds in deionized water; I: 20 mg·L^{-1} IBA-K soaked; S: 150 mmol·L^{-1} NaCl stress + soaked seeds in deionized water; SI: 150 mmol·L^{-1} NaCl stress + 20 mg·L^{-1} IBA-K soaked; Data represent means ± SD, $n = 3$. According to Duncan's test, different lowercase letters indicate significance between treatments ($P < 0.05$).

compared to CK. Additionally, the impact of NaCl stress on the plant height of Huayouza 158R was particularly severe, with stem thickness also facing serious inhibition due to NaCl stress. S treatment had a more pronounced negative effect on the leaf area of both rapeseed varieties, particularly on the 14th day. The leaf area of Huayouza 158R and Huayouza 62 decreased significantly by 81.89% and 77.98%, respectively, compared to CK on the 14th day(Table 2). However, treatment with IBA-K improved plant height, stem thickness, and leaf area in both rapeseed varieties relative to saline treatment. Seedlings of both varieties exhibited enhanced growth patterns, manifesting taller heights, thicker stems, and larger leaf areas after IBA-K soaking. The SI treatment notably increased leaf area compared to saline treatment. During days 14–24, the leaf area of Huayouza 158R and Huayouza 62 rose by 21.35%, 33.65%, 12.87% and 81.48%, 12.70%, 30.56%, respectively(Table 2). On day 24, plant height increased by 11.27% and 11.16%, while stem thickness increased by 6.14% and 15.86%, respectively, for the two rapeseed varieties (Table 2).

Effects of IBA-K soaking on rapeseed biomass under NaCl stress

In Fig. 2, NaCl stress suppressed the biomass of both rapeseed seedling varieties. In comparison to CK, Huayouza 158R exhibited a significant reduction in root fresh weight and dry weight by 71.41% and 63.77%, respectively, at day 19 under S treatment (Fig. 2E, G). In the case of the Huayouza 62, both root fresh weight and dry weight decreased by 48.37% and 58.31%, respectively, at day 14 (Fig. 2F, H). When compared to the S treatment, both IBA-K treatments enhanced the biomass of seedlings for both rapeseed varieties and alleviated the inhibitory effects of NaCl stress on rapeseed (Fig. 2). Distinct promotional effects were noted between days 14 to 24 for both varieties., by day 19, the fresh and dry weights of shoot and root parts of Huayouza 158R in the SI treatment increased by 36.91%, 75.16%, 26.94%, and 93.40% (Fig. 2A, C, E, G), respectively, compared to the S treatment. Similarly, for Huayouza 62, there were increases of 27.37%, 48.23%, 23.18% (Fig. 2B, D, F, H), and 57.25% in fresh and dry weights of shoot and root parts. Overall, the biomass showed significant increases, except for the dry weight of Huayouza 158R, which saw an insignificant increase on the 19th day.

Effect of IBA-K soaking on membrane lipid peroxidation in rapeseed seedlings under NaCl stress

Compared to CK, the S treatment notably increased the MDA content in both rapeseed seedlings. The MDA content in both varieties reached its peak on the 14th

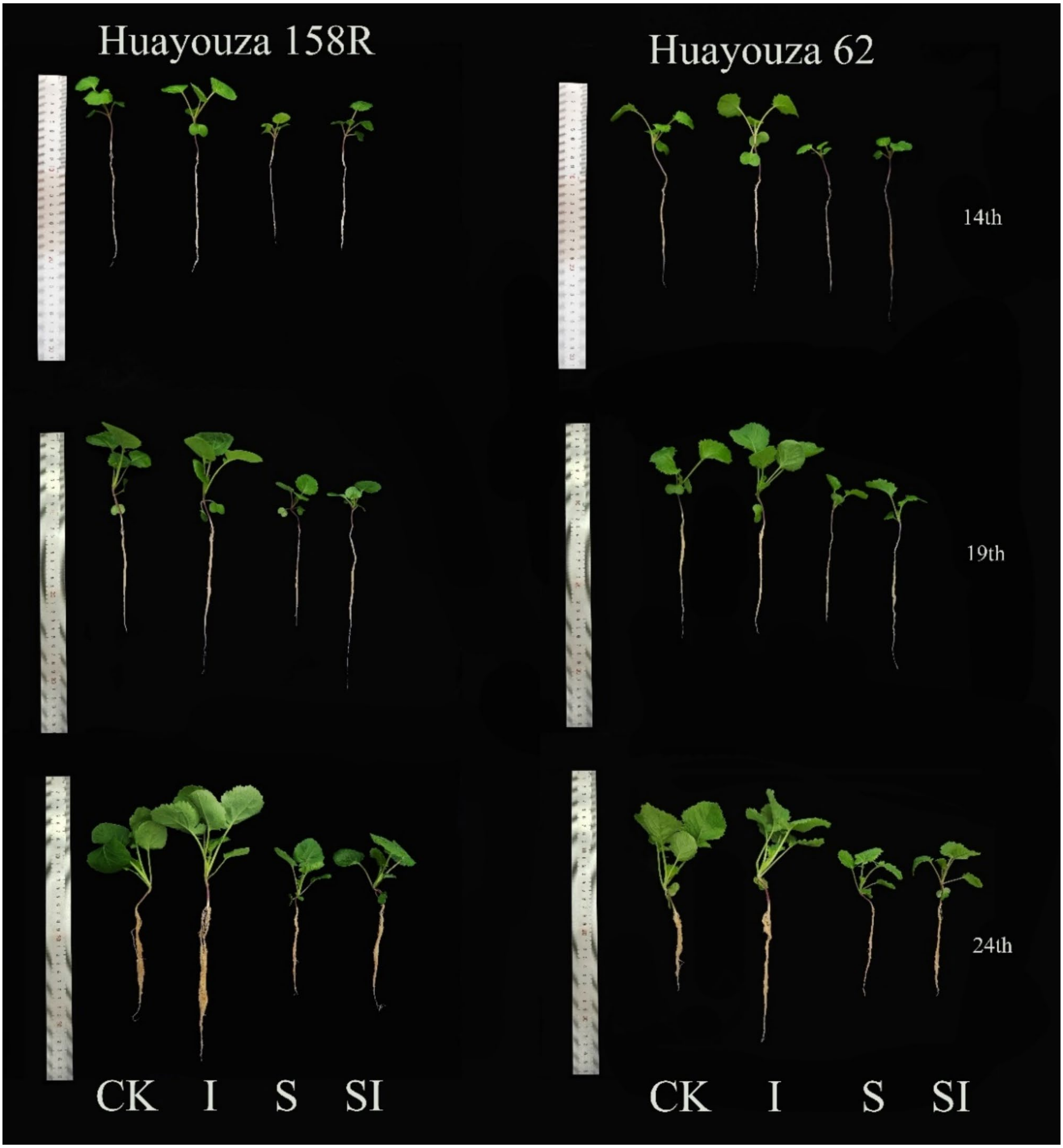

Fig. 1 Effects of IBA-K soaking on rapeseed plant morphology at the 14th, 19th, and 24th days under salt stress. CK: Control, soaked seeds in deionized water; I: 20 mg·L^{-1} IBA-K soaked; S: 150 mmol·L^{-1} NaCl stress + soaked seeds in deionized water; SI: 150 mmol·L^{-1} NaCl stress + 20 mg·L^{-1} IBA-K soaked

day under the S treatment and then decreased as the duration of stress increased. They exhibited increases of 15.01% and 28.47% when compared to the CK (Fig. 3). Furthermore, MDA levels diminished as stress duration increased. In comparison to the control, the IBA-K treatment significantly reduced the MDA levels in Huayouza 158R. Conversely, Huayouza 62 exhibited an increase in MDA levels with the I treatment from days 14 to 24. Both SI treatments significantly reduced the MDA content of Huayouza 158R by 23.24%, 10.75%, and 15.29% at d 14–24, respectively, compared with S treatment (Fig. 3A); Huayouza 62 was reduced by 2.96%, 5.24%, and 20.65%, respectively, in comparison with S treatment (Fig. 3B). Figure 3C illustrates a decrease followed by an

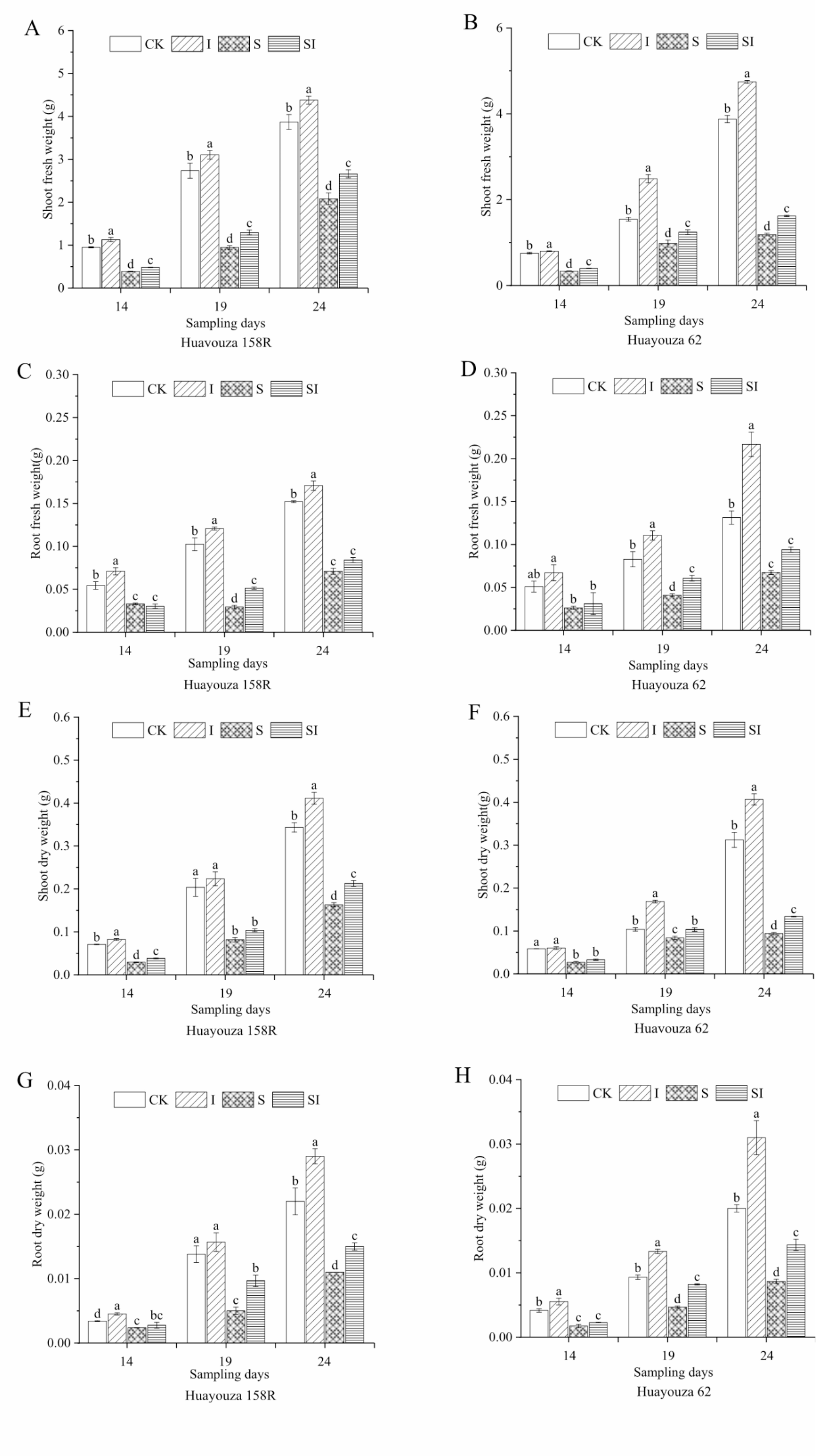

Fig. 2 Effects of IBA-K on shoot fresh weight (**A**, **B**), root fresh weight (**C**, **D**), shoot dry weight (**E**, **F**) and root dry weight (**G**, **H**) rape seedlings under NaCl stress. CK: Control, soaked seeds in deionized water; I: 20 mg·L^{-1} IBA-K soaked; S: 150 mmol·L^{-1} NaCl stress + soaked seeds in deionized water; SI: 150 mmol·L^{-1} NaCl stress + 20 mg·L^{-1} IBA-K soaked; Data represent means ± SD, *n* = 3. According to Duncan's test, different lowercase letters indicate significance between treatments ($P<0.05$)

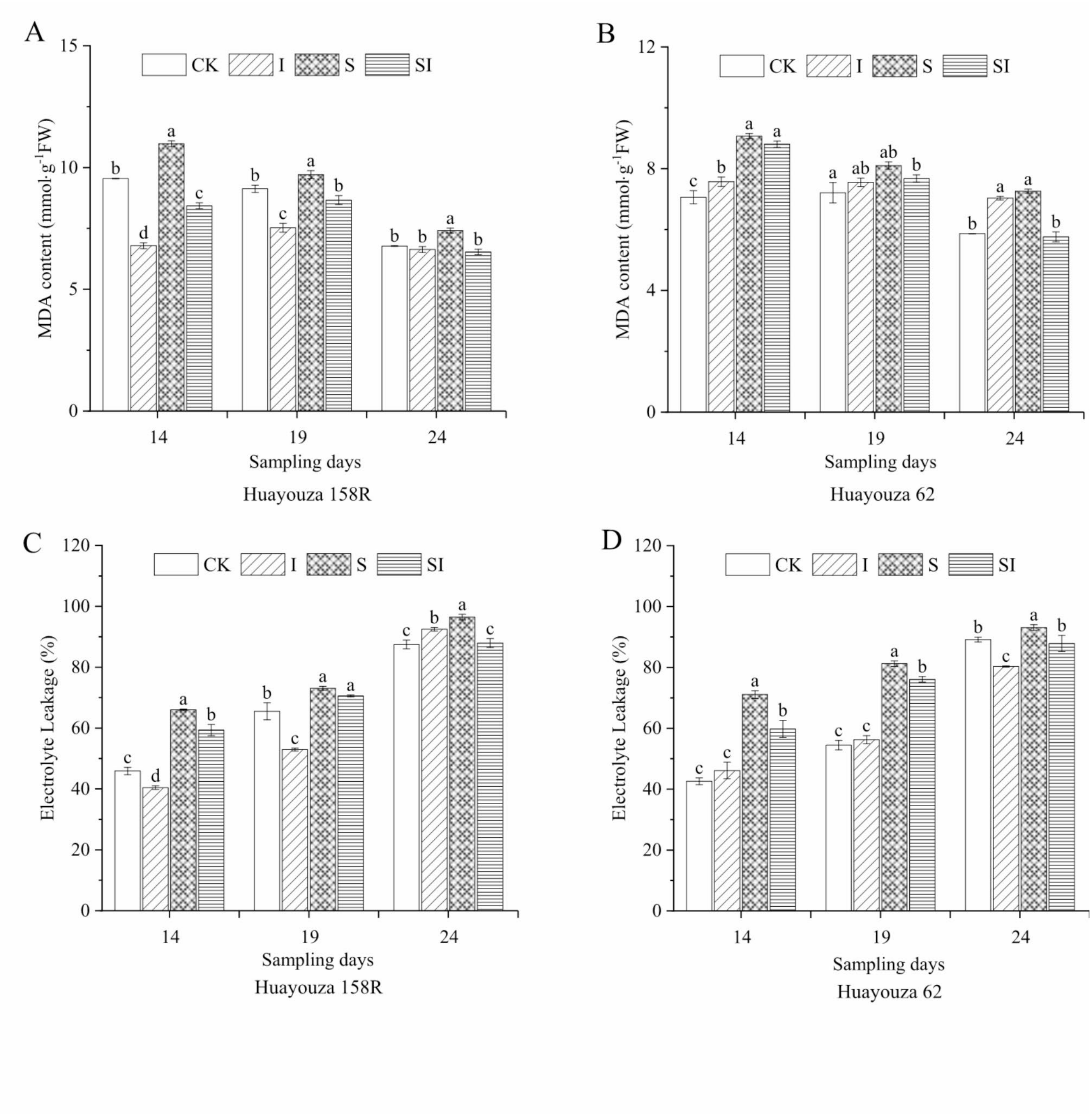

Fig. 3 Effects of IBA-K soaking seed on the contents of malondialdehyde (**A**, **B**) and Electrolyte Leakage (**C**, **D**) in rapeseed under NaCl stress. CK: Control, soaked seeds in deionized water; I: 20 mg·L^{-1} IBA-K soaked; S: 150 mmol·L^{-1} NaCl stress + soaked seeds in deionized water; SI: 150 mmol·L^{-1} NaCl stress + 20 mg·L^{-1} IBA-K soaked; Data represent means ± SD, *n* = 3. According to Duncan's test, different lowercase letters indicate significance between treatments ($P<0.05$)

increasing trend in EL for Huayouza 158R under I treatment, whereas Huayouza 62 displayed an opposite trend, first increasing and then decreasing (Fig. 3D). Under the S treatment, the EL of rapeseed seedlings from both varieties significantly increased compared to CK and continued to increase up to the 24th day. During the 14th to 24th day period, the EL of Huayouza 158R under SI treatment decreased by 10.08%, 3.47%, and 8.81% compared to the S treatment (Fig. 3C). On the other hand, the EL of Huayouza 62 under SI treatment decreased by 15.89%, 6.32%, and 5.64% compared to the S treatment (Fig. 3D).

Effect of IBA-K soaking on photosynthetic parameters of rapeseed seedlings under NaCl stress

Under salt stress, the chlorophyll a (Chl a) and chlorophyll b (Chl b) levels in Huayouza 158R rapeseed seedlings decreased significantly in comparison to the control (Fig. 4A, C). However, on day 24, their Chl a and Chl b levels increased markedly. Relative to the CK, salt stress lowered the Chl a and Chl b levels in Huayouza 62 by an average of 7.16% and 11.52%, respectively, between days 14–24 (Fig. 4B, D). After IBA-K treatment resulted in substantial increases in Chl a and Chl b levels for both rapeseed varieties, Huayouza 158R and Huayouza 62, at day 24, with increases of 31.79%, 26.10%, 24.04%, and 27.18%, respectively, compared to the salt treatment (Fig. 4). The I treatment yielded similar enhancements in Chl a and Chl b content for both varieties of rapeseed seedlings, relative to the CK (Fig. 4).

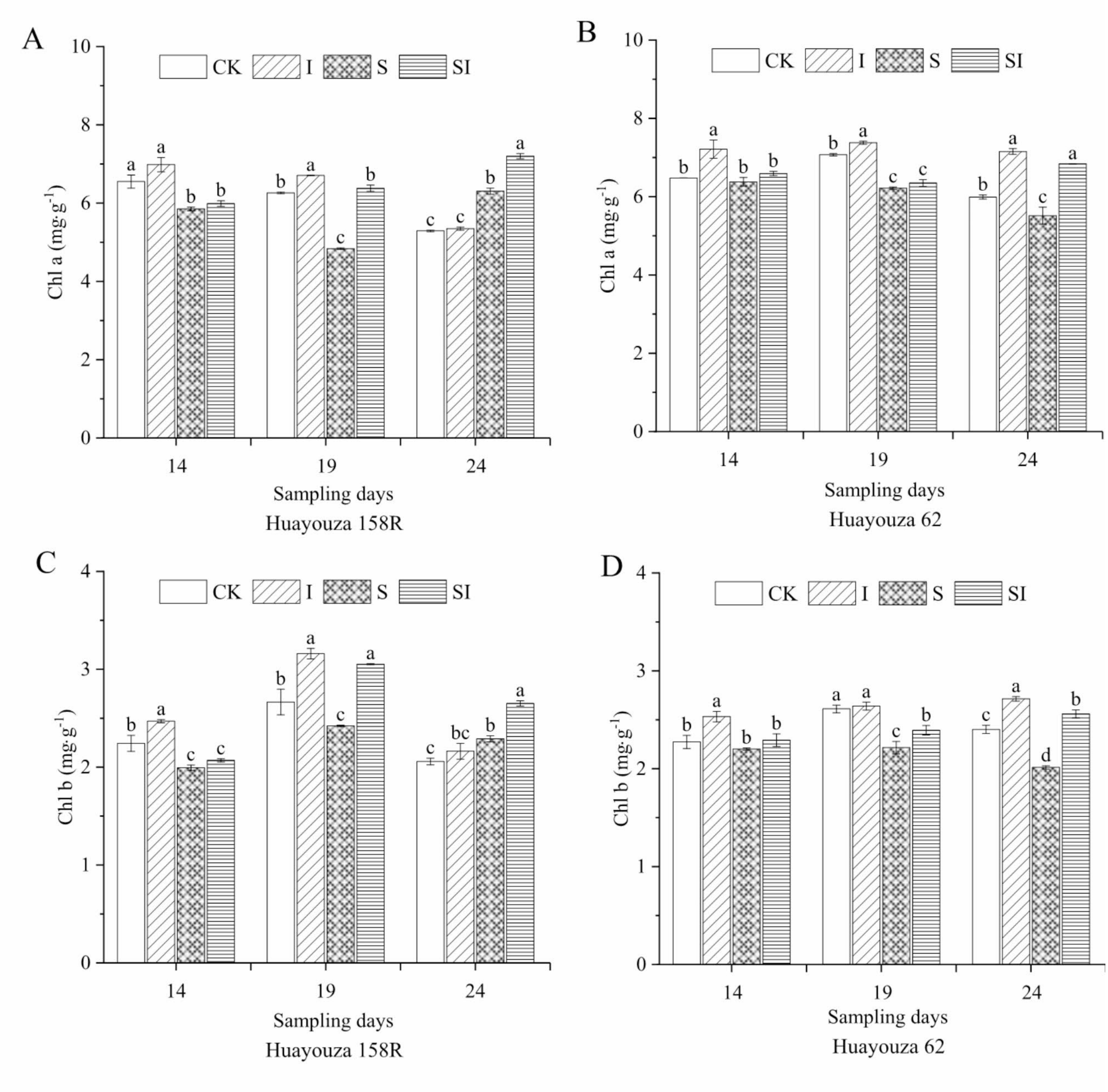

Fig. 4 Effects of IBA-K soaking of seeds on chlorophyll a (**A**, **B**) and chlorophyll b (**C**, **D**) in rapeseed seedlings under NaCl stress. CK: Control, soaked seeds in deionized water; I: 20 mg·L^{-1} IBA-K soaked; S: 150 mmol·L^{-1} NaCl stress + soaked seeds in deionized water; SI: 150 mmol·L^{-1} NaCl stress + 20 mg·L^{-1} IBA-K soaked; Data represent means ± SD, *n* = 3. According to Duncan's test, different lowercase letters indicate significance between treatments ($P<0.05$)

The results illustrated in Fig. 5 reveal the impact of NaCl stress on photosynthesis-related characteristics in rapeseed seedlings. An evident correlation was observed between time, NaCl stress treatment, IBA-K soaking, and photosynthetic traits. The stress treatment resulted in a decrease in photosynthetic traits, such as transpiration rate (*Tr*), net photosynthetic rate (*Pn*), stomatal conductance (*Gs*), and intercellular CO_2 concentration (*Ci*) in both varieties of rapeseed seedlings compared to the control CK. Compared to CK, Photosynthetic indicators of both rapeseed seedling varieties showed a marked reduction under salt NaCl stress, with decreases *Tr* by 56.51%, 39.48%, 22.71% (Fig. 5A) and 28.95%, 38.00%, 48.70% (Fig. 5B), *Ci* by 5.52%, 9.04%, 5.00% (Fig. 5E) and 1.83%, 7.41%, 14.81% (Fig. 5F). 50.10%, 4.79%, 4.77% (Fig. 5C) and 28.28%, 6.97%, 22.41% (Fig. 5D) in *Pn*. *Gs* significantly declined as rapeseed plants matured. For Huayouza 158R, *Gs*'s inhibitory effect lessened over time under stress, resulting in a significant reduction of 75.65% on day 14 (Fig. 5G). Conversely, for Huayouza 62, *Gs* inhibition due to NaCl stress positively correlated with stress duration, peaking at day 24 with a notable decrease of 57.52% (Fig. 5H). Compared to the S treatment, the SI treatment enhanced photosynthesis in Huayouza 158R and Huayouza 62. *Pn* rose by 62.33%, 50.86%, 28.14% (Fig. 5C) and 30.83%, 24.32%, 12.37% (Fig. 5D). *Tr* increased by 33.63%, 39.57%, 22.70% (Fig. 5A) and 40.06%, 9.79%, 45.51% (Fig. 5B), respectively, across both rapeseed varieties at days 14–24. *Gs* showed an increase of 54.39%, 84.72%, and 59.77% (Fig. 5G) for Huayouza 158R, showed an increase of 107.94%, 14.61%, and 57.66% (Fig. 5H) for Huayouza 62. Notably, the *Gs* of Huayouza 62 improved significantly on day 14, surpassing twice the value observed in the experiment. When compared to the S treatment, *Ci* in IBA-K treated Huayouza 158R decreased by 0.55% on day 14, but increased by 5.51% and 1.71% on days 19 and 24 (Fig. 5E). For Huayouza 62, *Ci* decreased by 1.36% on day 19, while showing increases of 2.39% and 13.38% on days 14 and 24 (Fig. 5F).

Effect of IBA-K soaking on antioxidant enzyme activities of rapeseed seedlings under NaCl stress

Compared to the CK, the S treatment led to increased activities of Superoxide Dismutase (SOD), Peroxidase (POD), and Catalase (CAT) enzymes in rapeseed. Specifically, there was a 7.85% increase in SOD activity, a 59.28% increase in POD activity, and a 70.88% increase in CAT activity for Huayouza 158R on the 24th day (Fig. 6A, C, E). By the 19th day, Huayouza 62 exhibited significant increases of 26.62%, 50.12%, and 30.73% in SOD, POD, and CAT activities, respectively (Fig. 6B, D, F). The I treatment also resulted in enhanced antioxidant enzyme activities in both rapeseed varieties, with activity levels progressively rising with plant development. The antioxidant enzyme activities in the two rapeseed seedling varieties increased further with IBA-K soaking compared to the S treatment, with better promotion observed on day 14. The activities of SOD, POD, and CAT in Huayouza 158R rose by 11.58%, 20.43%, and 18.14% respectively (Fig. 6A, C, E), while Huayouza 62 increased by 13.13%, 62.46% and 46.13% (Fig. 6B, D, F). Throughout the growth of rapeseed, both varieties exhibited enhanced SOD and POD activities, peaking on day 24 for the SI treatment (Fig. 6A, B, C, D). At this time, the CAT activity of Huayouza 158R also achieved its maximum, whereas Huayouza 62's CAT activity peaked on day 19, showing a significant increase of 47.17% compared to the S treatment.

Effect of IBA-K soaking on the soluble protein of rapeseed seedlings under NaCl stress

Compared to the CK, NaCl stress significantly decreased the soluble protein levels in Huayouza 158R by 10.36%, 14.56%, and 24.57% at different time points, peaking on day 24 (Fig. 7A). In contrast, soluble protein content in Huayouza 62 rapeseed seedlings displayed varying responses to NaCl stress. Under saline treatment, the protein content increased by 3.00% relative to the CK at day 14 (Fig. 7B) but subsequently decreased significantly by 16.73% and 0.82% at days 19 and 24, respectively (Fig. 7B). Furthermore, when comparing IBA-K treatment to saline treatment, the soluble protein content of both rapeseed varieties increased markedly by 72.03%, 2.66%, 15.42%, and 58.03%, 30.07%, 21.63%, respectively((Fig. 7A, B).

Discussion

Morphological traits of plants serve as vital indicators for assessing plant stress. A reduction in both size and biomass plays a crucial role in the adaptive strategies of plants under stress conditions. This study found that NaCl significantly diminished growth parameters, including plant height, stem diameter, leaf area, and biomass in rapeseed seedlings (Table 2 and Fig. 1). These findings underscore the inhibitory impact of salt stress on the growth of rapeseed, aligning with previous research by Zhang et al. [35] and Sanjaya et al. [36], whose their research confirms demonstrated substantial decreases in the growth parameters of rapeseed under high salt stress. Further results indicated that IBA-K soaking treatment enhanced growth characteristics and increased above-ground and root biomass in rapeseed (Table 2; Fig. 2). This suggests that IBA-K mitigates the detrimental effects of NaCl stress on the plant. Notably, Yu et al. [37] reported that IBA-K treatment resulted in greater biomass accumulation in soybeans, particularly in root fresh weight, compared to salt stress alone. However, this study revealed that IBA-K application under salt stress

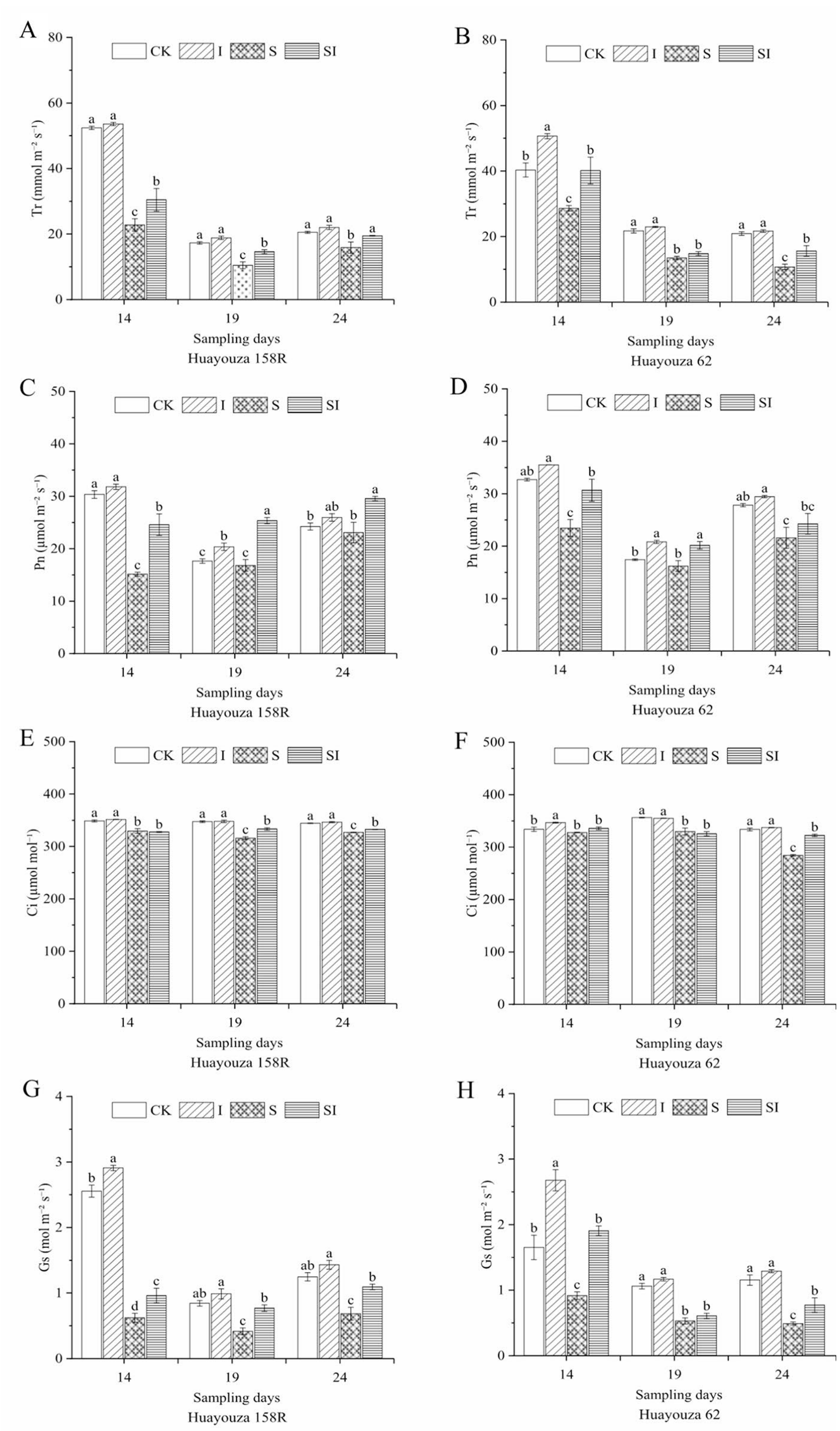

Fig. 5 Effects of IBA-K soaking on *Tr* (**A**, **B**), *Pn* (**C**, **D**), *Ci* (**E**, **F**) and *Gs* (**G**, **H**) of rape seedlings under NaCl stress. CK: Control, soaked seeds in deionized water; I: 20 mg·L^{-1} IBA-K soaked; S: 150 mmol·L^{-1} NaCl stress + soaked seeds in deionized water; SI: 150 mmol·L^{-1} NaCl stress + 20 mg·L^{-1} IBA-K soaked; Data represent means ± SD, *n* = 3. According to Duncan's test, different lowercase letters indicate significance between treatments ($P<0.05$)

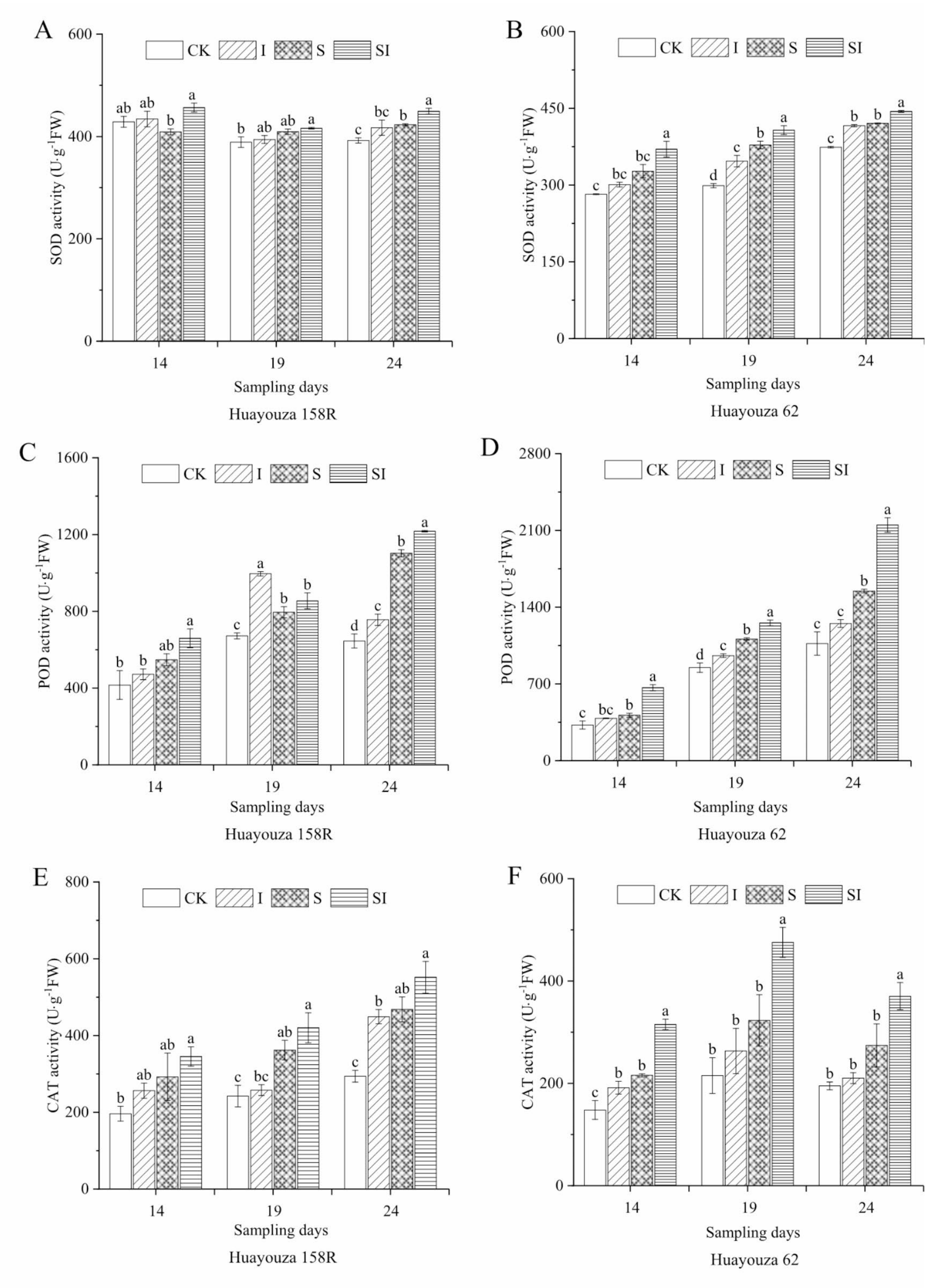

Fig. 6 Effects of IBA-K soaking on SOD (**A**, **B**), POD (**C**, **D**), CAT (**E**, **F**) of rape seedlings under NaCl stress. CK: Control, soaked seeds in deionized water; I: 20 mg·L^{-1} IBA-K soaked; S: 150 mmol·L^{-1} NaCl stress + soaked seeds in deionized water; SI: 150 mmol·L^{-1} NaCl stress + 20 mg·L^{-1} IBA-K soaked; Data represent means ± SD, *n* = 3. According to Duncan's test, different lowercase letters indicate significance between treatments (*P*<0.05)

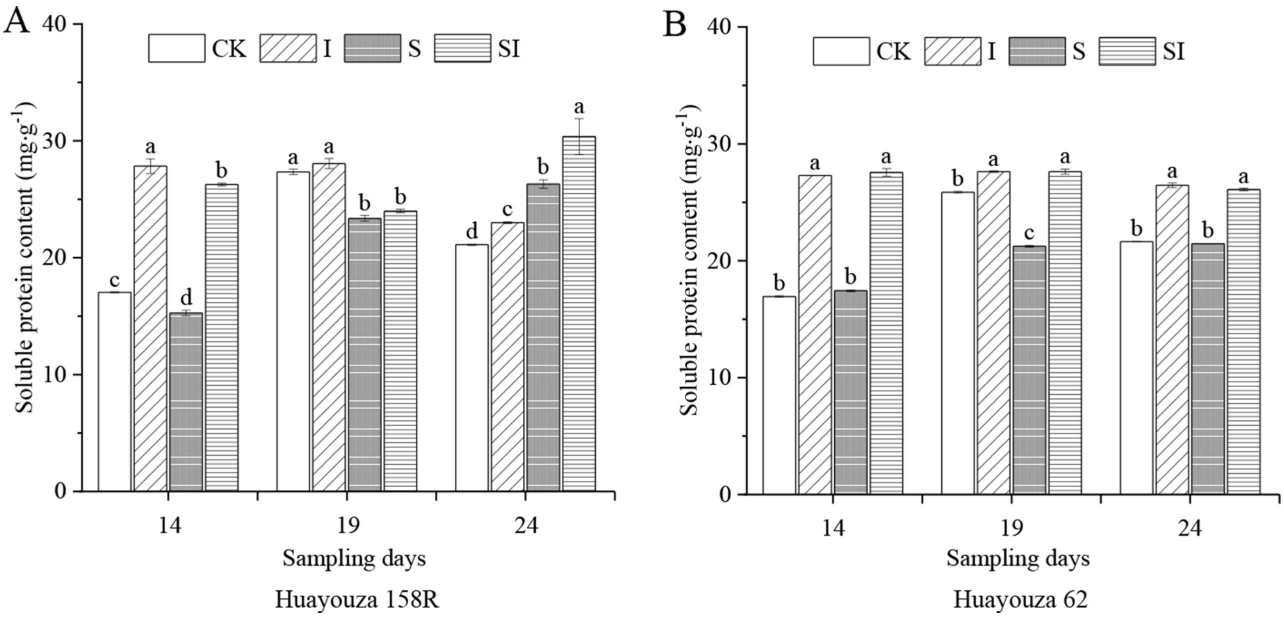

Fig. 7 Effects of IBA-K soaking on soluble protein content（**A**, **B**）of rape seedlings under NaCl stress. CK: Control, soaked seeds in deionized water; I: 20 mg·L^{-1} IBA-K soaked; S: 150 mmol·L^{-1} NaCl stress+ soaked seeds in deionized water; SI: 150 mmol·L^{-1} NaCl stress +20 mg·L^{-1} IBA-K soaked; Data represent means ± SD, n = 3. According to Duncan's test, different lowercase letters indicate significance between treatments ($P < 0.05$).

led to a marked increase in leaf area and below-ground dry weight of both varieties of rapeseed, with average increases of 22.62%, 41.58%, 48.88%, and 51.25% relative to other morphological traits and biomass (Fig. 2). This enhancement may be due to the ability of IBA-K to alleviate the adverse effects of salt stress on root growth, thereby promoting nutrient uptake and increasing dry matter accumulation in rapeseed [38].

The findings of the research indicated that the concentrations of chlorophyll a and chlorophyll b were diminished in the seedlings of both varieties of rapeseed subjected to NaCl stress (Fig. 4), which was consistent with the results of Tang et al. [39] that salt stress reduced the chlorophyll content of cucumber. This reduction may be attributed to diminished chlorophyll biosynthesis as well as structural damage to the chloroplasts under saline stress [40]. However, the difference was that on day 24, the Chl a and Chl b contents of Huayouza 158R increased (Fig. 4), while photosynthesis decreased, which might be because high salt stress reduced the leaf area of rapeseed, the leaves thickened and the photochemical processes were altered [37, 41], and to maintain the normal physiological activities and photosynthesis, Huayouza 158R mitigated the damage caused by the increase in the chlorophyll content. Both IBA-K increased the contents of Chl a and Chl b in seedlings of both varieties of rapeseed (Fig. 4), which may be because IBA-K soaking treatment mitigated the damage to chloroplasts caused by NaCl stress and promoted the accumulation of photosynthetic pigments. In the findings of this investigation, *Tr*, *Pn*, *Ci*, and *Gs* exhibited reductions averaging 39.57%, 19.88%, 6.52%, and 57.15% for Huayouza 158R, and 38.55%, 19.22%, 8.02%, and 50.69% for Huayouza 62, respectively, when compared to the control group under NaCl stress (Fig. 5). Notably, Gs demonstrated the most significant decrease, highlighting that the primary factor contributing to diminished photosynthesis under saline conditions is associated with stomatal opening and closing mechanisms. Our observations align with the findings reported by Dhokne et al. [42] and Moradi et al. [43], which indicated a marked decrease in the photosynthetic efficiency of pea and rice plants subjected to high salinity. This may be due to elevated soil salinity resulting in water loss from protective cells, thus reducing cell expansion rates and leaf stomatal openings, consequently leading to a decrease in internal CO_2 concentration, which could not satisfy photosynthetic demands, thereby causing a decline in net photosynthesis rates, inhibiting plant growth and ultimately diminishing biomass [44, 45]. In this study, the application of IBA-K treatment markedly enhanced *Tr*, *Pn*, and *Gs* across both rapeseed varieties (Fig. 5), implying that IBA-K treatment may promote photosynthesis in rapeseed by reducing water loss, facilitating stomatal opening, and allowing for greater CO_2 ingress into the cells, and sustaining elevated levels of *Tr* and *Gs* [46]. Furthermore, IBA-K soaking applied in this investigation significantly improved Chl a and Chl b concentrations (Fig. 4), leaf surface area (Table 2), above-ground biomass (Fig. 2A, B, E, F), and antioxidant enzyme activities (Fig. 6) across both cultivars of rapeseed seedlings. This indicates that IBA-K may also enhance photosynthetic efficiency by facilitating the development of photosynthetically active leaf area, increasing leaf biomass, boosting chlorophyll levels, stimulating antioxidant enzyme activities in rapeseed, and mitigating the formation of reactive oxygen species in leaves, and enhancing water use efficiency by elevating transpiration rates and stomatal conductance, thereby reducing the adverse effects of saline stress on rapeseed and fostering growth under such conditions [47, 48]. The application of IBA-K, when soaked, promotes photosynthetic activity in salt-stressed rapeseed, which is critical for augmenting the accumulation of photosynthetic products and ultimately enhancing the yield of rapeseed in saline environments.

In this investigation, the build-up of EL and MDA levels in young rapeseed plants exposed to NaCl stress rose significantly (Fig. 3). Indicating a high rate of membrane lipid peroxidation in rapeseed. That is likely due to alterations in lipids and proteins in the cytoplasmic membranes induced by salt stress, leading to compromised permeability of the plasma membranes [49, 50]. SOD, POD, and CAT were crucial protective enzymes in plants, and their activity was enhanced by NaCl stress (Fig. 6). The antioxidant enzyme activities of the two rapeseed plant varieties gradually increased as the duration of NaCl stress prolonged, indicating an improved antioxidant capacity that effectively reduced oxidative stress and alleviated membrane lipid oxidation [51]. Comparable findings were reported by Jovicic et al. [52] and Dai et al. [53], who established a strong correlation between antioxidant enzyme activity and salt tolerance. The SOD, POD, and CAT activities in the SI treatment exhibited additional enhancements compared to the S treatment (Fig. 6), At the same time, MDA and EL levels decreased significantly in both cultivars(Fig. 3). Our research findings are supported by Huang et al. [38], who demonstrated that IBA-K substantially increases the activity of antioxidant enzymes while concurrently lowering MDA levels, this phenomenon may be ascribed to IBA-K's potential to directly neutralize reactive oxygen species (ROS) through the enhancement of antioxidant enzyme function, or by suppressing the pathways responsible for the production of ROS and MDA, thus reducing lipid peroxidation and preserving plasma membrane integrity. These findings illustrate the potent anti-stress properties of IBA-K, underscoring its ability to scavenge reactive oxygen species, improve rapeseed's antioxidant capacity, increase stress resistance, and reduce lipid oxidation to enhance membrane stability, combatted salt-induced oxidative stress, maintaining cellular functions at equilibrium [27].

Soluble proteins are vital osmoregulatory compounds, and nutrients are intricately linked to cellular water retention capacity, sustenance, and biofilm stability, their synthesis and significant accumulation during salt stress play a crucial role in mitigating stress-induced damage to plants [54, 55]. In our research, the soluble protein levels of Huayouza 158R under NaCl stress (Fig. 7) exhibited a pattern of initial decrease followed by an increase as rapeseed plants grew, peaking on the 24th day. Conversely, Huayouza 62 showed a decline in soluble protein content over time under stress conditions (Fig. 7). The observed variations are likely attributable to the differential salt tolerance exhibited by various cultivars, each possessing distinct response mechanisms. This aligns with the findings of Zhao et al. [56], in which the soluble protein content in Huayouza 158R subjected to NaCl stress decreased from day 4 to day 10, followed by a significant increase on the 13th day. Additionally, prior research indicates that salt stress adversely affects the protein synthesis pathway, hastening protein hydrolysis and resulting in a considerable accumulation of amino acids, ultimately bringing about a reduction in protein content [57]. This may be the reason for the decrease in soluble protein content in rapeseed. In this investigation, both IBA-K treatments markedly elevated the soluble protein content in seedlings of both rapeseed varieties (Fig. 7). This suggests that IBA-K soaking facilitates the ongoing accumulation of soluble proteins in rapeseed, thereby supporting the natural operation of osmotic regulation mechanisms, ensuring water balance within plants, and bolstering their salt tolerance. Comparable findings were reported by Hang Zhou et al. [27], reinforcing the conclusion that IBA-K enhances soluble protein levels and augments protein synthesis, thereby boosting the salt tolerance of rice. This phenomenon may be attributed to IBA-K's role in facilitating the synthesis and accumulation of osmoregulatory compounds in rapeseed when subjected to salt stress, consequently enhancing the cells' water absorption and retention capabilities. This mechanism aids in preserving cellular osmotic pressure, thereby supporting the normal physiological development of rapeseed [58]. Nonetheless, the influence of IBA-K on other osmoregulatory substances requires additional examination. Figure 8 illustrates the associated regulation of the bolstered antioxidant system, preservation of osmotic equilibrium, enhancement of photosynthetic activity, and the mitigation of plant growth inhibition in rapeseed seedlings following IBA-K soaking under NaCl stress.

Conclusion

In conclusion, 20 mg·L^{-1} concentration IBA-K soaking had the most obvious promotional effect on rapeseed, which significantly increased the growth parameters and biomass of rapeseed seedlings. Salt stress inhibited the natural growth of rapeseed. It also reduces the biomass by disrupting osmotic homeostasis and by reducing photosynthesis through oxidative stress. Through 20 mg·L^{-1} IBA-K soaking treatment, accumulated soluble

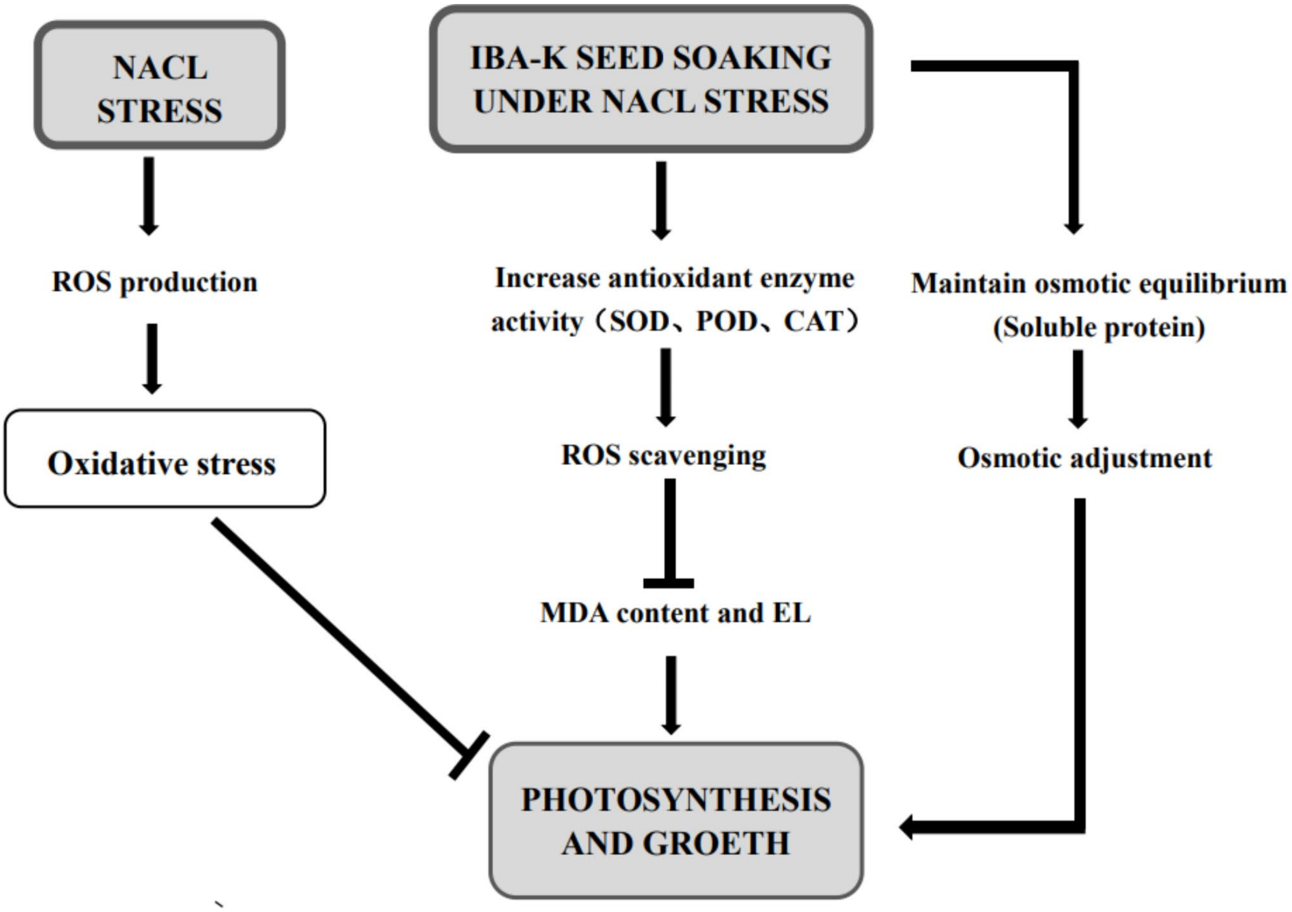

Fig. 8 IBA-K improves photosynthesis and alleviates the growth of rape under salt stress by enhancing the antioxidant system and maintaining osmotic balance. The arrows in the figure represent lifting, and the suppress arrows represent suppressing

proteins of rapeseed seedlings, enhanced the activities of antioxidant enzymes such as CAT, POD, and SOD, significantly reduced salt stress-induced oxidative stress, increased chlorophyll content, improved photosynthesis, and alleviated the inhibition of salt stress on the growth of rapeseed, and improved the salt tolerance of rapeseed. Consequently, the findings of this research indicated that IBA-K significantly contributed to the growth enhancement of rapeseed under NaCl stress and improved its salt tolerance. This insight is anticipated further to elucidate the mechanisms of salt tolerance in rapeseed and establish a basis for its cultivation in saline environments.

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Author contributions
J.L. conducted the experiment and wrote the manuscript. X.D., J.W. and X.W. help collect data. N.F. and D.Z. advise on the scientific method and provide background knowledge. N.F. oversees and funds all experiments. All authors contributed to the article and reviewed the submitted version.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable. All experimental studies on plants were complied with relevant institutional, national, and international guidelines and legislation.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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