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Response of Different Exogenous Phytohormones to Rice Yield Under Low-Temperature Stress at the Filling Stage

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Abstract: This paper aims to clarify the effects of different exogenous phytohormones on the physiological traits of rice (*Oryza sativa* L.) at the early stage of irrigation under low-temperature stress. In this study, two types of rice varieties with different temperature sensitivities screened out previously, namely, a cold-tolerant variety (Nan Jing 9108) and a low-temperature-sensitive variety (Hui Liang You 898), were used in pots to simulate the process of low-temperature stress in rice at the early stage of grouting (6–9 days after anthesis) with artificial low-temperature treatments. The experimental treatments were 450 mg L^{-1} Methyl jasmine acid (MJ), 46 mg L^{-1} Melatonin (MT), 69 mg L^{-1} Salicylate (SA), 40 mg L^{-1} Erythromycin (GA_3), 25 mg L^{-1} Zeatin (Z), 145 mg L^{-1} Spermidine (SPD), and 5 mg L^{-1} Abscisic acid (ABA) sprayed on rice before low-temperature stress, while low-temperature treatment without spraying (DK) and conventional planting without spraying (CK) were added as the control. The results showed that compared with the room temperature control (CK, sprayed with deionized water), the low-temperature control (DK, low-temperature treatment, and sprayed with deionized water) all significantly reduced the rice grain yield. Different exogenous hormones sprayed before low-temperature stress could increase rice yield, among which, Z and SPD spraying treatments had a better effect on the yield of Hui Liang You 898, while different exogenous hormone treatments increased the yield of Nan Jing 9108 in an average manner. The Z and SPD treatments increased the yield of Hui Liang You 898 by 24.87% and 26.16% and that of Nan Jing 9108 by 15.87% and 17.80%, respectively. This was mainly attributed to the significant increase in thousand-grain weight and fruiting rate, while there was no significant difference in the number of spikes and number of grains. The different exogenous hormone treatments were able to delay leaf senescence, enhance the photosynthetic production capacity of plants by increasing leaf chlorophyll content, and thus increase the accumulation of photosynthetic assimilation products and population growth rate after flowering. Among them, both Z and SPD treatments resulted in a population growth rate of more than 30% from spike flushing to maturity, which led to a higher dry matter accumulation of the plant at maturity. In addition, in the dry matter distribution of the plant at maturity, the seeds occupied a higher accumulation amount and proportion compared with the respective DK; the SPD treatment resulted in the maximum distribution rate of seeds at maturity of Hui Liang You 898, with an increase of 8.27%, and the Z treatment resulted in the maximum distribution rate of seeds at maturity of Nan Jing 9108, with an increase of 7.34%. At the same time, the Z treatment significantly increased the activities of adenosine diphosphate glucose phosphorylated enzyme (AGP) and starch branching enzyme (SBE) in the grains of both varieties, which resulted in the accumulation of more starch and ultimately increased the rice grain yield. The results verified that different exogenous phytohormones could be used to regulate the insufficiency of grouting caused by low-temperature stress during the grouting and fruiting stages of rice and enriched their agronomic and physiological traits in response at the same time.

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1. Introduction

Extreme global climate change has been one of the serious challenges of agriculture in recent years, especially in 2023, which is the second year of El Niño and also the year of La Niña development; as such, the frequency, degree and duration of extreme temperature occurrences are intensifying. For efficient physiological functioning, all plant species have an optimum temperature range for growth, development and reproduction, above or below, which can negatively affect plant performance and result in economic yield losses [1]. Rice (*Oryza sativa* L.) is a thermophilic crop that is sensitive to low temperatures, which, during growth and development, can cause physiological and metabolic disorders in the plant, decrease photosynthetic capacity, inhibit normal development of rice, affect yield formation, and thus limit the play of its yield potential [2,3]. In particular, low temperatures at the beginning of irrigation will cause carbon metabolism dysfunction in the plant, lack of energy and material supply to the organs, imbalance of the source library, growth and development hindered, and ultimately cause a decline in rice yield [4].

Natural phytohormones in plants, as well as synthetic compounds with physiological activity that play a role in regulating plant growth and development, are known as plant growth substances [5], which play an important role in regulating low-temperature stress in crops [6]. It was found that most of the phytohormone biosynthesis and signal transduction genes were up-regulated under low-temperature stress, and hormone-mediated defense mechanisms played an important role in low-temperature stress. These complex physiological processes may be both interconnected or independent of each other, constituting a self-regulatory network of rice under low-temperature adversity [7]. External regulation of phytohormones can effectively improve the chlorophyll content, aboveground biomass and grouting rate, thereby improving rice yield and quality [8]. The common plant growth substances are auxin (AUX), gibberellin (GA), abscisic acid (ABA), cytokinin (CTK), salicylic acid (SA), ethylene (ETH), jasmine acid (JA), flavonoid sterols (BR) and peptide hormones [9], whereas AUX, CTK, GA, SA, and BR are usually considered as hormones promoting plant growth and development [10]. MJ is involved in the regulation of plant growth and development as well as the alleviation of plant heavy metal stress, extreme temperature stress, etc. [11]. Exogenous ABA-soaked root treatments can induce the expression of ABA-synthesizing genes and inhibit ABA-catabolizing genes with a view to enhancing the cold tolerance of rice seedlings [12].

Moreover, some studies found that melatonin (MT) and spermidine (SPD), as new types of phytohormones involved in plant growth and development, have the ability to scavenge free radicals and improve plant stress tolerance [13] and that the seed-free SPD content was significantly or highly significantly positively correlated with the rate of grouting [14]. Previous studies have mainly focused on the effects of individual phytohormones on agronomic and physiological traits in rice [15–17], and less attention has been paid to the comparison between the responses of multiple exogenous hormones to low-temperature stress. Are there consistent responses of different exogenous phytohormones to the yields of hybrid and conventional Japonica rice during the grouting period under low-temperature stress? What are the physiological mechanisms of high yield? There are a few reports so far [18–21].

In order to address the above problems, the present study took two varieties of low-temperature-sensitive (Hui Liang You 898) and cold-resistant (Nan Jing 9108) as materials, simulated cold damage during the critical fertility period of rice yield formation through potting experiments, investigated the agronomic and physiological traits under low-temperature stress with different exogenous phytohormones, such as MT, MJ, SPD, Z, SA, ABA, and GA_3, and observed the relationship between the morpho-physiological indicators and yield formation during the filling period. We also observed the relationship between morpho-physiological indexes and yield formation during the filling period. It is hoped that the most suitable exogenous phytohormone to enhance the grain quality of rice under low-temperature stress can be identified. This study explores the suitable hormone-spraying method to mitigate the reduction of rice yield under low-temperature stress during the filling stage with a view to provide a theoretical basis and practical guidance for the application of anti-stress and stable yield cultivation techniques in rice.

2. Materials and Methods

2.1. Test Site and Test Material

The experiment was carried out in 2022 and 2023 at the Chengnan Experimental Base of Huaiyin Institute of Agricultural Science, Xuhuai District, Jiangsu Province (33°52′ N, 119°03′ E). The study area is located in the Jianghuai Plain, and the terrain is extremely flat, with an average elevation of 11 m. It has a northern subtropical humid monsoon climate, abundant rainfall and four distinct seasons, with an annual average temperature of 14.7 °C, an annual average of 2055 h of sunshine, and an average of 927 mm of precipitation. The varieties tested were the representative rice varieties of Hui Liang You 898 and Nan Jing 9108, and exogenous plant hormones were purchased from Shanghai Aladdin Biochemicals (Shanghai, China) and Shanghai Macklin Biochemicals & Technology Co., Ltd. (Shanghai, China). The exogenous plant hormones were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China).

The experiment was carried out in a pot-planting trial. Seedlings were planted in the open field in a wet nursery, sown on 12–15 May, and transplanted into a potting bowl every year on 13–14 June. Seedlings were planted in three holes per pot; all of them were double-planted, 9 pots for each treatment, a total of 144 pots, with a diameter of 32 cm, a height of 34 cm, and filled with 23 kg of sifted fine soil (taken from the soil of a nearby field, sandy loam with medium acidity), with soil organic matter of 21.3 g kg^{-1}, quick-acting nitrogen (QN) of 100.6 mg kg^{-1}, quick-acting phosphorus (QAP) of 24.7 mg kg^{-1}, and quick-acting potassium (K) of 97.2 mg kg^{-1}.

The soil was applied according to the standard of nitrogen and fertilizer transportation for local high-yield cultivation. Referring to the local high-yield cultivation nitrogen fertilization standard, the base fertilizer was 10 g of compound fertilizer (N, P, K mass ratio of 15:15:15) per pot, and the tiller fertilizer was 1.2 g of urea per pot. Field management was the same as local conventional high-yield cultivation, and the dates of each major fertility period during rice growth were recorded in real time. Hui Liang You 898 was harvested on 20 October 2022 and 18 October 2023, and Nan Jing 9108 was harvested on 24 October 2022 and 23 October 2023, respectively.

2.2. Exogenous Phytohormone Treatment

In 2022 and 2023, the experimental design was the same. A total of 70 days after rice transplanting (6–9 days after flowering and at the early stage of irrigation), seven exogenous phytohormone spray treatments were applied to the whole rice leaves using a sprayer. These spray treatments included methyl jasmine acid (MJ), melatonin (MT), salicylic acid (SA), gibberellin (GA_3), zeatin (Z), spermidine (SPD), and abscisic acid (ABA), with the aim of spraying the whole leaves with the following phytohormone treatments, and deionized water sprayed as control (CK). Based on the previous study, the spraying concentrations are shown in Table 1, which were pre-dissolved in ethanol before spraying and then diluted to a final ethanol concentration of 0.2% (v/v), and the final solution used for spraying contained 0.02% (v/v) of Tween-20 as the surfactant [10]. The control was an equal volume of deionized water, which also contained the same concentrations of ethanol and Tween-20. Hormone spray treatments were applied at a rate of 100 mL^{-1} for 3 consecutive days after 6:00 p.m. daily, and the spraying process was covered using baffles to prevent cross-impact.

Table 1. Exogenous plant hormones for testing.

Exogenous Plant Hormone	Dosage
MJ	450 mg L^{-1} [22]
MT	46 mg L^{-1} [23]
SA	69 mg L^{-1} [24]
GA_3	40 mg L^{-1} [25]
Z	25 mg L^{-1} [10]
SPD	145 mg L^{-1} [26]
ABA	5 mg L^{-1} [27]

2.3. Low-Temperature Treatment

After the spraying treatment, the potted plants were moved to the artificial climate chamber for low-temperature treatment, where different temperatures were set for daytime and nighttime (15 °C during the day and 10 °C at night). One set of filler lights was on during the daytime (6:00–18:00) with the relative humidity set at about 70%, and the filler lights were turned off during the nighttime (18:00–6:00) with the relative humidity set at about 80%. The treatment was continued for 5 days, and then the plants were removed from the artificial climatic chamber and placed outdoors at room temperature (Figures 1 and 2).

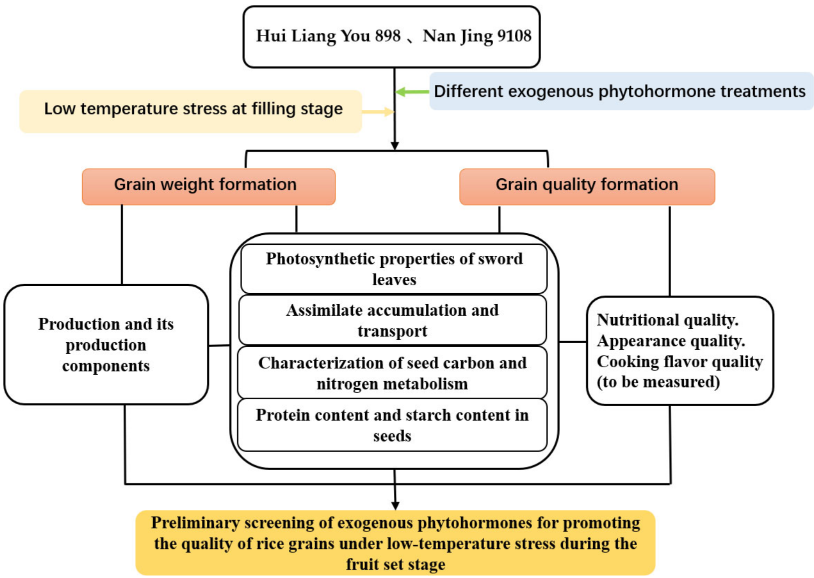

Figure 1. Experimental technical design drawings.

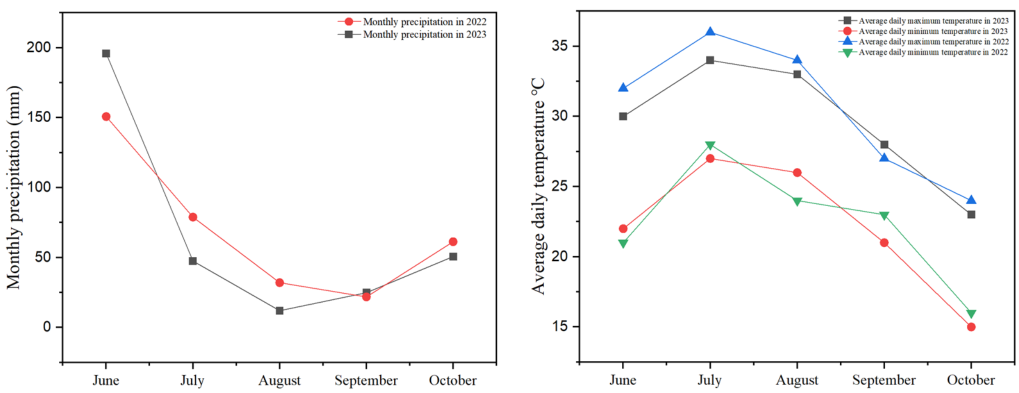

Figure 2. Average temperature and precipitation during the rice-growing season.

2.4. Determination of Yield and Yield Components

Three pots of each treatment were sampled at maturity, and the seeds were tested indoors to determine and calculate the number of primary pedicels, number of secondary pedicels, number of empty shells of primary pedicels, number of empty shells of secondary pedicels, number of effective spikes, number of kernels per spike, thousand-grain weight, and fruiting rate.

2.5. Determination of SPAD Value

The chlorophyll content was measured by a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan) on the 7th (D1), 14th (D2), and 21st (D3) days of low-temperature treatment during the filling period. The chlorophyll content was measured three times—at the top, middle and bottom of the sword leaf, respectively. The average was taken as the SPAD value of this leaf; each treatment was measured nine times, and the average was taken as the SPAD value of this treatment [22].

2.6. Determination of Dry Matter Accumulation and Transportation

At the flush and maturity stages, three pots were sampled for each treatment. All sampled plants were divided into three parts: stems (sheaths and culms), leaves, and seeds after removing the underground parts. They were heated at 105 °C for 30 min, and then weighed and dried at 80 °C to a constant mass, and then calculated with reference to the methods of [28,29]:

Pre-flowering nutrient organ assimilate transfer = dry matter weight of nutrient organs at flush stage—dry matter weight of nutrient organs at maturity stage;

Contribution of pre-flowering assimilate translocation to seed yield = pre-flowering dry matter translocation/seed dry matter weight at maturity × 100%;

Post-anthesis dry matter accumulation = dry matter accumulation at maturity—dry matter accumulation at flush;

Contribution of post-flowering dry matter accumulation to seed yield = post-flowering dry matter accumulation/seed dry matter weight at maturity × 100%;

Population growth rate [g/(m^2 d)] = (W2 − W1)/(t2 − t1): where W1 and W2 are the amount of dry matter measured before and after two times, and t1 and t2 are the time of the two measurements before and after.

2.7. Determination of Total Starch Content of Seeds

Starch is the main storage form of sugar in plants, and its content determination is important for investigating the sugar metabolism in plants [30]. For starch content determination, 0.1 g of the sample was weighed, and three replicates, using the Suzhou Ke Ming kit (Keming Company, Suzhou, China), 50 µL of the sample and 250 µL of working solution were taken into EP tubes. The sample was water-bathed at 95 °C for 10 min (capped tightly to prevent water dispersion), naturally cooled to room temperature, and then washed with water. Then, 50 µL of the sample and 250 µL of working solution were put into an EP tube placed in a water bath at 95 °C for 10 min (covered tightly to prevent water dispersion). Then, the tub was cooled to room temperature naturally, 200 µL was put into a micro-quartz cuvette or 96-well plate, and the absorbance value was recorded at the wavelength of 620 nm.

2.8. Determination of Soluble Protein Content

After the harvested rice was stored for 3 months, the quality indicators were measured after the physical and chemical properties of the rice were stabilized. An amount of 0.1 g of the sample was weighed for soluble protein content determination with three repetitions. Then, the Suzhou Ke Ming BCA method protein content determination kit was used and calculated according to Cp (mg/g fresh weight) = 0.5 × (A assay tube − A blank tube) ÷ (A standard tube − A blank tube) ÷ 0.1. Under alkaline conditions, cysteine, cystine, tryptophan, tyrosine, and peptide bonds in proteins were reduced from Cu^{2+} to Cu^{+};

two molecules of BCA were bound to Cu^+ to form a purple complex with absorption peaks at 540–595 nm, with the strongest at 562 nm.

2.9. Determination of Seed Adenosine Diphosphate Glucose Phosphorylated Enzyme (AGP) Activity

Adenosine diphosphate phosphorylated enzyme (AGP) activity was determined using the corresponding kit with reference to the methods of Douglas et al. [31] and Cheng Fang Min et al. [32]. The main assay procedure was as follows: 20 µL of crude extract of the enzyme was added to 110 µL of reaction solution (the final concentration was 100 mmol/L Hep NaOH (pH = 7.4), with 1.2 mmol/L AGPG, 3 mmol/L PPI, 5 mmol/L $MgCl_2$, and 4 mmol/L DTT). After 20 min at 30 °C, the reaction was terminated for 30 s in boiling water at 10,000× *g* for 10 min. An amount of 100 µL of supernatant was taken, and 5.2 µL of colorimetric solution (5.76 mmol/L NAGP, 0.08 unit glucose converting enzyme, 0.07 unit G6P-dehydrogenase) was added, and the reaction was carried out at 30 °C for 10 min, and then the 340 nm OD value was determined.

2.10. Determination of Seed Starch Branching Enzyme (SBE) Activity

Refer to the method of Cheng Fang Min et al. [33] to determine the starch branching enzyme activity. An amount of 20 µL of crude enzyme solution was added to 20 µL of the reaction solution, and the final concentration of the reaction solution was 50 mmol/L Hep-NaOH (pH = 7.4), 5 mmol/LGIP, 1.25 mmol/L AMP, and phosphorylase (5.4 units). The solution sat for 30 min at 30 °C following the termination of the reaction, then 10 µL of 1 mol/L HCl, 100 µL of Dimethyl sulfoxide, 140 µL of 0.1% I_2, and 1% KI were added, and the 540 nm OD was determined. After 30 min at 30 °C, the reaction was terminated with 10 µL of 1 mol/L HCl, then 100 µL of Dimethyl sulfoxide, 140 µL of 0.1% I_2, and 1% KI were added, and the 540 nm OD was determined.

2.11. Statistics Analysis

Microsoft Excel 2016 was used for data entry and calculation, and the SPSS 22.0 (IBM Corp, Armonk, NY, USA) data processing system was used for statistical analysis. An analysis of variance (ANOVA) was performed, followed by multiple comparison tests ($p < 0.05$). Different letters represent significant differences between different exogenous phytohormone treatments of the same variety. The graphs were created using Origin 8.0 (Origin Lab, Northampton, MA, USA).

3. Results

3.1. Effect of Different Exogenous Hormones on Yield and Yield Structure of Rice

Analyzing the yield and its components, it can be found (Table 2) that both Hui Liang You 898 and Nan Jing 9108's DK treatments significantly reduced their seed yields compared with CK. The use of different exogenous hormones for spraying treatments before reaching low temperatures could increase rice yield to different degrees. Compared with HDK plants, the seed yield of Hui Liang You 898 increased in all treatments, and the maximum yield was obtained in Z and SPD treatments, with significant yield increases of 24.87% and 26.16%, respectively, which were mainly attributed to the increases in thousand-grain weight and firmness, while there was no significant difference in the number of spikes and the number of grains in spikes. Compared with the NDK plants, the yield increase in Nan Jing 9108, except for MJ and MT treatments, amounted to 15.87–22.74%, which was also attributed to the significant increase in thousand-grain weight and fruiting rate, but the overall yield increase was smaller than that of Hui Liang you 898.

Table 2. Effect of different exogenous hormones on rice yield and its components under low-temperature stress.

Rice Varieties	Treatment	Grain Yield (g/Pen)	Panicles (Pen)	Number of Spike Lets per Panicle	1000-Grain Weight (g)	Percentage of Filled Grains (%)
Hui Liang You 898	MJ	171.97 b	39.00 a	193.07 a	26.10 c	86.30 c
	MT	178.63 b	40.33 a	200.87 a	26.90 abc	86.23 c
	SA	175.03 b	40.67 a	198.33 a	26.03 c	86.40 c
	GA_3	172.37 b	40.67 a	190.90 a	26.07 c	86.80 bc
	Z	193.13 a	42.00 a	200.80 a	27.27 ab	88.30 ab
	SPD	195.13 a	41.67 a	205.73 a	27.00 abc	88.23 ab
	ABA	178.43 b	40.33 a	202.63 a	26.67 bc	87.47 bc
	HDK	154.67 c	39.67 a	191.00 a	25.13 d	78.63 d
	HCK	188.53 a	41.67 a	191.23 a	27.77 a	89.80 a
	average	178.66	40.67	197.17	26.55	86.46
Nan Jing 9108	MJ	134.50 bcd	43.33 a	143.87 a	25.40 bc	89.54 ab
	MT	131.67 cd	43.33 a	136.87 a	25.27 bc	87.16 b
	SA	146.13 abc	44.33 a	146.60 a	25.93 ab	90.32 ab
	GA_3	150.07 a	45.00 a	145.20 a	26.60 a	91.63 a
	Z	141.67 abc	45.33 a	139.67 a	26.13 ab	90.97 a
	SPD	144.03 abc	43.00 a	145.97 a	25.97 ab	91.53 a
	ABA	148.60 ab	45.33 a	148.20 a	25.40 bc	89.85 ab
	NDK	122.27 c	44.00 a	140.20 a	24.53 c	82.03 c
	NCK	150.73 a	44.33 a	141.93 a	26.87 a	92.67 a
	average	141.07	44.22	143.17	25.79	89.52
Source of variation	df					
Rice varieties (V)	1	417.031 **	36.191 **	351.597 **	30.408 **	64.25 **
Exogenous hormone treatment (T)	8	12.986 **	0.716 ^{ns}	0.723 ^{ns}	10.973 **	30.216 **
V × T	8	3.439 **	0.398 ^{ns}	0.525 ^{ns}	2.484 *	0.882 ^{ns}

* and ** indicate significant effects at 0.05 and 0.01 levels, respectively. ^{ns} represents no significant difference. MJ, MT, SA, GA_3, Z, SPD, and ABA treatments were low-temperature treatments after spraying methyl jasmine, melatonin, salicylic acid, gibberellic acid, zeatin, spermidine, abscisic acid, respectively. HDK and NDK represent the low temperature planting treatments for Hui Liang You 898 and Nanking 9108, respectively. HDK and NDK were used for Hui Liang You 898 and Nan Jing 9108 low-temperature conventional planting treatments, respectively. No common letter indicates a significant difference at the p = 0.05 level within the same column.

The analysis of variance (ANOVA) of rice yield and its components under different treatments showed that variety and exogenous hormone treatments had significant or highly significant effects on rice grain yield. In terms of yield components, different rice varieties and different exogenous hormone treatments had highly significant effects on thousand-grain weight and fruiting rate. There were highly significant differences in yield components between the hybrid and conventional japonica rice, while different exogenous hormone treatments had no significant effect on the number of spikes and the number of grains in spikes. There were no significant differences in the effects of the two interactions on the yield components except for thousand-grain weight, but the effects on seed yield reached a highly significant level.

3.2. Effects of Different Exogenous Hormones on SPAD Values of Rice Rapeseed Leaves

Regardless of the treatments, there was an overall decreasing trend in the saber SPAD values of the two rice varieties with the increase in treatment duration (Figure 3). Compared with CK, the SPAD values of rapier leaves of two varieties of DK treatments were significantly reduced at 7–21 d after treatment. This indicated that the low-temperature treatment greatly reduced the chlorophyll content of both varieties. Both the Z and SPD treatments increased the SPAD values of both varieties. Among them, the highest SPAD value was found in Hui Two Excellent 898 variety with ABA treatment, indicating that ABA was able to resist low-temperature stress by significantly increasing leaf chlorophyll content.

The SPAD values of different exogenous hormone spraying treatments were significantly higher than those of HDK in Hui Liang You 898 at 7 d after treatment. The SPAD values of all treatments were higher than those of HDK at 14 d and 21 d after treatment except for the SA treatment, which was not significantly different from that of HCK. The SPAD values of Nan Jing 9108 were higher than those of HDK at 7 d, 14 d, and 21 d after treatment, except for SA treatment.

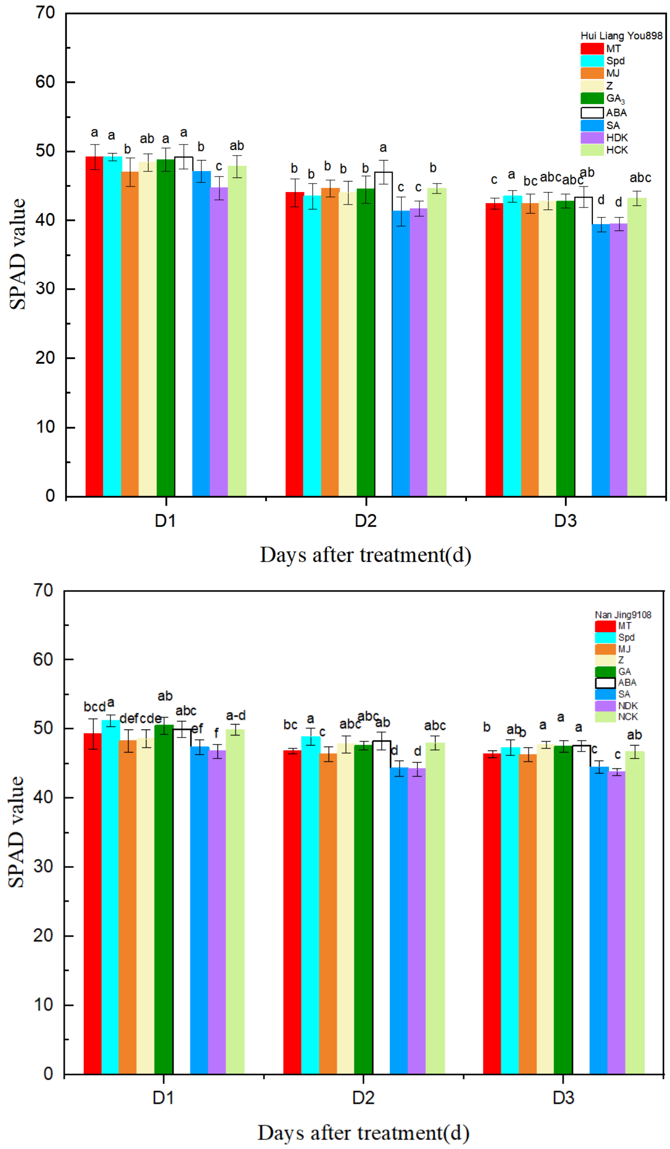

Figure 3. Effects of different exogenous hormones on SPAD values of rice leaves 7 days post-treatment (D1), 14 days post-treatment (D2), and 21 days post-treatment (D3). No common letter above the bars indicates the LSD at p = 0.05 within the same measurement time.

3.3. Effects of Different Exogenous Hormones on Aboveground Dry Matter Partitioning in Rice at the Maturity Stage

As shown in Table 3, compared with CK, the DK treatments all significantly reduced the dry matter accumulation of rice grains in the spike, and Hui Liang You 898 and Nan Jing 9108 reduced by 5.74% and 6%, respectively. Compared with the DK treatment, spraying different exogenous hormones at the early stage of irrigation could increase the dry matter accumulation of the spike grains at maturity; among them, the dry matter accumulation of the spike grains was higher than that of HCK under the Z and SPD treatments, and the SPD treatment reached a significant level in the case of Hui Liang You 898, and the maximum accumulation of the spike grains in the case of Nan Jing 9108 was obtained with the Z treatment, which was greater than that of the NCK treatment. In terms of accumulation ratio, compared with the DK treatment, the dry matter accumulation ratio of nutrient organs at maturity was significantly lower in all treatments, but the dry matter accumulation ratio of grains was significantly higher, indicating that the low-temperature stress adversely affected the growth and development of all organs of the plant, and the spraying of different exogenous hormones had a certain mitigating effect, which was favorable to the formation of yields, and the best effect was obtained with the Z and SPD treatments.

Table 3. Effects of different exogenous hormones on aboveground dry matter partitioning in rice at maturity under low-temperature stress.

Rice Varieties	Treatment	Leaf Distribution Ratio %	Stem Sheath Distribution Ratio %	Spike Distribution Ratio %
Hui Ling You 898	MJ	19.70 bc	32.42 bc	47.88 cd
	MT	19.66 bc	32.69 ab	47.65 d
	SA	19.78 bc	32.11 bc	48.11 cd
	GA3	19.83 bc	32.30 bc	47.87 cd
	Z	19.62 bc	31.69 bcd	48.69 abcd
	SPD	19.56 bc	31.22 cd	49.23 ab
	ABA	19.30 c	32.28 bc	48.42 abcd
	HDK	20.75 ab	33.78 a	45.47 e
	HCK	20.26 abc	31.66 bcd	48.08 cd
	Average	19.83	32.24 cd	47.93
Nan Jing 9108	MJ	20.55 abc	31.37 bcd	48.09 cd
	MT	20.12 abc	31.77 bcd	48.12 cd
	SA	20.36 abc	31.43 cd	48.21 bcd
	GA3	19.78 bc	31.69 bcd	48.53 abcd
	Z	19.31 c	31.29 cd	49.40 a
	SPD	20.20 abc	31.27 cd	48.53 abcd
	ABA	19.56 bc	31.91 bc	48.53 abcd
	NDK	21.23 a	32.75 ab	46.02 e
	NCK	20.56 ab	30.67 d	48.78 abc
	Average	20.18	31.57	48.24
Source of variation	df			
Rice varieties(V)	1	4.296 *	15.334 **	4.501 *
Exogenous hormone treatment (T)	8	3.511 **	6.1 **	19.087 **
V × T	8	0.487 ^{ns}	0.546 ^{ns}	1.049 ^{ns}

No common letter indicates a significant difference at the p = 0.05 level within the same column. * and ** indicate significant differences between the two rice varieties at p = 0.05 and p = 0.01 levels, respectively. ^{ns} means the difference is not significant.

3.4. Effects of Different Exogenous Hormones on Dry Matter Accumulation and Translocation in Rice

As shown in Tables 4 and 5, compared with CK, Hui Liang You 898, except for the Z and SPD treatments, it was found that DK treatments reduced the aboveground dry matter accumulation at maturity and the population growth rate from flushes to maturity to varying degrees, and increased the amount and proportion of pre-flowering dry matter translocation, and compared with the HDK treatments, the Z and SPD treatments could significantly increase the aboveground dry matter accumulation at maturity, the aboveground dry matter accumulation after flowering and the population growth rate, by 13.82%, 15.01%, 30.65%, 31.79%, 30.64%, and 31.77%, respectively. accumulation and population growth rate of 13.82%, 15.01%, 30.65%, 31.79%, 30.64% and 31.77%, respectively. All other exogenous hormone treatments increased dry matter accumulation at maturity and aboveground dry matter accumulation after flowering. Overall, spraying Z and SPD could better promote rice growth and development after low-temperature stress. The overall trends of the two varieties were the same, but the Z and SPD treatments did not have a significant effect on Nan Jing 9108. Compared with NDK, the SA and GA_3 treatments increased the dry matter accumulation and population growth rate at maturity and decreased the amount of nutrient organ transit aboveground before flowering. It indicated that the Z and SPD treatments could increase the accumulation of post-flowering photosynthetic assimilates in Hui Liang You 898 and increase the plant population growth rate from the spike flushing stage to the maturity stage, which led to an increase in the accumulation of photosynthetic assimilates at the maturity stage, which was conducive to the promotion of translocation and distribution of seeds to the spikes, and thus led to an increase in yield.

Table 4. Effect of different exogenous hormones on dry matter accumulation and population growth rate under low-temperature stress.

Rice Varieties	Treatment	Dry Matter Weight Accumulation During the Critical Period		Population Growth Rate from Flushes to Maturity (g (m^2 d)$^{-1}$)
		Jointing Stage (g/Pen)	Maturity (g/Pen)	
Hui Ling You 898	MJ	217.66 a	346.20 b	2.857 c
	MT	218.77 a	353.15 b	2.903 c
	SA	218.05 a	349.46 b	2.830 c
	GA3	218.11 a	347.97 b	2.823 c
	Z	215.07 a	380.62 a	3.450 ab
	SPD	214.25 a	385.91 a	3.507 a
	ABA	219.33 a	349.46 b	2.867 c
	HDK	210.15 a	327.97 c	2.393 de
	HCK	215.72 a	372.56 a	3.270 b
	Average	216.35	357.03	2.99
Nan Jing 9108	MJ	169.86 b	270.42 fg	2.100 fg
	MT	165.33 b	258.71 g	1.993 g
	SA	172.82 b	296.63 de	2.477 de
	GA3	175.85 b	298.20 de	2.447 de
	Z	174.63 b	279.40 ef	2.260 ef
	SPD	172.73 b	288.77 de	2.323 de
	ABA	175.44 b	294.68 de	2.387 de
	NDK	164.71 b	257.96 g	1.777 h
	NCK	173.78 b	300.09 d	2.527 d
	Average	171.68	282.76	2.25
	df			
Rice varieties (V)	1	774.312 **	714.375 **	516.611 **
Exogenous hormone treatment (T)	8	1.529 ^{ns}	12.634 **	29.628 **
V × T	8	0.697 ^{ns}	5.612 **	10.539 **

No common letter indicates a significant difference at the p = 0.05 level within the same column. ** indicate significant differences between the two rice varieties at p = 0.01 levels. ^{ns} means the difference is not significant.

3.5. Effect of Different Exogenous Hormones on Total Starch Content in Rice Grains

As can be seen from Figure 4, the growth rate of the total starch content in the grains of the two varieties of rice showed a trend of increasing and then decreasing, and the dynamics of total starch accumulation in the grains during the process of grouting were more or less the same. In addition, the different exogenous hormone treatments were able to increase the total starch content of the grains, and compared to the HDK, the Z and SPD treatments increased the total starch content of the grains of the Hui Liang You 898, indicating that the Z and SPD played a great regulatory role in the total starch content of the grains. The Z and SPD treatments significantly increased the total starch content of Hui Liang You 898, indicating that Z and SPD played a great role in regulating the total starch content of grains, of which the SPD treatment significantly increased the starch accumulation rate of Hui Liang You 898 in the middle of the pre-filling period, which promoted the filling of the grains, and thus increased the yield. For Nan Jing 9108, except for the MT treatment, other treatments showed the same trend in the growth rate of total starch content.

Table 5. Effects of different exogenous hormones on aboveground dry matter accumulation and translocation under low-temperature stress.

Rice Varieties	Treatment	Aboveground Dry Matter Accumulation After Flowering		Pre-Flowering Aboveground Nutrient Organ Translocation	
		Cumulative Amount (g/Pen)	Contribution to Seed Yield (%)	Transshipment Volume (g/Pen)	Contribution to Seed Yield (%)
Hui Ling You 898	MJ	137.173 c	82.71 def	28.66 abcdef	17.29 cde
	MT	139.370 c	82.75 def	28.90 abcdef	17.25 cde
	SA	135.743 cd	80.72 fg	32.41 ab	19.28 bc
	GA3	135.523 cd	81.36 f	31.12 abcd	18.64 c
	Z	165.547 ab	89.28 a	19.80 g	10.72 h
	SPD	168.323 a	88.63 ab	21.68 fg	11.37 gh
	ABA	137.563 c	81.29 f	31.68 abc	18.71 c
	HDK	114.813 fgh	77.00 gh	34.32 a	23.00 ab
	HCK	156.837 b	87.63 abc	22.31 efg	12.37 fgh
	Average	143.43	83.49	27.88	16.51
Nan Jing 9108	MJ	104.897 hi	80.65 fg	25.13 bcdefg	19.35 bc
	MT	99.710 i	80.13 fg	24.77 cdefg	19.87 bc
	SA	123.817 ef	86.59 abcd	19.18 g	13.41 efgh
	GA3	122.353 efg	84.56 bcdef	22.38 efg	15.44 cdefg
	Z	113.103 gh	81.95 ef	24.92 cdefg	18.05 cd
	SPD	116.037 efg	82.80 def	24.09 defg	17.20 cde
	ABA	119.240 efg	83.40 cdef	23.78 defg	16.60 cdef
	HDK	88.833 j	74.84 h	29.87 abcde	25.16 a
	HCK	126.310 de	86.29 abcde	20.08 g	13.71 defgh
	Average	112.70	82.36	23.80	17.64
Source of variation	df				
Rice varieties (V)	1	389.92 **	3.23 ^{ns}	14.79 **	3.23 ^{ns}
Exogenous hormone treatment (T)	8	30.344 **	11.97 **	4.353 **	11.97 **
V × T	8	10.646 **	5.025 **	3.096 **	5.025 **

No common letter indicates a significant difference at the p = 0.05 level within the same column. ** indicate significant differences between the two rice varieties at p = 0.01 levels. ^{ns} means the difference is not significant.

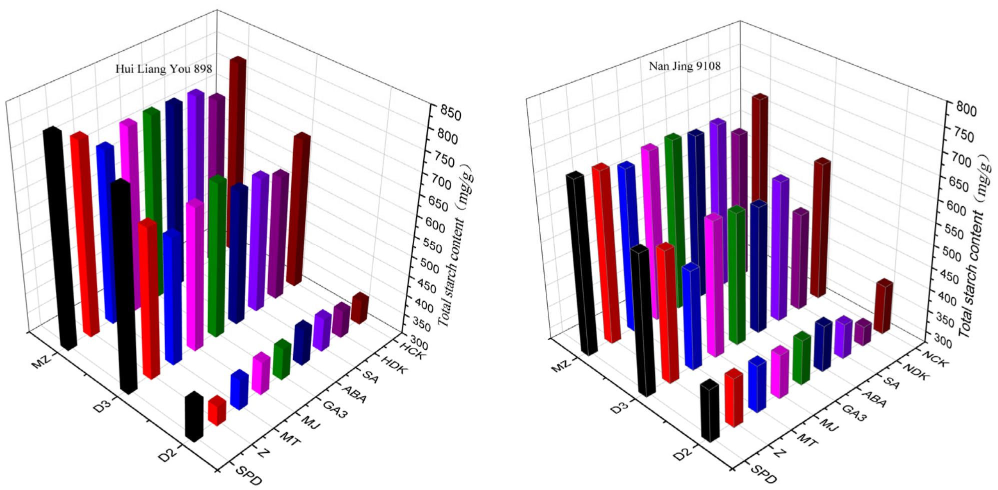

Figure 4. Effects of different exogenous hormones on the total starch content of rice grains under low-temperature stress. Different color bars represent different exogenous hormone treatments. From left to right (black to crimson) represent SPD, Z, MT, MJ, GA_3, ABA, SA, DK, CK.

3.6. Effects of Different Exogenous Hormones on Protein Content in Rice Grains at the Maturity Stage

Protein is the second-largest component of rice grain. From Figure 5, it can be found that Nan Jing 9108 has a lower protein content than Hui Liang You 898 in general, which may also be the reason why Nan Jing 9108 possesses a better flavor quality. Compared with the HDK treatment, the Z and SPD treatments significantly reduced the protein content, while compared with the HCK treatment, the HDK treatment did not significantly reduce the protein content, indicating that low-temperature stress had no significant effect on the protein content in Hui Liang You 898 variety, while the Z and SPD treatments might improve the flavor quality of rice due to the decrease in protein content, which can be investigated in the future. Compared with NDK treatment, the Z and SPD treatments also decreased the protein content in Nan Jing 9108 kernels, but the difference was that low-temperature stress significantly increased the protein content in Nan Jing 9108 kernels, which might have a serious effect on the flavor quality of its rice.

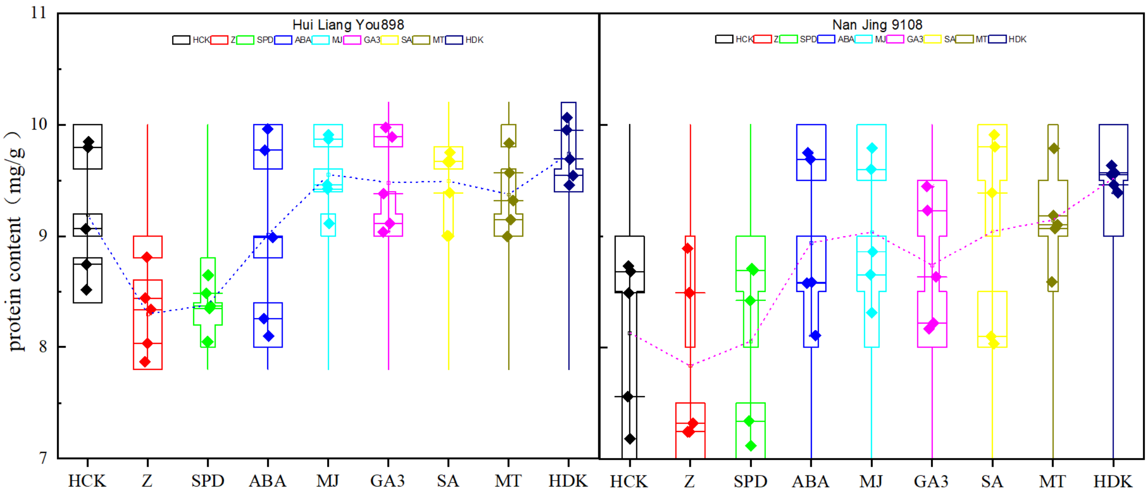

Figure 5. Effects of different exogenous hormones on rice grain protein content under low-temperature stress.

3.7. Effects of Different Exogenous Hormones on Adenosine Diphosphate Glucose Phosphorylated Enzyme (AGP) Activity in Rice Grains

As can be seen from Figure 6, the AGP activity of the two varieties during the filling and fruiting period showed an unimodal curve during the formation of grain filling, and the peaks all appeared 14 days after treatment. For Hui Liang You 898, the effect of low-temperature stress on seed AGP activity was more obvious, except for Z and SPD treatments, the rate of increase in AGP activity was lower than that of HCK before reaching the peak, and the peak value was also lower than that of HCK. The enzyme activity gradually declined after reaching the peak, which might be due to the imbalance of metabolism in the body of the plant under the low-temperature conditions, and the destruction of tissues and cellular structure. Compared with Hui Liang You 898, the effect of low-temperature stress on Nan Jing 9108 was smaller. It was still the Z and SPD treatments that obtained the maximum peak of AGP activity, and all the treatments increased the AGP activity of rice seeds compared with NDK.

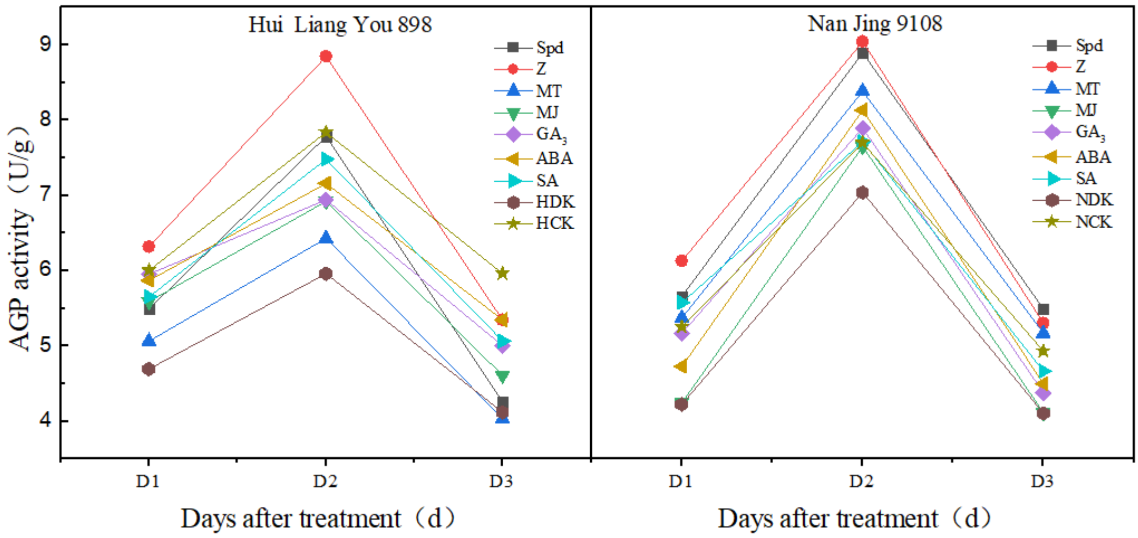

Figure 6. Effect of different exogenous hormones on adenosine diphosphate glucose phosphorylated enzyme (AGP) activity in seeds.

The AGP activity of different varieties differed in their stress capacity to low-temperature stress, and the ability of different exogenous hormone treatments to improve AGP activity also differed, but the general trend was the same. The peak AGP activity of Hui Liang You 898 decreased more, and the CRI varied between 107.88% and 148.48%, while that of Nan Jing 9108 was the next most affected, and the CRI varied between 107.91% and 125.89%. This indicates that Hui Liang You 898 is less cold-tolerant and can also be supplemented by the fact that japonica rice is better than hybrid rice in terms of cold tolerance (Table 6).

Table 6. Effect of different exogenous hormones on peak adenosine diphosphate glucose phosphorylated enzyme activity in seeds.

Treatment	Hui Liang You 898		Nan Jing 9108	
	Peak Adenosine Diphosphate Glucose Phosphorylated Enzyme Activity/U g^{-1}	CRI for Peak Adenosine Diphosphate Glucose Phosphorylated Enzyme Activity/%	Peak Adenosine Diphosphate Glucose Phosphorylated Enzyme Activity/U g^{-1}	CRI for Peak Adenosine Diphosphate Glucose Phosphorylated Enzyme Activity/%
DK	5.96 h	100.00	6.45 b	100.00
SPD	7.78 c	130.53	8.00 a	124.03
MT	6.43 g	107.88	7.57 ab	117.36
MJ	6.92 f	116.10	6.96 ab	107.91
GA_3	6.94 f	116.44	7.16 ab	111.01
ABA	7.16 e	120.13	7.36 ab	114.11
SA	7.48 d	125.50	7.01 ab	108.68
Z	8.85 a	148.48	8.12 a	125.89
CK	7.84 b	131.54	7.00 ab	108.53
F-value	1954.17 **	/	57.73 **	/

No common letter indicates significant differences at the p = 0.05 level within the same column. ** indicate significant differences between the two rice varieties at p = 0.01 levels.

3.8. Effects of Different Exogenous Hormones on Starch Branching Enzyme (SBE) Activity in Rice Grains

As shown in Figure 7, the SBE activity in rice grains of the two varieties showed an unimodal curve change of first increase and then decrease during the filling period, and both of them reached the peak value 14 days after treatment. Compared with HDK and NDK, the Z treatment obtained the maximum value, which increased the SBE activity in the grain by 26.54% and 20.53%, respectively. After the peak, compared with HCK, the SBE activity in the grain decreased in all treatments of Hui Liang You 898 except for the Z and

GA_3 treatments, which may be attributed to the destruction of the cell structure by the low temperature and the decrease in enzyme activity, which led to the slowing down of the rate of grouting.

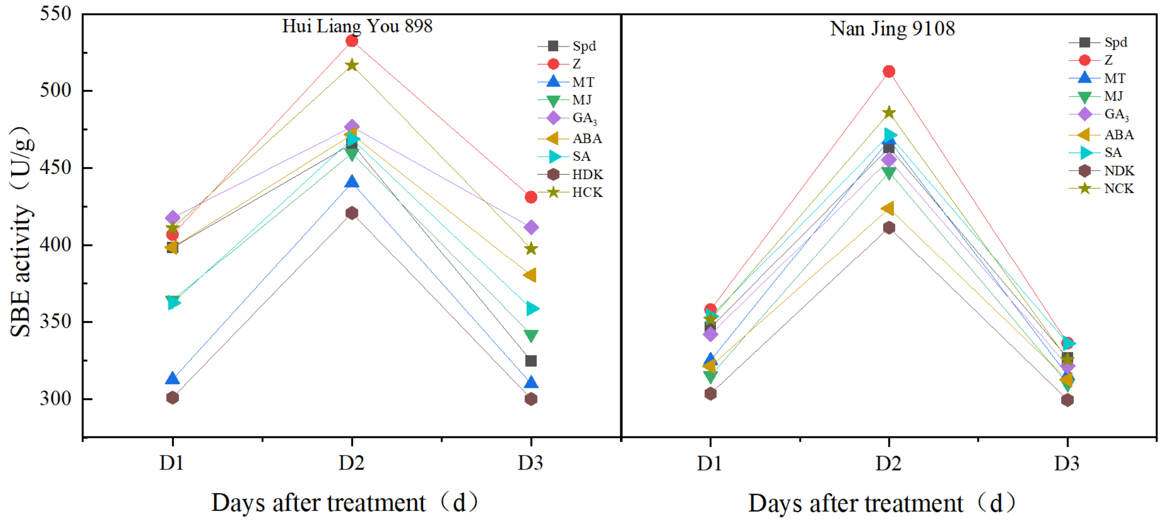

Figure 7. Effect of different exogenous hormones on seed starch branching enzyme (SBE) activity.

With the same trend of AGP activity, the peak SBE activity of Hui Liang You 898 decreased more, with the change of CRI ranging from 104.71% to 126.54%, followed by Nan Jing 9108, with the change of CRI ranging from 102.49% to 120.53%, which was less affected (Table 7).

Table 7. Effect of different exogenous hormones on peak starch branching enzyme activity in seeds.

Treatment	Hui Liang You 898		Nan Jing 9108	
	Peak Starch Branching Enzyme Activity/U g^{-1}	CRI for Peak Starch Branching Enzyme Activity/%	Peak Starch Branching Enzyme Activity/U g^{-1}	CRI for Peak Starch Branching Enzyme Activity/%
DK	420.91 h	100.00	449.12 bc	100.00
SPD	465.93 e	110.70	496.34 bc	110.51
MT	440.74 g	104.71	500.90 b	111.53
MJ	459.86 f	109.25	481.96 bc	107.31
GA_3	476.94 c	113.31	489.15 bc	108.91
ABA	471.82 d	112.10	460.31 c	102.49
SA	468.91 de	111.38	503.74 ab	112.16
Z	532.60 a	126.54	541.32 a	120.53
CK	516.94 b	122.81	516.93 ab	115.10
F-value	1032.51 **	/	64.65 **	/

No common letter indicates a significant difference at the p = 0.05 level within the same column. ** indicate significant differences between the two rice varieties at p = 0.01 levels.

4. Discussion

Plant hormones and their regulatory roles have been important in crop cultivation and physiological research. At present, the application of exogenous phytohormone regulation in rice has been very extensive, and there have been many reports on crop yield and quality in response to regulation. Many exogenous phytohormones have been discovered and widely used to investigate the molecular and physiological mechanisms of crop cultivation in adverse conditions [15,16,34]. However, there are fewer reports on the effects of many different exogenous phytohormones on physiological traits and yield in rice under specific periods. In particular, the occurrence of low-temperature stress during the rice grain filling and fruiting stage is highly probable and less studied. Two different rice varieties were used as materials in this study (Hui Liang You 898, Nan Jing 9108). Seven different exogenous phytohormone treatments were performed. Furthermore, the participation of exogenous phytohormones in the regulation of low-temperature adversity suffered by rice during the filling stage. The effects of spraying seven different exogenous phytohormones on agronomic and physiological traits of rice subjected to low-temperature stress during the filling stage were observed. Thus, we can explore the physiological effects of plant exogenous hormones on rice subjected to low-temperature stress during the period of grouting and fruiting and the effective ways to reduce yield loss and also enrich the physiological mechanisms of plant hormones to regulate low-temperature stress in rice and improve the quality of rice.

4.1. Effects of Different Exogenous Hormones on Chlorophyll Content, Seed Protein Content, and Total Starch Content and the Seed AGPase and SBEase Activities of Rice Leaves Under Low-Temperature Stress at the Early Stage of Irrigation

The results of this study show that different exogenous hormone treatments, except for SA treatment, could increase the chlorophyll content of rice saber leaves under low-temperature stress in the two varieties. These hormone treatments can also delay leaf senescence, enhance the photosynthetic production capacity of the plant, and favor the recovery and formation of yield after low-temperature stress. One researcher pointed out [35] that exogenous ABA can promote the synthesis and transportation of endogenous ABA in rice under low-temperature stress, improve chlorophyll content and photosynthetic capacity, and effectively mitigate the adverse effects of low-temperature stress on rice yield. CTK has been reported to regulate the development of chloroplast lamellae and chlorophyll synthesis. The authors also observed that Z (a species of CTK) treatment increased leaf chlorophyll content. It has been shown that the endogenous SA content in the leaf blades of mutant ospls1 was significantly higher than that of the wild type, and its premature senescence characteristics may be attributed to changes in SA signaling-related pathways [36]. This is consistent with the results of this study. Low-temperature stress treatment reduced the chlorophyll content, and spraying different exogenous hormones could increase chlorophyll content and delay leaf senescence in low-temperature stress-treated rice leaves.

The general study concluded that high temperatures during the filling period increase rice protein content, while low temperatures decrease it [37]. This is generally consistent with the present results. The Z and SPD treatments obtained lower protein content between both varieties; they could possibly be used as exogenous hormones for improving rice quality.

This needs to be explored further. In addition, the total starch content in the strong and weak grains of rice tended to increase with the advancement of the reproductive process [38]. In the present study, the total starch content increased gradually, and the increase appeared to be faster and slower. The Z and SPD treatments significantly increased the total starch content of Hui Liang You 898. This may be related to the fact that the Z and SPD treatments significantly increased the AGP and SBE activities in seeds. The scientists in [14] pointed out that endogenous SPD could promote rice grain filling and increased grain weight, which were attributed to the fact that the exogenous spraying of SPD during the pre-flowering period significantly increased the activity of starch synthesis-related enzymes in weak grains [39]. In this study, spraying different exogenous hormones increased the AGP and SBE activities in rice grains, which in turn increased grain starch accumulation and grain weight, with the Z and SPD treatments showing the greatest enhancement and the peak activity of Hui Liang You 898 showed a greater decrease in activity peak compared with that of Nan Jing 9108, which corroborated that Hui Liang You 898 was less cold-tolerant.

4.2. Effects of Different Exogenous Hormones on Dry Matter Accumulation and Translocation in Rice Under Low-Temperature Stress at Early Filling Stage

Chlorophyll content was significantly correlated with plant photosynthetic capacity [40], and photosynthesis is the main link in the formation of rice yield, and the increase in photosynthetic capacity makes the plant produce more photosynthetic products, which in turn produces a higher accumulation of aboveground dry matter weight. Previous studies have shown that [41] low-temperature stress during the grouting and fruiting period caused a decline in rice yields because a low temperature reduces the photosynthesis of leaves and photosynthetic products to the spike, affecting the process of aboveground dry matter accumulation and distribution, the second soluble sugar synthesis and decomposition is blocked, the key enzyme activity of starch synthesis of the grains is inhibited, the organic compounds cannot be efficiently converted to starch, resulting in insufficient grouting of the grain. This resulted in the inadequate filling of grains and a decrease in the thousand-grain weight and fruiting rate. In addition, spraying exogenous salicylic acid (SA) could significantly increase the plants' soluble sugar, proline, and betaine content under low-temperature stress and promote the transfer and distribution of aboveground dry matter accumulation from plants to seeds [42–44]. The results of this study showed that low-temperature stress treatment reduced chlorophyll content, and spraying different exogenous hormones could increase the chlorophyll content of low-temperature stress-treated rice leaves, delay leaf senescence, promote an accumulation and increase in post-flowering photosynthetically assimilated products of the plant, improve the population growth rate, and increase the allocation of seeds in the dry matter mass of the plant at the maturity stage and its proportion, and then improve the yield, which is basically in line with the results of the previous study. This is basically consistent with the results of previous studies.

In terms of enzymes related to plant dry matter translocation accumulation and seed starch synthesis, the activities of key enzymes of starch metabolism pathways in the seed grain directly affect the amount of starch accumulated in the seed grain and grain weight [45]. The results of a large number of studies have shown that the amount of post-flowering dry matter accumulation in rice is positively correlated with seed yield, and it has also been shown that the final formation of seed yield is the result of the joint action of pre-flowering nutrient organ storage and post-flowering photosynthetic product accumulation. Spermidine (SPD) and spermine (SPM) can promote rice grain filling and increase grain weight [46]. On the whole, spraying different exogenous hormones on both low-temperature-sensitive and cold-tolerant rice varieties used in this study could improve the plant's post-flowering photosynthetic material production capacity, increase the accumulation of photosynthetic assimilation products and post-flowering dry matter and its proportion at maturity, and facilitate the translocation of photosynthetically active products to grains at the spike, which ultimately led to the increase in the amount and proportion of grains accumulated at maturity and the increase the yield. Among the different exogenous hormone treatments, the spraying of Z and SPD was the most effective, and the exogenous spraying of Z and SPD under low-temperature stress was able to increase the chlorophyll content, the activity of AGP and SBE in rice grains, which was favorable to the accumulation of starch and grain weight.

4.3. Effects of Different Exogenous Hormones on Rice Yield and Its Components Under Low-Temperature Stress at the Early Stage of Irrigation

As a temperature-loving crop, the growth and development of rice can be adversely affected by low-temperature stress [47]. The results of this study showed that the filling and fruiting stage is a critical period for the formation of rice yield and quality, and low-temperature or low-light stress at this time will cause insufficient accumulation of photosynthates, slow down the grain filling rate and decrease the starch accumulation rate and content, which will lead to a reduction in the grain weight and poor quality, and seriously restrict the increase in rice yield and quality [48–50]. This is basically consistent with the results of this study; the rice yield of a low-temperature treatment at the early stage of irrigation was significantly lower than that of conventional planting treatment. Previous studies have concluded [51,52] that low-temperature stress during the gestation period significantly affects yield components such as the number of grains in a spike, thousand-grain weight, and fruiting rate of rice. One study [53] showed that the earlier the time of post-sprouting stress, the greater the effect on the fruiting rate, and the effect of stress on the number of solid grains attached to the primary and secondary branch pedicels was roughly the same as the effect on the fruiting rate, which further clarified that the decrease in the fruiting rate caused by low-temperature stress at the early stage of irrigation was the main reason for the reduction of yields, which was the same as the purpose of the treatment time of the present study. Another study [54] pointed out that low-temperature stress at the glume differentiation stage significantly reduced the number of grains in the spike, the fruiting rate, and the thousand-grain weight of rice, which in turn affected the final yield.

In research on low-temperature stress tolerance in rice, studies have shown that the seed free spermidine (SPD) content was significantly or highly significantly positively correlated with grain filling rate, suggesting that SPD can be used as a chemical regulator to promote grain filling and increase grain weight and fruit set [55]. The results of this study show that spraying different exogenous hormones before low temperature can reduce the adverse effect of low temperature on rice yield to a certain extent, and the overall Z and SPD can be selected as suitable exogenous hormones for spraying. Melatonin (MT) and spermidine (SPD) have the ability to scavenge free radicals, improve plant resistance, and promote the growth and development of rice by increasing its photosynthetic capacity, thereby alleviating the adverse effects of low-temperature stress on yield [56,57]. In addition, some studies have used [58–60] methyl jasmine acid (MJ), abscisic acid (ABA), and other spray treatments for rice under low-temperature stress conditions in different periods, and all achieved better results in low-temperature stress tolerance. The results of the present study showed that spray treatments at the pre-grouting stage could withstand low-temperature stress and thus increase rice yield, which was mainly attributed to the increase in thousand-grain weight and fruit set, which was basically in agreement with the results of the previous studies on the enhancement of cold tolerance in rice.

5. Conclusions

The results showed that the DK treatment significantly reduced the rice seed yield compared with CK. Different exogenous hormones for spraying treatments before low-temperature stress can improve rice yield, in which the Z and SPD spraying treatments of Hui Liang You 898 are more effective compared with HDK treatment, and its yield significantly increased, and even reached the level of HCK, while the yield increase in Nan Jing 9108 is more average. The main reason for the increase in the yield of exogenous spray treatments was the increase in the thousand-grain weight and fruiting rate.

Moreover, the chlorophyll content of sword leaves after treatment was higher than that of DK treatment, so the accumulation of the photosynthetically assimilated products in the aboveground part of the plant after flowering and the population growth rate increased along with the increase in the photosynthetic production capacity of the plant, which led to the higher dry matter accumulation of the plant at the maturity stage. Moreover, in the distribution of dry matter in the plant at maturity, the seeds occupied a higher accumulation amount and proportion, and the higher activity of adenosine diphosphate glucose phosphorylated enzyme (AGP) and starch branching enzyme (SBE) led to a higher accumulation of starch in the seeds, which ultimately led to an increase in the yield of rice. In addition, exogenous spraying of Z and SPD under low-temperature stress was able to obtain a lower seed protein content, which may improve rice quality and can be investigated specifically in the future.

In summary, the exogenous spraying of Z and SPD can increase the thousand-grain weight and fruiting rate of rice during the grouting period under low-temperature stress, thus increasing its yield, which can provide a theoretical basis and technical support for high-yield, high-quality, and stress-resistant cultivation of rice. In addition, focusing on the stability of the area and increasing yields can help to guarantee food security's bottom line and the stable production and supply of important agricultural products.

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