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Exogenous Diethylaminoethyl Hexanoate Highly Improved the Cold Tolerance of Early *japonica* Rice at Booting

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Abstract: Rice (*Oryza sativa* L.) is highly sensitive to cold stress, which leads to large reductions in rice yield at the booting stage. In this study, Kongyu131 and Kenjiandao6 rice cultivars with different levels of cold stress sensitivity were sprayed with diethylaminoethyl hexanoate (DA-6) concentrations of 500, 200, 20, 2, 0.2, and 0 mg/L one day before undergoing cold water stress (CWS). We analyzed changes in yield and its factors, dry matter production, stem characteristics, and physiological and biochemical characteristics of the rice plants. The results showed that DA-6 increased peroxidase activity, delayed nitrogen and chlorophyll degradation, maintained soluble protein and potassium contents, and suppressed the accumulation of malondialdehyde in the leaves of both cultivars under CWS. DA-6 also increased the phosphorous content and superoxide dismutase activity in Kenjiandao6 under CWS; however, in Kongyu131, DA-6 increased the soluble sugar content. In addition, DA-6 treatment increased the weight of the panicle at maturity, and of the leaf, panicle, and stem-sheath at heading in both cultivars. The lengths of the panicle, the top first internode, the export rate of stem-sheath, translocation rate of stem-sheath, and export of stem-sheath from heading to maturity were increased in Kenjiandao6; however, in Kongyu131, DA-6 increased the dry weight ratio of panicle to total plant and reduced the dry weight ratio of stem-sheath to total plant at maturity. Furthermore, DA-6 improved yield in both cultivars, mainly by increasing the grain weight in the inferior grains (IG) and middle grains (MG) under CWS. DA-6 increased the grain weight in the IG and MG in Kenjiandao6 mainly by enhancing the seed setting rate and number of filled grains (NFG) in the IG and MG, and in Kongyu131 by improving the NFG in MG and IG. The optimal concentration of DA-6 to alleviate CWS was 2 mg/L. In conclusion, exogenous DA-6 was effective for maintaining dry matter production and physiology in two early *japonica* rice cultivars under CWS at booting, thereby improving cold tolerance and enhancing yield. The less cold-tolerant cultivar Kenjiandao6 was more sensitive to the effects of DA-6 and displayed better results than the more cold-tolerant cultivar Kongyu131.

Keywords: diethylaminoethyl hexanoate; cold water stress; early *japonica* rice; physiological and biochemical characteristics; yield; dry matter production

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1. Introduction

Around 4×10^6 ha of early *japonica* rice was planted in the past year in Heilongjiang Province, which makes an important contribution to food security in China by contributing around 12.5% of the country's annual rice output [1]. However, Heilongjiang Province has the lowest annual average temperature and the shortest frost-free period in China, with cold stress occurring frequently at the booting stage. At this stage, cold stress can cause floret degradation or pollen sterility, which leads to a reduction of the seed setting rate [2]. These factors analyze the effect of chilling injury on rice yield composition at the booting stage of great practical significance for cultivating chilling-tolerant rice cultivars to ensure a high and stable grain yield in China. Cold stress at booting has become a research hotspot, as evidenced by recent studies on the effect of cold stress on yield, grain quality, phenotype, physiology and biochemistry, transcriptome, and proteome [3–7]. Salicylic acid, 6-benzyladenine, brassinosteroids, and abscisic acid have been shown to alleviate cold stress in rice at booting [8–11]. However, there are no published studies on whether diethylaminoethyl hexanoate (DA-6) can mitigate chilling injury in early *japonica* rice at booting.

DA-6 is a cytokinin-like compound with a plant growth regulator that promotes seed vigor [12] and seedling establishment [13], improves antioxidant enzyme activity [14] and photosynthesis [15], promotes key enzyme-encoding genes for sucrose metabolism [12] and the expression of photosystem I (PSI) complex-related genes [16], and enhances stress resistance [14,16] and yield [17]. Under low-temperature treatment, soaking seeds in a solution of 10 μg/L DA-6 increased rice seedling survival rate and catalase (CAT) activity, promoted proline content, and improved the cold resistance of rice seedlings [18]. Guan [16] (2020) showed that the 10 mol/L DA-6 treatment increased tomato stem diameter and plant height and reduced the hydrogen peroxide (H_2O_2) content exposed to cold stress. Zhang [19] (2001) found that DA-6 improved the contents of soluble protein (SP), chlorophyll (Chl), and CAT activity in rice seedlings. At present, studies on improving the chilling tolerance of early *japonica* rice with exogenous DA-6 have mainly focused on the seedling stage, and the effect of DA-6 on chilling tolerance at booting remains to be examined.

This study aimed to analyze whether yield components (including spike positions, dry matter production, culm traits, nutrient uptake, Chl, protein, carbohydrates, and antioxidant activity) in early *japonica* rice played important roles in alleviating cold injury under exogenous DA-6 at booting. We also explored the differences in the pathways of DA-6 to improve the yield of Kongyu131 and Kenjiandao6 under CWS.

2. Materials and Methods

2.1. Experimental Site

The experiment was carried out in the Rice Science and Technology Park (130° 22′ 08″ E and 46° 48′ 35″ N) of Heilongjiang Academy of Land Reclamation Sciences in 2020 and 2021, during the mid-temperate continental monsoon climate. This area has an average annual sunshine duration of 2525 h, an average annual frost-free period of 140 days, and an annual active accumulated temperature of 2590 °C. The temperature changes during the whole growth period of the rice in the experimental area are shown in Figure 1. The average daily temperature was below 16 °C for 9 days during the tillering stage of vegetative growth of rice in 2020 and 2021, including 12.53 °C on 23–27 May 2020 and 14.58 °C on 24–30 May 2021. The average daily temperature was lower than 17 °C for 3 days (2020) and 2 days (2021) in the reproductive growth stage of rice. The planting system in this area is one crop per year, and the soil type is a meadow albic bleach. The black soil layer extends from the soil surface to 26 cm deep and is a dark brown layer rich in organic matter with good soil aggregate structure. Soil chemical properties of the experimental site are shown in Table 1. In both years, the seeds were sown on 12 April. The rice seedlings were transplanted on 22 May, and four seedlings were planted in each hole with a hill-row spacing of 12 × 30 cm. The rice was harvested on 16 September.

Table 1. Soil chemical properties of the experimental site.

Total N (%)	Available P (%)	Available K (%)	pH	Organic Matter (%)
10.53	2.12×10^{-3}	1.12×10^{-2}	6.3	3.84

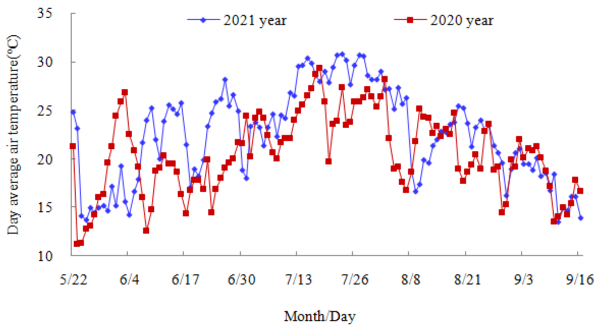

Figure 1. Changes in daily average air temperature during rice growing season in 2020 and 2021.

2.2. Plant Material and Exogenous DA-6 Treatment

The experimental rice cultivars were the chilling-sensitive Kenjiandao6 and the chilling-tolerant Kongyu131. We used a split-plot design with the experimental cultivars as the main treatment with 2 levels, and the application amount of exogenous DA-6 was the secondary treatment with 6 levels. Six DA-6 concentrations were used: 500, 200, 20, 2, 0.2, and 0 mg/L. Plants of both cultivars were sprayed with the DA-6 solutions of each concentration and the control (CK) of pure water in the morning under windless conditions before the first day of CWS to ensure that the leaves were covered with the medicinal liquid. There was no rainfall within 24 h after spraying. The plants in the paddy field were irrigated with 16 °C water for 5 days from 8 a.m. to 4 p.m. as the cold treatment during the rice booting stage. Then the temperature of the irrigation water returned to normal temperature (22.9 °C in 2020; 25.6 °C in 2021). At the same time, the plants in the paddy field were irrigated with water at 22.2 °C in 2020 and 24.1 °C in 2021 as normal temperature treatment (Figure 1).

2.3. Yield and Yield Components

The effective panicle number (EPN) of representative 30-hole plants was investigated in each plot at the maturity stage, and the average EPN per hill was calculated. According to the average EPN per hill, nine hills were harvested for each treatment. The panicles were divided into three parts: lower, middle, and upper (Figure 2). The dominant grains (DG) and inferior grains (IG) were the grains on the primary branch of the upper panicles and grains on the secondary branch of the lower panicles, respectively (Figure 2), and the middle grains (MG) were the remaining grains on the panicles (Figure 2). The grain weight and the empty and filled grains numbers were investigated for IG, MG, and DG, and the thousand-grain weight (TGW), seed setting rate (SSR), and yield (Y) were calculated.

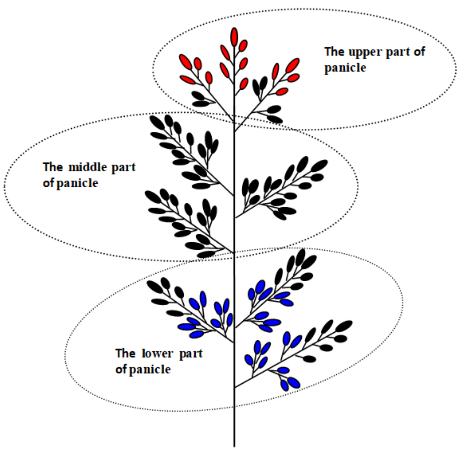

Figure 2. The rice panicle structure. The red, black, and blue grains represent the dominant grains (DG), middle grains (MG), and inferior grains (IG), respectively.

2.4. Dry Matter Production and Culm Traits

The tiller number (TN) of representative 30-hole plants was investigated in each plot at the maturity and heading stages, and the average TN per hill was calculated. According to the average TN, nine hills were harvested for each treatment to measure the take out the length of panicle (TP), top first internode length (TF), top second internode length (TS), and top third internode length (TT) at maturity. The plants were decomposed into three parts: stem-sheath, leaf, and panicle. Tissue samples were dried at 75 °C to measure the leaf, stem-sheath, and panicle dry weights at heading and maturity. We calculated the biological yield (BY), translocation rate (TRS), export rate (ERS), and exportation (ES) of the stem-sheath, as well as the dry weight ratio of stem-sheath (RSH) and panicle (RPH) to total plant at heading and the dry weight ratio of stem-sheath (RSM) and panicle (RPM) to total plant at maturity.

2.5. Physiology

The second leaves from the plant tops were cut off and put into zip lock bags in each treatment before the first day, on the second and fourth days during CWS, and on the first, third, and fifth days after the return to normal temperature. The leaves were immediately frozen in liquid nitrogen and stored in a −80 °C refrigerator for the determination of physiological indexes. The activities of peroxidase (POD) and superoxide dismutase (SOD), the malondialdehyde (MDA), Chl, SP, and soluble sugar (SS) contents were determined using the methods of Zhang [20] et al. (1997), Chen and Wang [21] (2002), Zhang [22] (1992), and Li [23] et al. (2000), respectively. The rice leaves were washed thoroughly with deionized water, deactivated in a 105 °C oven for half an hour, then kept at 80 °C to maintain constant weight while being crushed and passed through a 0.5-mm sieve. The samples then underwent the H_2SO_4-H_2O_2 elimination cooking method, and then a continuous flow analytical method was applied to assay the total nitrogen (N) content [24], the MoAb anti-spectrophotometry method was applied to assay the total phosphorus (P) content [25], and the flame photometry method was applied to assay the total potassium (K) content [26].

2.6. Statistical Analysis

Microsoft Excel 2016 was used for data statistics [27], and DPS software was used for one-way ANOVA and correlation analysis [28]. If there were significant differences between treatments, Duncan's method was used for multiple comparisons at the 0.05 or 0.01 level.

3. Results

3.1. Changes in Rice Yield Components and Yield in Response to DA-6 Applications

Exogenous DA-6 treatment enhanced the Y, SSR, and filled grain numbers (NFG) in both cultivars at booting under CWS compared to the control group (CK) (Figure 3A,B,D). The highest levels of Y, SSR, and NFG occurred under the 2 mg/L DA-6 condition in both cultivars in two different years. There was a significant benefit over the control condition in the yield components occurring in 2020, with the values higher by 11.50 and 26.22%, 4.40 and 11.77%, and 17.27 and 17.54% in Kongyu131 and Kenjiandao6, respectively. Only Kenjiandao6 showed a significant benefit over the control in 2021. Thus, DA-6 increased Y mainly by improving the NFG and SSR under CWS at booting, with the effects of treatment at the 2 mg/L level on SSR, NFG, and Y more obvious in Kenjiandao6 than in Kongyu131.

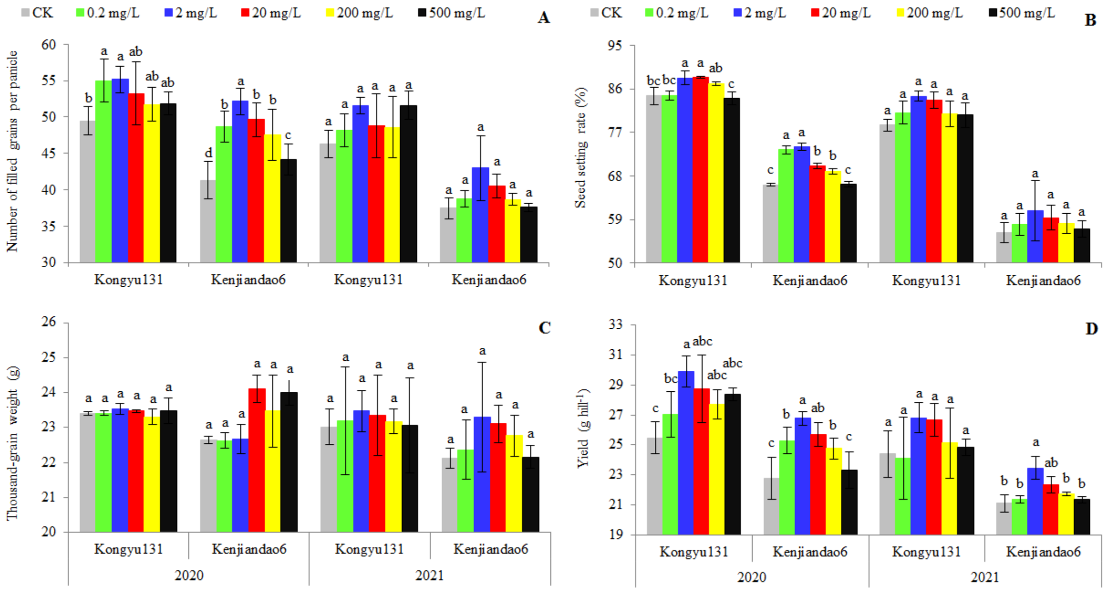

Figure 3. The changes of the (**A**) number of filled grains per panicle, (**B**) seed seting rate, (**C**) thousand-grain weight, and (**D**) yield in both cultivars treated with diethylaminoethyl hexanoate (DA-6) under cold water stress (CWS) at booting in 2020 and 2021. The letters above the columns indicate significant differences ($p < 0.05$).

Figure 4A,B,D indicates that DA-6 treatment enhanced the NFG, SSR, and Y of the different spike positions in both cultivars at booting under CWS compared to the CK. The NFG and Y in the IG and MG, and the SSR in DG and MG in Kongyu131 were greater in the 2 mg/L DA-6 condition compared to the CK, with the values increasing by 6.03 and 58.38%, 12.13 and 2.68%, and 11.51 and 68.76%, respectively. In Kenjiandao6, the NFG and Y in the IG, MG, and DG, and the SSR of the IG and MG under CWS were significantly higher in the 2 mg/L DA-6 group compared to the control groups, with the values increased by 22.81, 26.71, 27.38, 15.18, 8.75, 14.04, 18.21, and 18.62%, respectively. Thus, DA-6 enhanced the Y mainly by enhancing the SSR of MG and DG, the NFG and Y of IG and MG in Kongyu131, and also the SSR of IG and MG and the NFG and Y of IG, MG, and DG in Kenjiandao6. Overall, the effects of 2 mg/L DA-6 treatment on the NFG and Y of MG and the SSR of IG and MG were more obvious in Kenjiandao6 than in Kongyu131.

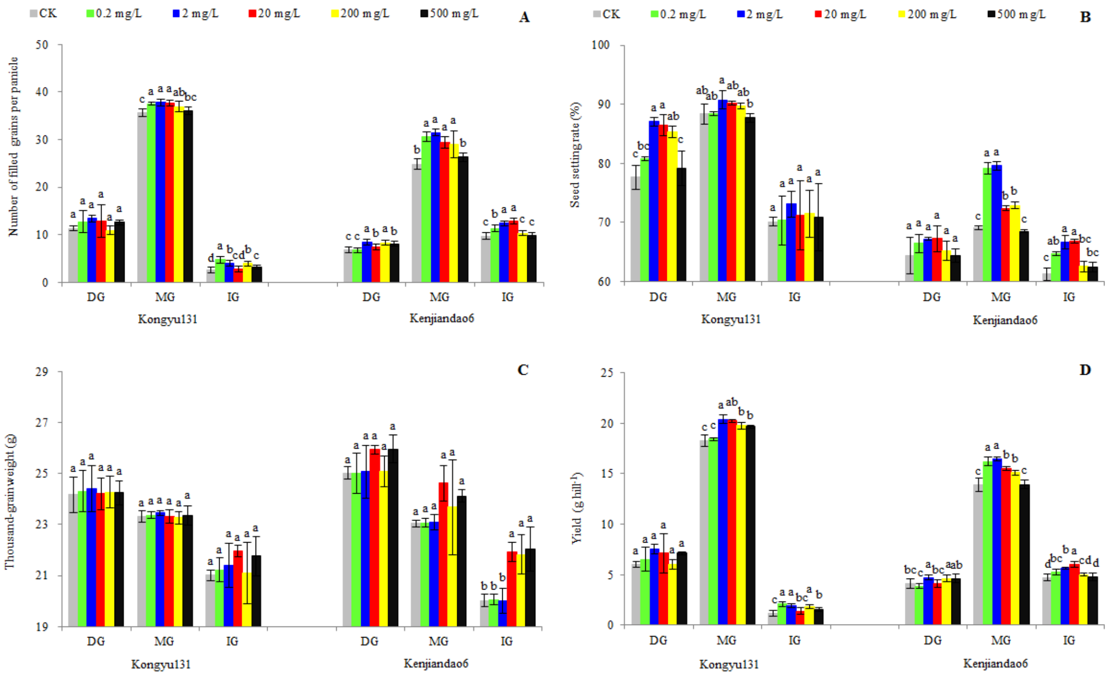

Figure 4. Effects of the (**A**) number of filled grains per panicle, (**B**) seed seting rate, (**C**) thousand-grain weight, and (**D**) yield under diethylaminoethyl hexanoate (DA-6) treatment in the inferior grains (IG), middle grains (MG), and dominant grains (DG) under cold water stress (CWS) on both cultivars. The letters above the columns indicate significant differences ($p < 0.05$).

3.2. Rice Culm Trait Changes in Response to DA-6 Application under Cold Stress

DA-6 improved the culm length in Kenjiandao6 mainly by improving the TP and TF under CWS as these metrics were greater in the Kenjiandao6 cultivar under the 2 mg/L DA-6 condition than in the control groups (Figure 5).

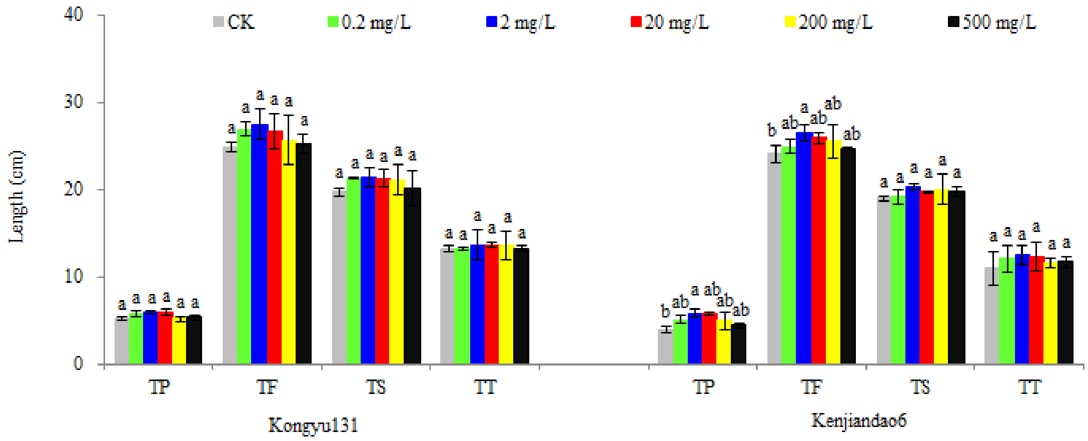

Figure 5. Effects of diethylaminoethyl hexanoate (DA-6) on internode length and take out the length of panicle at booting in both cultivars. TP—take out the length of panicle; TF—top first internode length; TS—top second internodes length; TT—top third internodes length. The letters above the columns indicate significant differences ($p < 0.05$).

3.3. Changes in Dry Matter Production in Response to DA-6 Application under Cold Stress

DA-6 increased the leaf dry weight (DL), stem-sheath dry weight (DS), panicle dry weight (DP), and BY in both cultivars at maturity and heading under CWS compared to the CK (Figure 6A,B). DA-6 at 2 mg/L treatment yield significantly higher DP in both cultivars at heading and maturity, BY and DL in two cultivars at heading, DS in Kongyu131 at heading, and BY in Kongyu131 at maturity compared with the control groups. DA-6 therefore improved BY mainly by improving the DP at maturity. The effects of 2 mg/L DA-6 treatment on BY and DL at heading were more pronounced in Kenjiandao6 than in Kongyu131. However, the effects of the treatment on DP at both heading and maturity were more obvious in Kongyu131 than in Kenjiandao6.

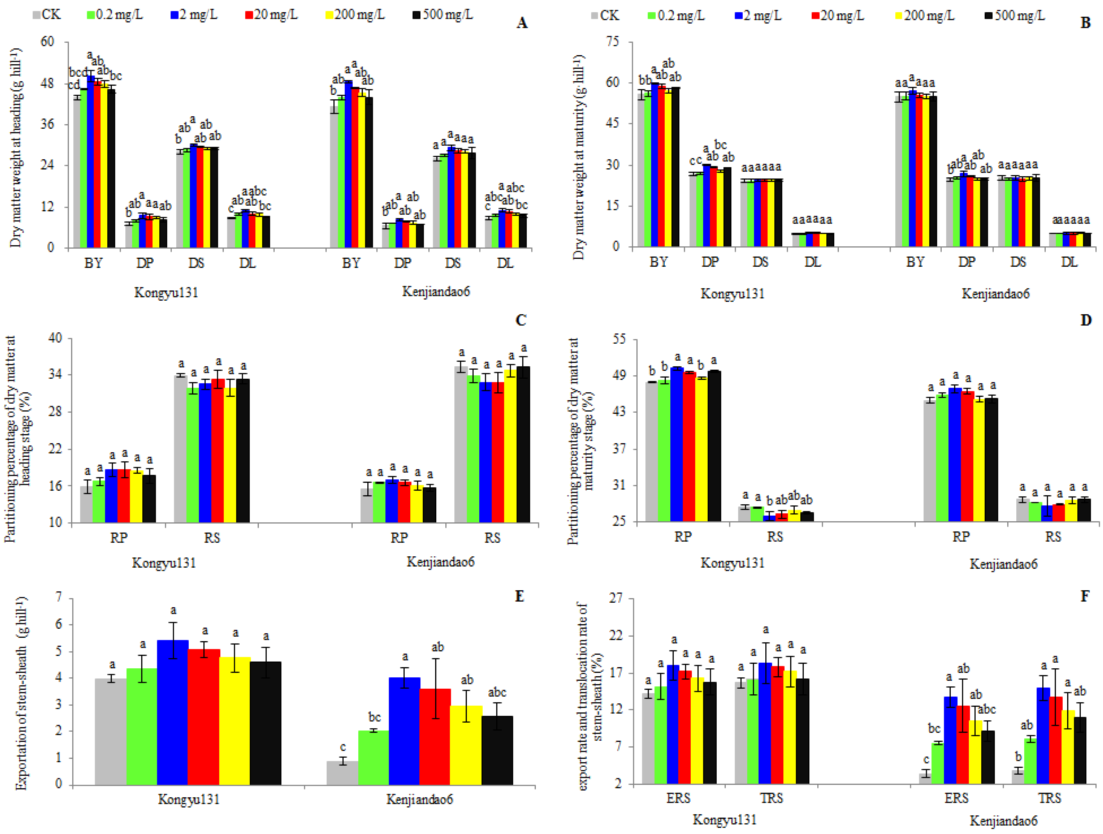

Figure 6. Effects of diethylaminoethyl hexanoate (DA-6) treatment on the (**A**) dry matter weight at heading, (**B**) dry matter weight at maturity, (**C**) partitioning percentage of dry matter at heading stage, (**D**) partitioning percentage of dry matter at maturity stage, (**E**) exportationg of stem-sheath, and (**F**) export rate and translocation rate of stem-sheath under cold stress in Kongyu131 and Kenjiandao6. BY—biological yield; DP—panicle dry weight; DL—leaf dry weight; DS—stem-sheath dry weight; TRS—stem-sheath translocation rate; ES—stem-sheath exportation; RP—dry weight ratio of panicle to total plant; ERS—stem-sheath export rate; RS—dry weight ratio of stem-sheath to total plant. The letters above the columns indicate significant differences ($p < 0.05$).

Treatment with DA-6 improved the dry weight ratio of panicle to total plant (RP) in both cultivars at maturity under CWS compared to the CK and decreased the dry weight ratio of stem-sheath to total plant (RS) (Figure 6C,D). In Kongyu131, the RP was greater, and the RS was lower in the 2 mg/L DA-6 treatment at maturity compared with the CK, and all differences were significant. RP was greater and the RS was lower in 2 mg/L DA-6-treated at heading and maturity compared with the CK in Kenjiandao6. Thus, the effect of the 2 mg/L DA-6 was better in Kongyu131 than in Kenjiandao6.

Figure 6E,F shows that the ERS, TRS, and ES from heading to maturity increased initially and then decreased under CWS with increasing DA-6 concentration. The ES, ERS, and TRS in Kenjiandao6 with 2 mg/L DA-6 were significantly higher than in the controls, whereas the difference was not obvious in Kongyu131. Thus, the effect of the 2 mg/L DA-6 was better in Kenjiandao6 than in Kongyu131.

3.4. Physiological Changes in Rice Leaf in Response to DA-6 Application under Cold Stress

Application of 2 mg/L exogenous DA-6 significantly ($p < 0.01$) improved SOD activity in Kenjiandao6 by 6.16% and POD activity in both cultivars' leaves relative to the CK on the second day after CWS by 7.79 and 9.25%, respectively (Figure 7A,B). DA-6 treatment also increased SOD and POD activities in both cultivars after the recovery period.

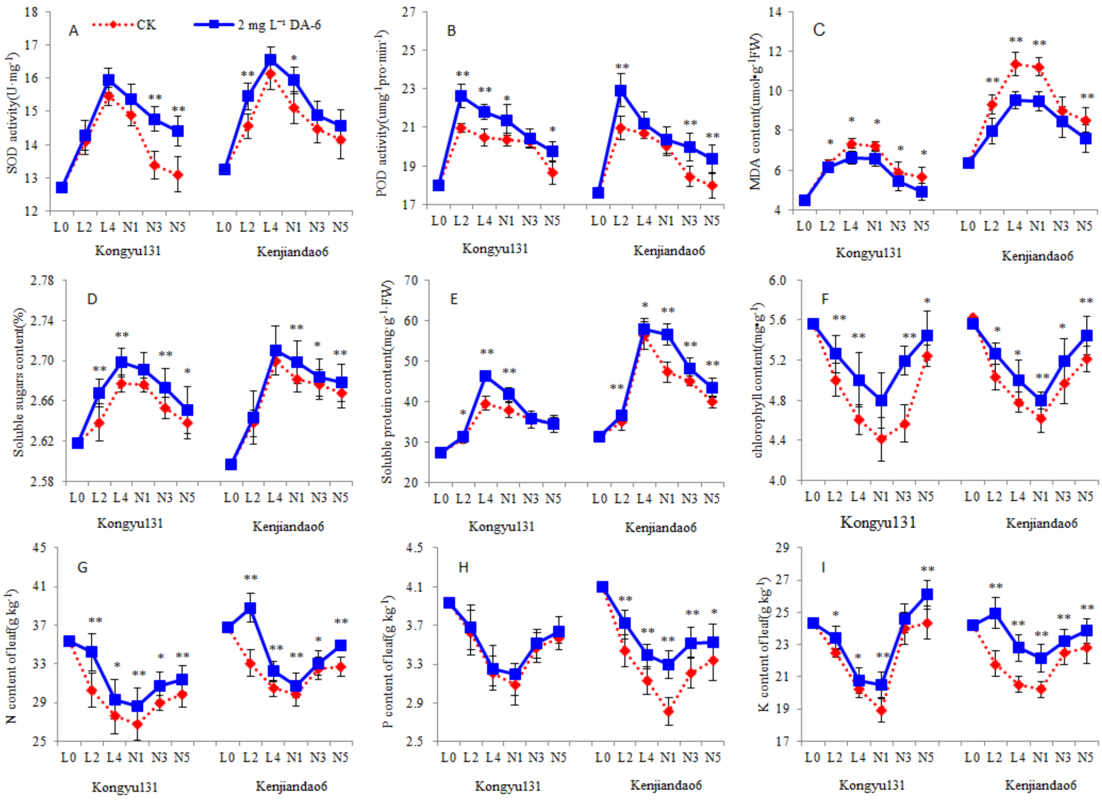

Figure 7. Effects of diethylaminoethyl hexanoate (DA-6) treatment on the activities of (**A**) superoxide dismutase (SOD) and (**B**) peroxidase (POD), and on (**C**) alondialdehyde (MDA), (**D**) soluble sugars, (**E**) soluble protein, (**F**) chlorophyll, (**G**) nitrogen (N), (**H**) phosphorus (P), and (**I**) potassium (K) contents in both cultivars under cold water stress (CWS) at booting. L0, L2, and L4 indicate before day 1, on the second day, and on the fourth day of the cold water stress (CWS), respectively. N1, N3, and N5 indicate the first, third, and fifth days after recovery to normal temperature, respectively. **, $p > 0.01$; *, $p > 0.05$.

The MDA content of plants under the 2 mg/L DA-6 treatment was significantly lower than that of the control group plants on the second and fourth days in both cultivars, decreasing by 2.50 and 14.62%, and 9.56 and 15.98%, respectively (Figure 7C). MDA content in the DA-6 treated plants was also significantly lower than control at the first and fifth days after moving to normal water temperature. The effect of DA-6 treatment on MDA content and the protective enzymes system was more obvious in Kenjiandao6 than in Kongyu131.

DA-6 also improved the contents of SS and SP (Figure 7D,E). The SS content in DA-6-treated Kongyu131 was significantly higher than in the control on the second and fourth days after CWS, whereas there was no significant effect ($p > 0.05$) on Kenjiandao6. In the DA-6 treatment, SP content in both cultivars was significantly higher than in the controls on the second and fourth days after CWS, with the values improving by 1.12 and 0.22%, and 0.80 and 0.40%, respectively. Following the application of DA-6, SP and SS contents decreased with time during the recovery period. The Chl content of leaves treated with DA-6 declined with time under CWS and then increased during the recovery period (Figure 7F). The Chl content in the DA-6 treatment group was significantly higher than in the control group on the second and fourth days after CWS in both the cultivars, with the values increasing by 5.18 and 4.62%, and 8.56 and 4.62%, respectively. Overall, the effect of DA-6 treatment on SS, SP, and Chl contents was more obvious in Kongyu131 than in Kenjiandao6.

The K, P, and N contents in Kongyu131 and the P content in Kenjiandao6 treated with DA-6 decreased with time after CWS, whereas the N and K contents in Kenjiandao6 increased and then decreased (Figure 7G–I). The P content in the DA-6-treated Kenjiandao6 was significantly higher than in the CK on the second and fourth days after CWS, whereas there was no corresponding difference in Kongyu131. The N and K contents of DA-6 were significantly higher than in the CK on the second day after CWS in both cultivars, and the values increased by 13.13 and 17.25%, and 4.27 and 14.49%, respectively. The N and K contents in DA-6-treated Kongyu131 were significantly higher than in the control condition on the first and fifth day of recovery in normal water temperature. The contents of K, P, and N in DA-6-treated Kenjiandao6 were significantly higher than in the control on the first, third, and fifth days of recovery. Overall, the effect of exogenous DA-6 on N, P, and K contents was more obvious in Kenjiandao6 than in Kongyu131.

3.5. Correlations of Yield Factors and Dry Matter Production after DA-6 Application under Cold Stress

DA-6-treated SSR had significant positive correlations with ERS, DLM, SSRD, SSRM, BYH, DPH, DSH, TRS, ES, and RPH in Kongyu131 under CWS at booting (Table 2). SSR in the DA-6-treated Kenjiandao6 showed significant positive correlations with NFGM, DLM, YM, SSRM, SSRD, RPM, TF, DSH, DSM, and SSRI.

Table 2. Correlations of yield factors and dry matter production after DA-6 treatment in both cultivars under CWS.

		NFGD	NFGM	SSRD	SSRM	SSRI	YD	YM	TP	TF	BYH	DPH	DSH	DLH	BYM	DPM	DSM	DLM	RPH	RPM	RSM	ES	ERS	TRS
Kongyu131	NFG	0.78 *	0.89 **	0.57	0.46	0.53	0.63	0.38	0.81 *	0.96 **	0.72	0.57	0.61	0.88 **	0.51	0.49	0.50	0.50	0.46	0.47	−0.47	0.63	0.58	0.63
	SSR	0.24	0.72	0.96 **	0.98 **	0.75	0.41	0.79 *	0.53	0.60	0.85 *	0.85 *	0.83 *	0.74	0.70	0.65	0.71	0.91 **	0.80 *	0.55	−0.70	0.86 *	0.95 **	0.85 *
	TGW	0.90 **	0.30	0.20	0.24	0.49	0.92 **	0.46	0.71	0.49	0.45	0.36	0.55	0.40	0.73	0.74	0.58	0.58	0.25	0.76 *	−0.72	0.53	0.41	0.54
	Y	0.68	0.66	0.76 *	0.63	0.85 *	0.87 *	0.92 **	0.59	0.66	0.93 **	0.93 **	0.97 **	0.76 *	0.96 **	0.96 **	0.97 **	0.80 *	0.88 **	0.95 **	−0.95 **	0.95 **	0.86 *	0.96 **

		NFGM	NFGI	TP	TF	BYH	DPH	DSH	DLH	BYM	DPM	RPH	RSH	RPM	RSM	ES	ERS	TRS	SSRD	SSRM	SSRI	KGWI	YM	YI
Kenjiandao6	NFG	0.97 **	0.87 *	0.97 **	0.94 **	0.92 **	0.97 **	0.82 *	0.95 **	0.78 *	0.86 *	0.97 **	−0.91 **	0.88 **	−0.87 *	0.86 *	0.81 *	0.86 *	0.92 **	0.82 *	0.90 **	−0.05	0.93 **	0.81 *
	SSR	0.95 **	0.75	0.79 *	0.71	0.66	0.80 *	0.51	0.71	0.73	0.79 *	0.94 **	−0.81 *	0.79 *	−0.79 *	0.54	0.47	0.54	0.85 *	0.99 **	0.79 *	−0.46	0.99 **	0.62
	TGW	−0.18	0.07	0.12	0.10	0.21	0.03	0.31	0.19	−0.30	−0.19	−0.18	−0.01	−0.06	0.09	0.38	0.45	0.38	−0.05	−0.55	0.07	0.96 **	−0.33	0.22
	Y	0.96 **	0.89 **	0.97 **	0.93 **	0.90 **	0.97 **	0.79 *	0.94 **	0.81 *	0.89 **	0.99 **	−0.94 **	0.91 **	−0.91 **	0.82*	0.76 *	0.82 *	0.94 **	0.84 *	0.91 **	−0.15	0.95 **	0.83 *

NFGI, NFGM, and NFGD: Filled grains numbers of IG, MG, and DG; SSRI, SSRM, and SSRD: Seed setting rate of IG, MG, and DG. YI, YM, and YD: Yield of IG, MG, and DG; DPH, DSH, DLH, and BYH: Dry weight of panicle, stem-sheath, leaf, and total plant at heading; BYM, DPM, DSM, and DLM: Dry weight of the total plant, panicle, stem-sheath, and leaf at maturity; RPH, RSH, RPM, and RSM: Dry weight ratio of panicle and stem-sheath to total plant at heading and maturity; TGWI: Thousand-grain weight of grains from the IG. The notes in Figure 5 have the TP and TF. The notes in Figure 6 have the ES, ERS, and TRS. **, $p > 0.01$; *, $p > 0.05$.

4. Discussion

4.1. Response of Rice Yield to Exogenous DA-6 under CWS

DA-6 can increase plant yield under abiotic stress [16]. We analyzed the effects of DA-6 concentration levels of 500, 200, 20, 2, 0.2, and 0 mg/L on dry matter production, yield, and yield factors in two cultivars and discovered the best DA-6 treatment to be 2 mg/L. In two previous studies, DA-6 alleviated cold stress on tomato seedlings and heat stress on eggplant seedlings, and the optimal concentrations were 10 mg/L [16] and 20 mg/L [29], respectively. We found DA-6 at 2 mg/L to significantly improve yield mainly by enhancing the NFG of IG and MG in both cultivars, the SSR of DG in Kongyu131, and the NFG of DG and the SSR of MG and IG in Kenjiandao6 under CWS. Li [30] (2016) also reported that the yield of rice treated with DA-6 at 20 mg/L could be increased by manipulating the number of panicles per hill at normal temperatures. We found the effect of the 2 mg/L DA-6 treatment to be stronger in Kenjiandao6 than in Kongyu131. DA-6 concentrations with 30 mg/L have been shown to increase the dry weight of rice seedlings under normal air temperature [30]. In this study, DA-6 increased the DP, DS, and DL at heading, the DP at maturity in both cultivars, the TP and TF in Kenjiandao6 (thus improving that cultivar's ERS and TRS), and at maturity, increased the RP and reduced the RS in Kongyu131.

4.2. Physiological Responses in Rice Leaves to DA-6 under CWS

Cold stress breaks the balance system of ROS production and clearance in cells, produces excessive ROS [31], accumulates a large number of membrane peroxidation products such as MDA, damages the structure of cell membrane, protein, and nucleic acid [32], and seriously affects the physiological and biochemical functions of cells [33]. SOD is the primary substance in the body that removes free radicals, and the oxidation of POD is dominant at high concentrations of oxygen, and they both can remove excess ROS, which allows the plant to grow and develop normally. DA-6 has been shown to induce SOD activity in *Capsicum chinense* Jacq [34] and eggplant seedlings [29] under high-temperature stress and enhance SOD and POD activities in tomatoes after cold stress [35]. We found that the application of DA-6 at 2 mg/L significantly increased POD activity in both cultivars and SOD activity in Kenjiandao6 under CWS and also reduced the content of MDA in both cultivars. Therefore, DA-6 can increase the activity of antioxidant enzymes in rice leaves under CWS, thus scavenging free radicals and reactive oxygen species, thereby maintaining the integrity of membrane function and structure and the normal physiological functions of rice. These results are consistent with existing research [18,19]. POD and SOD activities in Lixinjing and Sujing No. 2 (cold-tolerant rice cultivars) improved more rapidly under cold stress, while the activity of two key enzymes did not significantly change in Yangkenuo and Sanbaili (cold-susceptible cultivars) [36]. The enzymatic activity in sugarcane seedlings was higher in GT28 (chilling-tolerant cultivar) compared to ROC22 (chilling-sensitive cultivar) under cold stress [37]. In our study, the effect of DA-6 treatment on MDA content and SOD activity was more obvious in Kenjiandao6 than in Kongyu131, perhaps because DA-6 regulated the different expression of genes in rice cultivars with different cold tolerance. These mechanisms in both cultivars need to be further studied.

Cold stress can break the balance between plants' energy sources and metabolic pools, destroy chloroplast structure [38], reduce pigment synthase activity [39], and increase cell osmotic potential by increasing the content of osmotic regulatory substances such as SP and SS, thus improving plant cold tolerance [40,41]. Zhang [19] (2001) and Liang [18] (2003) found that DA-6 enhanced Chl, SS, and SP contents in rice seedlings under cold stress [18,19]. We found DA-6 at 2 mg/L increased the Chl, SP, and SS contents of rice under CWS at booting compared to the untreated controls. The increase in Chl, SP, and SS contents resulting from treatment with DA-6 was higher in Kongyu131 than in Kenjiandao6. We speculate that the stronger the cold tolerance of rice cultivars, the more easily they are induced by DA-6. Jiang [42] (2014) found that chill-tolerant wheat cultivar leaves had a higher SS content at the jointing stage under low temperatures. Other studies have shown that the SS content of sugarcane is higher in GT28 (cold-resistant variety) than in ROC22 (cold-susceptible cultivar) under cold stress [37]. In the current study, the SS content under 2 mg/L DA-6 treatment increased more in Kongyu131 than in Kenjiandao6, indicating DA-6 was more likely to increase the SS content of a chilling-tolerant cultivar under CWS, alleviating the increase of cell osmotic potential and thus improving the cold tolerance of rice. Ch1 content has also been shown to be higher in Bainong207 and AK58 (chilling-tolerant wheat cultivars) than in Zhengmai366 (chilling-sensitive cultivar) under cold stress [43]. In the current study, DA-6 was more likely to stabilize the pigment synthase activity of a chilling-tolerant rice cultivar under CWS, thereby alleviating the Ch1 decline and maintaining the normal chloroplast structure and photosynthetic mechanism.

N, P and K, the three major nutrient elements in rice, play an important role in the plant's growth [44]. Cold stress is not conducive to the accumulation of N, P, and K nutrients in rice [45]. DA-6 has been shown to increase P, N, and K contents in corn under normal temperatures [46] and enhance root activity in rice under cold stress [18,19]. In the current study, 2 mg/L DA-6 enhanced the P, N, and K contents in both cultivars after CWS. This may be because DA-6 alleviated the decreased metabolic activity of rice roots under CWS, maintained the uptake and utilization of nutrients by roots, and ensured the rational distribution of N, P, and K in roots, stem-sheaths, and leaves. Thus, exogenous DA-6 treatment enhanced cold tolerance in rice plants, and the effect of DA-6 treatment on P, N, and K contents was more obvious in Kenjiandao6 than in Kongyu131. This may be because DA-6 regulated the N, P, and K transfer in the roots, stem-sheaths, and leaves of early *japonica* rice with different cold tolerance under CWS at the booting stage or because DA-6 may have a different effect on root vitality, a topic which warrants further study.

4.3. Differences in Cold Tolerance of Both Cultivars Treated with DA-6 under CWS

We found that DA-6 activated antioxidant protect ion systems quickly and early, enhanced POD and SOD activities in Kenjiandao6 and the POD activity in Kongyu131 on the second day after CWS, and prevented MDA accumulation. Thus, DA-6 scavenged ROS in Kenjiandao6 mainly by improving POD and SOD activities under CWS and in Kongyu131 mainly by enhancing POD activity. These different mechanisms in both cultivars need to be further studied. We also found that DA-6 enhanced the contents of the osmotic adjustment substances SS and SP in Kongyu131 and the SP content in Kenjiandao6, thereby decreasing the osmotic potential in the plants' cells. This in turn enhanced the cells' ability to absorb water and regulate water equilibrium in response to cold stress. This might be due to DA-6 increasing sucrose phosphate synthetase activity and decreasing invertase activity in Kongyu131, however, DA-6 did not affect these enzymatic activities in Kenjiandao6. DA-6 might have promoted root growth and absorption in Kenjiandao6 and increased leaf P content, however, DA-6 did not affect P content in Kongyu131 (Figure 8).

In our study, DA-6 significantly improved the DP, DS, and DL, as well as the BY at heading, the TF, and TP, thereby improving the TRS, ERS, and ES of stem-sheath from heading to maturity. In Kongyu131, DA-6 did not affect ES, ERS, and TRS, perhaps because DA-6 might have little effect on the stem-sheath dry weight at maturity. This difference between cultivars requires further study. DA-6 increased Kenjiandao6 Y mainly by enhancing NFG and SSR. In Kongyu131, DA-6 increased Y mainly by enhancing the NFG of MG and IG, and the SSR of DG and MG, but the treatment did not affect SSR in the IG or NFG in the DG. Thus, DA-6 significantly enhanced the grain weights of the whole panicles in Kenjiandao6 and the grain weights in the MG and IG in Kongyu131 (Figure 8).

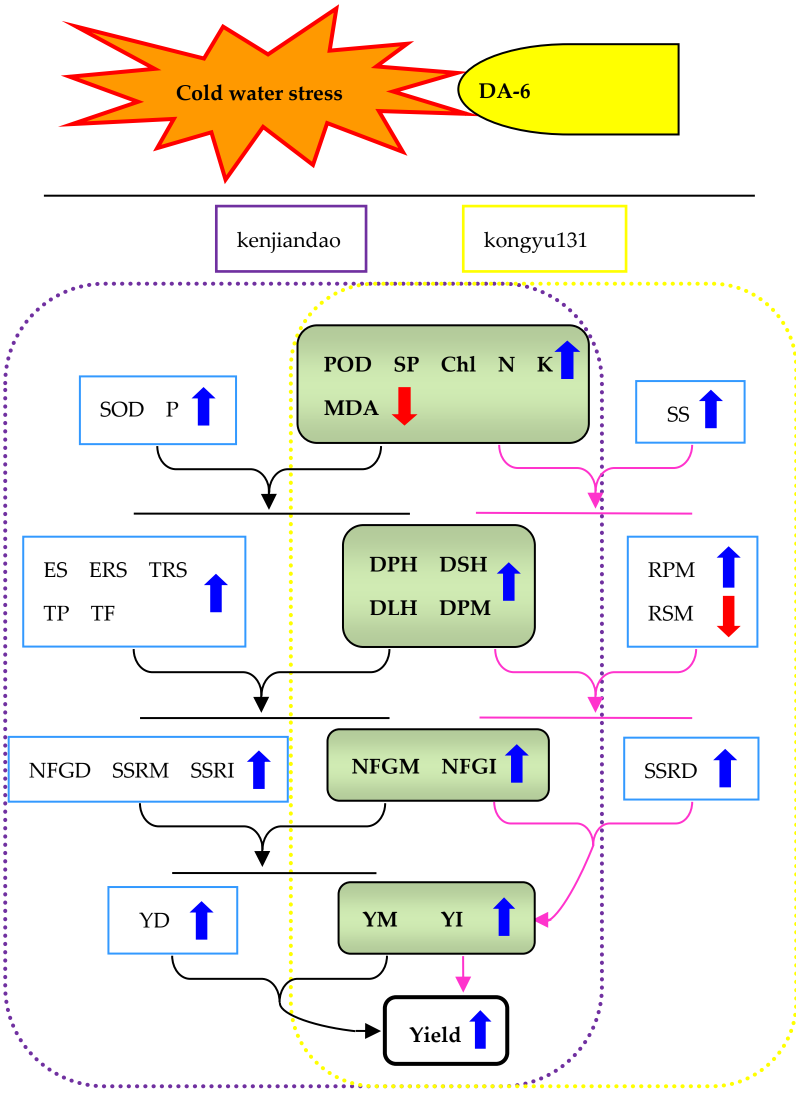

Figure 8. The proposed model of how exogenous diethylaminoethyl hexanoate (DA-6) affects the panicle and leaf response of Kongyu131 and Kenjiandao6 under cold water stress (CWS). The red and blue arrows indicate a decrease and increase, respectively. SOD—superoxide dismutase; POD—peroxidase; MDA—malondialdehyde; SS—soluble sugar; SP—soluble protein; Chl—chlorophyll; N—nitrogen; P—phosphorus; K—potassium; ES—stem-sheath exportation; ERS—stem-sheath export rate; TRS—stem-sheath translocation rate; TP—take out the length of panicle; TF—top first internode length; DPH, DSH, and DLH: Dry weight of panicle, stem-sheath, and leaf at heading; DPM—panicle at maturity; RPM and RSM: Dry weight ratio of panicle and stem-sheath to total plant at maturity; NFGI, NFGM, and NFGD: Filled grains numbers of IG, MG, and DG; SSRI, SSRM, and SSRD: Seed setting rate of IG, MG, and DG; YI, YM, and YD: Yield of IG, MG, and DG.

5. Conclusions

Exogenous DA-6 significantly enhanced POD activity, delayed N and Chl degradation, maintained K and SP contents, and suppressed the accumulation of MDA in the leaves of both rice cultivars under CWS at booting, thereby improving the cold tolerance of early *japonica* rice. DA-6 also increased the P content and SOD activity in Kenjiandao6 under CWS; however, in Kongyu131, DA-6 increased the SS content. Furthermore, DA-6 treatment enhanced the weight of the panicle at maturity, and of the leaf, panicle, and stem-sheath at heading in both cultivars, thereby improving the yield of early *japonica* rice. The length of the panicle, the top first internode, as well as the ERS, TRS, and ES from heading to maturity were increased in Kenjiandao6; however, in Kongyu131, DA-6 increased the dry weight ratio of panicle to total plant and reduced the dry weight ratio of stem-sheath to total plant at maturity. DA-6 improved yield in both cultivars, mainly by increasing the grain weight in the IG and MG under CWS. DA-6 increased the grain weight in the IG and MG in Kenjiandao6, mainly by enhancing the SSR and NFG in the IG and MG, and in Kongyu131 by improving the NFG in MG and IG. The optimal concentration of DA-6 to alleviate CWS in rice was 2 mg/L.

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