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Effect of Low Temperature on Photosynthetic Characteristics, Senescence Characteristics, and Endogenous Hormones of Winter Wheat "Ji Mai 22" during the Jointing Stage

Fengyin Zhang [1,2], Nan Jiang [1], Hanqi Zhang [1], Zhiguo Huo [1,2,*] and Zaiqiang Yang [1,*]

[1] Collaborative Innovation Center of Forecast and Evaluation of Meteorological, Nanjing University of Information Science & Technology, Nanjing 210044, China; 20211208020@nuist.edu.cn (F.Z.); 202211080002@nuist.edu.cn (N.J.); 202212080025@nuist.edu.cn (H.Z.)

[2] State Key Laboratory of Severe Weather (LASW), Chinese Academy of Meteorological Sciences, No.46, Zhongguancun South Street, Haidian District, Beijing 100081, China

* Correspondence: huozg@cma.gov.cn (Z.H.); yzq@nuist.edu.cn (Z.Y.); Tel.: +86-13-60106-4240 (Z.H.); +86-13-40196-3706 (Z.Y.)

Abstract: To investigate the effects of low-temperature (LT) stress on photosynthetic properties and senescence characteristics of winter wheat leaves during the jointing stage, an environmental temperature control experiment was designed at Nanjing University of Information Science and Technology in 2023, using *Triticum aestivum* L. *cv.* "Ji Mai 22" as the test material. Four different temperature levels were set: 18 °C/8 °C (daily maximum/daily minimum temperature; CK), 13 °C/3 °C, 10 °C/0 °C, and 7 °C/3 °C. The duration of each treatment was 2, 4, and 6 days, respectively. The experimental findings reveal that the changes in physiological parameters of winter wheat leaves under low-temperature stress treatments are nonlinear. Under the 3 °C LT treatment, the photosynthetic parameters and endogenous hormone levels of wheat leaves significantly decrease after 6 days of stress. Under the 0 °C LT treatment, the photosynthetic parameters, leaf pigment content, and endogenous hormones of wheat decrease significantly, while under the −3 °C LT treatment, all the parameters of winter wheat leaves show a significant decline. Generally, the "Ji Mai22" wheat cultivar has a lower growth temperature limit of −3 °C during the jointing stage.

Keywords: wheat (*Triticum astivum* L. *cv.*); net photosynthetic rate; stomatal conductance; transpiration rate; chlorophyll content; protective enzyme activity; endogenous hormone levels

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1. Introduction

Wheat (*Triticum astivum* L. *cv.*) belongs to the genus *Triticum* in the Poaceae family, which is classified into two types based on sowing time: spring wheat and winter wheat. Winter wheat is sown in autumn and is known for its strong cold resistance. Wheat is the world's largest cereal crop and is widely distributed globally due to its strong adaptability [1]. Winter wheat accounts for about 75% of the total global wheat area. In particular, China is the largest producer of wheat in the world, with winter wheat accounting for over 80% of the total wheat area and over 85% of the total wheat production [2]. Among them, the Huang-Huai-Hai wheat-growing region in China is the largest wheat-producing area, with a wheat planting area of 240 million acres, accounting for 68% of China's wheat area. Therefore, safe production of winter wheat plays a vital role in ensuring China's—and global—food security [3]. The experimental materials in this study were selected from the largest winter wheat variety, "Ji Mai 22," in the Huang-Huai-Hai region of China. With its strong tillering ability and high yield, "Ji Mai 22" has an average annual expansion area of 15 million acres, with a total planting area exceeding 2.35 billion acres, ranking first in China.

Low-temperature (LT) stress is one of the primary meteorological disasters that affect the growth and development of winter wheat [4]. In recent years, with the backdrop

of global climate instability, extreme temperature events have become more frequent. The Huang-Huai-Hai region in China has witnessed frequent occurrences of extreme LT disasters, such as hail and snow, during spring. These events have inflicted substantial losses on local agriculture and have led to significant reductions in wheat production in some areas [5]. After the jointing stage, the cold resistance of winter wheat decreases, making it highly sensitive to LT stress [6,7]. The frequent occurrence of low-temperature stress during the jointing stage has become a major threat to the safe production of winter wheat in China. Therefore, by studying the changes in various physiological parameters of the predominant variety "Ji Mai 22" leaves under different LT stresses in the Huang-Huai-Hai region, which is prone to frequent LT disasters, we can not only separate the low-temperature disaster indicators during the jointing stage of "Ji Mai 22" but also provide a basis for scientific cultivation management of wheat in China and global winter wheat production [8].

Photosynthesis is an important physiological process in crop metabolism, and it is also one of the first physiological functions of plants to be affected under LT stress [7]. The slightly lower temperatures can activate subcellular antioxidant systems and enhance the tolerance of winter wheat to subsequent LT stress, while excessive LT stress can inhibit wheat growth by suppressing oxidative bursts in the photosynthetic apparatus [9]. Scholars have found that under LT stresses, the net photosynthetic rate, transpiration rate, and stomatal conductance of winter wheat leaves decrease with decreasing stress temperature, while the intercellular carbon dioxide concentration shows a trend of initially decreasing and then increasing with decreasing stress temperature [10]. Photosynthesis in wheat leaves is weakened at 0 °C, and photosynthetic organs are damaged at temperatures below −2 °C [11]. Under LT stress, the photosynthetic activity of "Ji Mai 22" winter wheat decreased more significantly with the increasing severity of the stress, reaching its limit at −7 °C [12]. LT stress also limited the degree of stomatal opening in wheat, resulting in an increase and then a decrease in photosynthetic rate, while photosynthetic pigment content tended to decrease [13,14].

The senescence characteristics of winter wheat leaves have a significant impact on yield formation, and many scholars have conducted extensive research on chlorophyll, protective enzyme activity, and endogenous hormones [15–17]. LT stress significantly reduces the activity of pigment synthesis enzymes in winter wheat, leading to a decrease in chlorophyll content [18]. Using spectral monitoring techniques for diagnosing leaf chlorophyll content under LT stress, it was observed that with increasing severity of LT stress, the chlorophyll content in winter wheat leaves showed a trend of initially increasing and then decreasing [19]. LT can cause the accumulation of a large amount of reactive oxygen species in winter wheat leaves during the jointing stage, leading to lipid peroxidation of cell membranes [20]. Research has shown that the activity of superoxide dismutase (SOD) and peroxidase (POD) in the leaves of cold-resistant winter wheat varieties significantly increases with the increasing severity of LT stress [21]. Under three LT stresses, the activities of SOD, POD, and CAT protective enzymes in winter wheat leaves significantly increased, while the number and weight of grains per year decreased significantly, and the changes in the number of wheat plants and thousand-grain weight were not significant [22].

Endogenous hormones play a critical regulatory role in plant growth, development, and response to stress. Previous studies on the relationship between endogenous hormones and cold resistance in winter wheat have shown that under stress conditions, the levels of indole-3-acetic acid (IAA) and gibberellins (GA) in the roots and floral organs during the booting stage decrease [23], while abscisic acid (ABA) significantly accumulates [24], exerting a significant impact on wheat yield. Cold stress leads to a significant accumulation of ABA in the leaves during the flowering stage of winter wheat, and with increasing severity of LT stress, the levels of ABA, IAA, and GA in the leaves show a trend of initially increasing and then decreasing [25].

The adaptability of plants to LT stress has always been a global research topic, and LT stress is a challenge faced by agricultural development in China [26]. Currently, research

on the growth and development of winter wheat mainly focuses on the economic yield formation stage after heading and flowering, while there is less research on the sensitive jointing stage. Studies on endogenous hormones in winter wheat are mainly related to tillering and growth, with limited research on leaf stress resistance. We hypothesize that the physiological parameters of winter wheat leaves do not continuously decrease as the severity of LT stress increases. Within a certain range of LT, leaves may trigger their self-protection mechanisms, leading to an increase in some physiological parameters. However, when the temperature stress reaches the limits that the leaves can withstand, growth ceases and the leaves begin to senesce. Therefore, this study aims to (1) explore the effects of LT stress on the photosynthetic properties of winter wheat leaves; (2) study the changes in protective enzyme activity in winter wheat leaves under LT stress; (3) investigate the variations of endogenous hormones (ABA, IAA, CTK, GA) in winter wheat leaves under LT stress; (4) use correlation to analyze the effects of different LT stresses on the photosynthetic and senescence characteristics of "Ji Mai 22" winter wheat, and propose the minimum temperature suitable for its growth, so as to provide a scientific basis for the prevention and control of LT disasters during the wheat jointing stage.

2. Materials and Methods

2.1. Experimental Designs

The winter wheat variety "Ji Mai 22" (semi-winter variety) from China was selected as the experimental material. Winter wheat seeds were procured from greenhouse growers in Nanjing, China, and were planted in mid-October 2022 in a Venlo-type greenhouse at Nanjing University of Information Science and Technology (NUIST). The dimensions of the greenhouse were 5.0 m (height) × 9.6 m (width) × 30.0 m (length), with a north–south orientation. Scholars have conducted extensive research on the suitable temperature, humidity, soil environment, planting density, and other factors in the Huang-Huai-Hai wheat-growing region [27,28]. In this study, we have taken into consideration these indicators to establish our parameters. The planting density was 300 plants·m^2. The soil medium consisted of peat soil: perlite: vermiculite 2:1:1 ($v/v/v$), with pH ranging from 7.5 to 8. At the time of sowing, compound fertilizer (N-P-K: 30%-10%-30%) as a basal fertilizer was applied at 2500 g/m^2, diluted with water (fertilizer: water = 1:100). The winter wheat was irrigated once every 7 days, ensuring that each plant was irrigated to 70% of the field capacity (monitored by a soil moisture meter) to avoid water deficiency.

According to the suitable growth conditions for winter wheat [29], the temperature inside the greenhouse was set at 8 °C (minimum)/18 °C (maximum). The humidity was set at 75% ± 5, and the light intensity was set at 800 μmol·m^{-2}·s^{-1}. The photoperiod was set at 12/12 h (daytime from 6 a.m. to 6 p.m.). When the base internode of the wheat started elongating and was exposed 2 cm above the soil surface (jointing stage), the winter wheat was transplanted into nutrient pots with a height of 25 cm, upper diameter of 20 cm, and lower diameter of 18 cm. In each nutrient pot, five holes were dug near the middle position, and three plants were transplanted in each hole. The soil medium in the pots consisted of peat soil: perlite: vermiculite in a ratio of 2:1:1 ($v/v/v$). Two days after transplantation, a foliar fertilizer was applied, using a compound fertilizer (N-P-K: 15%-15%-15%) and urea. The ratio of compound fertilizer to urea was 4:1, and the diluted fertilizer was applied at a rate of 2000 g per square meter, diluted with water at a ratio of 1:600 (fertilizer: water). During the experiment, the plants were watered at least every two days (most of the time, watering was done once or twice a day). All plants were irrigated to 80% of the field capacity (monitored using a soil moisture meter) to avoid water deficiency. The potted plants were placed in a controlled environment chamber (TPG1260, Australia) for the environmental control experiment (Table 1).

Table 1. Experimental design.

Marker	Treatments	Temperature [°C]	Duration [d]
T1	CK-2d	18/8	2
T2	CK-4d	18/8	4
T3	CK-6d	18/8	6
T4	3–2d	13/3	2
T5	3–4d	13/3	4
T6	3–6d	13/3	6
T7	0–2d	10/0	2
T8	0–4d	10/0	4
T9	0–6d	10/0	6
T10	−3–2d	7/-3	2
T11	−3–4d	7/-3	4
T12	−3–6d	7/-3	6

(1) To make it easier to analyze the study's results, "minimum temperature-stress days" represent each treatment in the subsequent expressions. (2) To make the experimental results easy to observe, the T1, T2, and T3 treatments at the control temperature were collectively referred to as CK.

The environment chamber temperatures were designed according to the range of low temperatures that occur in northern China during the post-spring winter wheat jointing stage [30,31], as shown in Figure 1. Four temperature levels (daily maximum temperature/daily minimum temperature) were set: 7 °C/−3 °C, 10 °C/0 °C, 13 °C/3 °C, and 18 °C/8 °C (CK). The light intensity was set at 800 μmol·m^{-2}·s^{-1}, and the duration was 2, 4, and 6 days, respectively. During the experiment, the relative humidity was set at 70 ± 5%, and the photoperiod was set at 12/12 h (daytime from 6:00 a.m. to 6:00 p.m.).

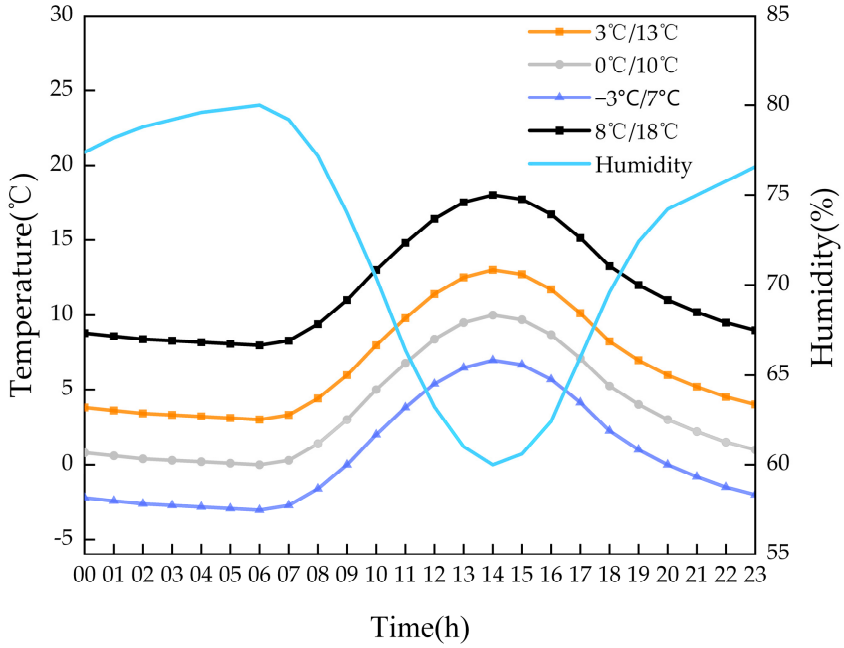

Figure 1. The setting of dynamic temperature and humidity of an artificial climate chamber.

2.2. Experimental Treatments and Leaf Sampling

On the morning of the day when the LT experiments commenced at 8 a.m., winter wheat at the jointing stage, exhibiting relatively uniform growth, was randomly selected and subjected to LT treatments within an artificial climate chamber. There was a total of 12 LT treatments. On the one hand, the recommended planting density for wheat is 200–300 plants·m^2. On the other hand, it was necessary to include a sufficient number of wheat plants in each LT treatment to measure various parameters. Therefore, for each LT treatment, 11 pots of winter wheat were placed (2 pots for measuring photosynthetic parameters,

2 pots for measuring protective enzyme activity, 2 pots for measuring pigment content, 2 pots for measuring endogenous hormones, and 3 spare pots), with each environmental chamber having an area of 2.25 m^2 and a total of 12 environmental chambers simultaneously conducting treatments.

At the end of the LT stress periods on day 2 (48 h), day 4 (96 h), and day 6 (144 h), winter wheat at the jointing stage, exhibiting relatively uniform growth, was selected for the measurements of photosynthetic parameters and pigment content. Additionally, fresh samples of the selected leaves were wiped clean and placed in zipper bags, then rapidly frozen in liquid nitrogen, and stored at −80 °C to measure protective enzyme activity and endogenous hormone content. Each of the measured indicators was sampled three times.

2.3. Testing Content and Methods of Measurement

2.3.1. Determination of Light Response Parameters

Light curve data of winter wheat leaves were measured using the LI-6400 Portable Photosynthesis System (LI-COR Biosciences Inc., Lincoln, NE, USA) between 9:00 a.m. and 11:00 a.m. on sunny days. Measurements were taken at the same blade position of the fully expanded penultimate leaf. During the measurements, the leaf chamber temperature in the LI-6400 was set at 24 °C, the CO_2 concentration was set at 400 μmol·mol^{-1}, and photosynthetically active radiation (PAR) was set at 1800, 1600, 1400, 1200, 1000, 800, 600, 500, 400, 300, 200, 100, 50, and 0 μmol·m^{-2}·s^{-1} [32]. The measured parameters included Pn (net photosynthetic rate), Gs (stomatal conductance to water vapor), Tr (transpiration rate), etc. When measuring the photosynthetic characteristics, three LI-6400 instruments were simultaneously used to measure three wheat leaves and three replicates were used for the determination of photosynthetic characteristics. The values are presented as "mean ± standard deviation (SD)".

The light response curve data of winter wheat leaf were fitted with a rectangular hyperbolic model after measurement [33]. The expression of the model is as follows:

$$p_n(i) = \alpha \frac{1 - \beta I}{1 + \gamma I} I - R_d \quad (1)$$

where α represents the slope of the light response curve of plant photosynthesis at I = 0, that is, the initial slope of the light response curve, also known as the initial quantum efficiency. β represents the correction coefficient, γ is a coefficient independent of light intensity, and Rd represents dark respiration.

2.3.2. Determination of Chlorophyll Content

The determination of chlorophyll content was conducted following the method proposed by Li [34] as follows. Select healthy and mature leaf samples from the top of winter wheat plants. Gently wipe the leaf surfaces to remove dust and remove the leaf veins. Weigh 0.2 g of leaf and then crush it into small pieces. Place the crushed leaf in 25 mL of 95% ethanol solution, seal it, and protect it from light for 48 h until the complete extraction of chlorophyll from the leaf. Measure the absorbance at 665 nm, 649 nm, and 470 nm wavelengths using a spectrophotometer (UV1800 Shimadzu) for colorimetric determination, with three replicates for each treatment. Calculate the chlorophyll content using the following formula:

$$\text{chla} = 13.95D_{665} - 6.88D_{649} \quad (2)$$

$$\text{chlb} = 24.96D_{665} - 7.32D_{665} \quad (3)$$

$$\text{chl(a + b)} = \text{chla} + \text{chlb} \quad (4)$$

$$\text{car} = (1000\text{D}_{470} - 2.05\text{Chla} - 114.8\text{Chlb})/245 \quad (5)$$

where chla, chlb, chl(a + b), and car represent chlorophyll a ($mg \cdot g^{-1}$), chlorophyll b ($mg \cdot g^{-1}$), total chlorophyll ($mg \cdot g^{-1}$), and carotenoids ($mg \cdot g^{-1}$), respectively. D665, D649, and D470 represent the absorbance values of the extracting solution at 665 nm, 649 nm, and 470 nm, respectively.

2.3.3. Determination of Protective Enzyme Activity

In each wheat plant, weigh 0.5 g of single-leaf samples from the same position at the top leaf and place them into a mortar. Add 5 mL of phosphate buffer solution with a pH of 7.8. Grind the mixture in an ice bath and then transfer the homogenate into a centrifuge tube. Freeze the tube and centrifuge for 20 min. Finally, transfer the supernatant (enzyme solution) into a test tube and store it at 0–4 °C.

The activity of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) is determined by the nitroblue tetrazolium (NBT) colorimetric method [35,36], the guaiacol method [37], and the UV spectroscopic method [38], respectively.

2.3.4. Determination of Endogenous Hormone Content

First, prepare 0.1 g/zeatin (ZT), 0.01 g/abscisic acid (ABA), 0.1 g/indole-3-acetic acid (IAA), and 5 g/gibberellin (GA) mother solutions separately. Mix these reagents to prepare a series of standard mixed solutions with different concentrations and store them in brown volumetric flasks at 4 °C for further use.

The second step involves referring to the method of Yang et al., with some modifications [39–41] to analyze the four endogenous hormones, zeaxanthin (ZT), gibberellin (GA), indole-3-acetic acid (IAA), and abscisic acid (ABA), using high-performance liquid chromatography (HPLC). Specifically, use 10 mL of 80% cold methanol for overnight extraction of frozen plant samples. Filter the extract and then perform two additional extractions using 10 mL of 80% cold methanol. Combine the clear extracts from each extraction. Deodorize the residue with an equal volume of petroleum ether. Discard the ether phase and retain the aqueous phase. Repeat this step three times. Evaporate the combined aqueous phase under reduced pressure at 37 °C until the volume is reduced to one-fourth of the original volume. Adjust the pH of the solution to 2.8 and then extract with an equal volume of ethyl acetate. Discard the aqueous phase, and combine the ester phase. Repeat this step three times. Evaporate the ester phase under reduced pressure at 37 °C until it reaches a volume of 1 mL. Adjust the volume to 2 mL with methanol, and after filtration through a 0.45 μm microporous membrane, the solution will be ready for analysis.

The chromatographic column used in the experiment is Agilent 5 HC-C18 (150 × 4.6 mm, 5 μm). The mobile phase used is a mixture of methanol and 0.075% acetic acid aqueous solution in a ratio of 45:55 with a flow rate of 0.7 mL/min. The column temperature is set at 35 °C, and each injection volume is 20 μL. The wavelength range for detection is 210 nanometers.

2.4. Statistical Analysis

For statistical analyses in this investigation, all data were the mean ± standard deviation (SD) of 3 biological replications. In order to investigate the effects of temperature, duration, and their interaction on various physiological parameters of leaf tissue, SPSS 24.0 (SPSS Inc., Chicago, IL, USA) was used for two-way ANOVA, Duncan's multiple comparison test ($p = 0.05$), and correlation analysis.

3. Results

3.1. Effect of LT on the Photosynthetic Characteristics of Wheat Leaves

3.1.1. Effect of LT Stress on Photosynthetic Rate (Pn) of Leaves

Figure 2 shows changes in the light response curve of winter wheat leaves under different LT stresses. From Figure 2a, it can be observed that except for the −3 °C treatment, the photosynthetic rate (Pn) of winter wheat leaves showed no significant difference during the first 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of PAR (photosynthetically active radiation) and then slightly

decreased after reaching the light saturation point. Under the CK treatments, the Pn of wheat leaves reached a maximum of 29.16 μmol·m^{-2}·s^{-1} at a PAR of 1600 μmol·m^{-2}·s^{-1}. Under the 3 °C treatments, the Pn of leaves reached a maximum of 25.67 μmol·m^{-2}·s^{-1} at a PAR of 1200 μmol·m^{-2}·s^{-1}, which was 88.03% of the CK value. Under the 0 °C treatment, the Pn of leaves reached a maximum of 18.15 μmol·m^{-2}·s^{-1} at a PAR of 1000 μmol·m^{-2}·s^{-1}, which was 62.24% of the CK value. Under the −3 °C treatment, the Pn of leaves reached a maximum of 2.24 μmol·m^{-2}·s^{-1} at a PAR of 400 μmol·m^{-2}·s^{-1}, which was only 7.70% of the CK value and showed an unclear changing trend. From Figure 2b,c, it can be observed that the trend of Pn in winter wheat leaves remained consistent with the 2d LT stress when subjected to 4d and 6d stress. However, the Pn at the light saturation point is lower in the prolonged stress conditions compared with the 2d stress. Furthermore, under −3 °C treatment, the maximum net photosynthetic rate of winter wheat tended toward 0.

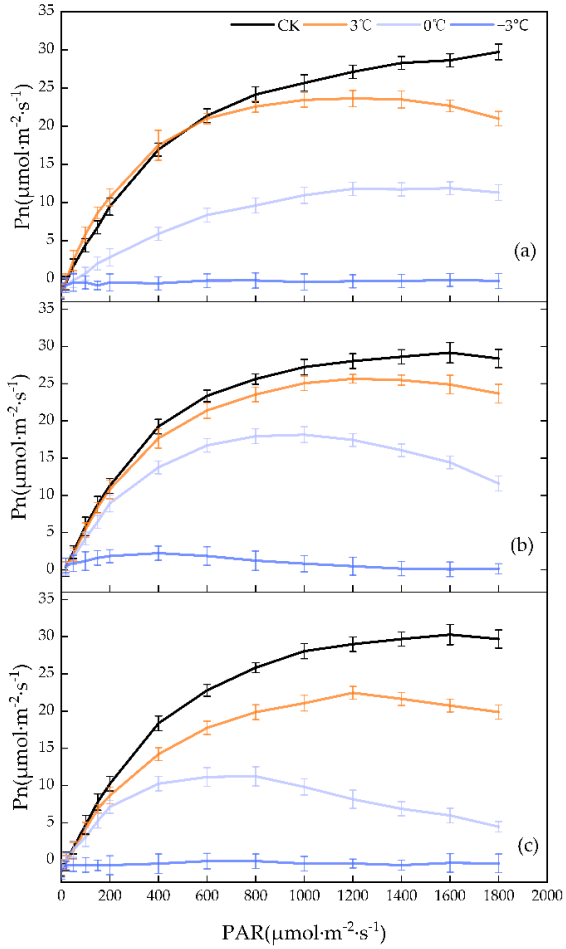

Figure 2. Changes in the light response curves of winter wheat leaves under different LT (low-temperature) stresses. (**a**) Light response curves under various LT stresses after 2 days of treatment. (**b**) Light response curves under various LT stresses after 4 days of treatment. (**c**) Light response curves under various LT stresses after 6 days of treatment. Three replicates were used for determination, and each value is presented as "mean ± standard deviation (SD)".

In conclusion, the photosynthetic rate of winter wheat leaves under different treatments increased to a saturation point and then slightly declined. As the severity of LT stress increased, the net photosynthetic rate of winter wheat leaves reached the light saturation point more quickly, but with a smaller maximum net photosynthetic rate. The −3 °C LT stress had a significant impact on the photosynthetic rate of winter wheat leaves, almost causing a complete cessation of photosynthesis in the leaves.

3.1.2. Effect of LT Stress on Stomatal Conductance (Gs) of Leaves

Stomata are the main channels through which plants exchange gases with the external environment. They play a crucial role in balancing water loss and gaining carbon for biomass production. Stomatal conductance (Gs) represents the degree of stomatal opening and is a major factor influencing plant photosynthesis, respiration, and transpiration [42]. Figure 3 shows changes in Gs of winter wheat leaves under different LT stresses. From Figure 3a, it can be observed that for the same duration of stress, Gs of winter wheat leaves decreased significantly with lower stress temperatures. Among them, Gs under T4 treatment showed little change compared with T1, while Gs of winter wheat leaves under T10 treatment reached a maximum of 0.03 mmol·m^{-2}·s^{-1} at a PAR of 1800 µmol·m^{-2}·s^{-1}, only 33.04% of the maximum value of T1. From Figure 3b, it can be seen that when PAR was between 100–200 µmol·m^{-2}·s^{-1}, Gs of leaves under T2, T5, T8, and T11 treatments rapidly increased, followed by a slow overall increase. Gs of leaves under the T8 treatment was significantly lower than that under the T5 treatment. From Figure 3c, it can be seen that after 6 days of stress, Gs of leaves under T10 treatment could still maintain a relatively high level, while Gs of leaves under T6 treatment decreased significantly compared with 2d and 4d stresses. Gs of leaves under T9 and T12 treatments remained at a lower level.

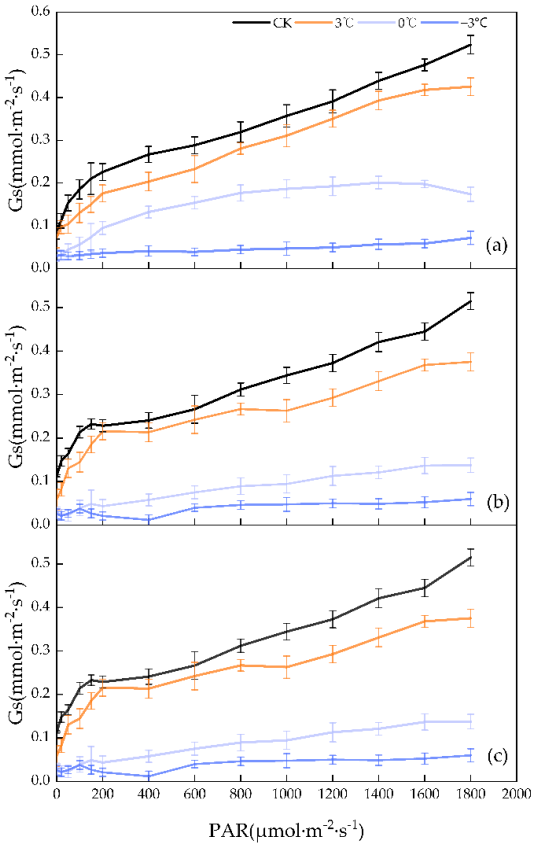

Figure 3. Changes in Gs (stomatal conductance to water vapor) of winter wheat leaves under different LT (low-temperature) stresses. (**a**) Gs variation under various LT stresses after 2 days of treatment. (**b**) Gs variation under various LT stresses after 4 days of treatment. (**c**) Gs variation under various LT stresses after 6 days of treatment. Three replicates were used for determination, and each value is presented as "mean ± standard deviation (SD)".

In summary, as the severity of LT stress increases, Gs of winter wheat leaves tended to decrease. Specifically, a significant decrease in Gs under 3 °C LT stress occurred after 6 days of stress, while a noticeable decrease in Gs under 0 °C LT stress occurred after 4 days of stress. Under −3 °C LT stress, Gs of leaves remained at a consistently lower level throughout the stress period.

3.1.3. Effect of LT Stress on Leaf Transpiration Rate (Tr)

Transpiration of winter wheat leaves can help regulate temperature [43]. Figure 4 shows changes in the leaf transpiration rate (Tr) of winter wheat leaves under different LT stresses. From Figure 4a, it can be observed that except for the T10 treatment, Tr of winter wheat leaves under the other LT treatments rapidly increased with increasing PAR and then stabilized. As the severity of LT stress increased, leaf Tr decreased. Under the T10 treatment, Tr of leaves remained constant at a level of 0.30 mmol·m^{-2}·s^{-1}, which was only 8.62% of the highest value under the T1 treatment. From Figure 4b, it can be seen that leaf Tr under the T2 treatment remained at a relatively high level, while Tr under the T5 treatment reached its peak and decreased significantly earlier than under the T4 treatment. Tr of leaves under T8 and T11 treatments remained at a lower level. From Figure 4c, it can be seen that leaf Tr under the T3 treatment remained at a higher level. Tr of leaves under the T6 treatment reached its peak earlier than under the T5 treatment and was higher than under the T3 treatment when PAR was between 100–600 µmol·m^{-2}· s^{-1}, then rapidly decreased. Tr of leaves under T9 and T12 treatments remained at a lower level.

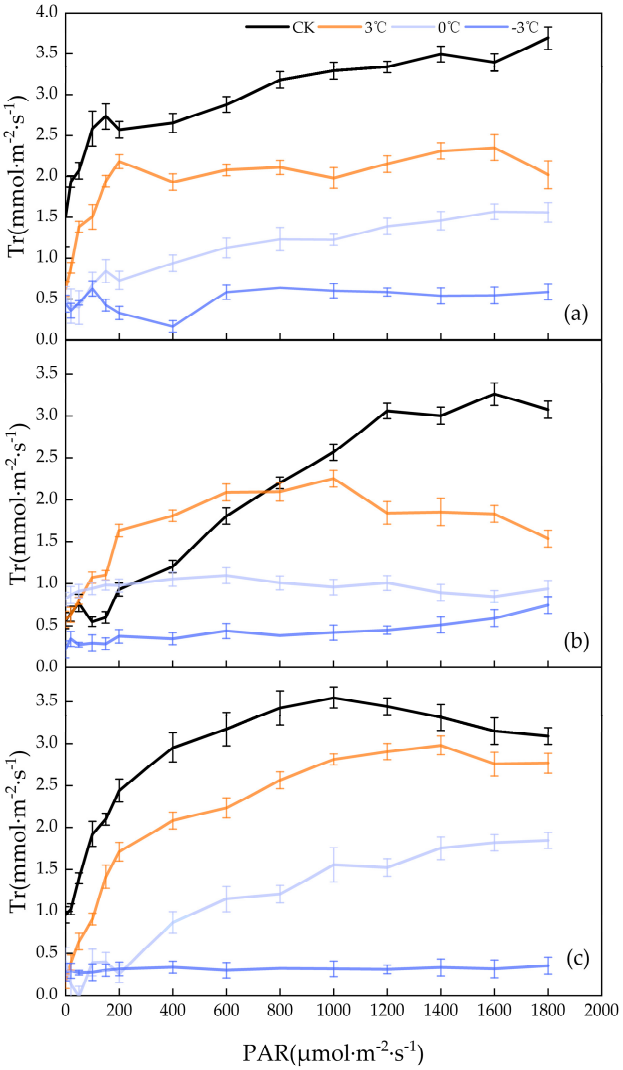

Figure 4. Changes in leaf Tr (transpiration rate) of winter wheat leaves under different LT (low-temperature) stresses. (**a**) Variation of Tr under various LT stresses after 2 days of treatment. (**b**) Variation of Tr under various LT stresses after 4 days of treatment. (**c**) Variation of Tr under various LT stresses after 6 days of treatment. Three replicates were used for determination, and each value is presented as "mean ± standard deviation (SD)".

In summary, as the severity of LT stress increases, the transpiration rate (Tr) of winter wheat leaves decreases significantly. Under CK treatment, the Tr of winter wheat leaves steadily increases. Under 3 °C treatments, the Tr of winter wheat leaves declines notably after 6 days of stress. Under 0 °C and −3 °C treatments, the Tr of winter wheat leaves remains consistently at a lower level.

3.2. Effect of LT Stress on the Pigment Content of Winter Wheat Leaves

The temperature stress duration and the interaction between temperature and stress duration have a highly significant impact ($p < 0.05$) on the content of chlorophyll a (chla), chlorophyll b (chlb), total chlorophyll content (chl(a + b)), and carotenoids (car) (shown in Table S1).

From Figure 5a, it can be observed that under T1, T2, and T3 treatments, the chla content in wheat leaves showed an increasing trend, indicating that the leaves were in the growth phase. In the early stage of stress (2d), there was not much difference in chla content among the different LT treatments compared with T1. With an increase in the severity of LT stress, under 3 °C and 0 °C treatments, the chla content in wheat leaves slightly increased during the middle stage of stress (4d) and significantly decreased during the later stage (6d). Under the T8 treatment, the chla content reached the maximum value of 3.22 mg·g^{-1}, showing a growth of 7.04% compared with the T2 treatment. Under the −3 °C treatment, the chla content in wheat leaves showed a continuous decreasing trend and reached the minimum value of 2.14 mg·g^{-1} under the T12 treatment, representing a decrease of 33.72% compared with the T3 treatment.

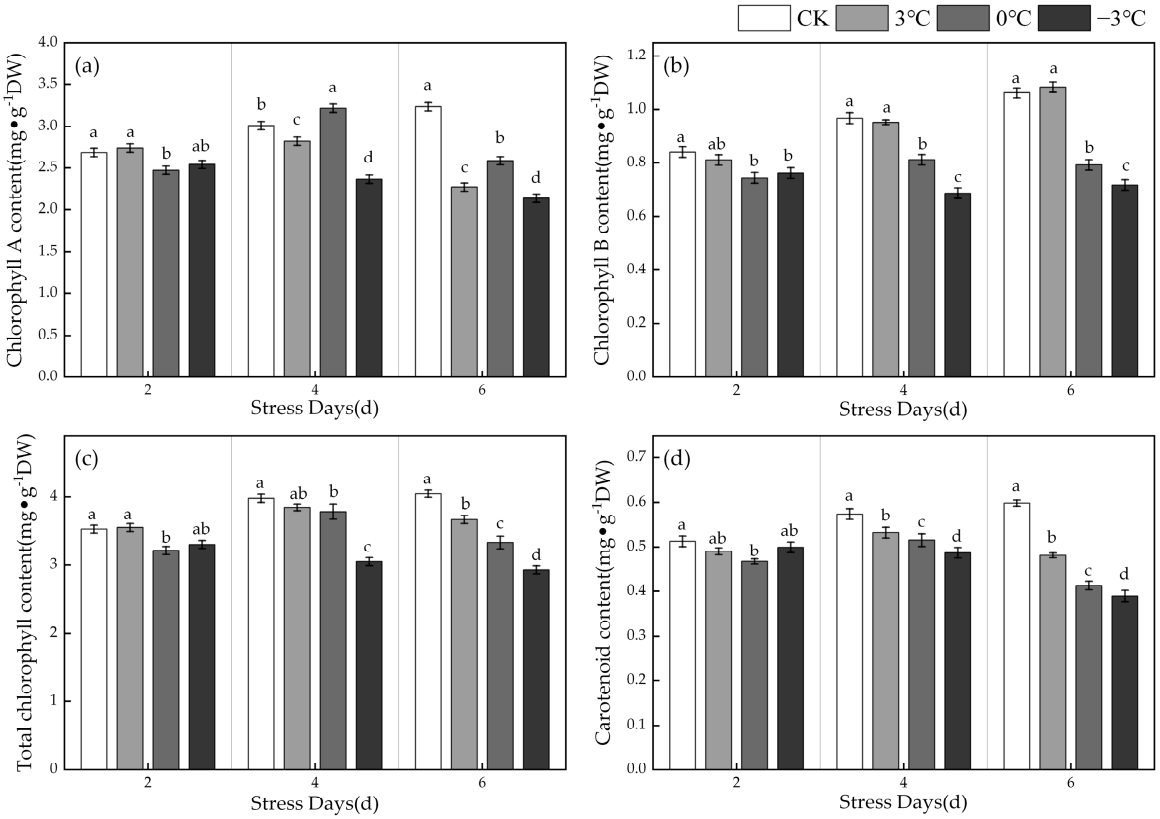

Figure 5. Changes in the leaf pigment content of winter wheat leaves under different LT (low temperature) stresses. (**a**) Chlorophyll a content. (**b**) Chlorophyll b content. (**c**) Total chlorophyll content. (**d**) Carotenoid content. Three replicates were used for determination, and each value is presented as "mean ± standard deviation (SD)". **Note:** Lowercase letters represent Duncan's test with $p < 0.05$.

Figure 5b shows changes in chlb content of winter wheat under different LT stress levels. From the figure, it can be observed that similar to chla, chlb in wheat leaves also exhibited an increasing trend under T1, T2, and T3 treatments. Under 3 °C treatment, there was not much difference in chlb content compared with T1, T2, and T3, as the severity of LT stress increased. Under 0 °C treatment, the chlb content in wheat leaves showed little change during the early to middle stages of stress (2–4d) but significantly decreased during the later stage (6d), compared with T1 and T2 treatments. Under the −3 °C treatment, the chlb content in wheat leaves continuously decreased with the increasing severity of LT stress. It reached the minimum value of 0.64 $mg \cdot g^{-1}$, representing a decrease of 40.72% under the T12 treatment, compared with the T3 treatment.

Figure 5c shows changes in the total chlorophyll content of winter wheat under different LT stresses. From the figure, it can be seen that under 3 °C and 0 °C treatments, the total chlorophyll content in wheat showed a slight increase during the early to middle stages of stress (2–4d) and a decrease during the later stage (6d), with a more pronounced decrease under 0 °C treatment compared with 3 °C treatment. Under the −3 °C treatment, the total chlorophyll content in wheat continuously decreased with the increasing severity of LT stress.

Figure 5d shows changes in car content of winter wheat under different LT stress levels. From the figure, it can be seen that during the early stage of stress (2d), there was not much difference in car content among the different LT treatments compared with T1. During the middle stage of stress (4d), the carotenoid content in wheat showed little change under different LT treatments compared with the early stage, but there was a significant difference compared with T2. As the severity of LT stress increased, the car content in wheat under all treatments decreased significantly. Under the T12 treatment, it reached the minimum value of 0.36, representing a decrease of 41.90% compared with the T3 treatment.

3.3. Effect of LT Stress on the Activity of Protective Enzymes in Winter Wheat Leaves

The temperature, stress duration, and the interaction between temperature and stress duration have a highly significant impact ($p < 0.05$) on the activity of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) in winter wheat leaves (shown in Table S2).

The CAT activity in winter wheat leaves under different LT stress conditions is shown in Figure 6a. From the figure, it can be seen that CAT activity in winter wheat leaves significantly increases with the severity of LT stress under 3 °C and 0 °C treatments. The largest increase in CAT activity is observed under the 0 °C treatment, reaching a maximum value of 8.02 $U \cdot g^{-1} \cdot min^{-1}$ at T9, which is a 40.13% increase compared with the T3 treatment. Under −3 °C treatment, CAT activity in winter wheat leaves decreases significantly with the severity of stress. At T12, CAT activity is the lowest, which is 4.26 $U \cdot g^{-1} \cdot min^{-1}$, representing a 30.61% reduction compared with the T3 treatment.

The activity of SOD in winter wheat leaves under different levels of LT stress is shown in Figure 6b. From the graph, it can be observed that the LT stress at 3 °C has a minimal impact on the SOD activity in winter wheat leaves. However, as the severity of LT stress increases, there is a clear increasing trend in SOD activity. Under −3 °C treatment, the SOD activity in the leaves shows the most significant enhancement with the increasing severity of LT stress. In the T12 treatment, it reaches a maximum value of 296.5 2 $U \cdot g^{-1} \cdot min^{-1}$, which is a 66.14% increase compared with the T3 treatment. This indicates that winter wheat leaves are able to maintain effective scavenging of reactive oxygen species even under −3 °C LT stress.

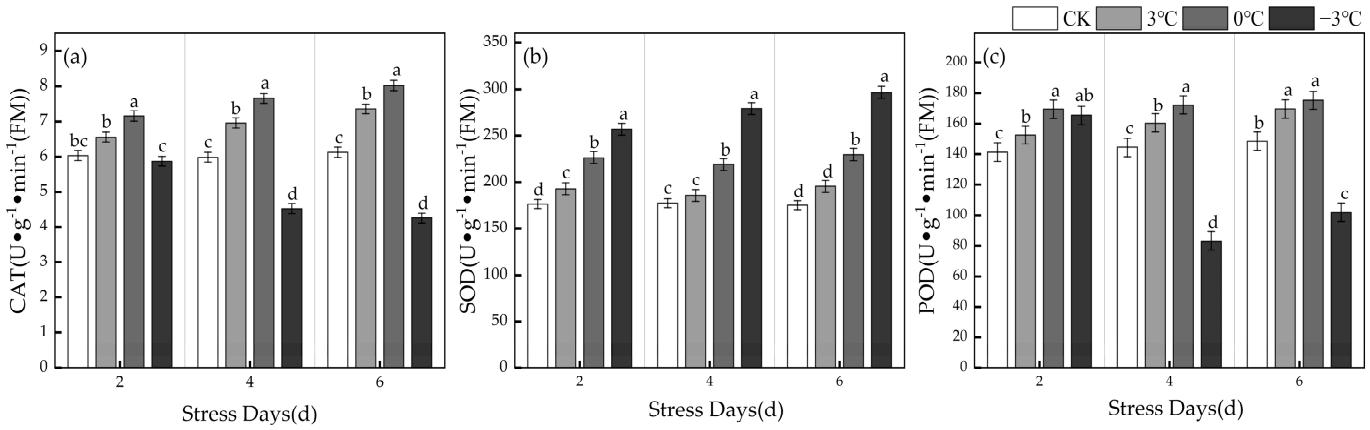

Figure 6. Changes in leaf protective enzyme activity of winter wheat leaves under different LT (low temperature) stresses. (**a**) The activity of CAT (catalase). (**b**) The activity of SOD (superoxide dismutase). (**c**) The activity of POD (peroxidase). Three replicates were used for determination, and each value is presented as "mean ± standard deviation (SD)". **Note:** Lowercase letters represent Duncan's test with $p < 0.05$.

The activity of POD in winter wheat leaves under different levels of LT stress is shown in Figure 6c. From the graph, it is evident that similar to CAT activity, there is a noticeable trend of increasing POD activity with the severity of LT stress, reaching a maximum value of 175.03 $U \cdot g^{-1} \cdot min^{-1}$ in the T9 treatment, which is an 18.21% increase compared with the T3 treatment. However, under −3 °C treatment, the POD activity in winter wheat leaves exhibits a significant increase in the early stages of stress (2d), followed by a notable decrease in the later stages of stress (4–6d), reaching its lowest value of 101.13 $U \cdot g^{-1} \cdot min^{-1}$ in the T12 treatment, representing a 31.85% reduction compared with the T3 treatment.

3.4. Effect of LT Stress on Endogenous Hormones in Winter Wheat Leaves

The temperature, duration of stress, and the interaction between temperature and stress duration have a significant impact on the content of zeatin (ZT), gibberellin (GA), indole-3-acetic acid (IAA), and abscisic acid (ABA) in winter wheat leaves ($p < 0.05$) (shown in Table S3).

Figure 7a shows changes in ZT content in winter wheat leaves under different LT stresses. From the figure, it can be observed that during the early to middle stages (2–4d) before cold stress, the ZT content in winter wheat leaves increased with the severity of LT stress and was significantly higher than in the control (CK) treatment. Under the T8 treatment, the ZT content in the leaves reached a maximum of 28.17 $\mu g \cdot g^{-1}$, showing a 27.16% increase compared with the T2 treatment. In the later stage of stress (6d), the ZT content in the leaves under all LT treatments sharply decreased. Under the T12 treatment, it reached the lowest value of 16.24 $\mu g \cdot g^{-1}$, showing a 35.58% decrease compared with the T3 treatment. This indicates that continuous 6d LT stress significantly affects the ZT content in winter wheat leaves.

Figure 7b shows changes in GA content in winter wheat leaves under different LT stresses. From the figure, it can be observed that during the early stage of LT stress (2d), the GA content in the leaves under various LT treatments did not differ significantly from the control (CK). However, as the severity of LT stress increased, there was a noticeable downward trend in the leaf GA content. Among them, the leaf GA content was most significantly affected by LT stress under the 3 °C treatment.

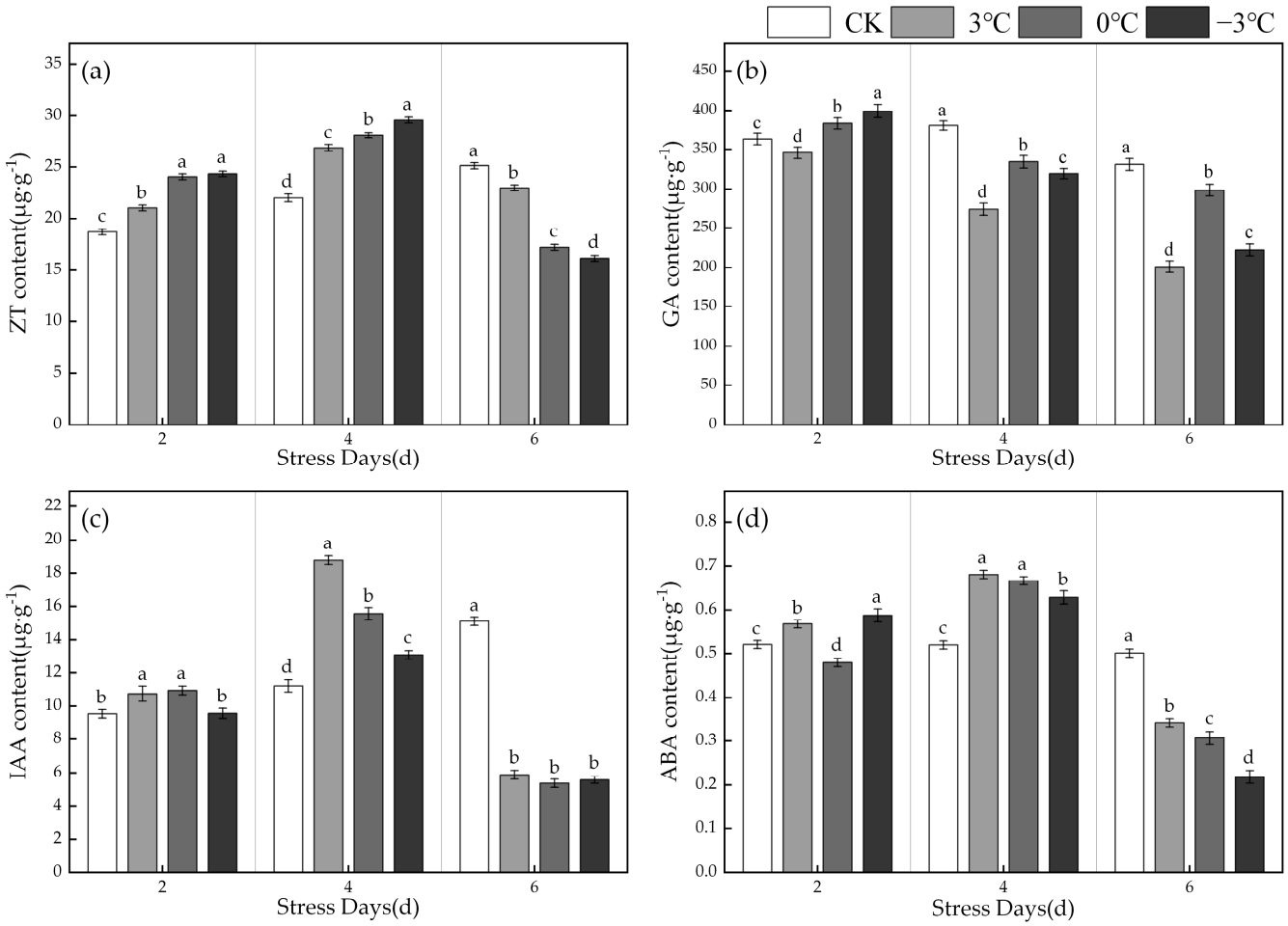

Figure 7. Changes in leaf endogenous hormone content of winter wheat leaves under different LT (low temperature) stresses. (**a**) The ZT (zeatin content) content. (**b**) The GA (gibberellin) content. (**c**) The IAA (indole-3-acetic acid) content. (**d**) The ABA (abscisic acid) content. Three replicates were used for determination, and each value is presented as "mean ± standard deviation (SD)". **Note:** Lowercase letters represent Duncan's test with $p < 0.05$.

Figure 7c illustrates changes in IAA content in winter wheat leaves under different LT stresses. From the figure, it can be observed that the IAA content in winter wheat leaves under T1, T2, and T3 treatments increased with time, indicating that the wheat leaves were in the growth stage. The IAA content in the leaves under various LT treatments showed an initial increase followed by a decreasing trend. Among them, the IAA content in the leaves under the T5 treatment reached a maximum value of 18.82 $\mu g \cdot g^{-1}$, an increase of 65.50% compared with T2. However, in the later stages of stress (6d), the IAA content in the leaves under all treatments remained at a lower level.

The effect of LT stress on the content of ABA in winter wheat leaves is shown in Figure 7d. It can be observed that with the increase in the LT stress, the leaf ABA content under each LT treatment shows a trend of increasing and then decreasing. Among them, the ABA content of winter wheat leaves under T5 treatment reached the maximum value of 0.68 $\mu g \cdot g^{-1}$, which increased by 30.83% compared with that of T2 treatment, while the ABA content of leaves under T12 treatment reached the minimum value of 0.22 $\mu g \cdot g^{-1}$, which decreased by 56.02% compared with that of T3.

3.5. Correlation Analysis of Various Physiological Indexes of Winter Wheat Leaves with Temperature and Duration Days

It can be seen that SOD activity and ZT content were negatively correlated with temperature, and all of them showed an increasing trend with the decrease in temperature. CAT activity, SOD activity, and chlb content were positively correlated with the number of days of duration, and all of them showed an increasing trend with the increase in the number of days of duration. Pn, Gs, and Tr showed highly significant correlations, and all of them decreased with the increase in the degree of LT stress. Also, chla content, chlb content, and chl(a + b) content showed highly significant correlations, all of which decreased with the increase in LT stress. CAT activity and POD activity showed highly significant correlations, all of which decreased with the increase in LT stress. ZT content, IAA content, and ABA content showed highly significant correlations, all of which decreased significantly with the increase in LT stress (Table 2).

Table 2. Correlation analysis.

Variants	Temperature	Stress Days	Pn	Gs	Tr	chla	chlb	chl (a + b)	car	POD	CAT	POD	ZT	GA	IAA	ABA
Temperature	1															
Stress days	0	1														
Pn	0.925 **	−0.134	1													
Gs	0.927 **	−0.265	0.943 **	1												
Tr	0.910 **	−0.247	0.929 **	0.901 **	1											
chla	0.242	−0.400	0.274	0.325	0.426	1										
chlb	0.054	0.291	0	−0.048	0.084	0.705 *	1									
chl(a + b)	0.199	−0.273	0.207	0.23	0.348	0.975 **	0.845 **	1								
Car	0.125	−0.326	0.088	0.167	0.292	0.848 **	0.710 **	0.862 **	1							
POD	0.224	−0.124	0.409	0.185	0.376	0.405	0.393	0.429	0.178	1						
CAT	0.199	0.214	0.397	0.135	0.352	0.447	0.501	0.494	0.221	0.910 **	1					
SOD	−0.898 **	0.116	−0.964 **	−0.888 **	−0.911 **	−0.422	−0.134	−0.360	−0.190	−0.543	−0.538	1				
ZT	−0.417	−0.469	−0.061	−0.044	0.071	0.243	−0.287	0.093	0.149	−0.021	0.050	−0.052	1			
GA	0.183	−0.749 **	0.157	0.262	0.382	0.446	0.125	0.376	0.570	0.180	0.029	−0.187	0.202	1		
IAA	0.236	−0.228	0.290	0.348	0.417	0.456	−0.098	0.314	0.480	0.030	0.068	−0.353	0.770 **	0.294	1	
ABA	0.122	−0.579 *	0.188	0.300	0.332	0.610 *	−0.042	0.447	0.535	0.079	0.082	−0.298	0.786 **	0.540	0.851 **	1

(1) ** denotes a highly significant correlation at $p < 0.01$; * denotes a significant correlation at $p < 0.05$. (2) Pn stands for net photosynthetic rate, Gs stands for stomatal conductance to water vapor, Tr stands for transpiration rate, chla stands for chlorophyll a content, chlb stands for chlorophyll b content, chl(a + b) stands for total chlorophyll content, car stands for carotenoid content, POD stands for peroxidase activity, CAT stands for catalase activity, SOD stands for superoxide dismutase activity, ZT stands for zeatin, GA stands for gibberellins, IAA stands for indole-3-acetic acid, and ABA stands for abscisic acid.

4. Discussion

Numerous studies have shown that during the jointing stages of winter wheat, its cold resistance is significantly reduced, making it unable to adapt to the adverse conditions of late spring frost [44,45]. The jointing stage is a critical period that affects the yield of winter wheat, and any LT damage occurring during this stage can cause irreparable losses to the yield [46,47]. In this study, we conducted experiments involving different LT stresses using the commonly cultivated winter wheat variety "Ji Mai 22" in China. We investigated the effects of different LT stresses on photosynthetic characteristics, senescence characteristics, and endogenous hormones of winter wheat leaves, and explored the relationship of each physiological index with temperature and days of stress through correlation analysis. This provides a scientific basis for the prevention of LT damage and the quantitative management of winter wheat growth and development.

Photosynthesis is an essential physiological process in the growth and development of winter wheat, which is affected by genetic and environmental factors, and its intensity can be reflected by various factors, such as stomatal conductance (Gs) and transpiration rate (Tr), which are closely related to the plant's status and metabolic level [48]. In this study, it was found that there was a highly significant positive correlation between Pn, Gs, and Tr in winter wheat leaves under LT stress, and they all decreased with the increase in the degree of LT stress. This is consistent with Fu's finding that LT stress limits the photosynthetic properties of winter wheat leaves [49]. This may be due to the fact that as the severity of LT stress increases, winter wheat decreases stomatal opening and transpiration rate to maintain temperature and leaf function, which leads to a decrease in net photosynthetic rate.

The leaf senescence characteristics of winter wheat can be observed from aspects such as chlorophyll content and protective enzyme activity [12]. In this study, it was found that

LT stress led to a decrease in chlorophyll content in winter wheat leaves, which is consistent with the results of recent studies that found a decreasing trend of chlorophyll with the increase in the degree of LT stresses [13,50]. On the one hand, LT stress reduces the activity of chlorophyll synthesis enzymes, thus inhibiting chlorophyll synthesis. On the other hand, LT slows down the metabolism of winter wheat, leading to insufficient raw materials for chlorophyll synthesis, resulting in a decrease in chlorophyll content. The contents of chla, chlb, and chl(a + b) were significantly correlated, and all of them decreased with the increase in the degree of LT stress, whereas the content of car was not significantly affected by LT stress, which may be attributed to the fact that LT stress is generally accompanied by drought, and drought will obviously cause car to rise [51].

Under LT stress, plants typically increase the activity of one or more antioxidant enzymes to adapt to the stress, and the increase in enzyme activity is generally associated with enhanced resistance [52]. Jin's research found that LT stress leads to an increase in SOD, POD, and CAT activity [53]. In this experiment, it was observed that with increasing severity of LT stress, SOD activity showed a continuous upward trend, while POD and CAT activities decreased in T11 and T12 treatments. In other words, POD and CAT exhibited an initial increase followed by a decrease with increasing LT stress severity. This may be attributed to the partial loss of leaf physiological functions when the severity of LT stress exceeds its tolerance limit, rendering the protective enzyme system ineffective. CAT activity and POD activity were highly correlated, while SOD activity was not significantly affected by LT stress, indicating that the SOD system in winter wheat leaves is more resistant to LT stress than CAT and POD.

The jointing stage is a crucial period for endogenous hormone regulation in winter wheat, and it is closely related to its yield [54]. This study found that as the severity of LT stress increased, the content of ZT in the leaves showed an initial increase followed by a decrease, while the GA content consistently decreased. This is consistent with the findings of Yang, who reported a significant decrease in ZT and GA content in winter wheat leaves under LT stresses [55]. The increase in ZT content in the early stages of leaf development was found to enhance the activity of SOD, thereby increasing the cold resistance of the leaves. However, as time progressed and temperature decreased, the growth of the winter wheat leaves slowed down, leading to a significant decrease in ZT synthesis. The decreasing trend of GA content indicated a reduction in the antiaging properties of the leaves under LT stress. In addition, this experiment also observed the content of IAA and ABA. The IAA content showed an initial increase (2–4d) followed by a decrease (4–6d) under LT stress. The increase in the early stage may be caused by the onset of LT stress when the leaves produce a certain degree of resistance, while the later decrease may be due to the fact that when the stress exceeds the leaf's ability to withstand stress, the leaf stops its metabolism. On the other hand, the enhancement of POD activity is due to the reduction of free radicals caused by LT stress at the same time and, also, to the degradation of the IAA. The ABA content also showed a trend of increasing and then decreasing, as well as the elevation of ABA content. On the one hand, LT promoted stomatal closure, which reduced water runoff and, at the same time, reduced the damage caused by LT stresses. On the other hand, the LT induced the production of some new proteins related to resistance, while the decrease might be due to the fact that the leaves did not metabolize when the temperature was low enough.

Although the study provides a detailed analysis of the changes in photosynthetic and senescence characteristics of winter wheat leaves under different LT stresses, there are still some limitations. In future experimental plans, we will continue to focus on selecting a more representative range of spring wheat, winter wheat, and winter wheat varieties. Additionally, we will attempt to conduct drought stress experiments. This will help overcome the limitations of using a single variety and not considering drought stress in our research. Through these experiments, we hope to gain a more comprehensive understanding and make comparisons of the physiological responses of winter wheat at the jointing stage to both low-temperature (LT) stress and drought stress.

5. Conclusions

With the decrease in temperature and the prolongation of stress, the changes in various winter wheat leaves were not linear. Among them, the photosynthetic parameters and carotenoids of wheat leaves showed a decreasing trend, while the chlorophyll b content showed an increasing trend. On the other hand, the chlorophyll a content, protective enzyme activity, zeaxanthin, auxin, and abscisic acid showed a trend of first increasing and then decreasing. The experiment discovered that, under 3 °C LT stress, the impact on the physiological parameters of the leaves was relatively minor, with a significant decrease observed only in pigment and endogenous hormone levels after 6 days of stress. Under 0 °C LT stress, the physiological parameters of wheat leaves significantly decreased after 4 days of stress, indicating that an LT environment of 0 °C or below for more than 4 days is not suitable for the growth of winter wheat. Under −3 °C LT stress, winter wheat leaves almost ceased photosynthesis, the protective enzyme system failed, and chlorophyll, carotenoids, and endogenous hormones remained at the lowest levels. This indicates that the temperature threshold for the growth of "Ji Mai 22" during the jointing stage is −3 °C. Considering the feasibility of future cold weather disasters, the research conducted in this paper focuses on the changes in photosynthetic parameters, aging characteristics, and endogenous hormone levels of wheat leaves under different levels of cold stress during the jointing stage. It provides an important theoretical basis for the classification of low-temperature disasters in the winter wheat variety "Ji Mai 22" during the jointing stage.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13102650/s1. We provided additional information on the two-factor analysis of variance regarding the effects of temperature and duration on leaf chlorophyll content (Table S1), protective enzyme activity (Table S2), and endogenous hormone levels (Table S3) in winter wheat.

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Data Availability Statement: Data not readily available for public consumption due to privacy and other issues.

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