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Dynamic evaluation of winter wheat's freezing resistance under different low-temperature periods and durations

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Winter extreme low temperature events have been occurring frequently both before and after the winter season. The freezing resistance temperature of wheat is far lower than the intensity of low temperatures during the mid-winter period. Therefore, it is necessary to further quantify and evaluate the impact of low-temperature periods and durations during the early winter and the green-up period on the freezing resistance of wheat, based on different evaluation indicators. Through conducting experiments in an artificial low-temperature control chamber, this study investigates the critical temperature thresholds for the impact of different low-temperature periods and durations on the tiller and yield of winter wheat, as well as the critical temperature thresholds for soil effective negative accumulated temperature. The results demonstrate that (1) the tiller mortality rate (R_T) and yield reduction rate (R_Y) of winter wheat during the winter increase with the severity and duration of low temperatures, showing an S-shaped curve. The winter wheat mortality rate during the early winter is related to the soil effective negative accumulated temperature in an exponential function, while the mid-winter and green-up stages have a linear relationship. (2) The freezing threshold temperatures for the R_T, R_Y and soil negative accumulated temperature (SENAT) in different low-temperature periods (early winter, mid-winter, and green-up periods) range from – 11.7 to -17.9 °C, -9.4 to -15.6 °C, and 15.9 to 131.7 °C·h (2.2 to 16.8 °C·d), respectively. (3) The freezing threshold temperatures for the R_T and R_Y in different low-temperature durations (1 day, 2 days, and 3 days) range from – 2.8 to -17.9 °C and – 9.4 to -15.6 °C, respectively. The findings of this study provide technical support and scientific guidance for the global cultivation structure and variety layout of winter wheat under the background of climate warming, as well as for the prevention and reduction of freezing damage and yield losses.

Keywords Winter wheat, Freezing resistance, Low temperature period, Low temperature duration, Tiller mortality rate, Yield reduction rate, Soil effective negative accumulated temperature, Dynamic evaluation

Wheat (*Triticum aestivum* L.), the third-largest cereal crop in the world after corn and rice, with an annual global production exceeding 6 billion tons, provides 21% of the global food supply[1,2]. Winter wheat freeze injury refers to the damage caused by a prolonged period of strong low temperatures or drastic temperature changes below 0 °C during the winter dormancy to green-up period. It is a common phenomenon in wheat-growing areas worldwide[3,4]. In recent years, with the increase in extreme weather events under the background of global climate warming, the frequency and intensity of agricultural meteorological disasters have shown a significant upward trend[5,6], and winter wheat freeze injury has become more complex. Due to the significant reduction in the number of days with temperatures below 0 °C and the significant rise in extreme low temperatures in the North China Plain (NCP)[7], winter wheat tends to grow vigorously before winter, leading to the forward of reproductive stages[8]. This leads to a decrease in wheat's cold resistance and increases the risk of winter wheat freeze injury to some extent. Meanwhile, with the northward shift of the winter wheat planting boundary in the NCP[9,10], winter wheat varieties with strong cold resistance are gradually being replaced by semi-winter and even weak spring varieties. The springness trend of the winter wheat has reduced the freezing resistance of winter

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wheat[11,12]. Once there is a sharp drop in temperature during winter or green-up period, widespread winter wheat freeze damage will occur.

For most of the winter wheat freezing damage assessment research, a 50% tiller death rate was widely adopted as the indicator of different wheat varieties' freezing resistance[13–15]. However, the reduction in crop yield can be categorized into mild damage (11–30% yield reduction), moderate damage (31–50% yield reduction), severe damage (51–80% yield reduction), and total loss (above 80% yield reduction) based on the degree of yield reduction[16,17]. In practical agricultural production, a 50% of stem and tiller death rate is too devastating for evaluating the intensity of disasters, thus this temperature indicator has limited guidance for agricultural production. Therefore, freezing damage indicators revealing varied degree of injury is needed for guiding agricultural production.

The ability of winter wheat to withstand the freezing injury varied among different organs, specifically, the tiller crown is the most freezing tolerable organ of wheat[18], which is mainly reflected in the formation of new tillers[19]. As long as the tiller crown remains alive during winter, the stored nutrients in the tiller crown can support the growth of new tillers after regrowth. The tiller crown of winter wheat generally located in 2 ~ 3 cm below the soil surface[18]. Therefore, the soil temperature at this depth is a direct factor that affects the winter tiller mortality[20]. Additionally, the degree of ground coverage before winter varies at different growth stages of wheat, which can affect the efficiency of cold air transmitting to the soil. Besides, the extent of yield loss caused by different disasters is the most concerning issue in crop production. After experiencing extreme low temperatures, although the crown remained alive, but the development is delayed due to freezing injury, which ultimately affects the final harvest yield[19]. Therefore, a combination of tiller mortality rate (R_T)[21], yield reduction rate (R_Y)[22], and cumulative soil negative temperature[23] were used as evaluation indicators to quantify the freezing resistance of wheat.

The freezing resistance of winter wheat during the overwintering process changes dynamically. It gradually increases during the process of cold hardening and decreases during the process of dehardening[13]. Therefore, the critical freezing resistance temperature of winter wheat fluctuates from early to middle and late winter. Currently, research has explored the impact mechanisms of freezing damage on the growth and development of winter wheat, as well as the morphology and risk assessment of plant performance indicators and the maximum freezing resistance temperature index during middle-winter period[24]. However, frequent extreme low-temperature weather events occur before and after the winter in production[25]. The freezing resistance temperature of wheat is far lower than the low-temperature intensity during the severe winter period, making it susceptible to low-temperature freezing damage. Moreover, the freezing resistance of winter wheat continuously strengthens throughout the overwintering process, and its resistance gradually weakens after reaching the maximum freezing resistance temperature during the severe winter period. Therefore, further research is needed to quantitatively evaluate the impact of low temperatures on wheat freezing resistance during the early overwintering and green-up periods based on different evaluation indicators.

This study aims to investigate the critical temperature indicators for the effect of different low temperature periods and durations on the tillering and yield of winter wheat, as well as the critical indicator for soil effective negative accumulated temperature. Through conducting experiments in a low temperature control box, we will dynamically evaluate the freezing temperature threshold at different developmental stages of winter wheat. The objective of this research is to provide technical support and scientific guidance for the adjustment of global winter wheat planting structure and variety layout under the background of climate warming, as well as to prevent and reduce crop loss caused by freezing damage.

Materials and methods

Experiments design

The study was conducted in the Baodi District Meteorological Bureau of Tianjin City (39°45'N, 117°20'E, elevation is 5.1 m) from 2016 to 2017 to investigate the responses of winter wheat (Seed source is *Nongda 211*(ND211), which from China Agricultural University) to different low temperature levels and durations during the early winter, mid-winter, and green-up periods. Experimental research and field studies on plants (either cultivated or wild), including the collection of plant material, comply with relevant institutional, national, and international guidelines and legislation. The region is characterized by fluvial and coastal plain landforms, with a relatively flat topography and an annual average sunshine duration of 2566.5 h, average temperature of 11.6 °C, average precipitation of 612.5 mm, and a freezing-free period of 184 d. The soil type at the experimental site is sandy loam, with an organic matter content of 18.8 g/kg, total nitrogen content of 102.1 mg/kg, available nitrogen content of 94.4 mg/kg, available phosphorus content of 5.2 mg/kg, available potassium content of 219.2 mg/kg. Soil bulk density, field capacity, and wilting point at different soil depths are shown in Table 1.

Depth (cm)	Soil bulk density (g/cm^3)	Field water capacity (%)	Wilting point (%)
0–10	1.43	20.4	6.1
10–20	1.49	19.0	5.1
20–30	1.47	19.7	5.6
30–40	1.41	22.7	7.1
40–50	1.39	24.2	7.6

Table 1. Soil characteristics at different depths of the experimental site.

Early winter period (from November 13, 2016 to November 22, 2016)
In order to determine the critical freezing temperature of winter wheat during the early stage of overwintering (before completion of hardening progress), a pot experiment was conducted. The pots used were PVC pipes with an inner diameter of 30 cm and a height of 30 cm, without top covers or bottom bases, to ensure the plants root can access the field soil water after green-up period when the root develops quickly and the demand for soil water increase rapidly. To prevent the pots from being firmly frozen into the surrounding soil and being impossible to remove during mid-winter, the pits were lined with a layer of plastic mat. Each pot contained 25 wheat seeds buried in the experimental field at sowing, with the pot surface level with the field surface. After emergence, each pot was thinned to around 20 seeds, giving a planting density of around 280 plants/m[226]. The soil used in the experiment was obtained from the Meteorological Bureau in Baodi District, Tianjin City, and the soil texture was sandy loam. Each pot was filled with 15 kg of soil, and before sowing, a basal fertilizer (compound fertilizer) of 2.0 g was applied, with an N: P: K ratio of 15%: 15%: 15%. The temperature control range of the artificial climatic chamber (LTC-150, Shanghai Santeng Instrument Co., Ltd.) was – 40 to 100 °C, equipped with a fan and a temperature fluctuation ≤ ± 1 °C. The field experiment was conducted on October 1 in 2016, with a sowing depth of 4 cm. Watering was carried out according to the soil moisture conditions to ensure that the wheat was not drought-stressed before winter. During the overwintering period, when the forecast indicated temperatures ≤-10 °C, straw was used for covering and insulation to prevent freezing damage. During the early stage of overwintering, the potted plants were taken out from the field and placed on trays before being moved into a low-freezing damage chamber for testing. Two factors were used in the experiment, with 8 levels for temperature treatment and 3 levels for freezing days treatment, resulting in a total of 24 experimental treatments (Table 2). The low-freezing damage chamber had 3 layers, with 3 pots placed on each layer, and each treatment had 3 replicates, giving 9 pots per chamber at each low temperature treatment. During the low temperature treatment, after the potted plants were moved into the low temperature chamber, the temperature variation process in the chamber was pre-compiled, starting from 0 °C and decreasing at a rate of 2–4 °C/h until reaching the lowest temperature set by the experiment. The set minimum temperature was maintained at this level for 3 h. Then, the temperature was increased at the same rate until reaching 0 °C again and held for 2 h. A temperature probe was placed in the upper right corner of the low-temperature chamber to monitor the temperature inside the chamber in real-time. At the same time, a temperature sensor was inserted into the soil at a depth of 2.5 cm to monitor the changes in soil temperature. The instrument used for this purpose was an air temperature and humidity thermistor (AV-10TH, Beijing Yugen Technology Co., Ltd.), which had a measurement range of -45 to 65 °C. After the first low-temperature freezing cycle (1 day treatment), one pot from each layer in the low-temperature chamber was taken as a sample for the 1 day freezing treatment. Similarly, after the second low-temperature freezing cycle, three pots were taken out from the chamber (one pot from each layer) as the 2 days freezing treatment. At the end of the third freezing cycle, the remaining three potted plants were all taken out as the 3 days low-temperature treatment. After the freezing controlling experiment, the plastic mat was removed and all the pots were buried back to the filed. An additional control group (9 pots) remained buried in the field without undergoing artificial low temperature treatment to ensure no freeze damage occurred as CK. We counted the survival tiller after one week of green-up period and the survival proportion was calculated as the mean of the three replications. Additional 60 mm irrigation was applied at jointing and flowering stage, along with 160 kg urea /ha spread at jointing stage. The grain seed were harvested at maturity stage treated as yield.

Mid-winter period (from December 6, 2016 to January 12, 2017)
The critical freezing temperature of winter wheat during the tillering stage in late winter was determined. The experimental materials were consistent with those used in the early winter experiments described in Sect. 2.1.1. During the late winter period, potted plants were removed from the field and transferred to low freeze-damage boxes for testing. Two factors were manipulated in the experiment, including 6 temperature levels and 3 freezing duration levels, resulting in a total of 18 experimental treatments (Table 2). In the low temperature treatment for 3 days, 4 replicates were used, while 1 replicate was used for measuring plant height and dry weight during the regrowth period. The low temperature boxes were divided into 3 layers, with 3 pots placed on each layer and 4 pots on the bottom layer, giving a total of 10 pots for each temperature treatment. The temperature reduction method and temperature measurement at the soil tillering node were the same as described in Sect. 2.1.1. At the end of the experiment, potted plants were buried in the field. Other field management were the same as described in Sect. 2.1.1.

Green-up period (from February 25, 2017 to March 3, 2017)
During the winter wheat tillering stage, after the deactivation of the cold hardening effect, the critical freezing temperature of stem tiller emergence was determined. The experimental materials were consistent with those used in the early winter experiments described in Sect. 2.1.1. Pot-grown wheat plants were removed from the field during the green-up period and transferred to low-freezing chambers for experimentation. Two factors

Frozen stage	Minimum temperature (°C)	Freezing days (d)
Early winter period	– 8, – 9, – 10, – 11, – 12, – 14, – 16, – 20	1, 2, 3
Mid-winter period	– 10, – 12, – 14, – 16, – 18, – 20	1, 2, 3
Green-up period	– 9, – 10, – 11, – 12, – 13, – 14	1, 2, 3

Table 2. Experimental design for different degrees of low temperature and freezing days.

were manipulated in the experiment: temperature, with six levels, and duration of freezing, with three levels, resulting in a total of 18 experimental treatments (Table 2). The low-temperature freezing chamber was divided into three layers, each containing three pots, with three replicates for each treatment. During the experiment, the cooling treatment of the low-temperature chamber and the method for determining the temperature at the soil tillering node were consistent with those described in Sect. 2.1.1. After the experiment, the pot-grown plants were buried in the field. A control group consisting of nine pots was established, the survival proportion of tillers were investigated on 15th, March and the other management procedures were consistent with those described in Sect. 2.1.1.

According to the experimental data of the low-temperature freezing cabinet control test in Baodi District in 2016–2017, the critical soil temperatures for cold-resistant wheat variety ND211 during the periods of early winter, mid-winter, and green-up are set at -3, -8, and −3°C, respectively. Prior to low-temperature treatment and after green-up period, the number of surviving tillers per pot is counted, and the low-temperature level at the tiller node during the low-temperature treatment period is recorded. After maturity, the yield of all treated wheat is assessed.

Experiments method

Overwintering mortality rate (R_{OM})

R_T referred to freezing damage, is calculated by comparing the number of tillers before experimental treatment with the number of tillers after the cessation of low-temperature treatment and the resumption of growth in wheat (Eq. 1).

$$R_T = (F_1 - F_2)/F_1 \tag{1}$$

Where, F1 represents the number of tillers in wheat before the treatment, and F2 represents the number of tillers in wheat after the completion of low temperature treatment.

R_Y caused by freezing damage is calculated by comparing the yield under different treatments with the control group (CK). This comparison enables a precise assessment of the impact of freezing on crop production (Eq. 2).

$$R_Y = (Y_{ck} - Y_i)/Y_{ck} \tag{2}$$

Where, Y_{ck} represents the yield of the CK group, measured in kilograms per hectare (kg/hm^2), and Y_i denotes the yield of the i treatment, also measured in kilograms per hectare (kg/hm^2).

The present study utilizes the Logistic growth curve to evaluate the effects of different degrees of low temperature on growth indicators of winter wheat (Eq. 3).

$$R_{OM} = \frac{1}{1 + e^{(a \cdot T_m + b)}} \tag{3}$$

Where, R_{OM} represents the impact of freezing damage in terms of stem and R_T and yield loss rate, expressed as a percentage (%), T_m represents the minimum temperature in degrees (°C), *a* and *b* are model parameters. We can calculate the critical temperature indicators for mortality rates of 10%, 30%, and 50% respectively.

Soil effective negative accumulated temperature (SENAT)

Different low temperature durations have different effects on the mortality rate of stem tillers due to the differences in freezing time at the tillering nodes. Therefore, the soil effective negative accumulated temperature (SENAT) index is adopted to characterize the extent of freezing damage. During the low temperature freezing treatment, the soil temperature does not remain below the critical freezing temperature for wheat. In this study, the hourly soil effective negative accumulated temperature ($SENAT_h$) was calculated. Considering the challenges in obtaining data at an hourly resolution, the study also calculated the daily soil effective negative accumulated temperature ($SENAT_d$).

$$SENAT_h = \sum{}_{i=1}^{n}(T_c - T_i) \quad (T_i < T_c) \tag{4}$$

$$SENAT_d = \sum{}_{i=1}^{n}(T_c - T_{smini}) \quad (T_{smini} < T_c) \tag{5}$$

Where, T_c represents the critical soil temperature for wheat stem tillering resistance to freezing (°C), T_i denotes the actual soil temperature at the i_{th} hour (°C), T_{smini} signifies the minimum soil temperature for the i_{th} day (°C). It's important to note that $SENAT_h$ and $SENAT_d$ do not accumulate when both T_i and T_{smini} are above T_c.

Statistical analysis

The data were analyzed and processed in Excel 2016. T test was used to test the significance of the established equations.

Results

Determination of critical temperature for freezing damage based on RT

Based on the findings illustrated in Fig. 1, it is evident that the mortality rate of tiller buds in winter wheat increases with both the severity and duration of low temperatures. This trend follows an S-shaped curve, with

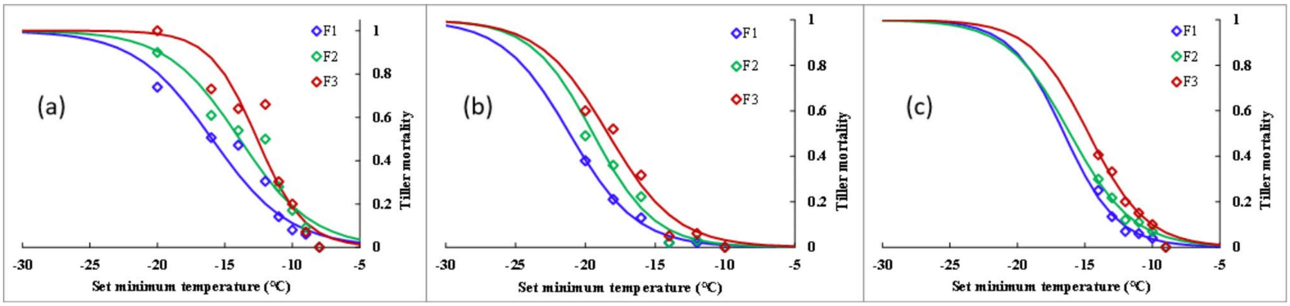

Fig. 1. Relationship between R_T and T_m during overwintering at different stages, where (**a**) represent in early winter period, (**b**) represent in mid-winter period, and (**c**) represent in green-up period. F1 indicated freezing for 1 day, F2 indicated freezing for 2 days and F3 indicated freezing for 3 days.

Freezing stage	Freezing days	Equations	R^2	R_{T10} (°C)	R_{T30} (°C)	R_{T50} (°C)
Early winter period	1	R_T=1/(1 + exp(0.350×T_m+5.587)	0.91**	– 9.4	– 12.5	– 14.0
	2	R_T=1/(1 + exp(0.371×T_m+5.165)	0.93**	– 7.7	– 10.7	– 12.1
	3	R_T=1/(1 + exp(0.571×T_m+7.207)	0.93**	– 8.6	– 10.5	– 11.4
Mid-winter period	1	R_T=1/(1 + exp(0.469×T_m+9.799)	0.93**	– 16.0	– 18.3	– 19.4
	2	R_T=1/(1 + exp(0.462×T_m+9.051)	0.96**	– 14.7	– 17.1	– 18.2
	3	R_T=1/(1 + exp(0.467×T_m+8.666)	0.89**	– 13.6	– 16.0	– 17.1
Green-up period	1	R_T=1/(1 + exp(0.505×T_m+8.355)	0.95**	– 12.0	– 14.2	– 15.2
	2	R_T=1/(1 + exp(0.429×T_m+6.907)	0.96**	– 10.7	– 13.3	– 14.5
	3	R_T=1/(1 + exp(0.468×T_m+6.889)	0.99**	– 9.8	– 12.1	– 13.2

Table 3. Fitting relationship between R_T and T_m under different treatments and critical indicators of freezing resistance. R_{T10} represents 10% R_T, R_{T30} represents 30% R_T, and R_{T50} represents 50% R_T. *Indicates significant at $P = 0.05$ level, and **indicates significant at $P = 0.01$ level. The table below is the same.

varying degrees of sensitivity to low temperatures observed during different stages of winter dormancy: early winter > mid-winter > green-up. Regarding the duration of exposure to low temperatures, the sensitivity follows the sequence: early winter > mid-winter = green-up.

Based on Table 3, it is evident that for different low-temperature periods, the critical freezing temperatures of winter wheat at different R_T (R_{T10}, R_{T30}, and R_{T50}) and varying durations of low temperatures (1 day, 2 days, and 3 days) are as follows: For the early winter period, the critical freezing temperatures are – 13.8, -12.8, and – 11.7 °C for R_T of 1 day, 2 days, and 3 days respectively. Correspondingly, the R_{T10}, R_{T30}, and R_{T50} critical freezing temperatures are – 10.8, -13.2, and – 14.3 °C respectively. Additionally, R_{T10}, R_{T30}, and R_{T50} span from – 7.7 to -9.4, -10.5 to -12.5, and – 11.4 to -14.0 °C respectively. During the mid-winter period, the critical freezing temperatures are – 17.9, -16.7, and – 15.6 °C for R_T of 1 day, 2 days, and 3 days respectively. The R_{T10}, R_{T30}, and R_{T50} critical freezing temperatures are – 14.8, -17.1, and – 18.2 °C respectively. Additionally, R_{T10}, R_{T30}, and R_{T50} span from – 13.6 to -16.0, -16.0 to -18.3, and – 17.1 to -19.4 °C respectively. For the green-up period, the critical freezing temperatures are – 13.8, -12.8, and – 11.7 °C for R_T of 1 day, 2 days, and 3 days respectively. The R_{T10}, R_{T30}, and R_{T50} critical freezing temperatures are – 10.8, -13.2, and – 14.3 °C respectively. Additionally, R_{T10}, R_{T30}, and R_{T50} span from – 9.8 to -12.0, -12.1 to -14.2, and – 13.2 to -15.2 °C respectively. According to the different durations of low temperatures, the critical freezing temperatures for the different stages of winter wheat (early winter, mid-winter, and green-up periods) in terms of stem and tiller death rates are – 12.0, -17.9, and – 13.8 °C for a 1 day low-temperature freezing period. Similarly, for the stem and tiller death rates of 10%, 30%, and 50% (R_{T10}, R_{T30}, and R_{T50}), the critical freezing temperatures during different periods are – 12.5, -15.0, and – 16.2 °C, respectively. For a 2 days low-temperature freezing period, the critical freezing temperatures for different stages of winter wheat in terms of stem and tiller death rates are – 10.2, -16.7, and – 2.8 °C. Similarly, for stem and tiller death rates of 10%, 30%, and 50% (R_{T10}, R_{T30}, and R_{T50}), the critical freezing temperatures during different periods are – 11.0, -13.7, and – 14.9 °C, respectively. Finally, for a 3 days low-temperature freezing period, the critical freezing temperatures for different stages of winter wheat in terms of stem and tiller death rates are – 10.2, -15.6, and – 11.7 °C. Similarly, for stem and tiller death rates of 10%, 30%, and 50% (R_{T10}, R_{T30}, and R_{T50}), the critical freezing temperatures during different periods are – 10.7, -12.9, and – 13.9 °C. These findings indicate the specific low-temperature thresholds required for winter wheat's survival during different stages of low-temperature exposure, providing valuable information for the management and protection of winter wheat crops.

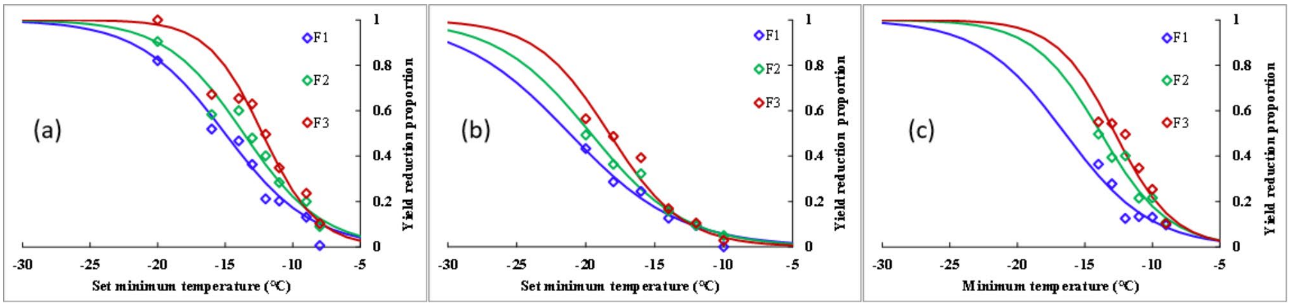

Fig. 2. Relationship between R_Y and T_m during overwintering at different stages, where (**a**) represent in early winter period, (**b**) represent in mid-winter period, and (**c**) represent in green-up period. F1 indicated freezing for 1 day, F2 indicated freezing for 2 days and F3 indicated freezing for 3 days.

Freezing stage	Freezing days	Equations	R^2	R_{Y10} (°C)	R_{Y30} (°C)	R_{Y50} (°C)
Early winter period	1	$R_Y=1/(1+\exp(0.312\times T_m+4.689)$	0.94**	− 7.6	− 11.2	− 12.8
	2	$R_Y=1/(1+\exp(0.346\times T_m+4.714)$	0.96**	− 7.0	− 10.1	− 11.6
	3	$R_Y=1/(1+\exp(0.496\times T_m+6.069)$	0.93**	− 7.6	− 9.8	− 10.8
Mid-winter period	1	$R_Y=1/(1+\exp(0.251\times T_m+5.316)$	0.98**	− 12.0	− 16.4	− 18.4
	2	$R_Y=1/(1+\exp(0.294\times T_m+5.774)$	0.98**	− 11.8	− 15.5	− 17.3
	3	$R_Y=1/(1+\exp(0.377\times T_m+6.903)$	0.95**	− 12.2	− 15.1	− 16.5
Green-up period	1	$R_Y=1/(1+\exp(0.316\times T_m+5.204)$	0.81**	− 9.2	− 12.7	− 14.3
	2	$R_Y=1/(1+\exp(0.40\times T_m+5.516)$	0.92**	− 8.0	− 10.8	− 12.1
	3	$R_Y=1/(1+\exp(0.463\times T_m+5.901)$	0.88**	− 7.8	− 10.1	− 11.2

Table 4. Fitting relationship between R_Y and T_m under different treatments and critical indicators of freezing resistance.

Determination of critical temperature for freezing damage based on RY

Based on Fig. 2, it can be observed that the R_Y of winter wheat during the overwintering period increases with the severity and duration of low temperatures. The trend follows an S– shaped curve, indicating that both the sensitivity to the degree of low temperature and the sensitivity to the duration of low temperature are highest during the early overwintering period, followed by the green-up and the mid-winter periods.

According to Table 4, it can be seen that the critical freezing temperatures for different R_Y (R_{Y10}, R_{Y30}, and R_{Y50}) and different durations of low temperature (1 day, 2 days, and 3 days) for winter wheat in the early winter period are − 10.5, -9.6, and − 9.4 °C respectively. For different durations of low temperature, the critical freezing temperatures for R_{Y10}, R_{Y30}, and R_{Y50} are − 7.4, -10.4, and − 11.7 °C respectively. Moreover, the critical freezing temperatures for R_{Y10}, R_{Y30}, and R_{Y50} range from − 7.0 to -7.6, from − 9.8 to -11.2, and from − 10.8 to -12.8 °C respectively. For winter wheat in the mid-winter period, the critical freezing temperatures for different durations of low temperature (1 day, 2 days, and 3 days) and different R_Y (R_{Y10}, R_{Y30}, and R_{Y50}) are − 15.6, -14.9, and − 14.6 °C respectively. The critical freezing temperatures for R_{Y10}, R_{Y30}, and R_{Y50} for different durations of low temperature are − 12.0, -15.7, and − 17.4 °C respectively. Additionally, the critical freezing temperatures for R_{Y10}, R_{Y30}, and R_{Y50} range from − 11.8 to -12.2, from − 15.1 to -16.4, and from − 16.5 to -18.4 °C respectively. In the green-up period, the critical freezing temperatures for different durations of low temperature (1 day, 2 days, and 3 days) and different R_Y (R_{Y10}, R_{Y30}, and R_{Y50}) for winter wheat are − 12.1, -10.3, and − 9.7 °C respectively. The critical freezing temperatures for R_{Y10}, R_{Y30}, and R_{Y50} for different durations of low temperature are − 8.3, -11.2, and − 12.5 °C respectively. Furthermore, the critical freezing temperatures for R_{Y10}, R_{Y30}, and R_{Y50} range from − 7.8 to -9.2, from − 10.1 to -12.7, and from − 11.2 to -14.3 °C respectively. According to the different durations of low temperatures, the critical freezing temperatures for different R_Y during different periods (early winter, mid-winter, and green-up periods) of winter wheat after being subjected to 1 day low-temperature freezing are − 10.5, -15.6, and − 12.1 °C respectively. At R_Y of 10%, 30%, and 50% (R_{Y10}, R_{Y30}, and R_{Y50}) during different periods at low temperatures, the critical freezing temperatures are − 9.6, -13.4, and − 15.2 °C respectively. Furthermore, after being subjected to 2 days low-temperature freezing, the critical freezing temperatures for different R_Y during different periods of winter wheat are − 9.6, -14.9, and − 10.3 °C respectively. At R_Y of 10%, 30%, and 50% (R_{Y10}, R_{Y30}, and R_{Y50}) during different periods at low temperatures, the critical freezing temperatures are − 8.9, -12.1, and − 13.7 °C respectively. Finally, after being subjected to 3 days low-temperature freezing, the critical freezing temperatures for different R_Y during different periods of winter wheat are − 9.4, -14.6, and − 9.7 °C respectively. At R_Y of 10%, 30%, and 50% (R_{Y10}, R_{Y30}, and R_{Y50}) during different periods at low temperatures, the critical freezing temperatures are − 9.2, -11.7, and − 12.8 °C respectively.

Determination of critical temperature for freezing damage based on SENAT

According to Fig. 3, when the low temperature lasts for 1 day, the soil tiller node's minimum temperature is from 7.3 to 9.7 °C higher than the air's minimum temperature. When the low temperature persists for more than 1 day, the soil tiller node's temperature at the low temperature treatment is about 1.8 to 5.4 °C lower than that after 1 day, aiming to quantify the relationship between the actual soil temperature at the tiller node and the stem and tiller mortality under different low temperature treatments, and obtain the critical temperature for freezing damage based on the soil effective negative accumulative temperature.

Figure 4 shows the relationship of soil accumulated negative effective temperature with mortality varied during the winter, exhibits an exponential relationship for early overwintering stage while a linear correlation for midwinter and green-up periods.

Based on Table 5, it can be observed that the critical temperatures for soil accumulative negative temperature freezing resistance for different R_T during different low temperature periods (early winter, mid-winter, and green-up periods) are 15.9, 56.9, and 131.7 °C·h, respectively. The critical temperatures for soil accumulative negative temperature freezing resistance for different stem and R_T (10%, 30%, and 50%) during different low temperature periods are 9.9, 65.9, and 128.8 °C·h, respectively. Furthermore, R_{Y10}, R_{Y30}, and R_{Y50} ranges from 2.2 to 20.6, 9.0 to 131.7, and 36.6 to 242.8 °C·h. In practical production, the available soil temperature data is usually obtained on a daily basis. The critical temperatures for soil accumulative negative temperature freezing resistance for different R_T during different low temperature periods (early winter, mid-winter, and green-up periods) are 2.2, 11.9, and 16.8 °C·d, respectively. The critical temperatures for soil accumulative negative temperature freezing resistance for different R_T (10%, 30%, and 50%) during different low temperature periods are 1.9, 10.0, and 19.0 °C·d, respectively. Moreover, R_{Y10}, R_{Y30}, and R_{Y50} ranges from 0.3 to 3.5, 1.2 to 16.8, and 5.1 to 30.1 °C·d.

Discussion

The freezing tolerance of winter wheat is closely related to both the extent and duration of low temperatures[27]. Previous studies have used the 50% lethal temperature (R_{T50}) to characterize synthetically the risk of crop damage stemming from cold spells, extended periods at low temperature, frequent occurrence of freeze-thaw cycles, and prolonged snow cover[28]. However, the 50% death criterion is too severe for practical production, and the R_{T50} index cannot accurately assess the extent of freezing damage. It can only determine whether freezing injury has occurred. Therefore, when assessing the risk of freezing damage based on this single criterion, it is impossible to differentiate the level of risk among different regions. Due to the long overwintering period of wheat, the degree of frost damage stress varies with different low temperature periods and durations. At the same time, due to the constantly changing process of cold resistance exercise, there are also significant differences in the frost resistance ability of wheat during the overwintering stage[19,29]. There is an urgent need for dynamic evaluation of winter wheat frost resistance under different low temperature periods and durations in production. Therefore,

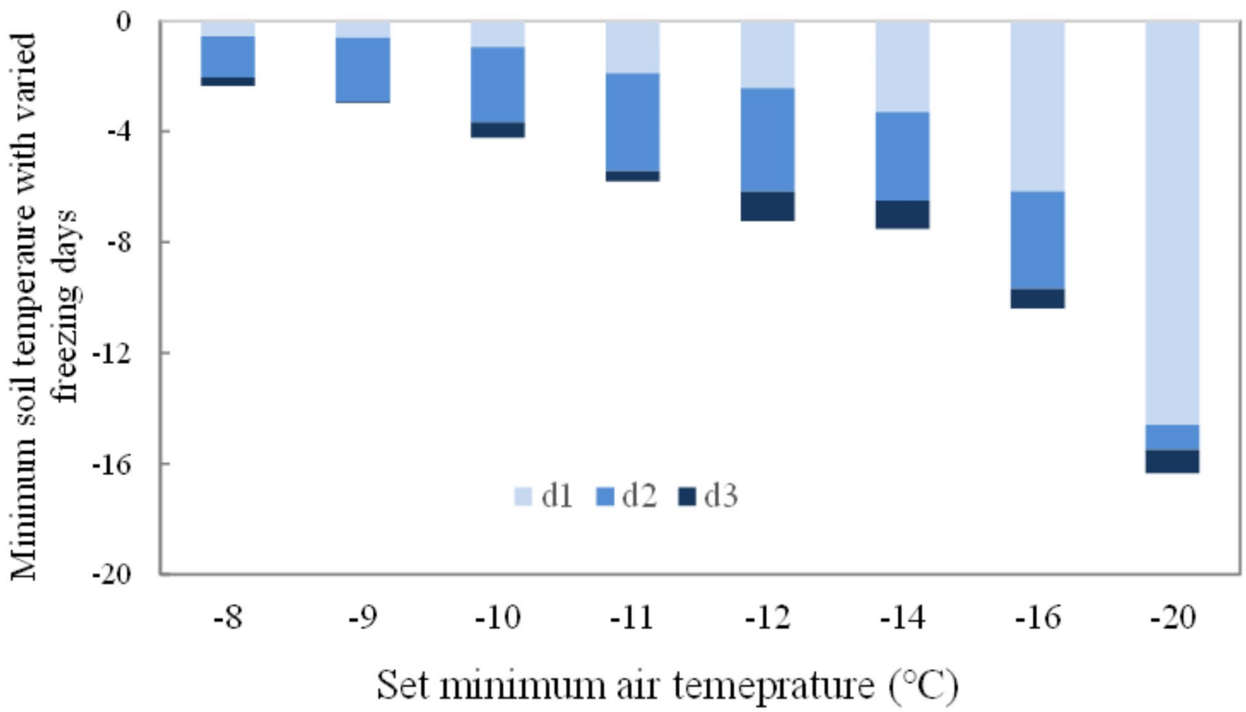

Fig. 3. Relationship between the lowest soil temperature and the lowest air temperature.

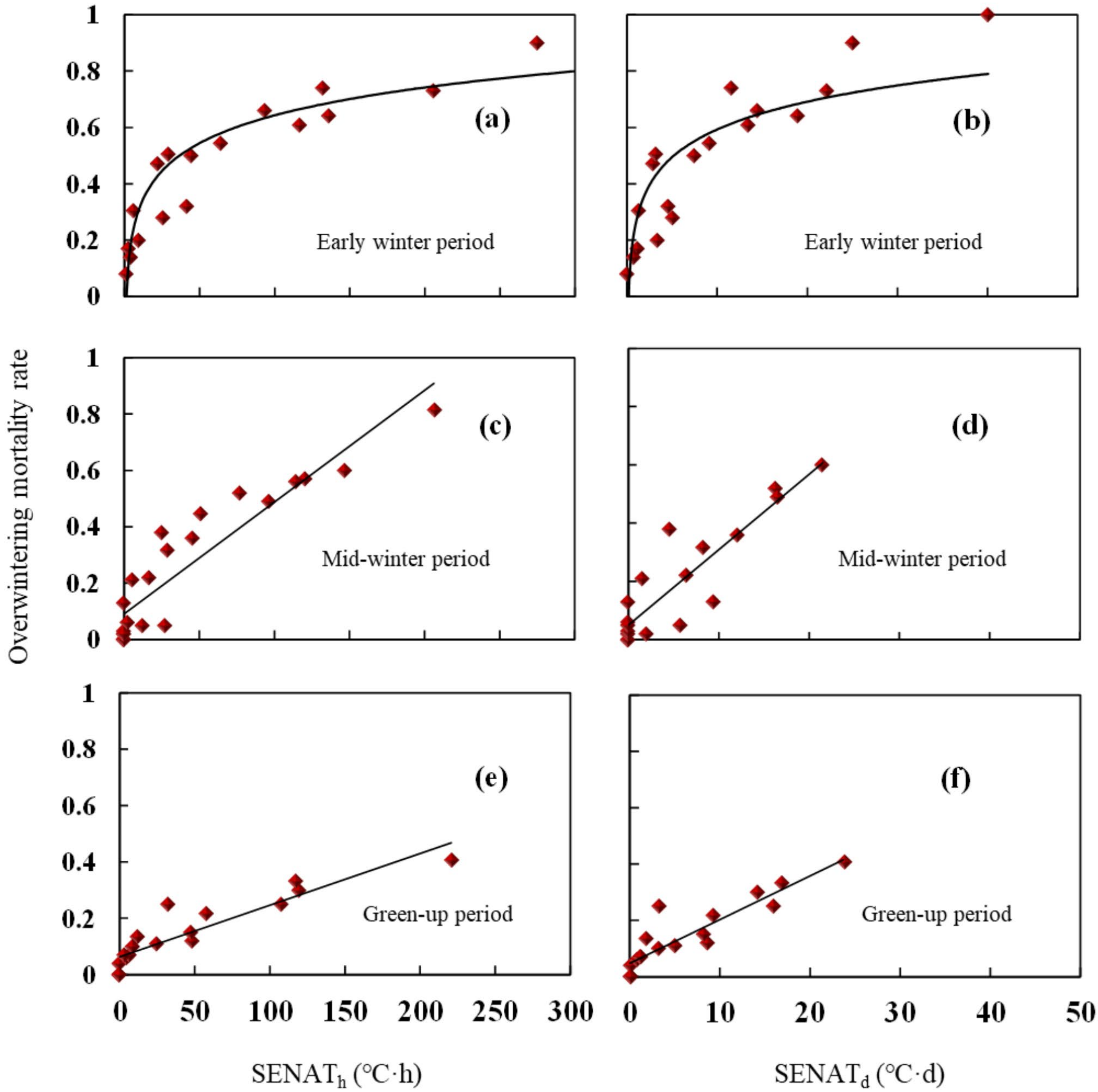

Fig. 4. Relationship between R_T and SENAT at different stages, where (**a**) represent R_T and $SENAT_h$ in early winter period, (**b**) represent R_T and $SENAT_d$ in early winter period, (**c**) represent R_T and $SENAT_h$ in mid-winter period, (**d**) represent R_T and $SENAT_d$ in mid-winter period, (**e**) represent R_T and $SENAT_h$ in green-up period, and (**f**) represent R_T and $SENAT_d$ in green-up period.

Treatment	Equations	R^2	$SENAT_{h10}$(°C h)	$SENAT_{h30}$(°C h)	$SENAT_{h50}$(°C h)
Early winter period	$R_T = 0.1425\times\ln(SENAT_h)$-0.0129	0.88**	2.2	9.0	36.6
Mid-winter period	$R_T = 0.0004\times SENAT_h + 0.0725$	0.85**	6.9	56.9	106.9
Green-up period	$R_T = 0.018 \times SENAT_h + 0.063$	0.83**	20.6	131.7	242.8
Treatment	Equations	R^2	$SENAT_{d10}$(℃·d)	$SENAT_{d30}$(℃·d)	$SENAT_{d50}$(℃·d)
Early winter period	$R_T = 0.1421\times\ln(SENAT_d) + 0.2658$	0.74**	0.3	1.2	5.1
Mid-winter period	$R_T = 0.020\times SENAT_d + 0.062$	0.84**	1.9	11.9	21.9
Green-up period	$R_T = 0.015\times SENAT_d + 0.048$	0.83**	3.5	16.8	30.1

Table 5. Equations between R_T and SENAT for different treatment.

this study considers a freezing damage index system based on tiller mortality rate (R_T), yield reduction rate (R_Y), and soil effective negative accumulated temperature (SENAT)[21–23,30]. It also takes into account the critical thresholds of 10%, 30%, and 50%. This system provides more meaningful guidance for practical freezing damage evaluation and prediction and can be applied to compare freezing damage hazards between different years and locations.

The R_{Y10} and R_{Y30} values are 1 to 3 ℃ higher than the R_{T10} and R_{T30} values in the northern winter wheat area, as shown in Tables 3, 4 and 5. This may be related to the degree of stem and tiller freezing injury and metabolite changes (disaccharides, monosaccharides, amino acids and lipids)[18]. In the case of R_{Y50}, the value is lower than R_{T50}, which may be attributed to the larger space occupied by surviving plants per plant, improved agricultural resource conditions, and effective cultivation management, which compensate for the lost productivity of dead seedlings[31]. Of course, further consideration needs to be given to the size of the small pot in future experiments, as the size of the small pot may affect crop growth (biomass, photosynthesis per unit leaf area), thereby affecting experimental results and objectives. In addition, research has indicated that the root freezing tolerance of winter wheat is the weakest, and exposure to temperatures ranging from − 5 to -9 ℃ can lead to soil albedo, water-holding capacity of the topsoil, the potential for storage and release of heat and potential attenuation of the effects of radiative frost, thereby affecting the root's ability to absorb moisture and nutrients, ultimately leading to root system death and reduced yield[32]. These effects cannot be measured by the critical temperature for stem and tiller, which underlines the effectiveness of yield-based freezing resistance indices as a complement to temperature-based freezing resistance indices. However, during field production, when the temperature falls below the freezing tolerance of the roots, significant effects of the air temperature on the underlying soil temperature, and at shallower layers (< 80 cm), the effects of the air temperature may be blocked by the snow cover, resulting in a poorly synchronous correlation between them. Therefore, the soil temperature where the roots are located remains higher than the air temperature[33], especially in wheat fields that have been irrigated with frozen water, and may affected vegetative growth and expanding storage capacity[34]. Therefore, in practice, leaf exposure is often the first to experience freezing, and if the root distribution is shallow and the upper soil layer is very dry, the upper roots can also freeze easily. The SENAT is suitable for assessing the risk of freeze damage in areas where surface soil temperature data are available[23]. Although soil temperature can be converted based on air temperature, the conversion methods differ depending on various weather conditions (sunny, cloudy, rainy, presence or absence of snow cover)[15,35,36]. In this study, the index for 10% mortality rate being smaller may be due to imbalanced nutrient proportions and interplant competition during winter wheat overwintering, or it could be attributed to the large error associated with the critical index values obtained from the regression equation[30]. Research has shown that when the harmful SENAT is 0 ℃·d, the mortality rate of tillering is 24%[37]. Therefore, the SENAT index may present significant deviations when assessing mild freeze damages, and further comprehensive evaluation of mild freezing damage to winter wheat based on other indicators is needed in the future.

This study has several aspects that need improvement. Firstly, the wheat used in this study was subjected to cold resistance training in a natural state, and after the low temperature treatment, it was placed in the field to recover until maturity. However, wheat growth in the field is a complex process[38]. For instance, wheat seedlings that have undergone low temperature treatment and are placed in the field may experience severe dehydration and death if exposed to strong winds or excessive sunlight. As a result, the cold resistance index obtained from field experiments is generally weaker compared to the control experiments[39]. Secondly, field production involves repeated freezing injuries during the winter. Due to fluctuating temperatures and nutrient depletion[40], the cold resistance training effect is constantly weakened and diminished throughout the overwintering period. Drought conditions and strong winds during the winter can cause plants to lose moisture, especially in areas with cracks or compacted soil, leading to winter death of seedlings[41]. Additionally, if unfavorable environmental conditions persist during the regreening stage, freeze damage may occur and the recovery of the wheat seedlings will be hindered, resulting in their gradual demise[25]. Therefore, even under conditions slightly higher than the critical lethal temperature identified in artificial low-temperature control boxes, severe freeze damage and seedling death can still occur in the field. Thirdly, this study is based on statistical results obtained from low-temperature treatments conducted in artificial control boxes. Furthermore, the low-temperature treatment process was carried out only once, with relatively small variations in SENAT values. On the other hand, the critical temperature values in the field are relatively high (-3 and − 8 ℃), and the accumulated SENAT during the winter is considerably higher[42]. Therefore, this index is only suitable for evaluating the impact of a single cold wave event. Finally, this study involves potted planting with a relatively high planting density[31], which may have some effects that were not quantified in this study. In addition, future work also needs to consider the significant impact of genotype variations on cold tolerance in order to draw more reliable conclusions[43].

Conclusion

1. During the winter, the death rate of winter wheat tillers and the reduction rate of yield both increase with the decrease in temperature and the duration of low temperatures, following an S-shaped curve. In the early winter, the death rate of winter wheat during dormancy shows an exponential function relationship with the effective accumulative negative temperature of the soil, while during the mid-winter and green-up periods, it demonstrates a linear function relationship.
2. The critical temperature for freezing resistance in terms of tiller mortality rate (R_T) at different low temperature periods (early winter, mid-winter, and green-up periods) of winter wheat is -11.7 to -13.8, -15.6 to -17.9, and − 11.7 to -13.8 ℃, respectively. The critical temperature for freezing resistance in terms of yield reduction rate (R_Y) at different low temperature periods is -9.4 to -10.5, -14.6 to -15.6, and − 9.7 to -12.1 ℃, respectively.

The critical temperature for freezing resistance in terms of soil negative accumulated temperature (SENAT) at different low temperature periods is 15.9 to 131.7 ℃·h (2.2 to 16.8 ℃·d).

3. The R_T under different durations (1 day, 2 days, and 3 days) varied of winter wheat, with critical freezing temperatures ranging from – 12.0 to -17.9, -2.8 to -16.7, and – 10.2 to -15.6 ℃, respectively. Similarly, the R_Y under different durations also exhibited variations, with critical freezing temperatures ranging from – 10.5 to -15.6, -9.6 to -14.9, and – 9.4 to -14.6 ℃ for the respective low temperature durations.

Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Declarations

Competing interests
The authors declare no competing interests.

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