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Analysis of the Physiological Parameters of Cold Resistance in Core Winter and Spring Wheat Cultivars

Yunhe Wang [1,†], Cunyao Bo [1,†], Xiaohua Wang [2], Xincheng Yang [1] and Hongwei Wang [1,*]

[1] College of Agriculture, Shandong Agricultural University, Taian 271018, China; yhwang0910@163.com (Y.W.); bocunyao1989@126.com (C.B.); xcyang0917@163.com (X.Y.)
[2] College of Agriculture and Forestry Science, Linyi University, Linyi 276000, China; wangxiaohua19880721@126.com
* Correspondence: wanghongwei@sdau.edu.cn; Tel.: +86-0538-8249278
† These authors contributed equally to this work.

Abstract: We selected 46 core winter–spring wheat cultivars from China's main wheat-producing areas as experimental materials to clarify the differences in the physiological parameters of their cold resistance and provide a theoretical basis and high-quality germplasm for cold resistance breeding. We investigated differences in their cold resistance under field conditions for two consecutive years, and determined the physiological parameters of the cold resistance, yield, and quality indicators of different winter–spring wheat cultivars. Our results showed that the cold resistance of winter wheat cultivars was higher than that of spring wheat cultivars. The chlorophyll (Chl), soluble sugar (SS), soluble protein (SP), and free proline (Pro) contents of different winter–spring wheat cultivars were positively correlated with cold resistance, and malondialdehyde (MDA) content was negatively correlated with cold resistance. The five physiological parameters can be used as physiological indicators for the breeding of cold-resistant cultivars. The cold resistance, yield, and quality indicators of different spring and winter wheat cultivars were comprehensively evaluated by using the average membership value and comprehensive score. It was found that the average membership value and comprehensive score of winter wheat cultivars were higher than those of spring wheat cultivars. Through classification using the K-means method, the cold-resistant, high-yield, and high-quality cultivars were screened out, namely, Jimai23 (JM23), Jimai44 (JM44), Shannong57 (SN57), and Jinmai 919 (JM919).

Keywords: wheat (*Triticum astivum* L.); frost damage; yield; quality; breeding

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the three major food crops around the world, accounting for 21% of the world's food supply [1]. China is the world's largest wheat producer and consumer, accounting for 17% and 16% of the world's total wheat production and consumption, respectively [2]. As one of the main food crops in China and around the world, high and stable yields, high quality, and stress resistance have remained important goals to be pursued in wheat production [3,4]. In recent years, with the increase in extreme weather events and the improper selection of wheat cultivars, frost damage has had a serious impact on wheat growth worldwide [5]. In particular, the major wheat-producing countries, namely, China, the United States, Australia, and Canada, have suffered the most severe production losses due to frost damage [6–8]. The screening and breeding of cold-resistant wheat cultivars are of great significance for coping with low-temperature frost damage and ensuring wheat yield and quality.

It is necessary for wheat to spend a certain amount of time at low temperatures during the vernalization stage. Based on the low-temperature conditions and duration required during the vernalization stage, wheat can be divided into strong-winter, winter, semi-winter, weak-spring, and spring cultivars. Wheat can survive under low-temperature conditions

during the vernalization stage; however, too low a temperature will have a certain influence on the growth and development of the wheat [9]. When the temperature drops below 0 °C, wheat suffers from low-temperature damage, the cell membrane structure is destroyed, the cell sap concentration and cytoplasm area increase, and the characteristics of the organelles also change, eventually leading to the death of cells and plants [10,11]. The results of other studies have shown that when the temperature is −10 °C, spring wheat will not survive; however, when the temperature is −15 °C or lower, winter wheat still shows a certain ability to survive [12].

The ability of wheat to survive winter conditions is referred to as cold resistance [13]. Plant cold resistance is a quantitative trait controlled by multiple genes and is closely related to physiological parameters such as plant photosynthesis, osmotic regulatory substances, membrane lipid peroxidation, and antioxidant enzyme activity [14,15]. Photosynthesis is the main material basis and energy source for the growth and development of all green plants. Roughly 90% of wheat yield is derived from the contribution of photosynthesis [16,17]. Low-temperature stress can affect the synthesis of photosynthetic pigments and the energy metabolism of plants [18]. When wheat is under low-temperature stress, the chlorophyll (Chl) content of wheat leaves shows a continuous downward trend [19,20]. Soluble sugar (SS) is widely considered to be a cold-resistant protective substance, and its presence has been confirmed in many cold-resistant plants [21,22]. Krasensky et al. [23] found that plants accumulate SS under low-temperature stress to increase the concentration of cell sap in order to prevent damage to protoplasts caused by freezing, thereby ensuring the stability and integrity of the cell membrane and reducing plant frost damage. As an important osmotic regulatory factor, soluble protein (SP) is dissolved in water in the form of a small molecule and has hydrophilic and water-retaining properties, which have a certain influence on the cold resistance of plants [24]. Free proline (Pro) plays a role in maintaining redox balance and cell homeostasis under low-temperature stress, removing the harmful effects of reactive oxygen species, and protecting cells from oxidative damage [25,26]. Malondialdehyde (MDA) is one of the key indicators of lipid peroxidation. Under low-temperature stress, a large number of active oxygen free radicals are produced in plants, thereby producing peroxidation products such as MDA, which cross-links with macromolecules such as proteins or nucleic acids to form polymers, destroying cell membranes and functional molecules in cells [27,28].

The yield and quality of wheat are a complex combination of various traits, which are mainly controlled by genotype and environmental factors [29]. Understanding the relationship between wheat cold resistance and physiological parameters can enable a more thorough scientific evaluation of wheat cold resistance and ensure wheat yield and quality. Conversely, physiological substances, such as the Chl, SS, SP, and Pro contents of wheat, also affect wheat yield and quality in the later stages [30,31]. Low-temperature stress can damage the functional leaves of wheat, thereby affecting the synthesis of carbohydrates, slowing down the transport of total SS and SP from stems and leaves to grains, reducing the number of grains and grain filling rate, and ultimately leading to a decrease in grain yield and quality [32,33].

A considerable amount of research has been performed on the cold resistance of wheat [34–36]; however, the authors of the referenced studies mainly focused on a few cultivars, or the studies were conducted at fixed temperatures in a laboratory, with such conditions differing from the frost damage that occurs under complex climatic conditions in the field [37]. Therefore, this study selected 46 core cultivars of winter and spring wheat as experimental materials to avoid the contingency caused by a few cultivars. The frost damage of different winter and spring wheat cultivars was investigated under complex climatic conditions in the field, which more realistically reflected the differences in cold resistance of different wheat cultivars. The novel use of physiological parameters to screen physiological indicators of cold resistance allowed for an analysis of the physiological and biochemical characteristics of wheat during cold resistance. The cold resistance, yield, and quality indicators of different cultivars of wheat were comprehensively evaluated by the

average membership and comprehensive values and classified by the K-means method. The purpose was to screen out cold-resistant, high-yield, and high-quality wheat germplasms, providing better germplasms and a theoretical basis for the future cold-resistant breeding of wheat more in line with actual production.

2. Materials and Methods

2.1. Experimental Design

This study was conducted in the experimental field of Zhaizi Village, Tai'an City, Shandong Province (36°1′51″ N, 117°15′53″ E), from October 2022 to June 2024. The test site is located in the temperate continental monsoon climate zone, with an average annual temperature of 14.5 °C, average annual sunshine of 2759.1 h, and average annual precipitation of 901.4 mm. The soil texture is loam, and the previous crop of the test site was corn, which is planted twice a year. The temperature during the wheat-growing season is shown in Figure 1. The monthly minimum temperature in the 2022–2023 growing season was −15.1 °C, and the monthly minimum temperature in the 2023–2024 growing season was −13.9 °C. The 0–20 cm soil layer contained 78.92 mg·kg^{-1} of alkaline nitrogen, 53.63 mg·kg^{-1} of available phosphorus, 140.00 mg·kg^{-1} of quick-acting potassium, and 25.62 g·kg^{-1} of organic matter.

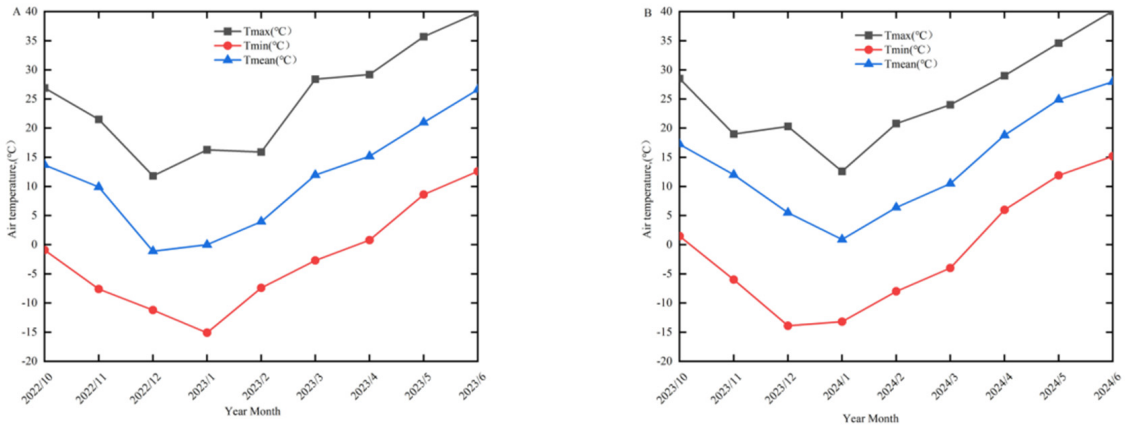


Figure 1. The monthly maximum temperature, monthly minimum temperature, and monthly average temperature during the wheat growing season ((**A**) 2022–2023; (**B**) 2023–2024).

In this study, we selected 46 core winter–spring wheat cultivars from China's main wheat-producing areas as experimental materials [38]. The cultivars were obtained from five provinces; namely, Shandong, Henan, Shanxi, Anhui, and Jiangsu (Table 1). The experiment adopted a randomized block design, with 3 replicate plots randomly set for each cultivar, a total of 138 experimental plots, and a plot area of 13.5 m^2. Each cultivar was planted in 6 rows in the plot, with a row length of 9 m and a row spacing of 0.25 m. A protection row was set near the experimental site. Two sampling points of 1 m in length were set for each plot with bamboo poles for later trait investigation. The base fertilizer (N-P-K = 15-15-15) input was 750 kg·hm^{-2} of compound fertilizer, and 750 kg·hm^{-2} of urea was applied during the wheat jointing stage. After wheat sowing, the emergence of seedlings was checked promptly, and the seedlings were quickly transplanted for the plots with severe seedling shortages and broken ridges to avoid field emergence differences. The wheat was irrigated after sowing and during jointing and flowering, with 60 mm of water used each time. We used 20% clofopyralid EC + 6.9% fenoxaprop-ethyl emulsion in water at the wheat jointing stage, diluted with water at a ratio of 1:1000, and sprayed for weed control. We used 40% prothioconazole + 10% imidacloprid + 98% potassium dihydrogen phosphate + 0.01% brassinolide at the wheat heading stage, diluted with water at a ratio of 1:1000, and sprayed for pest control.

Table 1. Wheat cultivars and their sources.

Cultivars	Code	Cultivar Source
Yannong31	YN31	Yantai Agricultural Science Research Institute, Shandong Province
Yannong33	YN33	
Yannong161	YN161	
Yannong301	YN301	
Yannong745	YN745	
Jimai23	JM23	Shandong Academy of Agricultural Sciences
Jimai44	JM44	
Jimai60	JM60	
Luyan128	LY128	Shandong Luyan Agricultural Variety Co., Ltd. Shandong, China
Luyan955	LY955	
Shannong38	SN38	Shandong Agricultural University
Shannong40	SN40	
Shannong48	SN48	
Shannong55	SN55	
Shannong56	SN56	
Shannong57	SN57	
Shannong59	SN59	
Shannong69	SN69	
Luliang1	LL1	
Zhengmai113	ZM113	Henan Academy of Agricultural Sciences
Zhengmai136	ZM136	
Zhengmai366	ZM366	
Zhengmai918	ZM918	
Zhengmai1860	ZM1860	
Zhengmai1905	ZM1905	
Zhengmai2118	ZM2118	
Zhengmai7698	ZM7698	
Zhengshi9170	ZS9170	
Zhoumai36	ZM36	Zhoukou Academy of Agricultural Sciences
Jinmai919	JM919	Shanxi Agricultural University
Wanke1838	WK1838	Anhui Academy of Agricultural Sciences
Quanmai725	QM725	
JIUhaomai2	JHM2	Anhui Luyan Seed Industry Co., Ltd. Anhui, China
Annong1589	AN1589	Anhui Agricultural University
Yangmai15	YM15	Jiangsu Lixiahe Agricultural Science Research Institute
Yangmai20	YM20	
Yangmai24	YM24	
Yangmai25	YM25	
Yangmai27	YM27	
Yangmai30	YM30	
Yangmai34	YM34	
Ningmai13	NM13	Jiangsu Academy of Agricultural Sciences
Ningmai35	NM35	
Ningmai36	NM36	
Ningzhongmai1	NZM1	
Zhenmai12	ZM12	Zhenjiang Agricultural Science Research Institute in Jiangsu Hilly Region

2.2. Testing Content and Methods of Measurement

2.2.1. Investigation of Frost Damage During the Winter Period of Wheat Under Field Conditions

According to the Rules of Field Investigation and Grading of Damage to Winter Wheat [39], the field frost damage levels of the tested wheat were classified during the wintering period.

2.2.2. Measurement of Chl Content

During the wheat greening period, a Chl meter (SPAD-502; Tokyo, Japan) was used to randomly select 10 representative leaves of different strains at each sampling point, with the front of the leaves facing upwards. The leaves were faced upwards and the relative chlorophyll content was measured at 1/3 of the distance from the stem, i.e., the SPAD value [40].

2.2.3. Measurement of SS Content

SS content was determined using the anthrone colorimetric method [41]. We used a plant SS content detection kit (Solarbio, Beijing, China). We weighed 0.1 g of wheat leaf tissue, added 1 mL of distilled water, and ground it into a homogenate. We poured it into a covered centrifuge tube and boiled it in water for 10 min. After cooling, it was centrifuged at 8000× *g* for 10 min at room temperature. We placed the supernatant in a 10 mL test tube and diluted it to 10 mL with distilled water. We pipetted 40 μL of the extract and mixed it with 40 μL of distilled water, 20 μL of working solution, and 200 μL of concentrated sulfuric acid, and placed this in a 95 °C water bath for 10 min. After cooling to room temperature, 200 μL was transferred it to a cuvette, and we measured the SS content at 620 nm by using a UV spectrophotometer (UV-30 Scan Spectrophotometer, Onda, Carpi, Italy).

2.2.4. Measurement of SP Content

SP content was determined by using the Coomassie Brilliant Blue G250 method [41]. We used a Bradford protein concentration determination kit (Solarbio, Beijing, China). We weighed 0.1 g of wheat leaf tissue, added 1 mL of extract, and ground it into homogenate. It was centrifuged at 8000× *g* for 10 min at room temperature, and then we took the supernatant and diluted it to 0.2 mg·mL^{-1} with distilled water. We pipetted 500 μL of the diluted supernatant and mixed it with 5 mL 1 × G250 staining solution, before it was kept at room temperature for 5 min. We measured the SP content by using a UV spectrophotometer (UV-30 Scan Spectrophotometer, Onda, Carpi, Italy) at 595 nm.

2.2.5. Measurement of Pro Content

Pro content was determined using the sulfosalicylic acid extraction and ninhydrin colorimetric method [41]. We used a Pro content detection kit (Solarbio, Beijing, China). We weighed about 0.1 g of wheat leaf tissue, added 1 mL of extract solution, and homogenized it in an ice bath. We placed it in a boiling water bath and shook it for 10 min. We then centrifuged it at 10,000× *g* for 10 min at room temperature and extracted the supernatant. Then, we pipetted 0.25 mL of the supernatant and mixed it with 0.25 mL of reagent 1 and 0.25 mL of reagent 2; then, we placed this in a boiling water bath for 30 min and shook it every 10 min. After cooling, we pipetted 0.2 mL into a cuvette and measured the Pro content at 520 nm by using a UV spectrophotometer (UV-30 Scan Spectrophotometer, Onda, Carpi, Italy).

2.2.6. Measurement of MDA Content

MDA content was determined using the thiobarbituric acid reaction method [42]. We used the MDA content detection kit (Solarbio, Beijing, China). We weighed about 0.1 g of wheat leaf tissue, added 1 mL of the extract solution, and homogenized this in an ice bath; then, we centrifuged it at 8000× *g* for 10 min at 4 °C, and extracted the supernatant. We pipetted 200 μL of the supernatant, 600 μL of the MDA detection working solution, and

200 μL of the reagent, mixed it thoroughly, kept it in a 100 °C water bath for 60 min, and cooled it in an ice bath. We centrifuged it at 10,000× *g* for 10 min at room temperature. We pipetted 1 mL into a cuvette and measured the MDA content at 532 nm and 600 nm in a UV spectrophotometer (UV-30 Scan Spectrophotometer, Onda, Carpi, Italy).

2.2.7. Measurement of Wheat Yield

After the wheat matured, it was harvested and threshed in different plots, and the yield of each cultivar of wheat was recorded after weighing [43].

2.2.8. Measurement of Wheat Grain Quality Traits

According to method AACC [44], the grain protein (GP) and wet gluten (WG) contents, grain hardness index (GHI), and sedimentation value (SV) of the wheat grains were determined using a near-infrared grain analyzer (DA7200; Stockholm, Switzerland), and the dough formation time (DFT) and dough stability time (DST) were determined using a farinograph (GWZJ/YS-26; Duisburg, Germany).

2.3. Statistical Analysis

Microsoft Excel (version 2020; Microsoft, Inc., Redmond, WA, USA) was used for data collation and chart preparation. SPSS statistical software (version 24; SPSS, Inc., Chicago, IL, USA) was used for one-way analysis of variance, and the average membership value and comprehensive score were calculated [45]. Cluster analysis was performed using the K-means method. Origin software (version 2021; Originlab, Inc., Northampton, MA, USA) was used for correlation analysis.

The membership value calculation formula is as follows

$$U_{ij} = (X_{ij} - X_{imin})/(X_{jmax} - X_{jmin}) \quad (1)$$

The calculation formula of the inverse membership value is as follows

$$U_{ij} = 1 - (X_{ij} - X_{imin})/(X_{jmax} - X_{jmin}) \quad (2)$$

The average membership value calculation formula is as follows

$$AU_i = \sum U_{ij}/n \quad (3)$$

In the formula, U_{ij} represents the membership function value of the i-th cultivar for the j-th index; X_{ij} represents the measured value of the j-th index of the i-th cultivar; $X_j$max and $X_j$min represent the maximum and minimum values of the j-th index, respectively; and AU_i represents the average membership value of the i-th cultivar. Formula (1) indicates that a certain indicator is positively correlated with the comprehensive value of wheat cultivars; Formula (2) indicates that a certain indicator is negatively correlated with the comprehensive value of wheat cultivars.

The standard deviation coefficient calculation formula is as follows

$$V_j = \sum i = 1nX_{ij} - X_j - 2n - 1X_{j\text{-}} \quad (4)$$

The weight calculation formula for each indicator is as follows

$$W_j = V_j \sum j = 1nV_j \quad (5)$$

The comprehensive score calculation formula is as follows

$$D = \sum j = 1nU_{ij} \times W_j \quad (6)$$

In the formula, $X_{j\text{-}}$ represents the average value of the j-th index of each cultivar; X_{ij} represents the measured value of the j-th index of the i-th cultivar; V_j represents the

standard deviation coefficient of the j-th index; W_j represents the weight of the j-th index; and D represents the comprehensive score.

3. Results

3.1. Winter–Spring Characteristics and Frost Damage Levels of Different Wheat Cultivars

The cold resistance of wheat is closely related to its spring and winter characteristics. Strong-winter, winter, and semi-winter cultivars have higher cold resistance than weak-spring and spring cultivars. Frost damage to different winter–spring cultivars of wheat in the 2022–2023 growing season was more severe than in the 2023–2024 growing season (Table 2).

In the 2022–2023 growing season, the frost damage of different winter–spring wheat cultivars ranged from level 2 to level 5. Specifically, seventeen level 2 frost-damaged cultivars, such as Yannong161 (YN161), Yannong31 (YN31), and Yannong33 (YN33), had good cold resistance. These cultivars are generally strong-winter, winter, and semi-winter cultivars. Seventeen level 3 frost-damaged wheat cultivars, such as Yannong301 (YN301), Luyan128 (LY128), and Luyan955 (LY955), had average cold resistance. These cultivars are generally winter, semi-winter, and spring cultivars. Six level 4 frost-damaged cultivars, such as Yangmai24 (YM24), Yangmai25 (YM25), and Yangmai34 (YM34), and six level 5 frost-damaged cultivars, such as Jiuhaomai2 (JHM2), Yangmai15 (YM15), and Yangmai20 (YM20), had poor cold resistance; these are all spring cultivars.

Table 2. Winter and spring characteristics and frost damage levels of different wheat cultivars.

Cultivars	Winter-Spring Characteristics	2022/2023 Frost Damage Level	2023/2024 Frost Damage Level
SN38	Strong-winter	2	2
YN161	Winter	2	2
YN301		3	3
SN56		2	2
SN59		3	2
LL1		2	2
YN31	Semi-winter	2	2
YN33		2	2
YN745		2	2
JM23		2	2
JM44		2	2
JM60		2	2
LY128		3	3
LY955		3	3
SN40		2	2
SN48		3	2
SN55		2	2
SN57		2	2
SN69		2	2
ZM36		3	3
ZM136		3	3
ZM366		3	3
ZM918		3	2
ZM1860		3	3
ZM1905		3	3
ZM2118		3	3
ZM7698		3	3
ZS9170		3	3
JM919		3	3
WK1838		2	2
QM725		3	3
AN1589		2	2
NZM1		3	3

Table 2. *Cont.*

Cultivars	Winter-Spring Characteristics	2022/2023 Frost Damage Level	2023/2024 Frost Damage Level
ZM113	Weak-spring	2	2
JHM2	Spring	5	4
YM15		5	4
YM20		5	4
YM24		4	3
YM25		4	3
YM27		5	4
YM30		5	4
YM34		4	3
NM35		5	4
NM36		4	3
NM13		4	3
ZM12		4	3

In the 2023–2024 growing season, the frost damage of the different winter–spring cultivars of wheat was distributed at levels 2 to 4. Specifically, twenty level 2 frost-damaged cultivars, such as YN161, YN31, and YN33, had good cold resistance, with these being generally strong-winter, winter, and semi-winter cultivars; twenty level 3 frost-damaged wheat cultivars, such as YN301, LY128, and LY955, had average frost resistance, with these being generally winter, semi-winter, and spring cultivars; six level 4 frost-damaged cultivars, such as JHM2, YM15, and YM20, had poor frost resistance, and all are spring cultivars.

3.2. Comparison of the Physiological Parameters of Different Winter–Spring Wheat Cultivars

The Chl, SS, SP, Pro, and MDA contents of the different winter–spring wheat cultivars vary significantly (Table 3).

Table 3. The chlorophyll content (CC), soluble sugar content (SSC), soluble protein content (SPC), free proline content (PC), and malondialdehyde content (MC) of the different wheat cultivars.

Cultivars	Year of 2022/2023					Year of 2023/2024				
	CC (SPAD)	SSC ($mg \cdot g^{-1}$ FW)	SPC ($mg \cdot g^{-1}$ FW)	PC ($\mu g \cdot g^{-1}$)	MC ($nmol \cdot g^{-1}$)	CC (SPAD)	SSC ($mg \cdot g^{-1}$ FW)	SPC ($mg \cdot g^{-1}$ FW)	PC ($\mu g \cdot g^{-1}$)	MC ($nmol \cdot g^{-1}$)
SN38	48.47 ^{abc}	39.59 ^{efg}	21.63 ^{def}	218.96 ^{ab}	21.72 ^{ijk}	58.70 ^{abc}	43.53 ^{bcd}	25.56 ^{bcd}	252.97 ^{a}	13.89 ^{lmn}
YN161	46.77 ^{def}	40.42 ^{def}	20.34 ^{fgh}	140.52 ^{fgh}	28.28 ^{bcd}	56.23 ^{abc}	48.03 ^{abc}	26.15 ^{bcd}	149.24 ^{ghi}	23.62 ^{bcd}
YN301	43.47 ^{lmn}	48.22 ^{abc}	19.85 ^{ghi}	82.48 ^{klm}	24.24 ^{efg}	52.73 ^{ghi}	53.47 ^{ab}	24.76 ^{fgh}	126.31 ^{ijk}	16.63 ^{ghi}
SN56	50.90 ^{ab}	34.98 ^{ijk}	21.30 ^{def}	200.52 ^{b}	24.60 ^{def}	58.00 ^{abc}	49.82 ^{abc}	24.75 ^{fgh}	210.68 ^{bcd}	16.56 ^{hij}
SN59	46.90 ^{cde}	38.24 ^{efg}	21.98 ^{def}	168.27 ^{de}	22.98 ^{ghi}	58.07 ^{abc}	50.05 ^{abc}	26.11 ^{bcd}	152.22 ^{fgh}	17.78 ^{ghi}
LL1	50.50 ^{abc}	37.01 ^{hij}	25.06 ^{ab}	212.12 ^{ab}	18.00 ^{pq}	56.77 ^{abc}	48.66 ^{abc}	29.64 ^{a}	231.53 ^{ab}	12.78 ^{n}
YN31	42.37 ^{nop}	51.19 ^{a}	20.95 ^{def}	88.09 ^{jkl}	20.36 ^{klm}	51.83 ^{hij}	57.26 ^{a}	26.02 ^{bcd}	102.86 ^{mno}	16.63 ^{ghi}
YN33	44.60 ^{hij}	43.83 ^{cde}	21.21 ^{def}	144.04 ^{efg}	19.88 ^{mno}	53.60 ^{def}	50.54 ^{abc}	26.72 ^{bcd}	180.15 ^{def}	14.77 ^{klm}
YN745	41.60 ^{opq}	33.87 ^{klm}	19.33 ^{jkl}	150.89 ^{def}	20.50 ^{jkl}	51.47 ^{ijk}	43.56 ^{bcd}	25.34 ^{cde}	184.80 ^{cde}	16.13 ^{ijk}
JM23	47.70 ^{bcd}	38.66 ^{efg}	21.34 ^{def}	174.77 ^{cd}	22.18 ^{hij}	57.63 ^{abc}	47.21 ^{abc}	26.09 ^{bcd}	89.27 ^{op}	15.63 ^{ijk}
JM44	47.57 ^{bcd}	42.60 ^{cde}	21.67 ^{def}	202.65 ^{b}	17.35 ^{q}	57.40 ^{abc}	51.51 ^{abc}	26.75 ^{bcd}	131.34 ^{hij}	13.45 ^{mn}
JM60	48.23 ^{abc}	38.03 ^{fgh}	21.61 ^{def}	199.96 ^{b}	22.52 ^{hij}	56.20 ^{abc}	48.44 ^{abc}	26.94 ^{bcd}	138.98 ^{op}	19.28 ^{efg}
LY128	50.13 ^{abc}	35.55 ^{ijk}	24.35 ^{bc}	80.89 ^{klm}	25.23 ^{def}	55.97 ^{abc}	50.67 ^{abc}	24.16 ^{ijk}	87.78 ^{op}	19.14 ^{efg}
LY955	41.10 ^{pqr}	50.83 ^{ab}	26.13 ^{a}	114.77 ^{hij}	19.28 ^{opq}	59.13 ^{ab}	31.74 ^{jkl}	23.95 ^{jkl}	109.90 ^{klm}	18.42 ^{fgh}
SN40	49.90 ^{abc}	43.27 ^{cde}	20.85 ^{def}	170.94 ^{cd}	19.52 ^{nop}	60.33 ^{a}	43.97 ^{bcd}	25.14 ^{efg}	219.80 ^{ab}	15.38 ^{jkl}
SN48	47.13 ^{cde}	38.07 ^{fgh}	20.73 ^{efg}	208.76 ^{b}	27.56 ^{bcd}	56.37 ^{abc}	44.81 ^{bcd}	27.11 ^{bcd}	205.28 ^{bcd}	18.67 ^{fgh}
SN55	51.53 ^{a}	32.68 ^{klm}	21.45 ^{def}	235.38 ^{a}	24.66 ^{def}	58.00 ^{abc}	41.35 ^{cde}	24.80 ^{fgh}	249.73 ^{a}	20.65 ^{cde}
SN57	48.47 ^{abc}	33.24 ^{klm}	21.58 ^{def}	215.61 ^{ab}	23.45 ^{fgh}	58.93 ^{abc}	40.29 ^{def}	27.71 ^{b}	226.31 ^{ab}	18.78 ^{fgh}
SN69	45.93 ^{fgh}	42.18 ^{def}	21.49 ^{def}	194.98 ^{bc}	27.37 ^{bcd}	55.93 ^{abc}	45.31 ^{bcd}	27.52 ^{bcd}	201.55 ^{bcd}	16.25 ^{ijk}
ZM36	50.33 ^{abc}	45.72 ^{bcd}	21.28 ^{def}	209.51 ^{b}	30.02 ^{bcd}	59.87 ^{ab}	52.90 ^{ab}	25.44 ^{bcd}	215.97 ^{bc}	22.08 ^{bcd}
ZM136	45.07 ^{ghi}	33.31 ^{klm}	22.43 ^{de}	121.49 ^{ghi}	28.89 ^{bcd}	53.77 ^{def}	45.82 ^{bcd}	26.74 ^{bcd}	143.97 ^{hij}	18.28 ^{fgh}

Table 3. *Cont.*

Cultivars	Year of 2022/2023					Year of 2023/2024				
	CC (SPAD)	SSC ($mg \cdot g^{-1}$ FW)	SPC ($mg \cdot g^{-1}$ FW)	PC ($\mu g \cdot g^{-1}$)	MC ($nmol \cdot g^{-1}$)	CC (SPAD)	SSC ($mg \cdot g^{-1}$ FW)	SPC ($mg \cdot g^{-1}$ FW)	PC ($\mu g \cdot g^{-1}$)	MC ($nmol \cdot g^{-1}$)
ZM366	47.30 ^{bcd}	36.86 ^{hij}	22.47 ^{de}	130.22 ^{fgh}	29.60 ^{bcd}	57.20 ^{abc}	46.86 ^{bcd}	27.6 ^{bc}	108.07 ^{lmn}	19.21 ^{efg}
ZM918	42.90 ^{mon}	38.46 ^{efg}	21.28 ^{def}	121.55 ^{ghi}	25.90 ^{def}	51.07 ^{jkl}	45.7 ^{bcd}	25.35 ^{cde}	144.74 ^{hij}	22.08 ^{bcd}
ZM1860	44.27 ^{ijk}	44.30 ^{cde}	20.24 ^{fgh}	141.22 ^{fgh}	19.99 ^{lmn}	53.60 ^{def}	51.18 ^{abc}	25.61 ^{bcd}	106.95 ^{mno}	16.13 ^{ijk}
ZM1905	45.07 ^{ghi}	37.06 ^{hij}	19.66 ^{ab}	156.96 ^{def}	22.59 ^{hij}	49.10 ^{lmn}	46.70 ^{bcd}	24.14 ^{ijk}	112.71 ^{klm}	18.25 ^{fgh}
ZM2118	44.23 ^{jkl}	37.64 ^{ghi}	20.67 ^{efg}	143.53 ^{efg}	24.08 ^{efg}	53.93 ^{def}	47.44 ^{abc}	25.52 ^{bcd}	170.44 ^{efg}	16.48 ^{hij}
ZM7698	41.33 ^{pqr}	36.01 ^{ijk}	20.37 ^{fgh}	144.42 ^{efg}	25.54 ^{def}	49.77 ^{klm}	46.68 ^{bcd}	25.77 ^{bcd}	205.05 ^{bcd}	16.44 ^{hij}
ZS9170	49.93 ^{abc}	39.88 ^{def}	21.45 ^{def}	102.63 ^{ijk}	24.83 ^{def}	58.50 ^{abc}	49.47 ^{abc}	26.56 ^{bcd}	121.66 ^{jkl}	16.91 ^{ghi}
JM919	44.65 ^{hij}	42.28 ^{def}	20.81 ^{def}	141.56 ^{fgh}	28.78 ^{bcd}	53.40 ^{efg}	44.78 ^{bcd}	24.34 ^{hij}	180.70 ^{def}	24.93 ^{bcd}
WK1838	46.20 ^{fgh}	28.40 ^{pq}	20.83 ^{def}	114.91 ^{ghi}	29.65 ^{bcd}	55.67 ^{bcd}	44.24 ^{bcd}	26.34 ^{bcd}	102.67 ^{mno}	25.16 ^{bcd}
QM725	47.77 ^{bcd}	39.83 ^{def}	19.23 ^{lmn}	82.15 ^{klm}	21.03 ^{jkl}	56.27 ^{abc}	49.17 ^{abc}	23.77 ^{lmn}	158.75 ^{fgh}	15.64 ^{ijk}
AN1589	41.77 ^{opq}	28.57 ^{pq}	20.74 ^{efg}	97.29 ^{ijk}	25.18 ^{def}	51.37 ^{ijk}	35.10 ^{ijk}	26.24 ^{bcd}	87.41 ^{op}	21.36 ^{bcd}
NZM1	47.93 ^{bcd}	43.38 ^{cde}	20.63 ^{efg}	139.35 ^{fgh}	31.79 ^{abc}	56.60 ^{abc}	47.65 ^{abc}	26.18 ^{bcd}	83.10 ^{p}	24.66 ^{bcd}
ZM113	48.80 ^{abc}	31.35 ^{mno}	22.81 ^{cd}	139.51 ^{fgh}	32.85 ^{ab}	58.83 ^{abc}	45.47 ^{bcd}	26.06 ^{bcd}	154.97 ^{fgh}	26.74 ^{ab}
JHM2	40.44 ^{rs}	30.07 ^{nop}	18.98 ^{mno}	92.97 ^{jkl}	28.72 ^{bcd}	42.23 ^{o}	39.82 ^{efg}	25.33 ^{cde}	99.66 ^{nop}	25.05 ^{bcd}
YM15	42.73 ^{mno}	37.58 ^{ghi}	21.91 ^{def}	77.14 ^{klm}	32.49 ^{abc}	50.73 ^{jkl}	36.63 ^{hij}	26.89 ^{bcd}	126.66 ^{ijk}	23.51 ^{bcd}
YM20	40.73 ^{qrs}	29.46 ^{opq}	20.89 ^{def}	82.62 ^{klm}	28.31 ^{bcd}	50.47 ^{jkl}	39.20 ^{fgh}	25.76 ^{bcd}	101.34 ^{nop}	26.02 ^{abc}
YM24	43.47 ^{lmn}	29.44 ^{opq}	18.94 ^{mno}	85.22 ^{klm}	35.36 ^{a}	53.07 ^{fgh}	34.75 ^{ijk}	23.93 ^{jkl}	98.46 ^{nop}	30.97 ^{a}
YM25	45.17 ^{fgh}	38.78 ^{efg}	19.41 ^{ijk}	94.59 ^{jkl}	25.16 ^{def}	54.43 ^{cde}	47.05 ^{abc}	25.25 ^{def}	86.01 ^{op}	22.33 ^{bcd}
YM27	39.70 ^{s}	27.43 ^{q}	18.9 ^{nop}	80.46 ^{klm}	30.2 ^{bcd}	46.33 ^{n}	27.72 ^{m}	23.63 ^{mn}	75.92 ^{p}	23.55 ^{bcd}
YM30	40.77 ^{qrs}	28.70 ^{pq}	18.92 ^{mno}	66.11 ^{m}	29.00 ^{bcd}	50.27 ^{jkl}	29.49 ^{klm}	24.61 ^{ghi}	126.10 ^{ijk}	22.08 ^{bcd}
YM34	44.40 ^{ijk}	30.70 ^{nop}	17.97 ^{p}	82.68 ^{klm}	25.23 ^{def}	53.13 ^{dfg}	39.71 ^{efg}	23.12 ^{n}	130.20 ^{ijk}	17.67 ^{ghi}
NM35	40.47 ^{rs}	31.94 ^{lmn}	18.31 ^{op}	83.16 ^{klm}	28.62 ^{bcd}	47.37 ^{mn}	28.56 ^{lm}	24.25 ^{ijk}	76.49 ^{p}	20.36 ^{def}
NM36	45.97 ^{fgh}	31.84 ^{lmn}	20.04 ^{fgh}	88.06 ^{jkl}	25.99 ^{def}	54.40 ^{cde}	37.92 ^{ghu}	25.68 ^{bcd}	85.71 ^{op}	18.08 ^{fgh}
NM13	46.63 ^{edf}	30.74 ^{nop}	19.28 ^{klm}	68.05 ^{lm}	27.08 ^{cde}	57.23 ^{abc}	33.41 ^{jll}	23.89 ^{klm}	86.45 ^{op}	21.36 ^{bcd}
ZM12	43.77 ^{klm}	38.68 ^{efg}	21.1 ^{def}	89.47 ^{jkl}	32.48 ^{abc}	50.93 ^{jkl}	43.63 ^{bcd}	26.15 ^{bcd}	125.36 ^{ijk}	26.38 ^{ab}

Note: Different letters following the data within each column indicate significant differences at $p < 0.05$ ($n = 3$).

In the 2023–2024 growing season, the highest Chl content was found in the semi-winter cultivar Shannong55 (SN55), at 51.19 SPAD; the lowest value was found in the spring cultivar Yangmai27 (YM27), at 29.7 SPAD. The highest SS content was found in the semi-winter wheat cultivar YN31, at 51.19 $mg \cdot g^{-1}$ of fresh weight (FW); the lowest value was found in the spring wheat cultivar YM27, at 27.43 $mg \cdot g^{-1}$ of FW. The highest SP content was in found the semi-winter cultivar LY955, at 26.13 $mg \cdot g^{-1}$ of FW; the lowest value was found in the spring cultivar YM34, at 17.97 $mg \cdot g^{-1}$ of FW. The highest Pro content was found in the semi-winter cultivar SN55, at 235.38 $\mu g \cdot g^{-1}$; the lowest value was found in the spring cultivar Yangmai30 (YM30), at 66.11 $\mu g \cdot g^{-1}$. The highest MDA content was found in the spring cultivar YM24, at 35.36 $nmol \cdot g^{-1}$; the lowest value was found in the semi-winter cultivar JM44, at 17.35 $nmol \cdot g^{-1}$.

In the 2023–2024 growing season, the highest Chl content was found in the semi-winter cultivar Shannong40 (SN40), at 60.33 SPAD; the lowest value was found in the spring cultivar JHM2, at 42.23 SPAD. The highest SS content was found in the semi-winter wheat cultivar YN31, at 57.26 $mg \cdot g^{-1}$ of FW; the lowest value was found in the spring wheat cultivar YM27, at 27.72 $mg \cdot g^{-1}$ of FW. The highest SP content was found in the winter cultivar Luliang1 (LL1), at 29.64 $mg \cdot g^{-1}$ of FW; the lowest value was found in the spring cultivar YM34, at 23.12 $mg \cdot g^{-1}$ of FW. The highest Pro content was found in the semi-winter cultivar SN55, at 294.73 $\mu g \cdot g^{-1}$; the lowest value was found in the spring cultivar YM27, at 75.92 $\mu g \cdot g^{-1}$. The highest MDA content was found in the spring cultivar YM24, at 30.97 $nmol \cdot g^{-1}$; the lowest value was found in the winter cultivar LL1, at 12.78 $nmol \cdot g^{-1}$.

3.3. Relationship Between the Physiological Parameters of Cold Resistance of Different Winter–Spring Wheat Cultivars

As can be seen from Figure 2, there is a significant positive correlation between the cold resistance and Chl, SS, SP, and Pro contents of different winter–spring cultivars of

wheat in the 2022–2023 growing season. The correlation coefficients were 0.58, 0.39, 0.44, and 0.69; there was a significant negative correlation with MDA content, and the correlation coefficient was −0.52.

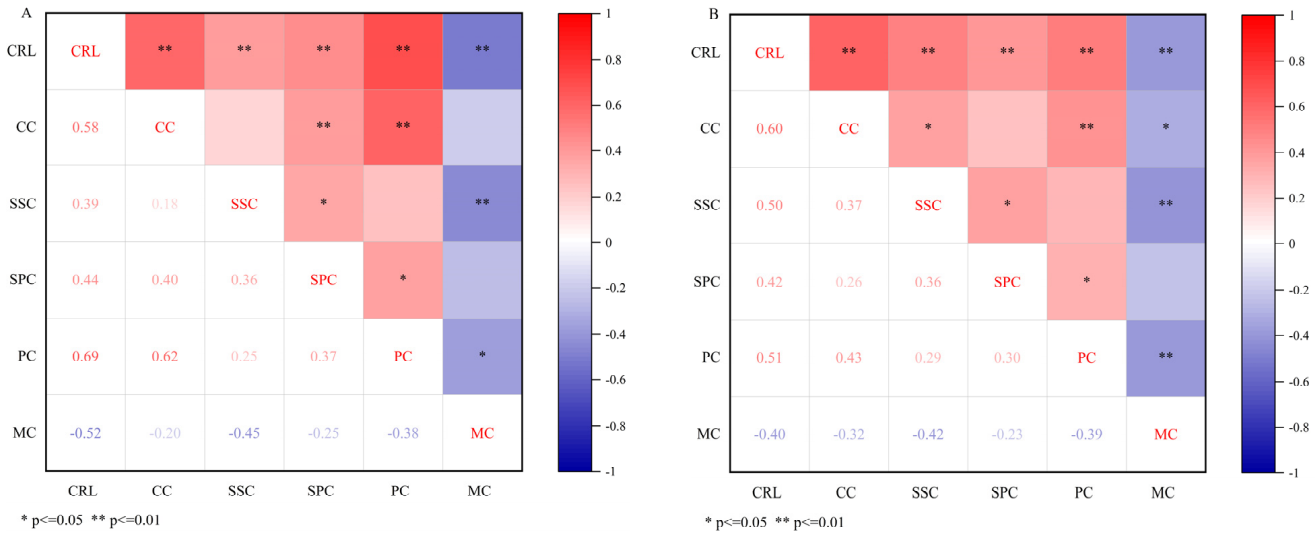


Figure 2. Correlation between cold resistance level (CRL) and chlorophyll content (CC), soluble sugar content (SSC), soluble protein content (SPC), proline content (PC), and malondialdehyde content (MC) in different winter–spring wheat cultivars ((**A**) 2022–2023; (**B**) 2023–2024).

In the 2023–2024 growing season, the cold resistance of different winter–spring cultivars of wheat had a significant positive correlation with the Chl, SS, SP, and Pro contents. The correlation coefficients were 0.60, 0.50, 0.42, and 0.51, respectively, with MDA content showing a significant negative correlation with a correlation coefficient of −0.40.

The results show that the higher the Chl, SS, SP, and Pro contents of wheat, the higher its cold resistance; the lower the MDA content, the higher the cold resistance of wheat. The five physiological parameters can all be used as indicators to evaluate the cold resistance of wheat.

3.4. Comparison of Wheat Yields of Different Winter–Spring Cultivars

The yields of different winter–spring wheat cultivars show significant differences (Figure 3).

In the 2022–2023 growing season, the wheat cultivars with the highest yields were the semi-winter cultivar LY 955, the winter cultivar Shannong38 (SN38), and the semi-winter cultivar YN31, with yields of 9635.71, 9422.10, and 9219.09 kg·hm^{-2}, respectively; the cultivars with the lowest yields were the spring cultivars YM15, YM20, and Ningmai35 (NM35), with yields of 1066.07, 1174.87, and 1321.10 kg·hm^{-2}, respectively.

In the 2023–2024 growing season, the wheat cultivars with the highest yields were the semi-winter cultivars LY955, SN57, and Zhengmai1860 (ZM1860), with yields of 10,417.12, 10,052.73, and 9750.982 kg·hm^{-2}, respectively; the cultivars with the lowest yields were the spring cultivars NM35, YM30, and JHM2, with yields of 6070.66, 6066.43, and 5952.492 kg·hm^{-2}, respectively.

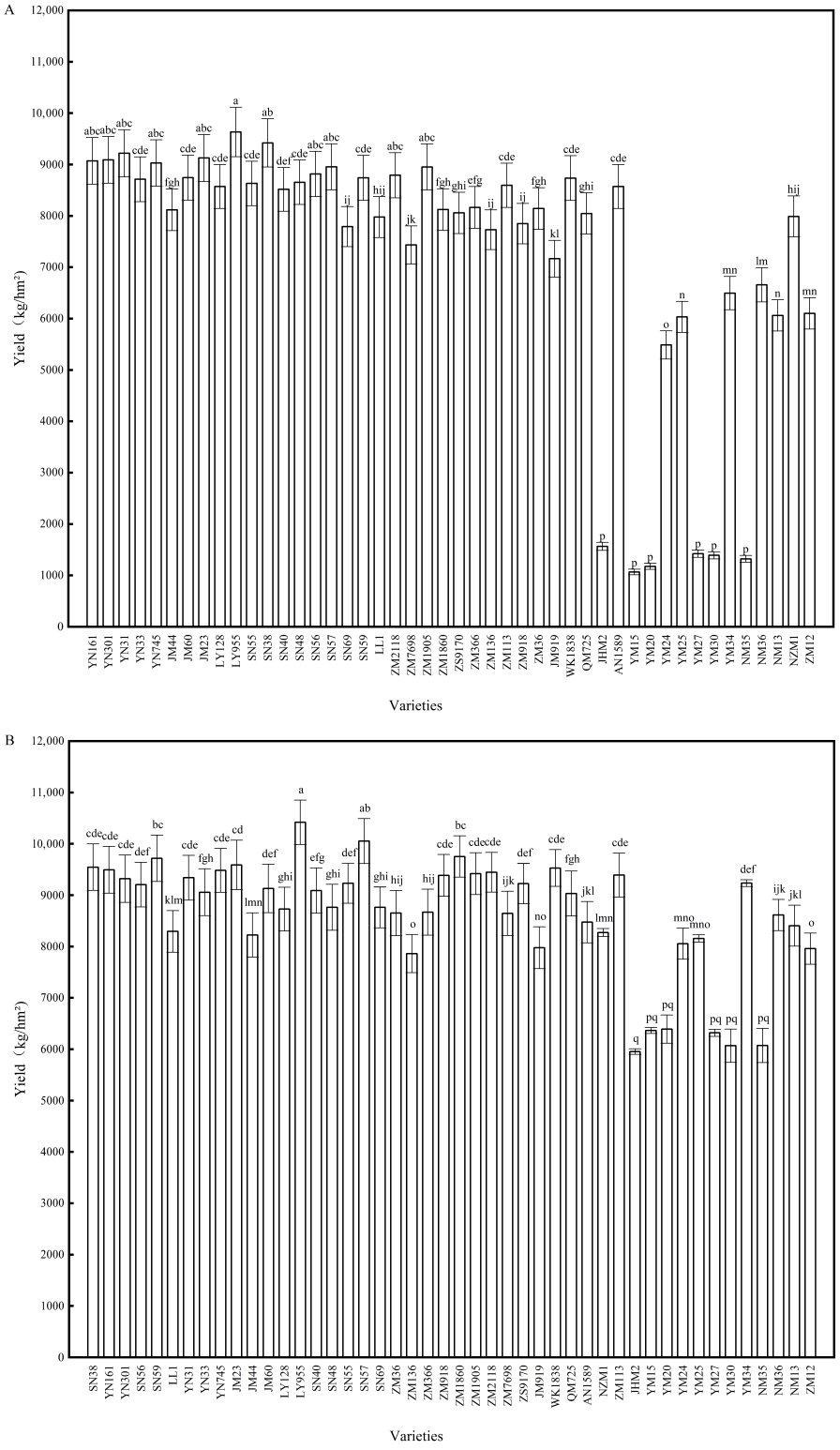


Figure 3. Yield of different winter–spring wheat cultivars ((**A**) 2022–2023; (**B**) 2023–2024). Note: Different letters following the data within each column indicate significant differences at $p < 0.05$ ($n = 3$).

3.5. Comparison of Quality Indices of Different Winter–Spring Wheat Cultivars

The GP content, WG content, DFT, DST, GHI, and SV of different winter–spring wheat cultivars are significantly different (Table 4).

Table 4. Grain protein content (GPC), wet gluten content (WGC), dough formation time (DFT), dough stability time (DST), grain hardness index (GHI), and sedimentation value (SV) of different winter–spring wheat cultivars.

Cultivars	Year of 2022/2023						Year of 2023/2024					
	GPC (%)	WGC (%)	DFT (min)	DST (min)	GHI	SV (mL)	GPC (%)	WGC (%)	DFT (min)	DST (min)	GHI	SV (mL)
SN38	12.87 ^{pqr}	27.80 ^{ijk}	4.03 ^{klm}	5.33 ^{hi}	74.92 ^{def}	32.24 ^{ghi}	14.00 ^{jkl}	25.02 ^{nop}	4.10 ^{klm}	6.97 ^{ghi}	73.08 ^{jkl}	28.40 ^{ijk}
YN161	13.79 ^{jkl}	34.54 ^{b}	4.37 ^{ghi}	7.40 ^{f}	70.67 ^{lm}	30.70 ^{ghi}	14.07 ^{ghi}	34.86 ^{a}	4.40 ^{ghi}	7.60 ^{f}	70.99 ^{mn}	29.58 ^{hij}
YN301	14.16 ^{hij}	24.65 ^{pq}	3.90 ^{nop}	4.00 ^{opq}	70.81 ^{lm}	27.42 ^{klm}	14.45 ^{def}	25.95 ^{lm}	3.87 ^{no}	4.63 ^{opq}	71.24 ^{mn}	27.04 ^{kl}
SN56	13.65 ^{klm}	29.52 ^{ef}	4.90 ^{def}	4.40 ^{klm}	71.95 ^{ijk}	39.05 ^{bc}	14.27 ^{efg}	25.92 ^{lm}	4.20 ^{jkl}	6.90 ^{ghi}	73.92 ^{ij}	29.74 ^{hij}
SN59	14.36 ^{ghi}	31.72 ^{d}	5.03 ^{cde}	4.77 ^{jkl}	67.20 ^{n}	37.25 ^{cde}	14.82 ^{cd}	33.00 ^{b}	3.50 ^{qr}	6.40 ^{jkl}	69.81 ^{n}	37.59 ^{a}
LL1	13.56 ^{lmn}	33.14 ^{c}	3.97 ^{mno}	14.60 ^{c}	70.08 ^{m}	43.97 ^{a}	14.03 ^{hij}	33.50 ^{b}	3.87 ^{no}	7.13 ^{fgh}	70.03 ^{n}	38.23 ^{a}
YN31	14.24 ^{ghi}	25.80 ^{no}	4.00 ^{lmn}	4.00 ^{opq}	73.80 ^{fgh}	25.55 ^{nop}	14.34 ^{efg}	25.82 ^{lm}	3.97 ^{mno}	4.10 ^{rst}	74.67 ^{ghi}	26.64 ^{klm}
YN33	13.28 ^{nop}	24.39 ^{pqr}	3.60 ^{rst}	4.17 ^{nop}	73.43 ^{ghi}	26.29 ^{mno}	13.87 ^{lmn}	24.29 ^{pqr}	3.77 ^{op}	4.10 ^{rst}	74.16 ^{hij}	23.96 ^{opq}
YN745	14.08 ^{hij}	25.29 ^{op}	3.53 st	4.27 ^{lmn}	73.05 ^{hij}	24.11 ^{qr}	14.21 ^{fgh}	25.29 ^{mno}	3.60 ^{pq}	4.07 ^{rst}	73.71 ^{ijk}	24.59 ^{nop}
JM23	14.93 ^{cde}	35.47 ^{b}	4.77 ^{def}	7.37 ^{f}	77.27 ^{bc}	30.41 ^{hij}	14.63 ^{def}	35.45 ^{a}	5.00 ^{b}	9.03 ^{d}	77.00 ^{bcd}	30.73 ^{fgh}
JM44	15.64 ^{b}	30.40 ^{e}	4.77 ^{def}	39.20 ^{a}	76.32 ^{cde}	30.10 ^{ijk}	15.43 ^{b}	29.99 ^{cd}	5.03 ^{b}	42.20 ^{a}	76.13 ^{def}	31.80 ^{efg}
JM60	13.47 ^{mno}	36.97 ^{a}	4.30 ^{hij}	3.53 ^{t}	72.53 ^{ijk}	29.60 ^{ijk}	14.29 ^{efg}	35.48 ^{a}	4.10 ^{klm}	3.57 ^{tu}	72.07 ^{klm}	25.33 ^{lmn}
LY128	13.69 ^{klm}	33.01 ^{c}	4.30 ^{hij}	5.40 ^{hi}	73.70 ^{fgh}	32.40 ^{ghi}	13.29 ^{pq}	33.71 ^{b}	4.40 ^{ghi}	6.63 ^{hij}	73.94 ^{ij}	34.93 ^{bc}
LY955	14.26 ^{ghi}	33.10 ^{c}	4.47 ^{ghi}	4.20 ^{mno}	75.71 ^{cde}	30.43 ^{hij}	14.48 ^{def}	35.36 ^{a}	4.50 ^{fgh}	8.23 ^{e}	75.78 ^{efg}	32.52 ^{def}
SN40	12.60 ^{qr}	27.10 ^{klm}	4.60 ^{fgh}	4.63 ^{klm}	71.10 ^{lm}	34.26 ^{def}	12.99 ^{qr}	27.51 ^{ij}	4.67 ^{def}	7.03 ^{fgh}	70.51 ^{mn}	32.16 ^{def}
SN48	14.11 ^{hij}	30.54 ^{e}	4.43 ^{ghi}	4.37 ^{klm}	78.97 ^{a}	33.70 ^{efg}	14.34 ^{efg}	28.08 ^{hi}	4.00 ^{lmn}	4.17 ^{qrs}	78.40 ^{abc}	27.62 ^{k}
SN55	13.45 ^{mno}	29.12 ^{fg}	4.47 ^{ghi}	4.88 ^{ijk}	79.60 ^{a}	31.76 ^{ghi}	14.79 ^{cd}	27.52 ^{ij}	4.40 ^{ghi}	8.63 ^{de}	78.60 ^{ab}	28.50 ^{1ijk}
SN57	15.22 ^{bcd}	33.13 ^{c}	5.47 ^{ab}	4.65 ^{klm}	71.72 ^{jkl}	42.43 ^{ab}	14.31 ^{efg}	24.87 ^{opq}	4.33 ^{hij}	5.97 ^{klm}	71.82 ^{lm}	25.23 ^{lmn}
SN69	13.46 ^{mno}	28.78 ^{fgh}	3.93 ^{mno}	4.98 ^{ijk}	71.46 ^{klm}	28.68 ^{jkl}	13.97 ^{klm}	27.54 ^{ij}	3.97 ^{mno}	5.97 ^{klm}	71.82 ^{lm}	25.23 ^{lmn}
ZM36	14.44 ^{fgh}	27.38 ^{jkl}	5.33 ^{abc}	18.52 ^{b}	75.63 ^{cde}	37.46 ^{cde}	14.25 ^{efg}	27.58 ^{ij}	5.70 ^{a}	19.56 ^{b}	76.06 ^{def}	37.29 ^{a}
ZM136	15.04 ^{cde}	30.47 ^{e}	5.60 ^{a}	7.43 ^{f}	75.81 ^{cde}	38.23 ^{cd}	15.36 ^{b}	29.94 ^{cd}	5.63 ^{a}	8.70 ^{de}	76.74 ^{cde}	37.71 ^{a}
ZM366	13.88 ^{ijk}	29.33 ^{fg}	4.30 ^{hij}	9.63 ^{d}	73.64 ^{fgh}	26.04 ^{nop}	13.87 ^{lmn}	29.22 ^{def}	4.40 ^{ghi}	9.03 ^{d}	73.23 ^{jkl}	26.78 ^{klm}
ZM918	14.32 ^{ghi}	27.47 ^{jkl}	3.77 ^{opq}	3.73 ^{rst}	61.04 ^{p}	26.51 ^{lmn}	14.15 ^{ghi}	26.29 ^{kl}	3.83 ^{no}	5.20 ^{mno}	60.54 ^{pq}	21.34 ^{rst}
ZM1860	13.68 ^{klm}	28.4 ^{ghi}	4.10 ^{jkl}	7.90 ^{ef}	76.49 ^{cde}	24.37 ^{pqr}	14.46 ^{def}	28.35 ^{ghi}	3.80 ^{nop}	7.57 ^{f}	75.06 ^{fgh}	25.06 ^{lmn}
ZM1905	13.53 ^{mno}	24.53 ^{pq}	4.13 ^{ijk}	5.77 ^{h}	71.04 ^{lm}	25.18 ^{opq}	13.45 ^{op}	24.65 ^{opq}	3.80 ^{nop}	6.50 ^{ijk}	71.36 ^{mn}	27.01 ^{kl}
ZM2118	14.17 ^{hij}	29.46 ^{efg}	3.97 ^{mno}	7.73 ^{ef}	76.47 ^{cde}	37.63 ^{cde}	13.95 ^{klm}	29.63 ^{cde}	4.20 ^{jkl}	7.57 ^{f}	77.54 ^{abc}	31.46 ^{efg}
ZM7698	14.73 ^{efg}	30.48 ^{e}	5.17 ^{bcd}	9.23 ^{d}	74.70 ^{efg}	37.40 ^{cde}	14.85 ^{cd}	30.20 ^{c}	4.97 ^{bc}	8.43 ^{e}	75.35 ^{efg}	33.23 ^{cde}
ZS9170	14.29 ^{ghi}	28.91 ^{fgh}	4.43 ^{ghi}	18.53 ^{b}	78.29 ^{ab}	33.48 ^{fgh}	14.44 ^{def}	28.61 ^{fgh}	4.30 ^{ijk}	19.18 ^{b}	78.05 ^{abc}	32.10 ^{def}
JM919	15.26 ^{bcd}	37.83 ^{a}	5.57 ^{a}	4.83 ^{ijk}	76.20 ^{cde}	34.60 ^{def}	15.12 ^{bc}	35.58 ^{a}	5.70 ^{a}	7.20 ^{fgh}	76.06 ^{def}	35.29 ^{b}
WK1838	14.48 ^{fgh}	27.01 ^{klm}	3.87 ^{nop}	4.60 ^{klm}	64.52 ^{o}	25.55 ^{nop}	14.79 ^{cd}	26.94 ^{jk}	3.97 ^{mno}	4.83 ^{op}	65.23 ^{o}	25.14 ^{lmn}
QM725	15.34 ^{cde}	33.40 ^{c}	4.67 ^{efg}	4.40 ^{klm}	60.29 ^{p}	28.54 ^{jkl}	15.39 ^{b}	28.33 ^{ghi}	4.73 ^{de}	4.73 ^{opq}	61.22 ^{p}	27.94 ^{jk}
AN1589	14.50 ^{fgh}	27.43 ^{jkl}	4.50 ^{fgh}	6.43 ^{g}	76.75 ^{bcd}	33.45 ^{fgh}	14.50 ^{def}	28.94 ^{efg}	4.80 ^{cd}	7.37 ^{fg}	78.91 ^{a}	33.87 ^{bcd}
NZM1	13.38 ^{mno}	26.45 ^{mn}	3.67 ^{pqr}	3.90 ^{pqr}	60.92 ^{p}	24.54 ^{opq}	13.20 ^{pq}	26.74 ^{k}	3.90 ^{mno}	5.47$^{l\ m}$	60.72 ^{pq}	24.01 ^{opq}
ZM113	13.40 ^{mno}	26.71 ^{lmn}	4.07 ^{jkl}	8.20 ^{e}	76.00 ^{cde}	22.45 ^{r}	14.06 ^{ghi}	26.41 ^{kl}	4.20 ^{jkl}	8.33 ^{e}	75.18 ^{fgh}	23.74 ^{opq}
JHM2	12.60 ^{qr}	25.32 ^{op}	3.67 ^{pqr}	4.73 ^{jkl}	57.98 ^{q}	23.39 ^{qr}	12.74 ^{r}	25.71 ^{lmn}	3.87 ^{no}	4.70 ^{opq}	58.56 ^{rs}	25.37 ^{lmn}
YM15	13.25 ^{nop}	24.49 ^{pq}	3.67 ^{pqr}	6.33 ^{g}	57.53 ^{qr}	23.44 ^{qr}	13.85 ^{mn}	24.58 ^{opq}	3.30 ^{r}	4.50 ^{pqr}	57.02 st	22.27 ^{qrs}
YM20	12.37 ^{r}	23.54 ^{qr}	4.10 ^{jkl}	4.23 ^{mno}	56.90 ^{qrs}	24.66 ^{opq}	12.74 ^{r}	25.71 ^{lmn}	3.87 ^{no}	4.70 ^{opq}	58.56 ^{rs}	25.37 ^{lmn}
YM24	13.63 ^{klm}	23.7 ^{qr}	3.67 ^{pqr}	3.83 ^{qrs}	55.33 ^{s}	18.73 ^{s}	13.78 ^{no}	23.62 st	3.47 ^{qr}	3.73 ^{stu}	56.58 ^{t}	20.17 ^{t}
YM25	13.53 ^{mno}	27.9 ^{hij}	3.53 st	5.33 ^{hi}	61.37 ^{p}	27.14 ^{klm}	14.11 ^{ghi}	24.13 ^{qrs}	3.60 ^{pq}	5.53 ^{lm}	62.11 ^{p}	20.78 st
YM27	16.39 ^{a}	34.85 ^{b}	4.57 ^{fgh}	4.53 ^{klm}	58.22 ^{q}	25.54 ^{nop}	16.22 ^{a}	33.46 ^{b}	4.50 ^{fgh}	5.40 ^{lmn}	60.66 ^{pq}	29.97 ^{ghi}
YM30	14.78 ^{def}	25.36 ^{op}	3.63 ^{qrs}	4.33 ^{lmn}	56.98 ^{qrs}	17.64 ^{s}	14.70 ^{de}	25.23 ^{mno}	3.33 ^{r}	4.33 ^{pqr}	58.05 ^{rst}	17.62 ^{u}
YM34	13.79 ^{jkl}	23.72 ^{qr}	3.47 ^{t}	3.63 st	56.14 ^{rs}	17.45 ^{s}	14.01 ^{ijk}	23.46 ^{t}	3.47 ^{qr}	3.47 ^{u}	57.15 st	17.45 ^{u}
NM35	12.99 ^{opq}	28.46 ^{fgh}	4.57 ^{fgh}	7.73 ^{ef}	64.96 ^{o}	25.59 ^{nop}	13.90 ^{klm}	28.98 ^{efg}	4.53 ^{fgh}	7.20 ^{fgh}	65.93 ^{o}	25.65 ^{lmn}
NM36	12.52 ^{qr}	23.38 ^{r}	3.97 ^{mno}	3.53 ^{t}	61.21 ^{p}	24.41 ^{pqr}	12.59 ^{r}	23.85 ^{rst}	3.97 ^{mno}	4.90 ^{nop}	59.53 ^{qr}	23.11 ^{pqr}
NM13	13.37 ^{mno}	26.91 ^{klm}	4.37 ^{ghi}	6.37 ^{g}	71.19 ^{lm}	32.23 ^{ghi}	13.29 ^{pq}	25.84 ^{lm}	4.60 ^{efg}	7.13 ^{fgh}	72.14 ^{klm}	30.70 ^{fgh}
ZM12	15.16 ^{bcd}	31.72 ^{d}	4.07 ^{jkl}	14.38 ^{c}	75.35 ^{def}	25.52 ^{nop}	15.31 ^{b}	29.69 ^{cde}	4.33 ^{hij}	14.86 ^{c}	77.81 ^{abc}	24.90 ^{mno}

Note: Different letters following the data within each column indicate significant differences at $p < 0.05$ ($n = 3$).

In the 2022–2023 growing season, the spring cultivar YM27 had the highest GP content, at 16.39%; in comparison, the spring cultivar YM20 had the lowest GP content, at 12.37%. The semi-winter cultivar JM919 had the highest WG content, at 37.83%; in comparison, the spring cultivar Ningmai36 (NM36) had the lowest, at 23.38. The semi-winter cultivar Zhengmai366 (ZM366) had the longest DFT, at 5.60 min; the spring cultivar YM34 had the shortest DFT, at 3.47 min. The semi-winter cultivar JM44 had the longest DST, at 39.20 min; the semi-winter cultivar JM23 had the shortest DST, at 3.53 min. The semi-winter cultivar SN55 had the highest GHI, at 79.60; in comparison, the spring cultivar YM24 had the lowest GHI, at 55.33. The winter cultivar LL1 had the highest SV, at 43.97 mL; the spring cultivar YM34 had the lowest SV, at 17.45 mL.

In the 2023–2024 growing season, the spring cultivar YM27 had the highest GP content, at 16.22; the spring cultivar NM36 had the lowest GP content, at 12.59%. The semi-winter cultivar JM919 had the highest WG content, at 35.58%; the spring cultivar YM34 had the lowest WG content, at 23.46%. The semi-winter cultivars Zhengmai918 (ZM918) and

JM919 had the longest DFT, both at 5.70 min; the spring cultivar YM15 had the shortest DFT, at 3.30 min. The semi-winter cultivar JM44 had the longest DST, at 42.20 min; the spring cultivar YM34 had the shortest DST, at 3.47 min. The GHI was the highest in the semi-winter cultivar Annong1589 (AN1589), at 78.91, and the lowest value was found in the spring cultivar YM24, at 56.58. The highest SV was found in the winter cultivar LL1, at 38.23 mL, and the lowest value was found in the spring cultivar YM34, at 17.45 mL.

3.6. Comprehensive Evaluation and Screening of Different Winter–Spring Wheat Cultivars

The average membership values and comprehensive scores of the cold resistance, yield, and quality indicators of the different winter–spring wheat cultivars were calculated. In general, the higher the average membership value and comprehensive score, the better the comprehensive traits of the cultivar. The K-means method was used to cluster the average membership values and comprehensive scores of the tested cultivars. After three clustering iterations, the cultivars were clustered into five groups. The average membership values and comprehensive scores of wheats in the first to the fifth groups decreased in sequence (Tables 5 and 6).

Table 5. Average membership values and comprehensive scores of the different winter–spring wheat cultivars.

Cultivars	Year of 2022/2023		Year of 2023/2024	
	Average Membership Value	Comprehensive Score	Average Membership Value	Comprehensive Value
SN38	0.51	1.92	0.5	1.42
YN161	0.59	2.03	0.62	1.83
YN301	0.42	1.53	0.42	1.21
SN56	0.61	2.11	0.53	1.52
SN59	0.58	1.9	0.62	1.82
LL1	0.62	2.09	0.59	1.8
YN31	0.49	1.8	0.5	1.41
YN33	0.42	1.64	0.43	1.19
YN745	0.44	1.67	0.45	1.25
JM23	0.69	2.3	0.72	2.17
JM44	0.76	2.3	0.77	2.16
JM60	0.58	2.04	0.57	1.68
LY128	0.54	1.85	0.54	1.75
LY955	0.58	1.98	0.65	1.99
SN40	0.5	1.86	0.52	1.56
SN48	0.57	1.97	0.53	1.57
SN55	0.57	2.08	0.6	1.74
SN57	0.74	2.4	0.51	1.4
SN69	0.47	1.73	0.47	1.33
ZM36	0.65	2.07	0.64	2
ZM136	0.67	2.13	0.65	2.07
ZM366	0.49	1.68	0.47	1.41
ZM918	0.37	1.28	0.38	0.97
ZM1860	0.46	1.65	0.47	1.36
ZM1905	0.41	1.5	0.38	1.1
ZM2118	0.55	1.92	0.53	1.64
ZM7698	0.62	2.01	0.59	1.82
ZS9170	0.6	1.96	0.58	1.74
JM919	0.7	2.2	0.68	2.19
WK1838	0.45	1.59	0.47	1.26
QM725	0.52	1.61	0.46	1.31
AN1589	0.59	2.07	0.63	1.93
NZM1	0.32	1.17	0.28	0.8
ZM113	0.48	1.77	0.51	1.42
JHM2	0.09	0.26	0.12	0.44

Table 5. *Cont.*

Cultivars	Year of 2022/2023		Year of 2023/2024	
	Average Membership Value	Comprehensive Score	Average Membership Value	Comprehensive Value
YM15	0.1	0.25	0.1	0.29
YM20	0.08	0.24	0.13	0.47
YM24	0.17	0.58	0.19	0.44
YM25	0.28	0.94	0.26	0.66
YM27	0.35	0.8	0.41	1.27
YM30	0.12	0.24	0.11	0.26
YM34	0.17	0.62	0.22	0.46
NM35	0.23	0.66	0.28	0.95
NM36	0.22	0.84	0.23	0.64
NM13	0.39	1.32	0.43	1.33
ZM12	0.49	1.5	0.53	1.56

Table 6. The results of cluster analysis.

Yeas	Class-Number	Group Classification	Final Cluster Center Average Membership Value	Final Cluster Center Comprehensive Value
2022/2023	1	JM23, JM44, SN57, JM919	0.72	2.30
	2	YN161, YN31, JM60, LY128, LY955, SN55, SN38, SN40, SN48, SN56, SN59, LL1, ZM2118, ZM7698, ZS9170, ZM136, ZM36, AN1589	0.58	1.99
	3	YN301, YN33, YN745, SN69, ZM1905, ZM1860, ZM366, ZM113, ZM918, WK1838, QM725, NM13, ZM12	0.45	1.57
	4	YM25, YM27, YM34, NM35, NM36, NZM1	0.26	0.84
	5	JHM2, YM15, YM20, YM24, YM30	0.11	0.31
2023/2024	1	JM23, JM44, LY955, ZM136, ZM36, JM919, AN1589	0.68	2.07
	2	YN161, JM60, LY128, SN55, SN40, SN48, SN56, SN59, LL1, ZM2118, ZM7698, ZS9170, ZM12	0.57	1.69
	3	YN301, YN31, YN33, YN745, SN38, SN57, SN69, ZM1905, ZM1860, ZM366, ZM113, WK1838, QM725, YM27, NM13	0.46	1.31
	4	ZM918, YM25, NM35, NM36, NZM1	0.29	0.80
	5	JHM2, YM15, YM20, YM24, YM30, YM34	0.14	0.39

In the 2022–2023 growing season, the average membership values of the examined wheat cultivars were distributed between 0.08 and 0.76, and the comprehensive scores were distributed between 0.24 and 2.40. The final cluster center values of the first group were the

highest, at 0.72 and 2.3, including the semi-winter cultivars JM23, JM44, SN57, and Jinmai 919 (JM919); the cluster center values of the fifth group were the lowest, at 0.11 and 0.31, including the spring cultivars YM24, JHM2, YM15, YM20, and YM30.

In the 2023–2024 growing season, the average membership values of the examined wheat cultivars were distributed between 0.10 and 0.77, and the comprehensive scores were distributed between 0.26 and 2.19. The final cluster center values of the first group were the highest, at 0.68 and 2.07, including the semi-winter cultivars JM23, JM44, LY955, ZM136, ZM36, JM919, and AN1589; the cluster center values of the fifth group were the lowest, at 0.14 and 0.39, including the spring cultivars JHM2, YM15, YM20, YM24, YM30, and YM34.

4. Discussion

Wheat cold resistance is a complex biological characteristic determined by multiple factors [46]. Different winter–spring wheat varieties have different levels of cold resistance [47]. In this study, we investigated the cold resistance of 46 core winter–spring wheat cultivars from China's main wheat-producing areas under field conditions; measured five physiological parameters of wheat during the greening period, including the Chl, SS, SP, Pro, and MDA contents; analyzed the relationship between wheat cold resistance and these physiological parameters; and screened out effective physiological indices of cold resistance. In addition, the level of physiological parameters also affects the formation of wheat yield and quality in the later stages.

The results of previous studies have shown that winter wheat cultivars have higher cold resistance than spring wheat cultivars [48]. Mu et al. [49] conducted an indoor freezing test and found that strong-winter wheat cultivars have the highest level of cold resistance, followed by winter and semi-winter cultivars, and spring wheat cultivars were found to have the lowest level of cold resistance. However, the authors of some studies have suggested that there is no necessary connection between the spring–winter characteristics of wheat and its cold resistance [50]. In this study, we also found that strong-winter, winter, and semi-winter wheat cultivars have a higher level of cold resistance than weak-spring and spring wheat cultivars.

Venzhik et al. [51] found that low-temperature stress has a direct impact on the synthesis and function of Chl in wheat leaves. Low-temperature stress reduces the activity of Chl synthase, thereby inhibiting the synthesis of Chl, which is similar to the results of this study in that the cold resistance of wheat is positively correlated with Chl content. After frost damage, the content of osmotic regulating substances such as SS and SP in wheat increases, increasing the concentration of cell sap and reducing plant frost damage [52,53]. Alexander et al. [54] found that wheat cultivars with strong frost resistance under low-temperature conditions will accumulate photosynthetic pigments, SS, and SP; in comparison, wheat cultivars with poor frost resistance will not accumulate SS, which is similar to the results of this study.

Low molecular weight compounds in the plant antioxidant system also participate in the plant's defense against low-temperature stress. Pro acts as an osmotic regulator and cell membrane protector, exerting antioxidant functions and improving the cold resistance of wheat [55,56]. Ali et al. [57] showed that wheat cultivars with strong cold resistance under low-temperature stress can accumulate more Pro and have higher levels of antioxidant enzyme activity, which can reduce the degree of damage to wheat under adverse conditions, maintain higher photosynthetic efficiency, and allow for higher yields. Zhang et al. [58] showed that winter wheat varieties accumulate more Pro than spring varieties under low-temperature stress, improving the ability of wheat to resist low-temperature environments. In this study, we found that winter wheat cultivars under low-temperature stress have higher Pro content than spring wheat cultivars, which plays an important role in wheat's resistance to low-temperature stress. The MDA content in plants can reflect the degree of damage to cell membranes under adverse stress conditions. The greater the degree of stress the plant suffers, the higher the MDA content [59,60]. In this study, we found that the cold resistance of different winter–spring wheat cultivars has a significant negative correlation

with MDA content. Low-temperature freezing damage causes a large amount of MDA to accumulate in spring wheat cultivars, accelerating freezing damage to wheat.

The authors of other studies have highlighted that low-temperature stress has a negative impact on both wheat yield and quality [61,62]. Low-temperature stress limits the production of nitrogen compounds and non-structural carbohydrates, thereby reducing the transport of SP and SS from the stem to the grain, resulting in a decrease in wheat yield and quality [63,64]. The results of this study show that the yield and quality indicators of winter wheat cultivars; namely, WG content, DFT, DST, GHI, and SV, were higher than those of spring wheat cultivars. Low-temperature frost damage caused leaf damage to spring wheat cultivars with weak cold resistance, reduced nutrient synthesis and transportation, and reduced yield and quality. In this study, we found that the spring variety YM27 had the highest GP content, which may be determined by variations in characteristics and cold resistance-related genes. Some wheat cultivars were damaged by frost in the early growth stage but had better recovery ability in the later stages and could still maintain high yield and quality [65–67].

The effects of different natural environments on wheat growth and development vary; however, because this study was conducted only in Shandong Province, the results of this study have certain limitations. This study spanned two growing seasons and may not capture the effects of long-term trends or changes in climate conditions on wheat cold resistance, as cold resistance may change significantly over a longer time frame or under more diverse conditions. In addition, there are potential interactions between wheat cold resistance and factors such as pests and diseases, drought and flood disasters, and adaptability to different soil conditions, which affect the growth and development of wheat. In future experimental plans, we will combine different climate and environmental conditions to conduct a more in-depth study of the relationship between wheat cold resistance and biological and non-biological factors, so as to provide a more practical theoretical basis for wheat stress resistance breeding.

5. Conclusions

In the 2022–2023 growing season, seventeen level 2 frost-damaged cultivars such as YN161, seventeen level 3 frost-damaged cultivars such as YN301, six level 4 frost-damaged cultivars such as YM24, and six level 5 frost-damaged cultivars such as JHM2 were found; in the 2023–2024 growing season, twenty level 2 frost-damaged cultivars such as YN301, twenty level 3 frost-damaged cultivars such as YN301, and six level 4 frost-damaged cultivars such as JHM2 were found. Level 2 and level 3 frost-damaged cultivars are generally strong-winter, winter, and semi-winter wheat cultivars, and level 4 and level 5 frost-damaged cultivars are generally weak-spring and spring wheat cultivars. The cold resistance of winter wheat cultivars is greater than that of spring wheat cultivars. The Chl, SS, SP, and Pro contents of the different winter–spring wheat cultivars are positively correlated with cold resistance; in comparison, MDA content is negatively correlated with cold resistance. These five physiological parameters can be used as physiological indicators for the breeding of cold-resistant cultivars. After comprehensively evaluating the average membership values and comprehensive scores of the cold resistance, yield, and quality indicators of the different winter and spring wheat cultivars, it was found that the average membership values and comprehensive scores of winter wheat cultivars were higher than those of spring wheat cultivars. The average membership values and comprehensive scores of the different winter and spring wheat cultivars were classified using the K-means method, and the cold-resistant, high-yield, and high-quality cultivars were screened out, namely, JM23, JM44, SN57, and JM919.

In the process of cold-resistance breeding, the five physiological parameters of cold resistance selected in this study can help breeders judge the cold resistance of different wheat cultivars from the perspective of physiological indicators of cold resistance, and provide a theoretical basis for cold-resistance breeding. In addition, the four wheat cultivars selected

in this study, namely, JM23, JM44, SN57, and JM919, can provide more cultivar options for growers and breeders who pursue high yields, high quality, and high cold resistance.

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