



## Hardening and frost resistance of winter common wheat under climate change conditions

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**Abstract:** The growth and development, the level of hardening and frost resistance of winter common wheat are strongly dependent on stability of climatic factors. The dynamic temperature regime observed in recent years worsens the conditions of overwintering of plants. A period of 21 consecutive years (2003-2024) was studied with the aim to follow the variation of hardening conditions during autumn-winter months and the frost resistance of winter common wheat in relation to climate change. Varieties from standard scale with known level of frost resistance were subjected to artificial freezing: Mironovskaya 808 (highest frost resistance), Bezostaya 1, № 301, Rusalka and San Pastore (lowest frost resistance). Temperatures of freezing were chosen based on the level of hardening to achieve good differentiation between standard varieties.

The lowest test temperature (-22°C) leading to good differentiation between standard varieties were applied in 2016-2017. For the period 2020-2024, related to the lack of conditions for good hardening, differentiating temperatures were relatively higher from -16°C to -11°C. According to the hardening conditions and the level of frost resistance, the analyzed years were divided into 4 groups.

**Key words:** winter wheat, hardening, frost resistance

### 1. INTRODUCTION

Resistance to low temperature is essential for survival in winter and is one of the many physiological factors influencing the winter hardiness of winter cereals (Jaškūnė et al., 2022). Frost resistance of common wheat varieties changes during the winter period as a consequence of complicated interactions between plants and various environmental factors (Bergjord et al., 2008; Veisz & Tischner, 1995). Among the environmental conditions influencing the development of genetically determined hardiness and frost

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resistance, the duration and temperatures of hardening have a major role (Veisz & Sutka, 1989). For hardening and developing the frost resistance, the plants need a certain period of growth at low, but positive (non-freezing) temperatures (Janda et al., 2007; Petcu & Terbea, 1995). The threshold temperature for the beginning of hardening of winter cereals is about 10°C, optimal about 3°C – first stage of hardening, and exposure to negative temperatures (-2/-4°C) has been proven increase acclimatization (Fowler et al., 1999) – second stage of hardening. The level of frost resistance acquired through the hardening processes is not static and it can be quickly lost by increasing the temperature (Gusta & Fowler, 1976; Mantri et al., 2012; Rizza et al., 1994). Winter warming as a major consequence of global climate change negatively affects the hardening of winter crops (Li et al., 2015). Unstable climatic regime in winter months with clear periods of warming and sudden colds worsens the conditions for overwintering of the plants (Sadykov et al., 2021).

The studied trends to climate changes in agricultural sector, expressed in high variability of conditions and an increase in the frequency of extreme weather events, lead to changes in agroclimatic characteristic of agricultural regions (Kazandzhiev & Georgieva, 2020) and need to create winter cereal varieties adapted to variable climatic conditions (Mihova et al., 2017).

The frost resistance is important aspect in breeding process in creating new winter common wheat varieties (Tsenov et al., 2009). The annual testing of hybrid materials from the individual breeding units in different combinations of meteorological and stress factors, follows their behavior and determines the risk of damage in real winter conditions in our country (Tsenov et al., 2012). The lack of suitable winter colds each year doesn't allow for an annual assessment of frost resistance under fields conditions, which requires the application of field-laboratory methods with controlled freezing of plants in cold chambers. Tsenov & Petrova (1984) described a method for testing the frost resistance of winter cereals lines and varieties, which is applying in the Physiology Laboratory at the Dobrudzha agricultural institute until nowadays. The differences of test temperature at which survival is achieved to differentiate the standard varieties, as in the individual years, as in each winter period, are due to the different level of plant hardening. The aim of the investigation is to follow the variability of hardening conditions during the autumn-winter months and the level of frost resistance in winter common wheat in relation to climate change.

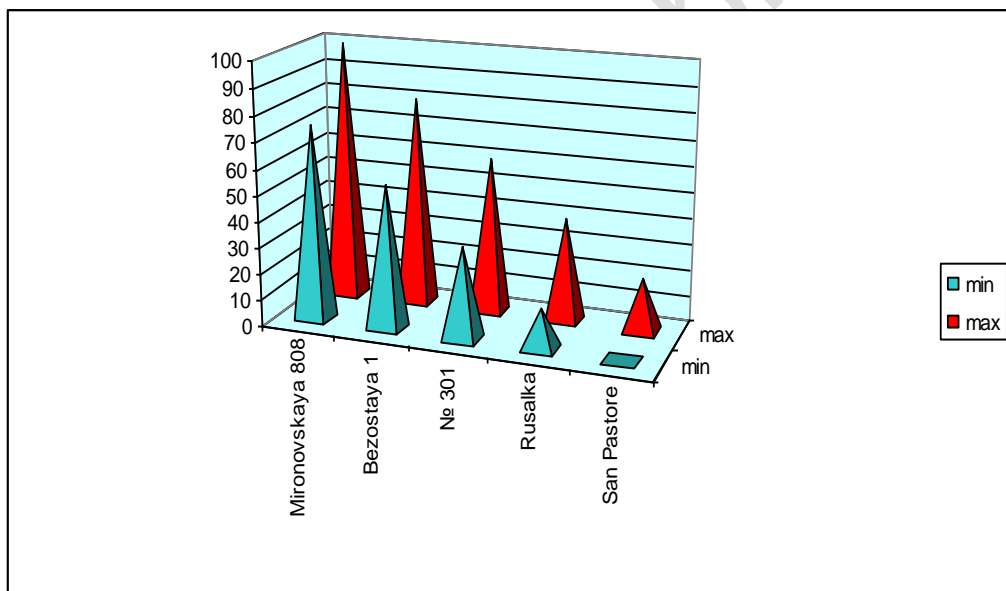
## **2. MATERIALS AND METHODS**

The experiment was carried out at the Physiology Laboratory at the Dobrudzha Agricultural Institute, General Toshevo. The investigation covers a period of 21 consecutive years (2003-2024). An analysis of temperature conditions by data of Hydrometeorological Observatory Dobrich, National Institute of Meteorology and Hydrology, Varna branch, Bulgaria, was performed. Meteorological indicators for the months of November, December and January, directly related with the preparation and survival of plants during the winter period, were studied.

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The frost resistance was assessed using a method described by Tsenov and Petrova (1984). The plants were grown in vegetation plates under natural conditions outside. During the period with the highest frost resistance the plants were frozen in cold chambers KTK 3000 to an appropriate negative temperature. After growing in a greenhouse, the survival rate was recorded.

The experiment included varieties from standard scale with known level of frost resistance: Mironovskaya 808 (highest frost resistance), Bezostaya 1, № 301, Rusalka and San Pastore (lowest frost resistance). Temperatures of freezing were chosen based on the level of hardening to achieve good differentiation between standard varieties. A differentiating temperature was one at which survival of each variety from standard scale was achieved within certain limits (Figure 1): from 0% to 20% live plants for the San Pastore variety, from 15% to 40% for the Rusalka variety, from 35% to 60% for the № 301 variety, from 55% to 80% for Bezostaya 1 variety and from 75% to 100% for Mironovskaya 808 variety.



**Fig. 1.** Limits of percentage of surviving plants defining the test temperature as well differentiating

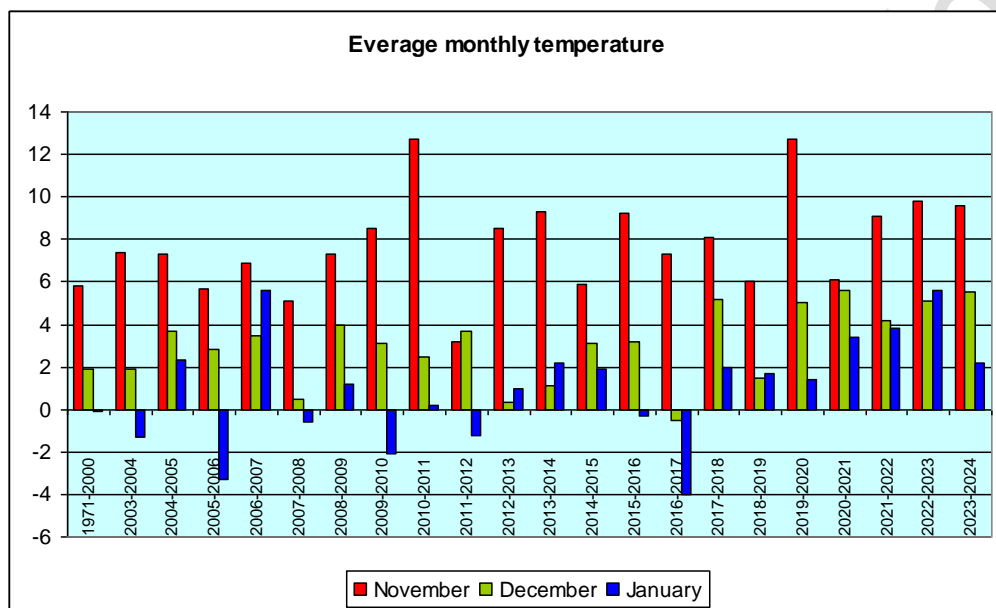
To follow the variability in the level of resistance within each year, 4 tests were performed, every 10-15 days – first one was in the last decade of December and the last one was around the beginning of February. For each freezing carried out in the individual periods (P1, P2, P3 and P4), a differentiating temperature was determined.

The relationships between meteorological factors and the level of frost resistance were determined through correlations ( $r$ ). The experimental data were processed with the help of Microsoft Excel<sup>XP</sup> and STATISTICA, release 7.0 (StatSoft Inc., 2004).

### 3. RESULTS AND DISCUSSION

#### 3.1. Temperature conditions

Increased average monthly, average minimum and average maximum temperatures were recorded for the months of November, December and January (Figure 2), compared to the average for a previous 30-year period (1971-2000).



**Fig. 2.** Average monthly temperature for the months of November, December and January by years, and average for a multi-year period (1971-2000)

The conditions in November determine the plant growth and development. Mean values of average monthly, average minimum and average maximum temperatures for the period 2003-2024 increased the average from the previous period 1971-2000 by +2.1°, +2.0° and +2.3°C respectively. The years with the largest deviations (up to +6.9°C) were 2010-2011 and 2019-2020. Increased temperatures in the initial stages of winter common wheat development hide a risk of accelerated growth and overgrowth of plants, which is unfavorable from the point of view of their hardening. Greater total biomass correlates with lower total sugar content and poorer frost resistance (Hanslin & Mortensen, 2010).

The conditions in December are crucial for the degree of plant hardening. For the month of December raised of the average monthly temperature in 15 out of 21 studied years was recorded, as for the period of 2019-2024 deviation was the biggest – to +3.7°C compared to the mean of multi-year period. The number of the days with minimum temperatures bellow 0°C on average for 2003-2024 was 17 (Table 1), with

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the lowest number in 2020-2021 (4 days), 2021-2022 (8 days), 2017-2018 and 2022-2023 (13 days). The number of the days with maximum temperatures above 10°C varied between 3 and 16, and with temperatures above 15°C between 0 and 10 days. The largest number of days with maximum temperatures above 15°C in December were recorded in 2017-2018 (10 days), 2023-2024 (9 days), 2022-2023 (8 days) and 2019-2020 (7 days). The period 2019-2024 is characterized with an increase in days with maximum temperatures above 10° and above 15°C, compared to the average for 2003-2018.

The conditions in January determine the dynamic of the level of resistance to low temperatures. Average monthly temperatures significant below the long-term average were recorded in 2016-2017 (-3.9°) and 2005-2006 (-3.2°C), and over the average in 2006-2007 (+5.7°C), 2022-2023 (+5.7°C) and 2021-2022 (+3.9°C). In the month of January, in recent years, an increase of the days with maximum temperatures above 10° and 15°C has also been observed. In cases of positive minimum and high daily temperatures, plants start processes of dehardening, so their resistance to cold drastically decreases. This subject the plants at risk in case of sudden colds, especially in the absence of snow cover, which has been a trend in recent years.

### **3.2. Differentiating temperatures**

Table 2 presents the four differentiating test temperatures for each year and the corresponding survival of individual varieties on average for all tests. The first test temperature, at which good differentiation was achieved, varied between -11° (2010-2011) to -22°C (2016-2017). In 2016-2017 the varieties were tested to the lowest temperatures, respectively -22°C, -20°C, -22°C and -17°C, with an average to -20.3°C. At the end of the analyzed period, for 4 consecutive years (2020-2024) the differentiating test temperatures were relatively high from -16°C to -11°C.

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**Table 1.** Deviations of average monthly, average minimum and average maximum temperatures from the average for multi-year data (1971-2000), number of days with minimums below 0°C, and maximums above 10° and above 15°C, for the months of December and January, by year

Month	December						January					
Year	Deviations from multi-year data (1971-2000)			days with T <sub>min</sub> < 0°C	days with T <sub>max</sub> > 10°C	days with T <sub>max</sub> > 15°C	Deviations from multi-year data (1971-2000)			days with T <sub>min</sub> < 0°C	days with T <sub>max</sub> > 10°C	days with T <sub>max</sub> > 15°C
	av. mon. T (°C)	av. T <sub>min</sub> (°C)	av. T <sub>max</sub> (°C)				av. mon. T (°C)	av. T <sub>min</sub> (°C)	av. T <sub>max</sub> (°C)			
1971-2000	<b>1.9</b>	<b>-1.1</b>	<b>5.7</b>				<b>-0.1</b>	<b>-3.5</b>	<b>3.7</b>			
2003-2004	+0.0	-0.5	+0.7	22	7	0	-1.2	-1.8	-0.3	20	3	0
2004-2005	+1.8	+1.6	+2.6	15	12	3	+2.4	+2.7	+3.3	15	9	2
2005-2006	+0.9	-0.8	+1.6	16	9	2	-3.2	-3.2	-2.6	26	2	0
2006-2007	+1.6	+0.9	+3.1	19	14	2	+5.7	+4.6	+8.1	12	21	9
2007-2008	-1.4	-0.6	-1.8	21	6	0	-0.5	+0.0	-0.1	22	2	1
2008-2009	+2.1	+2.9	+1.7	10	11	5	+1.3	+1.2	+2.0	19	8	0
2009-2010	+1.2	+1.5	+0.5	16	7	3	-2.0	-2.7	-2.1	22	6	2
2010-2011	+0.6	-0.9	+1.2	17	12	5	+0.3	+0.3	+0.9	20	3	0
2011-2012	+1.8	+0.7	+2.8	19	13	6	-1.1	-0.9	-1.2	27	3	0
2012-2013	-1.6	-2.4	-1.1	25	6	2	+1.1	+0.7	+1.7	21	8	2
2013-2014	-0.8	-1.6	-0.1	26	5	0	+2.3	+2.1	+3.3	17	14	4
2014-2015	+1.2	+1.1	+1.4	14	10	1	+2.0	+1.5	+2.6	14	10	5
2015-2016	+1.3	+0.1	+4.0	19	14	5	-0.2	-2.0	+0.1	19	8	2
2016-2017	-2.4	-3.6	-1.0	28	3	1	-3.9	-3.8	-4.1	30	0	0
2017-2018	+3.3	+2.3	+4.5	13	16	10	+2.1	+1.4	+3.1	22	10	3
2018-2019	-0.4	-0.4	-0.8	17	3	0	+1.8	+1.8	+1.4	21	7	1
2019-2020	+3.1	+1.8	+4.6	14	15	7	+1.5	+0.3	+3.6	28	11	1
2020-2021	+3.7	+4.4	+2.2	4	11	4	+3.5	+3.5	+3.5	14	13	3
2021-2022	+2.3	+2.2	+1.6	8	11	3	+3.9	+4.5	+3.7	14	7	2
2022-2023	+3.2	+2.3	+4.3	13	15	8	+6.1	+6.6	+6.3	10	16	7
2023-2024	+3.6	+1.8	+4.7	19	16	9	+2.7	+2.3	+2.0	22	6	4
<i>mean</i>	<b>+1.2</b>	<b>+0.6</b>	<b>+1.7</b>	<b>16.9</b>	<b>10.3</b>	<b>3.6</b>	<b>+1.5</b>	<b>+1.4</b>	<b>+2.1</b>	<b>19.8</b>	<b>8.0</b>	<b>2.3</b>
<i>min</i>	-2.4	-3.6	-1.8	4.0	3.0	0.0	-3.5	-3.3	-3.6	10.0	0.0	0.0
<i>max</i>	+3.7	+4.4	+4.7	28.0	16.0	10.0	+6.1	+6.6	+8.6	30.0	21.0	9.0

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**Table 2.** Differentiating temperatures (°C) in the four test periods (P1, P2, P3 and P4) and % of surviving plants of standard varieties (average for the four tests), by year

Year	Test temperature (°C)				mean T (°C)	Mir. 808	Bez. 1	№ 301	Rus.	San Past.
	P1	P2	P3	P4						
2003-2004	-20	-17	-20	-20	<b>-19.3</b>	86.6	73.5	46.7	27.2	8.3
2004-2005	-19	-15	-16	-14	<b>-16.0</b>	80.8	74.9	38.0	23.5	12.6
2005-2006	-14	-13	-21	-18	<b>-16.5</b>	92.3	79.6	40.9	26.6	14.3
2006-2007	-18	-17	-13	-15	<b>-15.8</b>	87.9	71.4	39.5	23.5	15.6
2007-2008	-19	-22	-21	-18	<b>-20.0</b>	96.5	68.0	40.0	24.5	10.0
2008-2009	-13	-17	-17	-15	<b>-15.5</b>	83.3	74.1	29.8	15.1	1.9
2009-2010	-17	-16	-15	-18	<b>-16.5</b>	77.8	63.6	43.3	32.0	19.0
2010-2011	-11	-15	-16	-15	<b>-14.3</b>	81.0	64.4	42.5	24.8	7.0
2011-2012	-16	-19	-19	-19	<b>-18.3</b>	83.3	68.5	51.5	37.8	18.8
2012-2013	-16	-19	-14	-13	<b>-15.5</b>	93.7	82.6	59.8	44.4	9.8
2013-2014	-19	-16	-19	-17	<b>-17.8</b>	77.6	61.5	41.0	25.6	10.6
2014-2015	-18	-16	-19	-11	<b>-16.0</b>	84.6	62.5	46.6	21.1	13.3
2015-2016	-18	-19	-12	-14	<b>-15.8</b>	88.4	67.9	47.3	39.0	17.6
2016-2017	-22	-20	-22	-17	<b>-20.3</b>	79.8	59.5	45.4	29.7	17.9
2017-2018	-15	-17	-16	-17	<b>-16.3</b>	81.0	55.3	38.0	24.0	7.8
2018-2019	-20	-20	-18	-14	<b>-18.0</b>	82.0	63.4	50.9	43.3	21.0
2019-2020	-14	-13	-18	-20	<b>-16.3</b>	78.8	63.5	46.8	35.0	16.8
2020-2021	-12	-15	-11	-15	<b>-13.3</b>	67.0	40.8	30.0	18.0	3.3
2021-2022	-13	-16	-14	-14	<b>-14.3</b>	71.0	50.5	40.0	32.3	17.5
2022-2023	-14	-14	-13	-14	<b>-13.8</b>	63.8	52.9	36.2	27.0	12.8
2023-2024	-12	-15	-14	-16	<b>-14.3</b>	67.9	57.4	45.6	29.1	17.9
<b>mean</b>	<b>-16.2</b>	<b>-16.7</b>	<b>-16.6</b>	<b>-15.9</b>	<b>-16.3</b>	<b>81.2</b>	<b>64.6</b>	<b>42.8</b>	<b>28.7</b>	<b>13.0</b>
<b>min</b>	<b>-22.0</b>	<b>-22.0</b>	<b>-22.0</b>	<b>-20.0</b>	<b>-20.3</b>	<b>63.8</b>	<b>40.8</b>	<b>29.8</b>	<b>15.1</b>	<b>1.9</b>
<b>max</b>	<b>-11.0</b>	<b>-13.0</b>	<b>-11.0</b>	<b>-11.0</b>	<b>-13.3</b>	<b>96.5</b>	<b>82.6</b>	<b>59.8</b>	<b>44.4</b>	<b>21.0</b>
<b>st. dev..</b>						<b>8.39</b>	<b>9.75</b>	<b>6.78</b>	<b>7.54</b>	<b>5.22</b>
<b>VC%</b>						<b>0.10</b>	<b>0.15</b>	<b>0.16</b>	<b>0.26</b>	<b>0.40</b>

The relationship between meteorological conditions during the months of November and December, and differentiating temperature of the first test is shown on Table 3. Temperature conditions in December are crucial for the frost resistance formation of common wheat. At higher average monthly, average minimum and average maximum temperatures for the month of December, the degree of hardening is reduced, which necessitates an increase in the testing temperature leading to good differentiation of the standard varieties. The number of the days with maximum temperatures above 10° and above 15°C in December also has a positive correlation with the differentiating temperature. At 10°C hardening begins, but the same temperature is not enough to maintain the resistance of the hardened plants (Gusta & Fowler, 1976). As higher are the daily maximum temperatures, as faster is the rate of

dehardening. The trend of increasing temperatures leading to an increase in the duration of the frost-free period and a shortening of the period with temperatures below 0°C (Kazandzhiev et al., 2011) was also observed in the last years of the analyzed period. This adversely affected the hardening processes, a lower level of cold resistance was achieved, and the differentiating temperature was higher, respectively.

**Table 3.** Correlation between main meteorological factor for the months of November and December, and the differentiating temperature of the first test (T1)

Month	Av. Mon. T	Av. T <sub>min</sub>	Av. T <sub>max</sub>	Abs. T <sub>min</sub>	Abs. T <sub>max</sub>	Days T<0°C	Days T>10°C	Days T>15°C
Nov.	0,392	0,266	0,427	0,290	0,056	-0,384	0,452*	0,277
Dec.	<b>0,683**</b>	<b>0,573**</b>	0,499*	0,239	<b>0,624**</b>	<b>-0,639**</b>	<b>0,612**</b>	<b>0,657**</b>

### 3.3. Grouping of the analyzed years

The analyzed years were divided into four groups depending on their hardening and level of frost resistance (Table 4).

**Table 4.** Distribution of years according to hardening conditions and level of cold resistance

Group	Characteristic	Year
<b>first</b>	good hardening conditions, high level of frost resistance, which maintained throughout the entire test period	2003-2004, 2007-2008, 2016-2017, 2018-2019
<b>second</b>	good hardening conditions, high level of frost resistance at the beginning of the test, dynamic conditions in the winter period, varying level of resistance	2004-2005, 2006-2007, 2009-2010, 2012-2013, 2013-2014, 2014-2015, 2015-2016
<b>third</b>	lack of good hardening conditions, low frost resistance at the beginning of the test, improving the conditions and gradually increasing the resistance	2005-2006, 2011-2012, 2019-2020
<b>fourth</b>	lack of good hardening conditions, low frost resistance throughout the entire test period	2008-2009, 2010-2011, 2017-2018, 2020-2021, 2021-2022, 2022-2023, 2023-2024

The first group included the years with stable winters 2003-2004, 2007-2008, 2016-2017, 2018-2019. Average monthly temperatures of the months of November, December and January were close to the average of the multi-year period. The number of days with minimum temperatures below 0°C in total for the three months varied between 46 and 56, and the days with maximum temperatures above 10°C for the months of December and January were from 0 to 7. The year 2016-2017 is characterized as the coldest one. Here the biggest deviation for the months of December and January from the average on long-term period, were registered. At this year, the lowest differentiating temperature (-22°C) of test for the entire analyzed period, were applied.

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The second group included the years 2004-2005, 2006-2007, 2009-2010, 2012-2013, 2013-2014, 2014-2015, 2015-2016. The years were distinguished by good hardening conditions. The average monthly temperature for the month of December varied between 1.1° and 3.7°C. The total number of the days with minimum temperature below 0°C was an average of 25 days. In the first test, the standards differentiated by temperatures between 17° and 19°C in the individual years. In this group the month of January is characterized with variable conditions. Less number of the nights (12-22) with negative minimum temperatures and more number of the days with maximum daily temperatures above 10°C (6-21), of which 0 to 5 days the average daily temperatures exceed 15°C, were observed. The lowest temperatures (-25.5°C) for the analyzed period, were registered in the end of January 2010. Unstable conditions subjected the plants to multiple hardening and dehardening, as a result differentiating temperatures within a single test period were strongly variable.

The third group included 2005-2006, 2011-2012 and 2019-2020. There were no suitable conditions for hardening in November and December. In the first test, the plants had low frost resistance with differentiating temperatures between -14° and -16°C. Subsequently, in January, conditions were improved and the level of resistance gradually increased. January was characterized as a cold month with 26 to 28 nights with temperatures below zero, supporting the additional hardening of the plants.

The fourth group, with inappropriate hardening conditions and a low level of resistance throughout the entire testing period, included 2008-2009, 2010-2011, 2017-2018, 2020-2021, 2021-2022, 2022-2023, 2023-2024. Characteristic for the group were the largest increases in average monthly, average minimum and average maximum temperatures for the winter months, with the most drastic deviations for the period 2019-2024. Strong decline in the number of nights with negative minimum temperatures, while increased number of days with maximum temperatures exceeding 15°C, were observed. Under these conditions, good hardening of the plants was not achieved, respectively, the differentiating temperatures were relatively high from -17° to -11°C, and for the last 4 consecutive years the lowest temperature for achieving differentiation of the standards was -16°C.

At the beginning of the analyzed period (2003-2012), an even distribution and alternation of the years belonging to the individual groups was established. For the period 2012-2024, the cases of years with not good hardening and varying conditions during the winter period significantly exceed the number of years with stable winters. Distinctive for the period is the occurrence of sequences of 4 consecutive years, once in the years from the second group (2012-2016), and a second time in the years from the fourth group (2020-2024).

#### **3.4. Varieties of winter common wheat, breeding of Dobrudzha Agricultural Institute**

With the predicted milder and snowless winters (Kazandjiev & Georgieva, 2020), as the study confirmed for the last 5 years (2019-2024), the risk of sudden frosts and

variability of the temperature regime during the winter months should not be ignored when choosing a common wheat variety. In climatically unstable winters, with frequent temperature fluctuations, plants have difficulty falling into dormancy, especially the varieties with low frost resistance, which in such a case are distinguished by more intensive growth during the winter months (Sadykov et al., 2021). Despite the warming trends, the recommendation of Tsenov et al. (2012) that common winter wheat varieties grown in agricultural production should have frost resistance between that of the standard varieties Bezostaya 1 and No. 301, remains valid.

Table 5 presents winter common wheat varieties, breeding of Dobrudzha Agricultural Institute, with a high level of frost resistance, showing adaptability to sudden variation in meteorological factors during the autumn-winter periods, provoking injury. Some of them combine productivity under different types of abiotic stress and a stable level of quality traits.

**Table 5.** Implemented in practice and new common wheat varieties created at DZI, distinguished by a high level of frost and winter resistance

Variety	Grain quality	Level of frost resistance, according to the standard scale
Pchelina	excellent	level Mironovskaya 808
Lazarka	excellent	level Mironovskaya 808
Merylin	excellent	level Mironovskaya 808
Shibil	very good	level Bezostaya 1
Chudomira	good	level Mironovskaya 808
Zara	very good	level Mironovskaya 808
Fani	very good	level Bezostaya 1

#### 4. CONCLUSION

During the analyzed period the temperature conditions strongly varied. From 2003 to 2012, an even distribution and alternation of years with stable and unstable winters was established. For the period 2012-2024, the years with a lack of conditions for good hardening and with a varying level of frost resistance of common winter wheat significantly prevail.

The test temperatures, at which good differentiation of the standards was achieved, varied between -11° (2010-2011 year with low level of frost resistance) and -22°C (2016-2017 year with high level of frost resistance). The period 2020-2024 was characterized with high differentiating temperatures from -16°C to -11°C. The deteriorated conditions for plant hardening and the corresponding low level of frost resistance were associated with increased average daily temperatures, a reduced number of days with minimum temperatures below 0°C and an increased number of days with maximum temperatures above 10°C during the winter months. The instability of conditions caused alternating hardening and dehardening of plants and

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exposed them to a real risk of winter frost in the event of a reversal of agrometeorological conditions. The study supported the recommendation that the level of cold resistance of winter common wheat varieties grown in agricultural production should be close to that of the Bezostaya 1 standard.

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