

Frost resistance of newly created soft winter wheat varieties of Myronivka breeding under different hardening conditions

T.V. Yurchenko¹, S.V. Pykalo², M.V. Kharchenko³

^{1,3}Candidate of Agricultural Sciences

²Candidate of Biological Sciences

The V.M. Remeslo Myronivka Institute of Wheat of NAAS

68 Tsentralna str., Tsentralne vil., Obukhiv dist., Kyiv reg., Ukraine, 08853

¹t.yurchenko978@gmail.com, ²pykserg@ukr.net,

³michail.kharch@gmail.com

ORCID: ¹0000-0003-0164-4003, ²0000-0002-3158-3830, ³0000-0002-4005-2134

Goal. To study the frost resistance of newly created soft winter wheat varieties of Myronivka breeding under different hardening conditions in the central part of the Forest-Steppe of Ukraine and identify varieties with consistently high levels of resistance. **Methods.** After hardening the plants in an open field using the standard technique, they were subjected to freezing in low-temperature chambers at a temperature of -18 °C. The reliability of the obtained data was verified using the Fisher's criterion. **Results.** The conducted research over three contrasting years confirmed that the frost resistance of wheat varieties depends on the conditions of their hardening. Due to the gradual decrease in air temperature, the process of plant hardening was much more efficient than with sudden fluctuations. As a result, under favorable conditions for the hardening phases in 2021/22, the percentage of live plants after freezing averaged 84.4% for all varieties, under satisfactory conditions in 2020/21 – 68.1%, and under unfavorable conditions in 2019/2020 - 51.9%. The frost resistance of the benchmark variety Myronivska 808 varied from 56% to 94% during the years of the study. In 2019/2020, the level of resistance exceeded the

benchmark for the variety Avrora Myronivska, and at the benchmark level were the varieties MIP Assol, MIP Fortuna, MIP Vidznaka, Vezha myronivska, MIP Yuvileina, Estafeta myronivska, MIP Nika. In 2020/21, the resistance was higher than the benchmark for the varieties MIP Vidznaka and MIP Feieriia, and at the benchmark level were the varieties MIP Roksolana, Estafeta myronivska, Vezha myronivska, MIP Yuvileina, Avrora myronivska, MIP Nika and MIP Fortuna. According to the results of freezing in 2021/2022, the percentage of live plants in the varieties MIP Fortuna, MIP Vidznaka, MIP Yuvileina, Estafeta Myronivska, Vezha Myronivska, MIP Nika was at the level of the benchmark Myronivska 808. A strong inverse correlation ($r = -0.77$) relationship was established between the percentage of live plants after freezing and the temperature regime of the hardening period. **Conclusions.** The following soft winter wheat varieties MIP Fortuna, MIP Vidznaka, Vezha Myronivska, MIP Yuvileina, Estafeta Myronivska and MIP Nika were identified as having a high level of resistance over the course of three years.

Key words: *Triticum aestivum L., percentage of live plants, weather conditions, air temperature, vegetation year, snow cover height, tillering node.*

An important task today is to ensure the food security of our country. With this in mind, one of the priority directions of agricultural production is considered to be stabilizing the production of high-quality food grain through the cultivation of winter wheat [1]. Modern varieties of winter wheat have a high potential for productivity, the realization of which largely depends on the growing conditions [1, 2]. In particular, the main limiting factor is the weather conditions throughout the year, with weather accounting for 82% of productivity formation [3]. The climate in recent years is characterized by rapid changes in weather conditions with significant fluctuations in precipitation and temperature [4, 5]. The recent increase in air temperature in Ukraine is characterized by unevenness: periods of sharp temperature rise alternate with slower or even cooling. Often, against the backdrop of general

warming, there are cold spells with frost, which poses a real danger to agriculture [6]. The degree and nature of climate change can significantly affect the productivity of winter wheat, as weather variability leads to significant (up to 40-60%) fluctuations in yields [7, 8]. Each phase of plant development contributes to its yield, and the duration depends on agroclimatic conditions. The maximum yield increase occurs at optimal values of agrometeorological factors that provide the biological optimum for plants in each period of the vegetation cycle [9].

The winter period is of great importance for the growth and development of winter wheat [8, 9]. During the wintering of winter wheat plants at low air temperatures, the growth rates and the intensity of physiological processes sharply decrease, making them very vulnerable to the negative factors of the winter period, such as low critical temperatures, especially in the absence of snow cover, sudden temperature increases, and to waterlogging and freezing [10, 11]. With the recent increase in the overall temperature background of the autumn-winter period, the level of its fluctuations has significantly increased, resulting in frequent prolonged thaws with sharp short-term temperature drops, the formation of ice crust, which becomes an additional factor in freezing [12]. Due to unfavorable conditions for wintering in various regions of Ukraine, a large number of winter wheat crops die almost every year. This is why frost resistance is of great importance - the ability of plants to withstand low temperatures without irreversible harmful consequences [8, 11]. The unsatisfactory condition of winter crop plantings emphasizes the urgent need for agronomic and breeding research in the direction of increasing their frost resistance [11, 13]. In general, frost resistance is one of the main components of the adaptability of winter wheat varieties [14, 15].

Winter crops are able to withstand adverse temperature conditions during the cold period due to a series of adaptive reactions, with adaptation to low temperatures being the most important [16, 17]. In the autumn period, with a decrease in air and soil temperatures, complex physiological

processes take place in winter crops, ensuring their preparation for wintering, known as plant hardening. It should be noted that the hardening of plants in the autumn-winter period significantly increases their resistance to the negative effects of weather conditions during the winter period. As is known, the hardening of winter crops occurs in two phases: Phase I occurs at daytime air temperatures from 8 to 10°C and nighttime temperatures from 0 to 4°C, and Phase II occurs when the average daily temperature fluctuates between 0 and -5°C [18].

In Phase I, plants harden due to active vegetation and photosynthesis processes. During this period, the plant accumulates a sufficient amount of sugars (carbohydrates) in the tillering nodes, which are not spent on respiration and growth during nighttime temperatures from 0 to 4°C [19]. As the sugar content in the tillering nodes continuously increases and can reach about 30% by the end of the phase, and due to the increase in the dry matter content in the plant's body, the decrease in temperature at the depth of the tillering node to -10...-12°C is tolerated by the plant normally [18].

Phase II involves the process of dehydration of plant cells, leading to an increased concentration of soluble sugars. As a result, the plant's body reduces the content of free water, which is capable of freezing rapidly, and instead, water with a high sugar concentration, which freezes only at critically low temperatures, appears [20]. After successful passage of Phase II, hardened plants become even more resistant to low temperatures. Well-hardened wheat can withstand temperatures of about -18...-19°C near the tillering node. The duration of the passage of both hardening phases by plants is 20-25 days [19, 20].

In the process of hardening, winter crop plants replenish their reserves of osmotically active substances, primarily soluble carbohydrates and proline amino acids in the tillering nodes, which allows them to achieve a high level of frost resistance [21, 22]. Less frost resistant varieties of winter crops contain fewer water-soluble carbohydrates than frost resistant ones, and in their tillering nodes, less cell sap is formed.

Considering the above, the study of the characteristics of modern genotypes of winter wheat in forming frost resistance under different hardening conditions is currently a relevant direction of research.

The **goal** of the research was to study the frost resistance of newly created soft winter wheat varieties of Myronivka breeding under different hardening conditions in the central part of the Forest-Steppe of Ukraine and identify varieties with consistently high levels of resistance.

Materials and methods of research. The study was conducted during 2019 – 2022 at the V.M. Remeslo Myronivka Institute of Wheat of NAAS. The research material consisted of 13 varieties of soft winter wheat from Myronivka breeding: Aurora myronivska, MIP Assol, MIP Fortuna, MIP Vidznaka, Vezha myronivska, MIP Yuvileina, Estafeta myronivska, MIP Nika, MIP Feieriia, MIP Roksolana, Hratiia myronivska, MIP Darunok, MIP Lada. The highly frost-resistant variety Myronivska 808 was used as the frost resistance standard.

Plant frost resistance was determined according to DSTU 4749:2007 [23], which involves conducting research using the following methodology. Crates measuring 30x40 cm and a depth of 12–15 cm were filled with sifted soil to 3–4 cm below the top edge. The soil surface was leveled and divided into rows with a spacing of 3–4 cm. The research material was sown in crates with 20–25 seeds in each row and covered with 3 cm of soil. In each crate, two rows of the reference variety were also sown. From autumn to the beginning of winter, the plants were left in natural conditions and underwent both phases of hardening. Care for the plants included systematic watering. During the first decade of January, the crates were placed in low-temperature chambers KNT-1. To evaluate frost resistance, each variety was exposed to a temperature of -18 °C. The temperature in the chambers (ambient temperature) was reduced by 2 °C per hour until the desired value was reached. The exposure to freezing was 24 hours. After gradual thawing (over 2 days), the crates with the crops were placed in rooms with temperatures ranging from 18 to 24 °C and prepared the plants for growth by trimming,

leaving a leaf blade of 0.5 cm in length. Preliminary counting of live and dead plants was done after 10–12 days, and the final count was conducted after 15–16 days.

The reliability of the obtained data was checked using Fisher's criterion. Pearson's correlation coefficient (r) was interpreted using Cheddok's scale [24]: $0 < r < 0.09$ – no connection, $0.10 < r < 0.29$ – weak, $0.30 < r < 0.49$ – moderate, $0.50 < r < 0.69$ – significant, $0.7 < r < 0.89$ – strong, $0.90 < r < 0.99$ – very strong, $r = 1.00$ – functional connection.

Research results. In 2019, the cessation of plant vegetation was observed on November 21, with an average daily temperature of -0.8°C , followed by a sharp decrease and subsequent gradual increase (Fig. 1). From December 15, due to the changing weather conditions, the plants resumed their vegetation, and it was finally halted on December 26, which was 50 days later compared to the previous year of 2018 (November 6) [25].

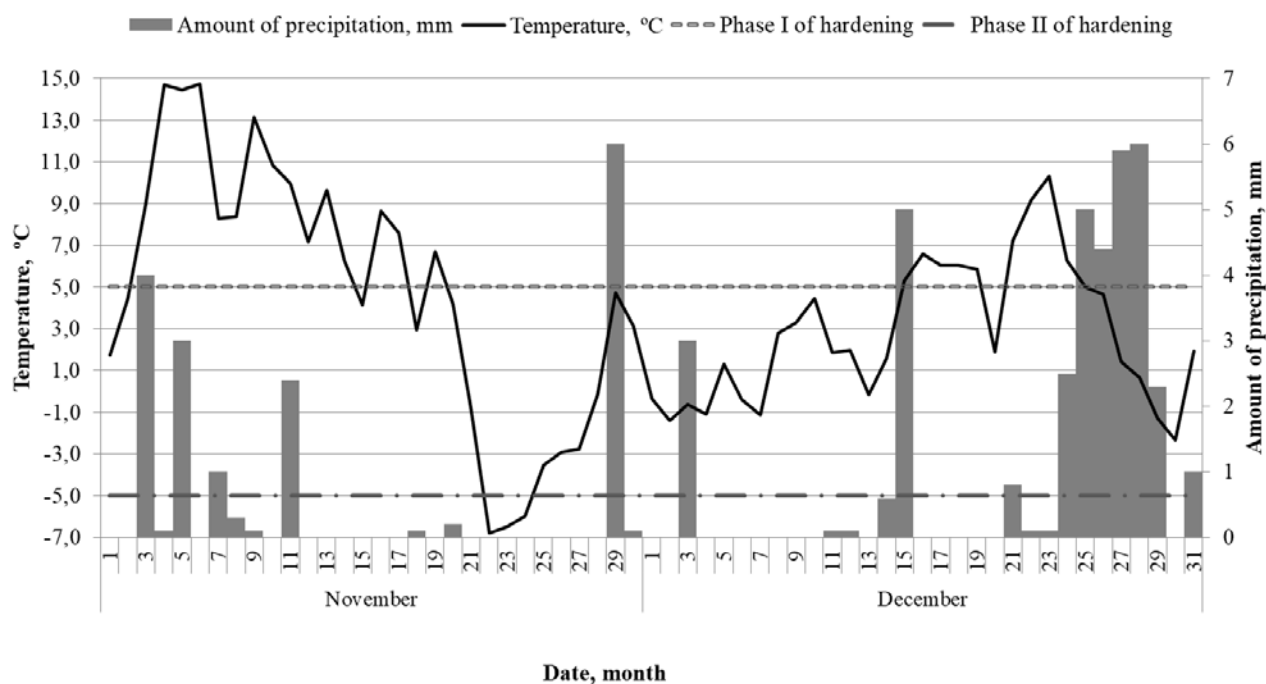


Fig.1. Hydrothermal conditions during the plant hardening period of 2019.

The lowest daily average temperature was recorded on November 22, 2019, at -6.8°C , with a temperature at the depth of node formation of -2.0°C [25]. Snowfall reached a depth of up to 5 cm but immediately melted (Fig.2).

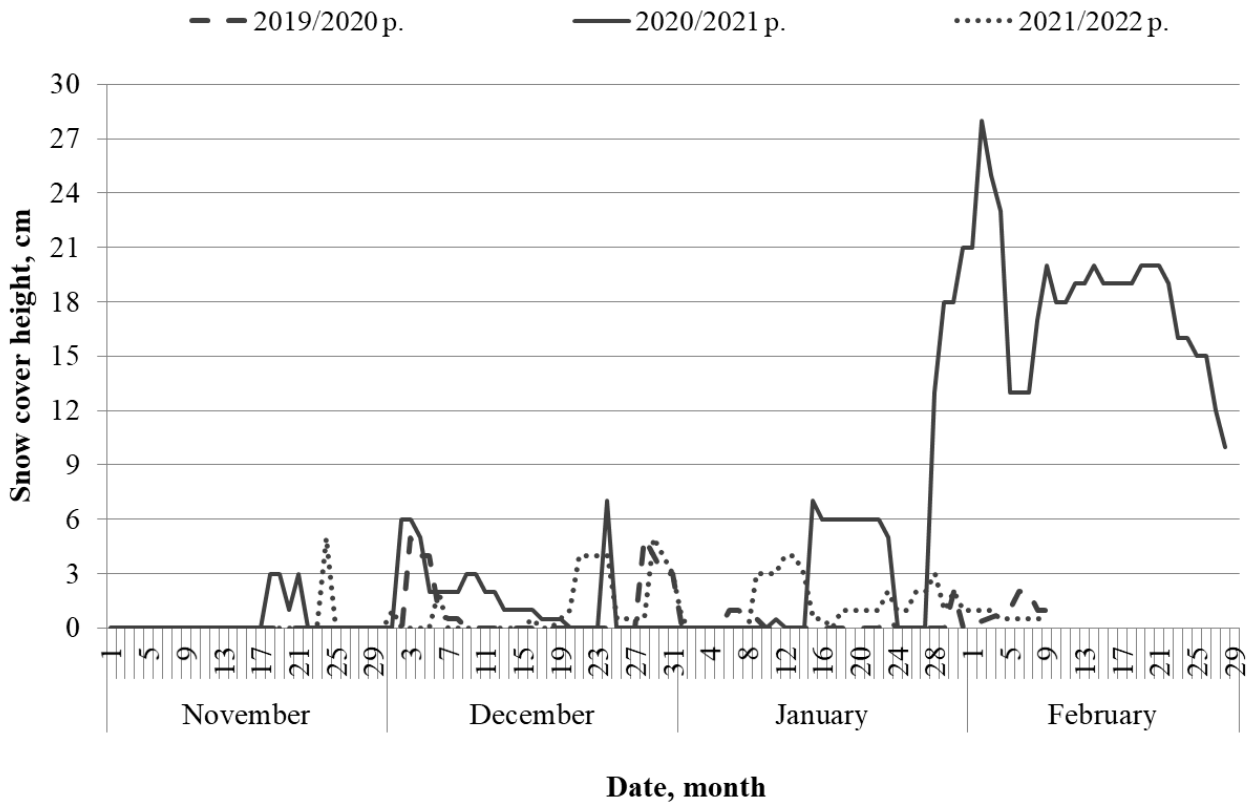


Fig.2. Snow covers height during the autumn-winter period of 2019/20 – 2021/2022.

Overall, in terms of temperature, the winter of 2019/2020 was relatively mild, but it was characterized by the absence of snow cover and sharp temperature fluctuations. As a result, the plants did not undergo the necessary hardening phases for winter survival and did not accumulate a sufficient amount of sugars in their nodes of growth, which led to their complete or partial death. However, this provided breeders with the opportunity to select forms with high adaptive capability under natural selection conditions.

The cessation of plant vegetation in 2020 was observed on November 11, with an average daily temperature of 4.4°C, which was 45 days earlier [25] than in the previous year of 2019 (December 26), followed by a gradual decrease (Fig. 3): 3.2°C on November 12 and 2.7°C on November 13.

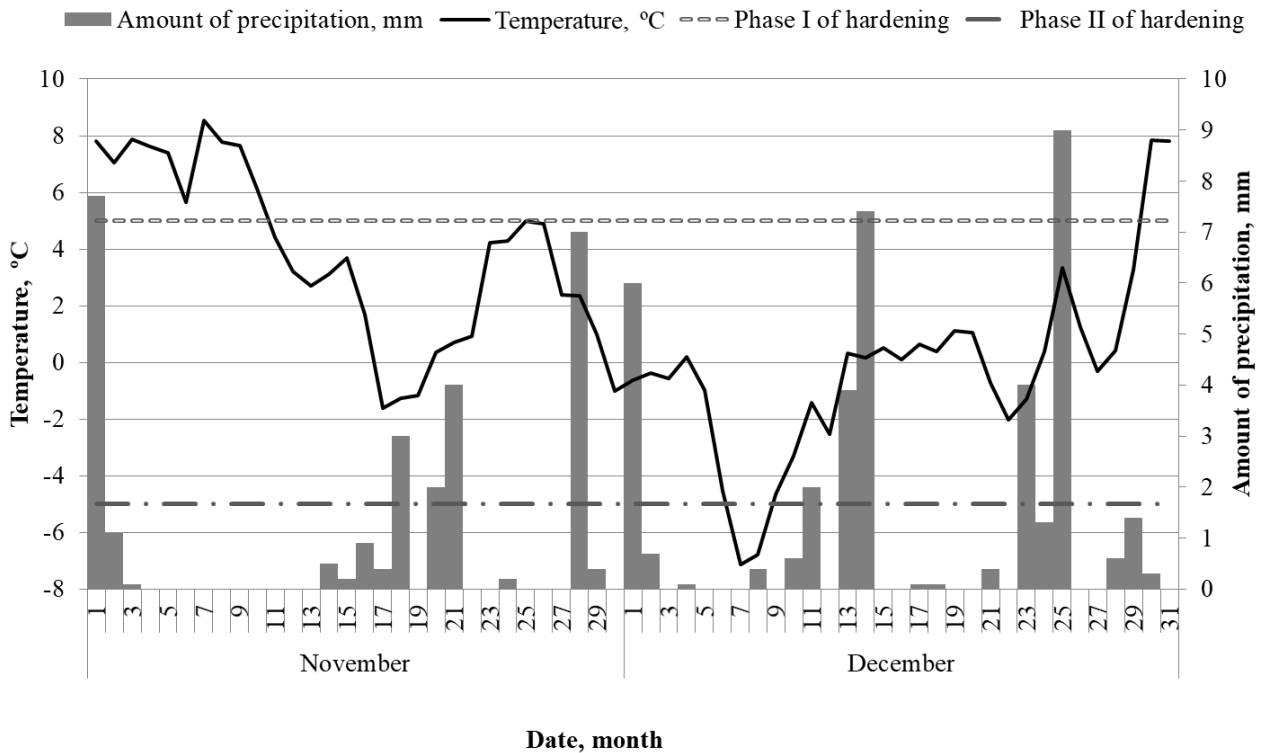


Fig.3. Hydrothermal conditions during the plant hardening period of 2020.

The lowest daily average temperature was recorded on January 17, 2021 (-17.6°C), with the temperature at the depth of the node of shrub freezing being -3.5°C [25]. The winter temperature in 2020/2021 was moderate, with only February having an average air temperature lower than the long-term average (-4.7°C). The average temperature in December and January exceeded these values by 2°C and 1.4°C, respectively. The minimum snow cover was established as early as December 1, and its maximum height during the winter period reached 28 cm (Fig. 2).

In 2021, the cessation of vegetation in winter crops was observed on November 9 when the average daily air temperature was +4.1°C, followed by a gradual decrease to -0.8°C on November 16 and -2.5°C on November 17. On November 20, due to the changing weather conditions, a reactivation of winter crop vegetation occurred, and on November 23, the final cessation of vegetation took place, which was 12 days later [25] compared to the previous year (November 11) (Fig. 4).

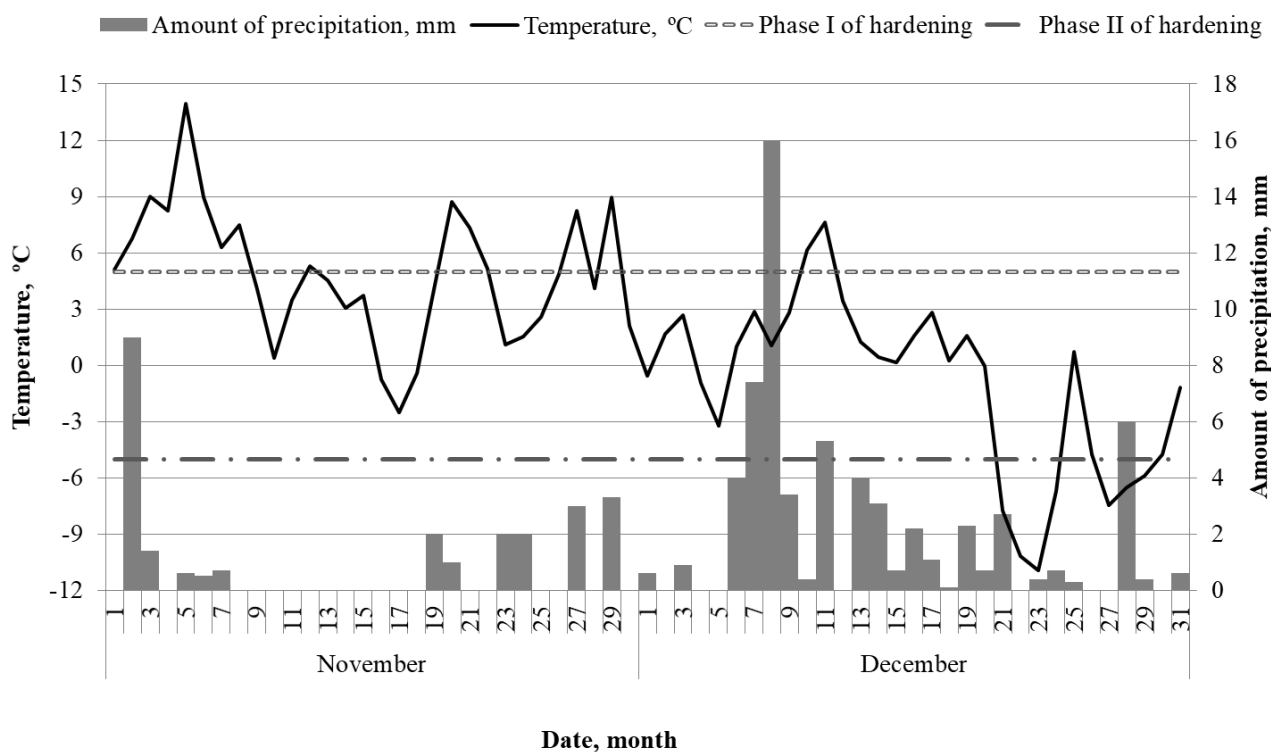


Fig.4. Hydrothermal conditions during the plant hardening period of 2021.

Elevated temperature conditions were observed in January and February of 2022, with temperatures being -1.2 and 1.7 °C, respectively, compared to the 30-year historical average for these months of -3.5 and -2.4 °C [25]. The maximum recorded temperature during the winter period was 9.6 °C on January 5, 2022, and 9.9 °C on February 21, 2022.

The winter of 2021/2022 was characterized by moderate air temperatures. In January and February, the average monthly temperatures were 2.3 and 4.1 °C higher, respectively, compared to the long-term multi-year averages. The lowest daily average temperature was recorded on January 12, 2022, at -13.0 °C, with the temperature at the depth of the node of tillering being -4.5 °C. The maximum snow cover height ranged from 3 to 5 cm, and the soil frost depth during periods of low temperatures was 16 cm [25].

In summarizing the analysis of hydrotechnical conditions during plant hardening, the following brief characterization can be provided for each year:

2019 – unfavorable (a sharp drop in air temperature during the phase I of hardening without snow cover; 2020 – satisfactory (plants hardened at an air temperature corresponding to phase I with a sharp temperature drop, and a 1 cm snow cover at that time); 2021 – relatively favorable (gradual decrease in air temperature during both phases of plant hardening, and a presence of a snow cover up to 5 cm).

Under these contrasting plant hardening conditions that occurred during the years 2019–2021, significant variability in frost resistance was observed among different varieties and research years. For example, the frost resistance of the standard variety Myronivska 808 ranged from 56% to 94% (Table 1). In the 2019/2020 research year, the variety Avrora Myronivska exhibited a high level of frost resistance (78%), with the percentage of live plants exceeding that of the standard variety according to Fisher's criterion. Among the varieties that performed at the standard level (50–58% of live plants), we observed MIP Assol (63%), MIP Fortuna (58%), MIP Vidznaka (58%), Vezha Mironivska (58%), MIP Yuvileyna (54%), Estafeta Mironivska (52%) and MIP Nika (50%).

1. Frost resistance of soft winter wheat varieties after plant freezing in boxes at a temperature of $-18\text{ }^{\circ}\text{C}$, 2019 – 2022.

Variety	Number of live plants ($\% \pm S_p$) after freezing		
	2019/2020	2020/2021	2021/2022
Myronivska 808 – standard	56±5.6	74±4.9	94±2.7
Avrora myronivska	78±4.8**	75±5.1*	57±8.3
MIP Assol	63±5.3*	30±5.2	78±5.6
MIP Fortuna	58±5.7*	63±5.5*	95±2.4*
MIP Vidznaka	58±5.8*	93±3.4**	95±2.8*
Vezha myronivska	58±5.7*	79±4.5*	92±3.2*
MIP Yuvileina	54±5.7*	77±4.6*	95±2.5*
Estafeta myronivska	52±5.5*	82±4.3*	95±2.5*
MIP Nika	50±5.4*	73±5.0*	84±4.7*
MIP Feieriia	48±5.7	91±3.3**	97±2.1*
MIP Roksolana	47±5.7	84±4.1*	93±3.0*
Hratsiia myronivska	42±5.2	35±4.1	72±5.7
MIP Darunok	33±4.8	45±5.8	76±4.9
MIP Lada	29±4.6	52±5.5	58±7.6

Average	51.9	68.1	84.4
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*Note: *the frost resistance of the variety does not differ significantly from the frost resistance of the Myronivska 808 variety according to the Fisher criterion; **the frost resistance of the variety significantly exceeds the frost resistance of the Myronivska 808 variety according to the Fisher criterion.*

In the 2020/2021 research year, the following wheat varieties exhibited resistance levels higher than the standard variety: MIP Vidznaka (93%) and MIP Feieria (91%), while at the standard level were MIP Roksolana (84%), Estafeta Myronivska (82%), Vezha Myronivska (79%), MIP Yuvileina (77%), Avrora Myronivska (75%), MIP Nika (73%) and MIP Fortuna (63%). Based on the results of winter freezing in 2021/2022, the percentage of live plants in the MIP Fortuna, MIP Vidznaka, MIP Yuvileina, Estafeta Myronivska, Vezha Myronivska, MIP Nika, MIP Feieria and MIP Roksolana varieties was at the level of the standard Myronivska 808.

Thus, the evaluation for frost resistance has identified soft winter wheat varieties: MIP Fortuna, MIP Vidznaka, Vezha Myronivska, MIP Yuvileina, Estafeta Myronivska and MIP Nika, whose resistance levels remained high throughout all three years of the study.

Analyzing the frost resistance of the varieties over the years, the average percentage of live plants after winter freezing was 51.9% in 2019/2020, 68.1% in 2020/2021, and 84.4% in 2021/2022. Therefore, it can be considered that the hydrothermal conditions in 2021/2022 were favorable for increasing the frost resistance of the plants.

As a result of the conducted research, it was established that the influence of weather conditions on the frost resistance of soft winter wheat was quite significant. A strong inverse correlation ($r = -0.77$) was found between the percentage of live plants after winter freezing and the average monthly temperature during November to January for the years 2019-2022. This correlation can be described by the regression equation:

$$y = 5.5218 - 0.0611x,$$

where y represents the percentage of live plants after winter freezing,

and x represents the average monthly air temperature during the specified period.

Therefore, our research has shown that unfavorable conditions during the hardening phases lead to a decrease in the frost resistance of winter wheat plants. Similar results were obtained by D.V. Blyshchyk and colleagues [6] when studying the impact of changing weather conditions in the Odessa region on the formation of frost resistance in winter wheat plants in 2012 and 2013, which had contrasting durations of the first hardening phase. The results of these studies indicate significant genetic variability in the response to the duration of the first hardening phase. The authors concluded that a promising direction for stabilizing winter wheat grain production is the development of varieties that can efficiently utilize limited conditions favorable for winter preparation. Researchers also identified a trend toward increased fluctuations in dynamics and a reduction in the duration of favorable temperature regimes for the first hardening phase of winter wheat plants in southern Ukraine, which negatively impacts the formation of frost resistance.

Conclusions. The research conducted over three contrasting years in terms of weather conditions confirmed that the frost resistance of different wheat varieties depends on the conditions of their hardening. Under gradually decreasing air temperatures, plant hardening was significantly more effective than under rapid fluctuations. Overall, under favorable hardening conditions in 2021/2022, the percentage of live plants after winter freezing averaged 84.4% across the varieties, while under satisfactory conditions in 2020/2021, it was 68.1%, and under unfavorable conditions in 2019/2020, it was 51.9%. A strong inverse correlation ($r = -0.77$) was established between frost resistance and the temperature regime during the hardening period. Certain soft winter wheat varieties were identified as having a high level of resistance throughout all three years of the study, including MIP Fortuna, MIP Vidznaka, Vezha Myronivska, MIP Yuvileina, Estafeta Myronivska and MIP Nika.

References

1. Lukashchuk, L.Ia. (2012). Vplyv zminy klimatu na produktyvnist pshenytsi ozymoi zalezho vid strokiv sivby [The impact of climate change on the productivity of winter wheat depending on periods of sowing]. *Bulletin of Sumy National Agrarian University. Agronomy and Biology*, 9(24), 91–94. [In Ukrainian].
2. Kalenska, S.M., Chubko, O.P., & Fedchuk, V.F. (2005). Vykorystannia zemelnykh uhid na osnovi provadzhennia adaptyvnykh tekhnolohii vyroshchuvannia zernovykh kultur [The use of land on the basis of adaptive technologies for growing grain crops]. *Collection of scientific works of the National Scientific Center "Institute of Agriculture of NAAS", Special issue*. 180–190. [In Ukrainian].
3. Kochmarskyi, V.S., Kyrylenko, V.V., Basanets, H.S., Khomenko, S.O., Humeniuk, O.V., Marynka, S.M., & Kharchenko, A.V. (2010). Zmina klimatychnykh umov ta adaptivni vlastyvosti suchasnykh sortiv pshenytsi ozymoi v zoni diialnosti Myronivskoho instytutu pshenytsi [Changes in climatic conditions and adaptive properties of modern varieties of winter wheat around the V.M. Remeslo Myronivka Institute of Wheat]. *Factors in Experimental Evolution Of Organisms*, 8, 154–161. [In Ukrainian].
4. Polovyi, A.M., Bozhko, L.Iu., & Dronova, O.O. (2011). Analiz tendentsii zminy termichnykh pokaznykiv ahroklimatychnykh resursiv v Ukraini za period do 2030–2040 rr. [Trend analysis of thermal indicators of agroclimatic recourses in Ukraine for the period up to 2030 – 2040 ys.] *Ukrainian Hydrometeorological Journal*, 9, 90–99. [In Ukrainian].
5. Chaudhry, S., & Sidhu, G.P.S. (2022). Climate change regulated abiotic stress mechanisms in plants: A comprehensive review. *Plant Cell Reports*, 41(1), 1–31. <https://doi.org/10.1007/s00299-021-02759-5>
6. Blyshchuk, D.V., Polovyi, A.M., & Feoktistov, P.O. (2014). Formuvannia morozostiikosti roslynamy ozymoi pshenytsi pid vplyvom zminy klimatu na pivdni Ukrainy [Formation of frost resistance of winter wheat under the influence of climate change in the southern part of Ukraine]. *Culture of Peoples of Black*

Sea, 274, 211–215. [In Ukrainian].

7. Kyrylenko, V.V., Voloshchuk, S.I., Dubovyk, N.S., & Blyzniuk, B.V. (2016). Retrospektyvnyi analiz pohodnykh umov u zoni diialnosti Myronivskoho instytutu pshenytsi. [Retrospective analysis of weather conditions in environs of Myronivka Institute of Wheat]. *Myronivka Bulletin*, 2, 87–97.

<https://doi.org/10.21498/2518-7910.0.2016.119548>

8. Pirykh, A.V. (2018). Morozostiikist novykh sortiv pshenytsi miakoi ozymoi myronivskoi selektsii [Frost resistance of new bread winter wheat varieties of Myronivka breeding]. *Myronivka Bulletin*, 7, 85–92.

<https://doi.org/10.31073/mvis201807-09>

9. Balabukh, V.O. Odnolietok, L.P., & Kryvoshein, O. (2017). Vplyv zminy klimatu na produktyvnist ozymoi pshenytsi v Ukraini u periody vechetatsiinoho tsykladu [Climate change impacts on the winter wheat productivity in Ukraine during vegetation cycle]. *Hydrology, Hydrochemistry and Hydroecology*, 3(46). 72–85. [In Ukrainian].

10. Samets, N.P., Hrytsevych, Yu.S., & Voronchak, M.V. (2021, May 27-28). Otsinka zminy klimatu na tryvalist periodiv vechetatsii ta spokoivu pshenytsi ozymoi [Assessment of climate change on the duration of growing and dormant periods of winter wheat]. In *Dopovidi uchasnykiv mizhnarodnoi naukovo-praktychnoi konferentsii Mizhnarodnogo forumu. Stratehiia intehtratsii ahrarnoi osvity, nauky, vyrobnytstva: hlobalni vyklyky prodovolchoi bezpeky ta zmin klimatu* [Reports of the participants of the International Scientific and Practical Conference of the International Forum. Strategy of integration of agricultural education, science, production: global challenges of food security and climate change]. (pp. 85–88). Mykolaiv, Ukraine. [In Ukrainian].

11. Kyrylenko, V.V., Humeniuk, O.V., Derhachov, O.L., Dubovyk, N.S., Blyzniuk, B.V., & Khomenko, S.O. (2015). Metody pidvyshchennia morozozymostiikosti pshenytsi miakoi ozymoi (*Triticum aestivum* L.) v umovakh Lisostepu Ukrainy [Methods to improve frost resistance of winter wheat (*Triticum aestivum* L.) in conditions of Forest-steppe of Ukraine]. *Factors in Experimental Evolution Of Organisms*, 16. 120–124. [In Ukrainian].

12. Feoktistov, P.O., Blyshchyk, D.V., & Nahuliak, O.I. (2013). Vplyv zmin pohodnykh umov na formuvannia morozostiikosti roslyn ozymoi pshenytsi v Odeskii oblasti [Influence of changes in weather conditions on the formation of frost resistance of winter wheat plants in Odesa region]. *Seed Production*, 6, 7–9. [In Ukrainian].

13. Lytvynenko M.A., & Lyfenko S.P. (2004). Vplyv strokiv sivyby i subletalnykh zymovykh temperatur na vyzhyvanist ta vrozhainist ozymoi pshenytsi [Influence of sowing dates and sublethal winter temperatures on survival and yield of winter wheat]. *Bulletin of Agricultural Science*, 5, 27–31. [In Ukrainian].

14. Olimpiieva, O.K. (2020). Mekhanizmy morozostiikosti roslyn [Mechanisms of frost resistance of plants]. *Student Scientific Bulletin [MNAU]. Agricultural Sciences*, 1(14), 190–197. [In Ukrainian].

15. Awasthi, R., Bhandari, K., & Nayyar, H. (2015). Temperature stress and redox homeostasis in agricultural crops. *Frontiers in Environmental Science*, 3(11), 1–24. <https://doi.org/10.3389/fenvs.2015.00011>

16. Kolupaev, Yu.E., Karpets, Yu.V., & Kabashnikova, L.F. (2019). Antioxidative system of plants: cellular compartmentation, protective and signaling functions, mechanisms of regulation (Review). *Applied Biochemistry and Microbiology*, 55, 441–459. <https://doi.org/10.1134/S0003683819050089>

17. Kolupaev, Y.E., Horielova, E.I., Yastreb, T.O., Ryabchun, N. I., & Reznik, A.M. (2020). Nitrogen oxide donor enhances cold-induced changes in antioxidant and osmoprotective systems of cereals. *Applied Biochemistry and Microbiology*, 56, 219–225. <https://doi.org/10.1134/S000368382002009X>

18. Morgun, V.V. & Mayor, P.S. (2009). Winter and frost resistance of winter cereal crops. In V.V. Morgun (Ed.). *Fiziolohiia roslyn: Problemy ta perspektyvy rozvytku* [Plant physiology: problems and prospects of development] (2, pp. 105–165). Kyiv: Logos. [In Ukrainian].

19. Maior, P.S., Kozina, H.Ia., & Slyvka, L.V. (2010). Vmist rozchynnykh tsukriv u roslynakh ozymoi pshenytsi protiahom osinno-zymovoho periodu [Soluble sugar content in winter wheat plants during autumn-winter period].

Physiology and Biochemistry of Cultivated Plants, 42(2), 174–182. [In Ukrainian]

20. Maior, P.S. (2010). Vzaiemozviazok mizh vmistom vilnoho prolinu, rozchynnykh tsukriv ta obvodnenistiu tkanyn u roslynakh ozymoï pshenytsi protiahom osinno-zymovoho periodu [Interrelation between free proline, soluble sugars and dry matter contents in plants of winter wheat during fall-winter period]. *Physiology and Biochemistry of Cultivated Plants*, 42(4), 298–305. [In Ukrainian]

21. Ghosh, U. K., Islam, M. N., Siddiqui, M. N., Cao, X., & Khan, M. A. R. (2022). Proline, a multifaceted signalling molecule in plant responses to abiotic stress: understanding the physiological mechanisms. *Plant Biology*, 24(2), 227–239. <https://doi.org/10.1111/plb.13363>

22. Hosseinifard, M., Stefaniak, S., Ghorbani Javid, M., Soltani, E., Wojtyla, Ł., & Garnczarska, M. (2022). Contribution of exogenous proline to abiotic stresses tolerance in plants: A review. *International Journal of Molecular Sciences*, 23(9), 5186. <https://doi.org/10.3390/ijms23095186>

23. Pshenytsia ozyma. Metody vyznachannia morozostiikosti sortiv: DSTU 4749:2007 [Winter Wheat. Method for Frost Resistance Determination of Varieties: State Standard 4749:2007]. (2008). Kyiv: Derzhspozhyvstandart Ukrainy. [in Ukrainian]

24. Chaddock, R.E. (1925). Principles and methods of statistics. Boston: Houghton Mifflin Company.

25. Pykalo, S.V., Demydov, O.A., Yurchenko, T.V., & Kharchenko, M.V. (2023). Osoblyvosti pohodnykh umov v tseñtralnomu Lisostepu Ukrainy vprodovzh 2019–2022 rokiv [Weather Conditions in the Central Forest-Steppe of Ukraine during 2019-2022]. *Ecological Sciences*, 3(48), 78–85. [in Ukrainian]. <https://doi.org/10.32846/2306-9716/2023.eco.3-48.12>