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RELATIONSHIP BETWEEN FROST RESISTANCE OF ETIOLATED SEEDLINGS AND GREEN WHEAT PLANTS (*Triticum aestivum*) IN THE TILLERING PHASE AND THE POSSIBILITIES OF ITS USE IN GENOTYPE EVALUATION

SUMMARY

Frost tolerance assessment of plants at the tillering stage is considered the most reliable method for evaluating winter wheat cultivars and lines as well as other winter cereals. However, this approach is labor-intensive, energy-consuming, and season-dependent, which significantly complicates its application for high-throughput screening of breeding material. This study aimed to explore the potential of using etiolated seedlings as a model for evaluating the frost tolerance of wheat genotypes. Nine winter wheat cultivars of diverse eco-geographical origins were assessed for frost tolerance at the tillering stage (the standard method) and using etiolated seedlings. In experiments conducted in 2022–2024, adult plants were outdoor-grown in soil-filled containers and underwent natural cold hardening in the autumn. Following hardening, the content of soluble carbohydrates in tillering nodes was determined, and the plants were frozen in climate chambers with temperatures gradually decreasing to –16°C or –18°C. Concurrently, the frost tolerance of etiolated seedlings of these wheat cultivars was investigated. Three-day-old seedlings were hardened in a thermostat at 3°C for 6 days. Subsequently, one group of seedlings was used to determine soluble carbohydrate content, while the other was frozen at –9°C or –12°C. There was a strong correlation between the survival of plants at the tillering stage and that of etiolated seedlings across all specified freezing temperatures (r=0.76 … 0.83). Furthermore, there was a significant correlation between post-freezing survival and post-hardening content of soluble carbohydrates both in tillering nodes (r=0.95 … 0.99) and in etiolated seedlings (r=0.77 … 0.79). It is

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concluded that etiolated seedlings can serve as a model for a preliminary assessment of the frost resistance of winter wheat genotypes.

Keywords: *Triticum aestivum*, etiolated seedlings, tillering phase, frost tolerance, cold hardening, soluble carbohydrates

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most essential cereal crops, accounting for 30% of global grain production (Hossan *et al.*, 2021). By 2050, the demand for wheat is projected to increase by approximately 60%, driven by global population growth. However, the production growth rate of this crop has slowed in recent decades, primarily due to constraints imposed by climatic factors (Ahad *et al.*, 2023; Nazarenko and Okselenko, 2026). Most wheat-growing regions worldwide frequently experience stress caused by low temperatures (Hassan *et al.*, 2021; Soleimani *et al.*, 2022). Cold stress can not only kill a portion of the plant population but also reduce the number of productive tillers, kernel number per spike, and final yield of the surviving plants (Thakur *et al*., 2010; Hassan *et al*., 2021; Ejaz *et al.*, 2023).

Milder winters in temperate latitudes do not diminish the relevance of frost tolerance in winter wheat. Furthermore, given the decreasing predictability of extreme cold events, research into the frost tolerance of different cultivars and lines remains crucial for both breeding and risk assessment in the crop production (Wu *et al.*, 2024; Li *et al*., 2025).

Frost tolerance is a core component of winter hardiness—the ability of winter plants to withstand a complex of unfavorable winter factors (Prášil *et al.*, 2025). Due to climate change, which has led to irregular occurrences or the complete absence of winter plant damage under natural conditions, winter field evaluations of winter wheat for frost tolerance, as well as phenotypic selection, are becoming ineffective (Jaškūnė *et al.*, 2022). This necessitates the use of specialized methods for genotype screening for frost tolerance (Gusta, Wisniewski, 2013).

Direct freezing of plants that have undergone cold hardening required for the development of frost tolerance is considered a reliable assessment of this trait (Zheng *et al*., 2018; Cheng *et al*., 2025; Musinov *et al*., 2025; Chipilski *et al.*, 2026). Such an effect can be achieved in the field or by growing plants in soil-filled containers on outdoor plots, provided the autumn temperatures are sufficiently low (typically below 5–10°C) for several weeks (Janmohammadi and Mahfo, 2012; Vaitkevičiūtė *et al*., 2022). However, freezing tillered plants in containers requires specialized large-capacity freezers and generally involves significant material, energy, and time costs.

Consequently, alternative approaches are employed to evaluate the frost tolerance of wheat cultivars. Some of these are based not on the assessment of post-freezing plant survival but on the determination of other physiological indicators associated with frost tolerance. For instance, a strong correlation was found between leaf growth parameters during the autumn period and frost

tolerance, allowing the use of growth indicators for screening wheat cultivars for tolerance to sub-zero temperatures (Jaškūnė *et al.*, 2022). In some studies, the quantum efficiency of photosystem II (the ratio of variable to maximum fluorescence—Fv/Fm) is used as an indicator of frost tolerance (Armonienė et al., 2013; Wu *et al.*, 2024). Specifically, it was demonstrated that in tolerant wheat genotypes, unlike susceptible ones, the Fv/Fm ratio does not significantly decrease after exposure to sub-zero temperatures under natural conditions (Wu *et al.*, 2024).

To faster screen wheat genotypes for frost tolerance, models such as etiolated cereal seedlings may be of significant interest. Substantial differences in the frost tolerance of seedlings were reported for different cereal species: *Secale cereale, Triticum aestivum, T. durum,* and *Hordeum vulgare* (Kolupaev *et al.*, 2015). There are data on relationships between soluble carbohydrate content and the frost tolerance of etiolated seedlings of different cold-hardened wheat cultivars (Yastreb *et al.*, 2023). It was also shown that etiolated seedlings of frost-tolerant wheat cultivars, unlike susceptible ones, are able to synthesize considerable amounts of low-molecular-weight protective proteins—15 kDa dehydrins (Yastreb *et al.*, 2026).

Thus, there are grounds for utilizing etiolated seedlings as a model for screening wheat genotypes for frost tolerance. However, until now, no comparative studies have been conducted on a sufficient number of wheat cultivars differing in frost tolerance, with evaluation of this trait in both tillered plants under near-natural conditions and etiolated seedlings subjected to cold hardening under controlled laboratory (growth chamber) conditions. Such a comparison between nine cultivars of diverse eco-geographical origins was the primary objective of this study. The article also presents methodological protocols for frost tolerance screening of wheat plants under conditions close to natural—which serve as the state standard (DSTU 4749:2007) for assessing this trait in Ukraine—and an express-method proposed by the authors using etiolated seedlings as a model.

MATERIAL AND METHODS

Nine winter bread wheat cultivars were studied (Table 1). The seeds of all studied cultivars were kindly provided by the National Center for Plant Genetic Resources of Ukraine (Kharkiv, Ukraine). According to data from the Ukrainian Institute for Plant Variety Examination and scientific literature, ‘Albidum 114’, ‘Antonivka’ and ‘Doskonala’ are classified as highly frost-tolerant cultivars, while ‘Nordika’ and ‘Zelenyi Hai’ exhibit above-moderate frost tolerance. Moderate and below-moderate frost tolerance is intrinsic to ‘Etana’ and ‘Tonnage’. Conversely, ‘Malibu’ and ‘Tobak’ exhibit low frost tolerance. It should be noted that most of the cultivars selected for the study are currently grown in Ukraine or other European countries. The exception was ‘Albidum 114’, which was excluded from the catalogs of recommended cultivars. Its inclusion in our study was motivated by the availability of data demonstrating its

very high frost tolerance. This cultivar has long been used as a reference for frost tolerance assessments (Riabchun *et al*., 2008).

Table 1. Data on the wheat cultivars used in the study

Cultivar	Country	Frost tolerance	Source
Albidum 114	Russia	very high	https://doi.org/10.1078/0176-1617-00642
Antonivka	Ukraine	high	http://sort.sops.gov.ua/cultivar/view/12198
Doskonala	Ukraine	high	http://sort.sops.gov.ua/cultivar/view/12253
Nordika	Czech Republic	above average	http://sort.sops.gov.ua/cultivar/view/4802
Zelenyi Hai	Ukraine	above average	http://sort.sops.gov.ua/cultivar/view/4798
Etana	Germany	medium	http://sort.sops.gov.ua/cultivar/view/4796
Tobak	Germany	low	http://sort.sops.gov.ua/cultivar/view/3599
Tonnage	Denmark	below average	http://sort.sops.gov.ua/cultivar/view/14669
Malibu	Germany	low	http://sort.sops.gov.ua/cultivar/view/3186

Plant hardening at the tillering stage and assessment of frost tolerance

The plant hardening experiments under near-natural conditions followed by controlled freezing of the hardened plants in climate chambers were carried out at a vegetation plot of the Laboratory of Plant Physiology and Biochemistry of the Yuriev Plant Production Institute of the National Academy of Agrarian Sciences of Ukraine (Kharkiv) in 2022-2024. The method is based on exposing plant samples to freezing temperatures that cause varying mortality rates depending on the cultivar's frost resistance.

The soil mixture was prepared from a fertile soil layer (0–30 cm) and washed river sand in a 3:1 ratio. The vegetation containers were placed on a hard-surfaced vegetation plot at a slight incline (1–2°) to allow for the drainage of rainwater and meltwater, and then filled with the soil mixture.

Sowing was performed in the second half of September. The sown seeds were covered with a 4 cm layer of the soil mixture and lightly compacted. After sowing, the containers were watered. Throughout the autumn and early winter, the plants remained on the vegetation plot under natural conditions, where they underwent cold hardening.

Plant care during this period consisted of regular watering and fertilization with complex NPK fertilizer (1.5 g per 10 kg of soil mixture). After cold hardening (exposure to natural temperatures of 0–5℃) and light frosts (down to –4℃), by January 10, the containers with plants were placed in climate chambers (Danfoss Optima, Netherlands). The temperature was reduced at a rate of 2℃ per hour until reaching the target temperatures of –16℃ or –18℃. The plants were exposed at the target temperatures for 24 hours. Subsequently, the temperature in the chambers was gradually increased at a rate of 2℃ per hour to 2–4℃. After thawing, damaged plants were trimmed, leaving leaf blades 0.5–2.0 cm in length and transferred to a greenhouse with a temperature range of 18–24℃ and an illuminance of at least 12 klx (12 h photoperiod). The leaf pruning procedure allows for a more accurate determination of survival based on growth capacity (maintaining the viability of meristematic cells in tillering nodes). The plants

were watered as needed. The survival rate was recorded 12–14 days after the regrowth start. Plants were considered viable if they remained turgid and green, with a leaf growth of at least 5 cm during the specified period (Riabchun *et al.*, 2008).

The content of soluble carbohydrates in the tillering nodes was determined at the end of the cold hardening period (January 1-15) in 2022-2024.

Hardening of etiolated seedlings and assessment of their frost tolerance

The seeds were disinfected for 15 minutes in a 5% NaClO solution, then rinsed eight rinses with sterile distilled water. Approximately 80 uniform caryopses were placed in Petri dishes on double sterile filter papers moistened with 8 mL of sterile distilled water. The seeds were germinated in a thermostat at 24℃ for 3 days. For cold hardening, the three-day-old seedlings were transferred to a cooling chamber at 3℃ for 6 days. The length of exposure to low positive temperatures required for the maximum development of frost tolerance in etiolated seedlings was established in our previous experiments (Yastreb *et al.*, 2023). After cold hardening, one group of seedlings was used to determine the content of soluble carbohydrates, while the other was used to evaluate the frost tolerance of the cultivars. The hardened seedlings were frozen in a dark chamber at –9℃ or –12℃ for 5 hours; to reach these target temperatures, the chamber temperature was reduced at a rate of 1℃/h. Following the freezing period, the samples were thawed in the chamber by gradually increasing the temperature to 5℃ (at a rate of 1℃/h). Subsequently, the seedlings were transferred to a chamber with a temperature of 24℃ and an illuminance of 12 klx, where they were grown for 3 days before their survival was assessed. Seedlings with green leaves that maintained their growth capacity were classified as viable.

Determination of soluble carbohydrate content

The concentration of total soluble carbohydrates in the plant tissues was determined by the Roe (1954) method, using an anthrone reagent, with modifications previously described (Kolupaev *et al.*, 2015). Soluble sugars were extracted from the plant tissues by heating the samples in distilled water within a boiling water bath for 10 minutes. For clarification of the resulting extracts, equal volumes (0.3 mL) of 30% zinc sulfate and 15% potassium hexacyanoferrate (II) trihydrate were added to each reaction tube. Following filtration through paper filters, the samples were diluted with distilled water as necessary. The assay mixture consisted of 3 mL of anthrone reagent and 1 mL of the filtrate; for the control, the filtrate was replaced with distilled water. After boiling the mixture in a water bath for 7 minutes, the samples were cooled, and the absorbance was measured at 610 nm against the control. D-glucose served as the external standard for the calibration curve.

Soluble carbohydrates content was expressed in mg per gram of dry weight (mg/DW). To determine the dry mass percentage, samples of tillering nodes (1 g) and seedling shoots (0.5 g) were initially dried in an oven at 103℃ for 40 min, followed by several-hour drying to a constant weight at 80℃.

Statistical analysis

All experiments were performed in triplicate. To assess plant survival, each replicate included at least 80 seedlings or established plants. For sugar content determination, each replicate used a pooled sample of at least 12 seedling shoots or tillering nodes of established plants.

The Shapiro–Wilk test was applied to evaluate whether the data followed a normal distribution. The statistical significance of differences between the studied parameters across various treatments was assessed using a two factor analysis of variance (ANOVA) followed by a Tukey's test for multiple comparisons. Data presented in the figures represent mean values from three biological replicates ± standard error of the mean. Statistically significant differences ($p \leq 0.05$) are denoted by different letters. Pearson's correlation coefficients (r) and their associated significance levels were calculated using Microsoft Excel.

RESULTS AND DISCUSSION

Frost tolerance of winter wheat plants at the tillering stage after hardening under natural conditions

Following exposure to –16°C, the studied cultivars showed differentiation in their frost tolerance (Figure 1). The highest survival percentage was recorded for 'Albidum 114', followed by slightly lower values for 'Antonivka', 'Doskonala' and 'Nordika'. However, it was not possible to detect statistically significant differences between the frost tolerance of the first three cultivars. Next came the group consisting of 'Zelenyi Hai,' 'Etana,' and 'Tobak,' among which no statistically significant differences in survival after exposure to -16°C were observed. 'Tonnage' exhibited somewhat lower tolerance, while 'Malibu' showed very low frost tolerance (Figure 1).

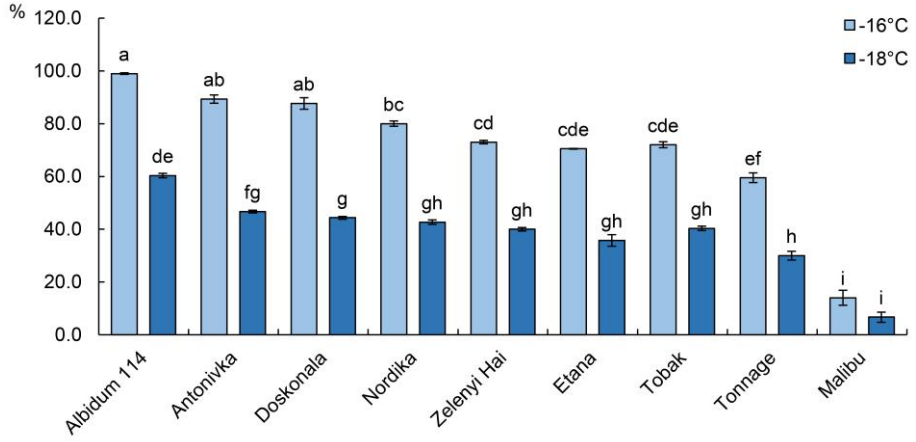

Figure 1. Plant survival (%) at the tillering stage following exposure to freezing temperatures (mean for 2022–2024). *Different letters denote significant differences at $p \leq 0.05$

The general pattern of inter-cultivar differences was maintained after freezing at –18°C. However, the differentiation between the group consisting of 'Antonivka', 'Doskonala', 'Nordika', 'Zelenyi Hai', 'Etana', and 'Tobak' was less pronounced than after the –16°C treatment. The survival rates for 'Tonnage' and, particularly, 'Malibu' were very low following exposure to –18°C (Figure 1).

Frost tolerance of hardened etiolated wheat seedlings

As previously established, etiolated wheat seedlings exhibit significantly lower frost tolerance compared to green plants at the tillering stage (Yastreb *et al.*, 2026). Consequently, to identify cultivar-specific differences, the seedlings were exposed to freezing temperatures of –9°C and –12°C. The ranking of cultivars based on seedling survival generally aligned with the results obtained for plants at the tillering stage (Figure 2). However, the relative positions of some moderately tolerant cultivars differed slightly. For instance, after freezing at –9°C, the survival rate of 'Nordika' was somewhat lower than that of 'Zelenyi Hai'—a trend not observed in the tillering stage assessment; nevertheless, these differences in relative survival were not statistically significant at $p \leq 0.05$ (Figure 2). The cultivar distribution following the –12°C exposure showed minimal variation compared to that after freezing at –9°C.

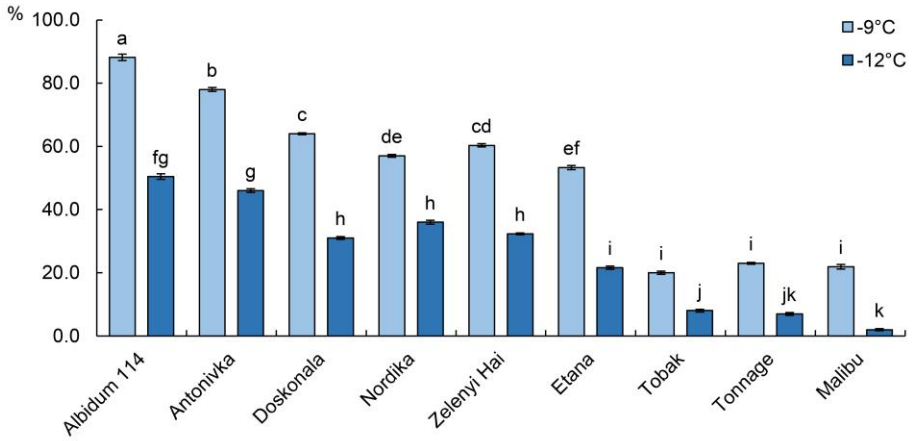

Figure 2. Survival of etiolated seedlings following exposure to freezing temperatures. *Different letters denote significant differences at $p \leq 0.05$

Soluble carbohydrate content in the tillering nodes and etiolated seedlings

At the end of the natural cold hardening period, the highest content of soluble carbohydrates was observed in the frost-tolerant group, namely 'Albidum 114', 'Antonivka' and 'Doskonala' (Figure 3). Slightly lower contents were recorded for 'Nordika', 'Etana', and 'Zelenyi Hai'. The carbohydrate content in the tillering nodes of 'Tobak' and 'Tonnage' was lower but comparable to the levels

found in the aforementioned three cultivars. Finally, in 'Malibu', the concentration of soluble carbohydrates after hardening was three times lower than that recorded for the frost-tolerant cultivars.

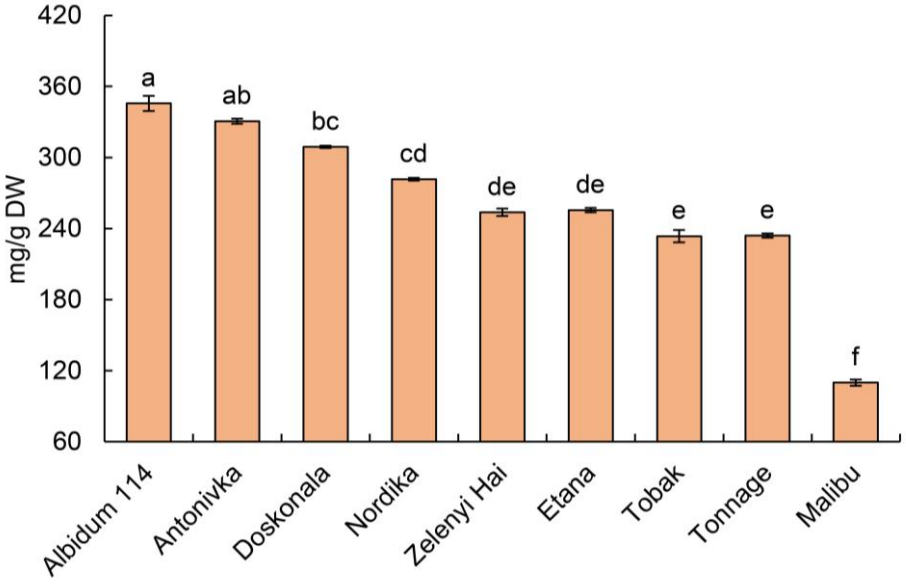

Figure 3. Soluble carbohydrate content (mg/g dry weight) in wheat tillering nodes after cold hardening under natural conditions (mean for 2022–2024). *Different letters denote significant differences at $p \leq 0.05$

In the experiments with etiolated seedlings, the concentration of soluble carbohydrates in the shoots was measured both before and after cold hardening. For non-hardened seedlings, there were no significant differences in carbohydrate content between most cultivars (Figure 4).

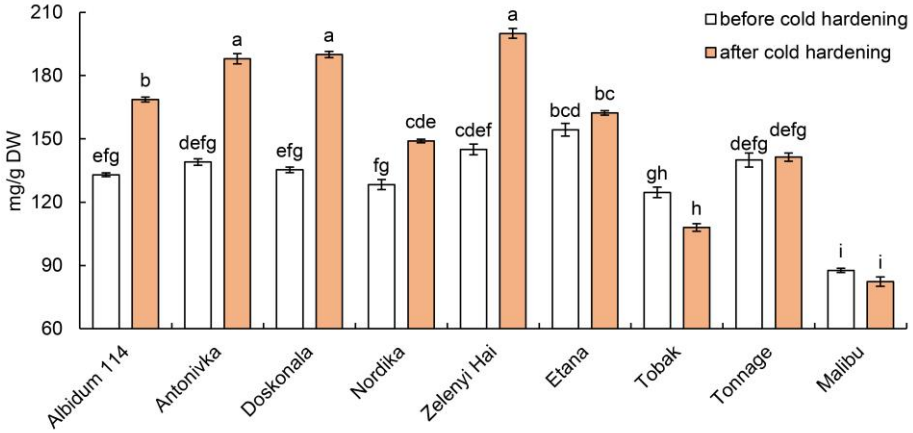

Figure 4. Soluble carbohydrate content in wheat seedling shoots (mg/g dry weight). Different letters denote significant differences at $p \leq 0.05$

Only 'Malibu' cultivar was distinguished by a very low constitutive level of soluble carbohydrates in its shoots. Cold hardening induced a significant increase in carbohydrate content in the shoots of 'Albidum 114', 'Antonivka', 'Doskonala', 'Zelenyi Hai', and 'Nordika'. Conversely, in the cultivars with lower frost tolerance ('Etana', 'Tobak', 'Tonnage', and 'Malibu'), no increase in soluble carbohydrate concentration was observed during the hardening period (Figure 4).

Correlation analysis

There was a very strong correlation (r=0.98, p≤0.001) between the survival rates of plants at the tillering stage after freezing at the two testing temperatures (–16°C and –18°C) (Figure 5). Similarly, there was a very strong correlation between the survival rates of seedlings after freezing at –9°C and –12°C (r=0.98, p ≤ 0.01). The correlation between the survival of plants at the tillering stage and etiolated seedlings after freezing at moderately low temperatures (–16°C for plants and –9°C for seedlings) was also relatively strong (r=0.76, p≤0.05) (Figure 5).

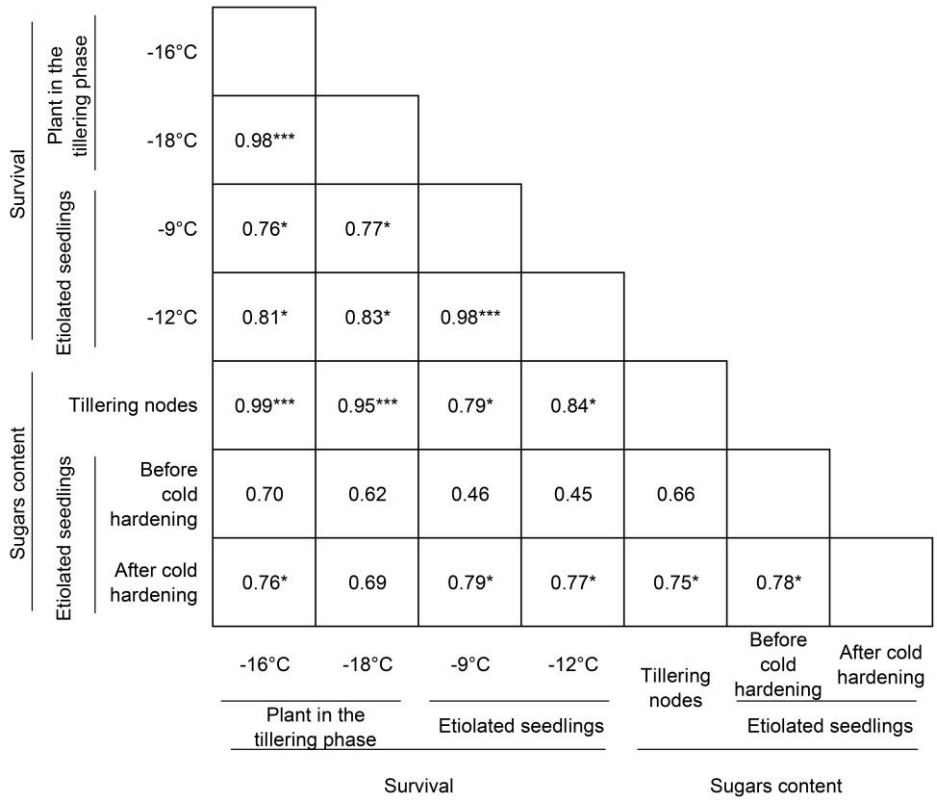

Figure 5. Correlations between the post-freezing survival of tillered plants and etiolated seedlings and soluble carbohydrate content in tillering nodes and seedling shoots. **Significant at p≤0.05, **Significant at p≤0.01, ***Significant at p≤0.001.*

When the data on tillered plants and seedlings for lower temperatures (–18°C and –12°C, respectively) were compared, the correlation between survival rates was even stronger ($r=0.83$, $p\leq0.05$). Overall, when calculating the correlations between the survival rates of plants at the tillering stage and etiolated seedlings across all freezing temperatures, we noted that the values remained significant at $p\leq0.05$ (Figure 5).

A very strong positive correlation was found between carbohydrate content in the tillering nodes at the end of the hardening period and plant survival after freezing ($r=0.99$ and $r=0.95$ for –16°C and –18°C, respectively) (Figure 5).

In contrast, no significant correlation was observed between soluble carbohydrate content in non-hardened etiolated seedlings and their survival after freezing at –9°C or –12°C (Figure 5). However, after the cold-induced increase in shoot carbohydrate levels in the frost-tolerant cultivars, the correlation between this parameter and seedling survival at both temperatures became significant at $p\leq0.05$ (Figure 5). Another noteworthy phenomenon is the significant correlation between sugar content in the tillering nodes and etiolated seedling shoots after low-temperature hardening ($r=0.75$, $p\leq0.05$) (Figure 5).

DISCUSSION

Overall, our results demonstrate that the proposed model (etiolated wheat seedlings after cold hardening) effectively represents the frost tolerance of wheat plants hardened at the tillering stage. As previously shown (Kolupaev *et al.*, 2015), non-hardened etiolated seedlings—those not exposed to low positive temperatures (0–5°C) for 5–7 days—exhibit extremely low frost tolerance and undergo nearly total mortality even under mild frost. In contrast, hardened seedlings are capable of surviving temperatures of –9°C and –12°C, at which a distinct differentiation of cultivars by their frost tolerance becomes evident (Figure 2). The low-temperature hardening of etiolated seedlings is associated with substantial activation of different components of their stress-protective systems. This includes shifts in antioxidant enzyme activities (Kolupaev *et al.*, 2015; Yastreb *et al.*, 2023), enhanced secondary metabolism evidenced by increased phenolic and anthocyanin contents (Kolupaev *et al.*, 2018), and accumulation of diverse osmolytes, specifically sugars and proline (Kolupaev *et al.*, 2015).

Among the osmolytes that are accumulated during cold adaptation, sugars play a pivotal role (Bilyavska *et al*., 2019; Fürtauer *et al*., 2019; Wang *et al.*, 2024). Sucrose and fructans are the dominant water-soluble compounds accumulated in wheat tillering nodes during cold hardening (Yoshida & Kavakami, 2013). Soluble carbohydrates are currently recognized as multifunctional protective agents in plants (Fürtauer *et al*., 2019). They serve as stabilizers for the structure of membrane protein-lipid complexes (Janska *et al.*, 2010; Deryabin, Trunova, 2021). It is well established that the phase transition temperature and fluid properties of biological membranes are highly dependent on sugar concentrations in the surrounding solution (Quinn, 1989; Fürtauer *et al.*,

2019). Another vital function of soluble carbohydrates under stress is the detoxification of reactive oxygen species (ROS). Currently, sugars are considered important components of the non-enzymatic antioxidant system (Gangola *et al.*, 2018). They can interact with ROS directly (e.g., by scavenging free radicals) or indirectly by stimulating the expression of genes encoding enzymes for syntheses of low-molecular antioxidants (Deryabin, Trunova, 2021).

In this study, there was a strong correlation for etiolated wheat seedlings of different cultivars between their post-hardening carbohydrate content and survival at two potentially lethal temperatures (r=0.79 and 0.77, respectively) (Figure 5). An even stronger correlation was observed between sugar content in the tillering nodes and survival of plants at the tillering stage. There was also a rather strong correlation between sugar levels in tillering nodes and etiolated seedling shoots (r=0.75) (Figure 5), further suggesting that the hardening mechanisms in tissues of tillering nodes and etiolated seedlings share common pathways. However, it should be noted that the relationship between soluble carbohydrate content in etiolated seedlings and their frost tolerance is not universal across all cereal species. For instance, in etiolated triticale seedlings, the sugar content in a susceptible cultivar ('Aleksandra') was found to be higher than that in frost-tolerant cultivars ('Rarytet' and 'Buket') (Kolupaev *et al.*, 2020). Consequently, cereal seedlings can be used as a model in studies of cold hardening processes and relationships between specific biochemical parameters and frost tolerance only with due account for species-specific characteristics. Nevertheless, the critical role of soluble carbohydrates in wheat adaptation to low temperatures has been further corroborated by molecular genetic studies. For example, it was demonstrated that a Chinese winter wheat cultivar, 'Dongnongdongmai 1', which is capable of overwintering at temperatures as low as –35°C (Bao *et al.*, 2021), exhibited differential expression of 1,715 genes involved in carbohydrate metabolism. These included genes regulating glycolysis/gluconeogenesis as well as starch and sucrose metabolism (Tian *et al.*, 2022).

Undoubtedly, wheat adaptation is not limited to changes in the metabolism of carbohydrates and other low-molecular compounds acting as osmoprotectants, antioxidants, and membrane stabilizers. It is well known that wheat and other cereals accumulate dehydrins in response to cold—proteins that exerting specific membrane-protective and antioxidant effects (Liu *et al*., 2017; Sun *et al.*, 2021). Notably, etiolated seedlings, similar to adult green plants, are capable of altering their protective protein profile during cold hardening. For instance, cold-induced accumulation of dehydrin-like proteins with molecular weights ranging from 41 to 209 kDa was demonstrated in etiolated seedlings of both winter and spring wheat (Borovskii *et al.*, 2002). Recently, we reported about a strong direct correlation between frost tolerance of wheat cultivars and hardening-induced accumulation of low-molecular dehydrins (~15 kDa) in etiolated seedlings (Yastreb *et al.*, 2026). This phenomenon further justifies the use of etiolated seedlings as a model for screening wheat cultivars and lines for frost tolerance as

well as for studying the specialized functions of stress-protective systems. A particular advantage of this model consists in a significant reduction in energy, labor, and time required for material assessments, especially when large samples of accessions are evaluated.

Nevertheless, the etiolated seedling model has certain limitations in studies of frost tolerance. Specifically, potentially lethal temperatures (causing death of considerable percentages of plants) for hardened seedlings are considerably higher than those for hardened tillered plants (Figures 1, 2). This indirectly suggests the presence of additional protective systems in tillered plants that are either absent or silent in etiolated seedlings. In our experiments, there were distinct differences in post-freezing survival rates between the susceptible cultivars at the tillering stage, whereas these differences were leveled off when using seedlings (Figures 1, 2). Thus, the use of seedlings does not completely replace the standard test, but can only serve as a preliminary screening method. Therefore, using seedlings as a model for screening wheat genotypes for frost tolerance is most expedient during the initial stages of evaluation of extensive amounts of breeding materials. Subsequently, once the selection is narrowed down, it is advisable to assess frost tolerance of tillered plants for those genotypes that exhibited the highest resilience in the seedling assays.

CONCLUSIONS

In this study, we compared the frost tolerance of nine winter bread wheat cultivars of diverse eco-geographical origins at the tillering stage after cold hardening under near-natural conditions with that of etiolated seedlings. We observed a strong correlation between survival rates of these two developmental stages. Furthermore, there was a significant correlation between frost tolerance and soluble carbohydrate accumulation during hardening both in tillered plants and etiolated seedlings. Thus, we can conclude that etiolated wheat seedlings can serve as a model for the primary (preliminary) selection of breeding material for frost tolerance.

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