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Variation in frost resistance of ten poplar clones tested in eastern Ukraine

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Abstract

Assessing frost tolerance of hybrid poplar clones in the temperate zone is a key factor to select genotypes used for plantation forestry to intensify wood production and to strengthen forests' adaptability to extreme weather conditions. This study aimed to evaluate frost resistance of ten poplar clones, aged 7 years, in eastern part of Ukraine. We analyzed frost resistance by comparing the average tissue damage indices of control plants with those exposed to frost temperatures of –25 °C and –30 °C. Cluster analysis further classified the clones into four distinct groups based on their frost resistance potential. Clones, which have been cultivated or intercrossed in Ukraine since the 1960s, are more resistant to frost due to the acclimatization of their parental individuals. Clones 'Gulliver' (*P. deltoides*), 'Lvivska' (*P.* × *euramericana* cv. 'Regenerata' × *P. trichocarpa*), and 'Nocturne' (*P. trichocarpa* × *P. lasiocarpa*) are characterized by the highest frost resistance in eastern Ukraine. Clones 'Strilopodibna' (*P.* × *euramericana* × *P. nigra* cv. 'Italica'), 'Perspektyvna' (*P.* × *euroamericana* cv. 'Regenerata' × *P. lasiocarpa*), 'Dorskamp' (*P.* × *euroamericana* cv. 'Dorskamp'), and 'Ghoy' (*P.* × *euroamericana* cv. 'Ghoy') were the most damaged by frost. The OLS regression model explained 64% of the variability in tissue damage, with clone and freezing temperature being the most influential factors ($R^2 = 0.640$, $F_{stat} = 17.49$, $p < 0.0001$). In conclusion, our study demonstrated the importance of testing cultivars under local climatic conditions before using them on a commercial scale.

Key words: *Populus* spp.; frost damage; frost hardiness; temperate zone; cluster analysis

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1. Introduction

Temperature is a limiting factor affecting forest trees' survival and growth in the temperate zone (Kreyling 2010). Trees in this zone are adapted to a range of temperatures that fluctuate between warm summers and cold winters (Vitasse et al. 2014; Harvey et al. 2020). Optimal temperatures for growth vary depending on the species, but generally, moderate temperatures in the spring and fall promote optimal growth, while extreme temperatures can be harmful (Shvidenko et al. 2018). As global temperatures continue to rise, many tree species may be at risk of increased frost damage due to changes in the timing and severity of winter frosts. Global climate change may result in changes in freeze-thaw cycles (Lindner 2010). Milder winters may reduce winter hardening in trees, increasing their vulnerability to frost (Hänninen 2006).

Many studies proved that poplars have economic, social, cultural, and environmental benefits to enhance the safety of food production and to ensure sustainable land management (Dickmann 2006; Gasol et al. 2009). Poplar trees are known for their fast growth and high productivity, making them an important commercial crop in many regions (Kutsokon et al. 2018; Niemczyk et al. 2018; Oliveira et al. 2020; Schiberna et al. 2021; Fürtner et al. 2022).

Nowadays, interest in poplars is increasing, as they not only form an important component of forestry and agriculture but also many of them are adapted to a wide range of climatic and soil conditions (Richardson et al. 2014; Fuchylo et al. 2016; Niemczyk, et al. 2019; Beule et al. 2020; Porro et al. 2021). Some species and hybrids are able in degraded, polluted, and waterlogged areas it is a priority for cultivation in conditions not suitable for other species (Zacchini et al. 2009; Polle & Chen 2015).

Poplar hybridization allows breeders to combine and mix desired traits from different species (Stettler et al. 1996; Hu & Thomas 2019). Hybrids between genetically diverse varieties demonstrate increased growth and overall biomass (Goff 2011). At the same time, hybrids may

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exhibit stress tolerance against various stressors (Abbruzzese et al. 2009; Harfouche et al. 2014).

In northern Europe, severe frost damages on introduced poplar clones were reported (Telenius 1999; Christersson 2006; Pliura et al. 2014). According to research conducted by Weber et al. in 1985, the bud set of genotypes that have been transferred to northern regions is generally delayed due to the longer periods of daylight. Conversely, genotypes originated from northern regions or higher elevations tend to set buds earlier in the autumn than trees from local populations.

In Ukraine, a large-scale experiment on breeding and hybridization of poplars was conducted in the late 1950s. Over 600 promising clones were obtained (Kutsokon et al. 2018). Currently, preservation of the poplar gene pool *ex-situ* is carried out in special nurseries (Balakliys'ky nursery, Kharkiv region; Lazirkivs'ky nursery, Poltava region) and collections (collection plantation of URIFFM, Kharkiv region; collection plantation of NULES of Ukraine, Kyiv region). A lot of poplar species and clones which were selected in Ukrainian and foreign breeding programmes have been tested in these field trials. Some of them have been cultivated in Ukraine for a long time, and new clones have been tested in local conditions for no more than 20 years (Fuchylo et al. 2016).

Hybrids of various poplar taxa such as *P. deltoids* W. Bartram ex Marshall, *P.* × *euramericana*, *P. trichocarpa* Torr. & A. Gray ex Hook., *P. laurifolia* Ledeb., *P. lasiocarpa* Oliv. have been selected as excellent materials for afforestation and plantations in Ukraine. Numerous studies have been conducted to evaluate hybrid poplars' characteristics, which have primarily focused on growth (Patlai & Rudenko 1990; Fuchylo et al. 2016) and biology (Krupei 1970; Kots 1972; Kutsokon et al. 2018). In the East and South of Ukraine, *Populus* hybrids appear specifically promising for use on degraded lands and those polluted (due to military actions) by toxic agents. However, extreme minimum temperatures and frequent freeze-thaw events can play an important role in the survival and long-term productivity of hybrid poplar plantations in eastern Ukraine.

The study of frost resistance of tree species is based on analyses of various physiological and biochemical indicators or by use of a laboratory freezing method. This method allows for determining the frost resistance of plants based on objective signs of damage caused by low temperatures in artificially controlled conditions and reproducing natural thermal regimes. The freezing method makes it possible to obtain a sufficient set of experimental data with the necessary repetition within one winter season, which provides a convenient opportunity to determine the biological limits of low temperatures at which plant survival is possible (Bublyk et al. 2013; Oksantyuk & Koldar 2021). However, there are no modern studies on the resistance of poplar hybrids in eastern Ukraine.
The study aims to assess the frost resistance of 10 hybrid poplar clones in the eastern part of Ukraine. We hypothesized that the clones introduced in the 1960s (cuttings from mature trees collected across Ukraine) are more resistant to frost damage due to their parent trees' appropriate adaptation to low temperatures.

2. Materials and methods

2.1. Study area

The experimental site is located on the territory of the State Enterprise "Kharkiv Forest Research Station", Kharkiv region, Ukraine (50°04'59" N, 36°18'25" W, 156 m a.s.l.). According to information obtained from the ClimateCharts.net web platform (Zepner et al. 2020), the average annual temperature of the research area for the period 2010–2019 amounted to +9 °C, the average annual rainfall was 528.8 mm. The average annual temperature for the period 2000–2009 amounted to +8.4 °C, average annual rainfall was 543.7 mm. According to the Köppen classification, climate is temperate continental (Dfb). According to the weather archive, the lowest temperature for the period 2010–2019 (–25.5 °C) was recorded in January 2014, and the highest (+38.4 °C) in July 2016. The average frost-free period in 1991–2010 was 163–209 days. The dates of the first frost in the air in the Kharkiv region were 30.09.–05.11. Frost end dates were 26.03.–07.05. (Oleksienko & Zatula 2011).

The planting material was 1-year-old rooted cutting used for the testing plot which was established in the spring period of 2014. In total 50 poplar clones have been tested on the plot, representing various taxa of *Aigeros*, *Tacamahaca*, and *Leucoides* sections, which were selected in Ukrainian and foreign breeding programmes. To evaluate frost resistance, we selected ten clones (Table 1). Clones 'Guliver', 'Druzhba', 'Lvivska', 'Novoberlinska', 'Nocturn', 'Roganska', 'Strilopodibna', 'Perspektivna' were selected and introduced in the 1960s. Clones 'Dorskamp' and 'Ghoy' have been introduced in the last 20 years.

Table 1. Information about ten selected poplar hybrid clones.

Clone	Parent trees	Section
'Gulliver'	*P. deltoides*	*Aigeiros*
'Druzhba'	*P. trichocarpa* × *P. laurifolia*	*Tacamahaca*
'Lvivska'	*P. euramericana* cv. 'regenerata' × *P. trichocarpa*	*Tacamahaca*
'Novoberlinska'	*P. pyramidalis* × *P. laurifolia*	*Aigeiros* and *Tacamahaca*
'Nocturne'	*P. trichocarpa* × *P. lasiocarpa*	*Tacamahaca* and *Leucoides*
'Rohanska'	*P. simonii* f. *fastigiata*	*Tacamahaca*
'Strilopodibna'	*P.* × *euramericana* × *P. pyramidalis*	*Tacamahaca*
'Perspektyvna'	*P.* × *euramericana* cv. 'regenerata' × *P. lasiocarpa*	*Tacamahaca* and *Leucoides*
'Dorskamp'	*P.* × *euramericana* var. Dorskamp	*Tacamahaca*
'Ghoy'	*P.* × *euramericana* var. Ghoy	*Tacamahaca*

2.2. Determination of frost resistance

The frost resistance of shoots was tested at the Laboratory of Plant Physiology and Microbiology of the Institute of Horticulture NAAS of Ukraine. Sample selection and anatomical-microscopic analysis were carried out according to the methodology of Bublyk et al. (2013). The dormant one-year old shoots of the 10 poplar clones (in size 30–40 cm) were sampled in 5 repetitions, in January 2021.

Freezing was conducted in a CRO/400/40 refrigerator at temperatures of –25 °C and –30 °C. The preparation for freezing involved a 4-hour pre-cooling process at –5 °C. During this phase, water was released from the cells into the intercellular space, leading to an increase in the concentration of cellular sap and a subsequent lowering of the freezing point of the cytoplasm. The temperature was gradually reduced at a rate of 5 °C per hour and maintained at the specified temperature for 4 hours. In the final stage of freezing, the temperature was gradually elevated to room temperature at a rate of +5 °C per hour to prevent potential damage to cell walls caused by the gradual transition of water from a solid to a liquid state. To observe the effects of freezing, the samples were stratified at room temperature for 7 days. Control samples remained unfrozen. Following stratification, anatomical microsections were obtained using a microtome, placed on a microscope slide, and covered with glycerine (Fig. 1).

The samples were scrutinized using the MBS-10 microscope with magnification in the range of 8-times. During microscopy, we estimated the degree of damage to the bark, cambium, xylem, and pith, as well as the tissues of the parenchymal mass under the apical bud and the buds in general. The degree of damage was assessed on a 6-point scale: 0 – no damage (0%); 1 – slight discoloration, damage to 20% of tissue; 2 – moderate tissue damage (40%); 3 – average damage (browning of tissue borders with others tissues (60%); 4 – severe damage, tissue completely browned, borders with other tissues black (80%); 5 – complete death, in some cases tissues cannot be separated from others (100%). Then, the total score of tissue and shoot damage was evaluated.

2.3. Data analysis

The normality of the data was assessed using the Shapiro-Wilk test, which showed that the data distribution was significantly different from normal ($p < 0.05$). Differences in frost tolerance between clones were evaluated with a Kruskal-Wallis test. Tukey's test was used for multiple comparisons between tree clones and tissue types under various temperature regimes. Total damage across all tissues under different temperature regimes was analyzed, with standard deviation included to represent data variability. Ordinary Least Squares (OLS) regression analysis produced an R-squared was used to determine the influence of tree variety and freezing temperature on the overall damage level. The model evaluated the impact of both factors on the damage index. A heatmap was used to visually represent the damage levels across tree varieties and tissue types, providing a clear comparison between varieties. Cluster analysis was used to group clones that had the most similar relative frost resistance into a hierarchical dendrogram (Sneath & Sokal 1973). An ordination diagram obtained using the fuzzy clustering method was used to verify the correctness of the cluster number determination. The analysis involved comparing the average tissue damage indices of control plants with those exposed to frost temperatures of –25 °C and –30 °C. All differences were considered significant at $P \leq 0.05$. For interactive computing and visualization, open-source Python packages were employed (Rossant 2018).

3. Results

During the dormancy and freezing period of experimental samples at temperatures of –25 °C and –30 °C, among the clones tested a significant variation in frost resistance was detected (*Kruskal-Wallis*, $\chi^2 = 30.11$, $P < 0.001$) (Fig. 2).

At a freezing temperature of –25 °C, the total damage score was the lowest in the clones 'Nocturne', 'Lvivska',

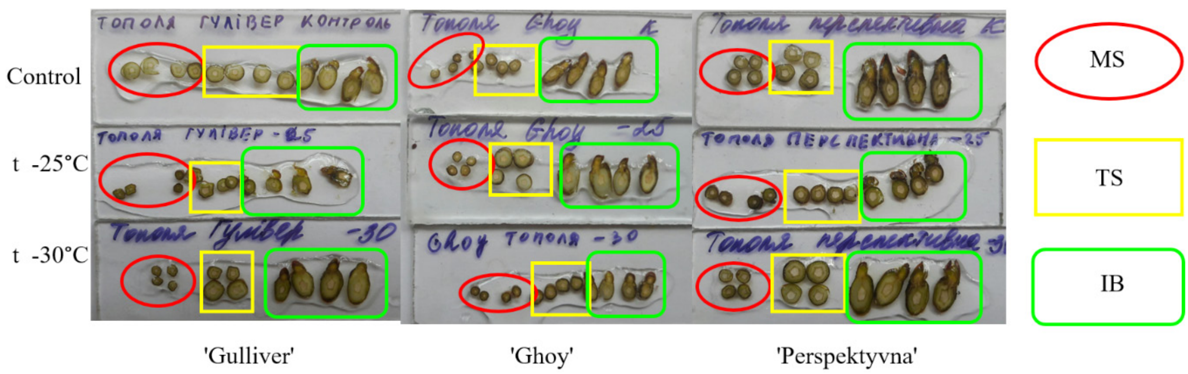

Fig. 1. The sample of anatomical microsections for determining the frost resistance of poplar clones by the direct freezing method: TS – top of the shoot; MS – middle of the shoot; IB – incision through the apical bud; t –25 °C – freezing temperature –25 °C; t –30 °C – freezing temperature –30 °C.

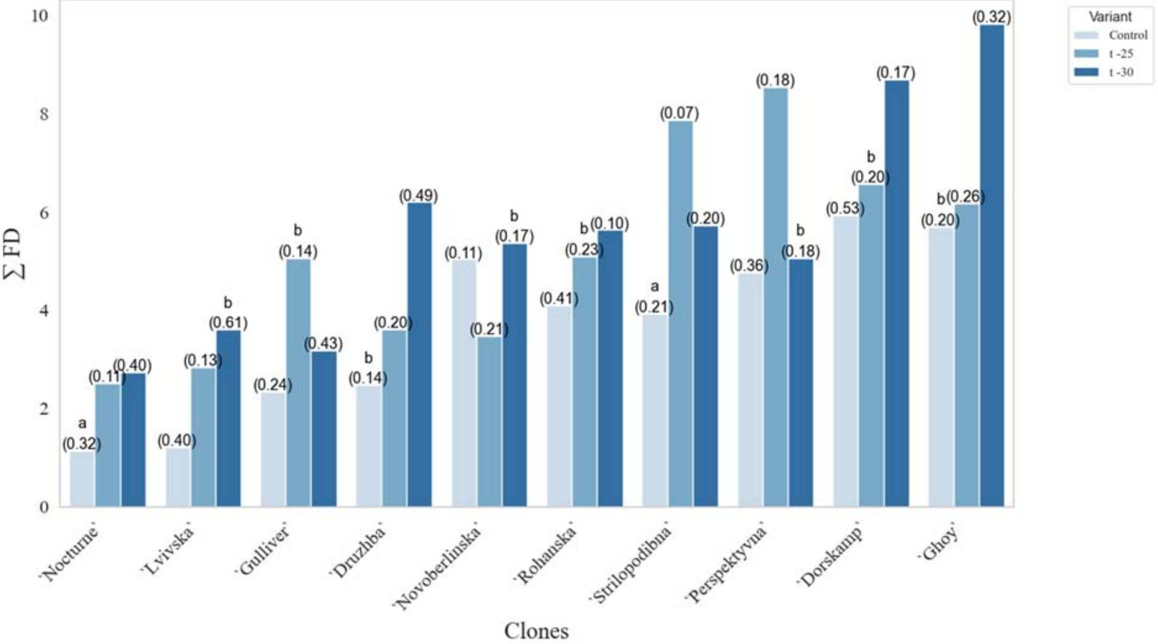

Fig. 2. The frost resistance of different poplar clones: ΣFD – total frost damage points; t –25 – freezing temperature –25 °C; t –30 – freezing temperature –30 °C. The results of multiple comparisons conducted using Tukey's test are indicated by letters. Clones marked with different letters exhibit statistically significant differences in damage levels ($p < 0.05$). The standard error of the mean is given in parentheses.

'Novoberlinska', and 'Druzhba' – 7.5–10.8. The highest damage index was observed in the clones 'Strilopodibna', 'Perspektyvna', 'Dorskamp', and 'Ghoy' – hybrids of *P.* × *euramericana* – 18.5.–25.6.

At a freezing temperature of –30 °C, the total damage index was the lowest in the clones 'Nocturne', 'Gulliver', and 'Lvivska' – from 8.2 to 10.8; and highest in the clones 'Dorskamp' and 'Ghoy' – 26.1 and 29.5, respectively.

Multifactor regression analysis OLS demonstrated that clone and freezing temperature significantly impact tissue damage levels ($p < 0.05$). The model explained 64% of the variance in the data ($R^2 = 0.640$), indicating a good fit. The model is statistically significant ($F_{stat} = 17.49$; $p < 0.0001$) (Table 2). The Durbin-Watson statistic of 1.60 suggests no significant autocorrelation in the residuals.

Clones 'Druzhba', 'Gulliver', 'Lvivska', 'Nocturne', 'Novoberlinska', 'Rohanska', and 'Strilopodibna' were all negatively associated with the dependent variable. Of these, 'Nocturne' had the largest negative coefficient (–1.24, $p < 0.001$), followed by 'Lvivska' (–1.13, $p < 0.001$). Clones with negative coefficients are expected to show lower levels of damage. Notably, the clones 'Ghoy' and 'Perspektyvna' did not have a strong influence on the dependent variable in this model ($p > 0.1$).

Table 2. Results of OLS Regression Analysis.

Dependent Variable	Mean	Dependent Variable	Mean
R-squared	0.640	Number of Observations	120
Adjusted R-squared	0.604	AIC	113.9
F-statistic	17.49	BIC	147.3
Prob (F-statistic)	2.17e-19	Df Model	11
Log-Likelihood	–44.937	Df Residuals	108

Variable	Coefficient	Std. Error	t-statistic	P-value	Confidence Interval
Constant	1.479	0.117	12.608	0.000	[1.246 – 1.711]
'Druzhba'	–0.74	0.151	–4.916	0.000	[–1.045 – –0.444]
'Ghoy'	0.04	0.151	0.275	0.784	[–0.258 – 0.342]
'Gulliver'	–0.89	0.151	–5.852	0.000	[–1.186 – –0.586]
'Lvivska'	–1.13	0.151	–7.466	0.000	[–1.431 – –0.830]
'Nocturne'	–1.24	0.151	–8.163	0.000	[–1.536 – –0.936]
'Novoberlinska'	–0.61	0.151	–4.036	0.000	[–0.911 – –0.311]
'Perspektyvna'	–0.24	0.151	–1.559	0.122	[–0.536 – 0.064]
'Rohanska'	–0.53	0.151	–3.504	0.001	[–0.831 – –0.230]
'Strilopodibna'	–0.31	0.151	–2.018	0.046	[–0.606 – –0.005]
t –25 °C	0.38	0.083	4.552	0.000	[0.213 – 0.542]
t –30 °C	0.49	0.083	5.858	0.000	[0.321 – 0.650]

These results suggest that different tree varieties and treatment conditions exert varying effects on the mean response variable. Further investigation is warranted to better understand the underlying factors driving these differences, especially in the case of varieties that showed non-significant results.

There was a linear correlation with the symptoms of frost damage along the length of the one-year-old shoot ($0.75 < R^2 < 0.97$). The least damaged tissues were observed in the lower part of the shoots, while the tips of the shoots were most affected by frost damage.

At a freezing temperature of –25 °C, the damage index of apical buds was the lowest in the case of clones 'Druzhba' and 'Gulliver' (1.2.–1.3.) and clones 'Nocturne', 'Strilopodibna' and 'Dorskamp' the highest (1.8.–2.9.), respectively ($P < 0.05$).

The analysis of bark, cambium, xylem, and pith provided insights into the range of damage (min and max values) as well as the mean damage level for each tissue type (Fig. 3).

For the clone 'Dorskamp', damage was most severe in the pith, with a mean damage level of 2.20, ranging from 1.90 to 2.50, while the bark had the lowest mean damage of 1.53. Similarly, 'Ghoy' showed high damage in both the bark (mean 2.10) and cambium (mean 2.13), while pith was less affected (mean 1.46).

The most resistant clone, 'Nocturne', had the lowest damage levels across all tissues from 0.10 to 0.90, the pith (mean 0.49) and xylem (mean 0.37). In contrast, 'Perspektyvna' showed high damage in the bark (mean 1.98) and cambium (mean 1.92), while pith exhibited lower damage (mean 0.99).

These results highlight significant variations in tissue vulnerability to freezing across different clones. Clones like 'Nocturne', 'Lvivska', and 'Gulliver' demonstrated greater resistance, particularly in the internal tissues (pith and xylem), 'Dorskamp', 'Ghoy', and 'Perspektyvna' were more susceptible, especially in the outer tissues (bark and cambium).

Tukey's test revealed significant differences between tree varieties for various tissue types and temperature regimes (Fig. 4). The most pronounced differences were observed between 'Ghoy', 'Lvivska', and 'Nocturne', particularly under the –30 °C freezing temperature.

This tissue-specific damage analysis provides important insights for selecting clones with greater tolerance to cold conditions, especially in terms of preserving core tissues, which are crucial for plant survival.

Cluster analysis based on the tissue damage index of shoots and buds at different temperatures revealed four clusters of potential frost resistance of poplar clones (Fig. 5). Cluster 1 includes mainly hybrids of

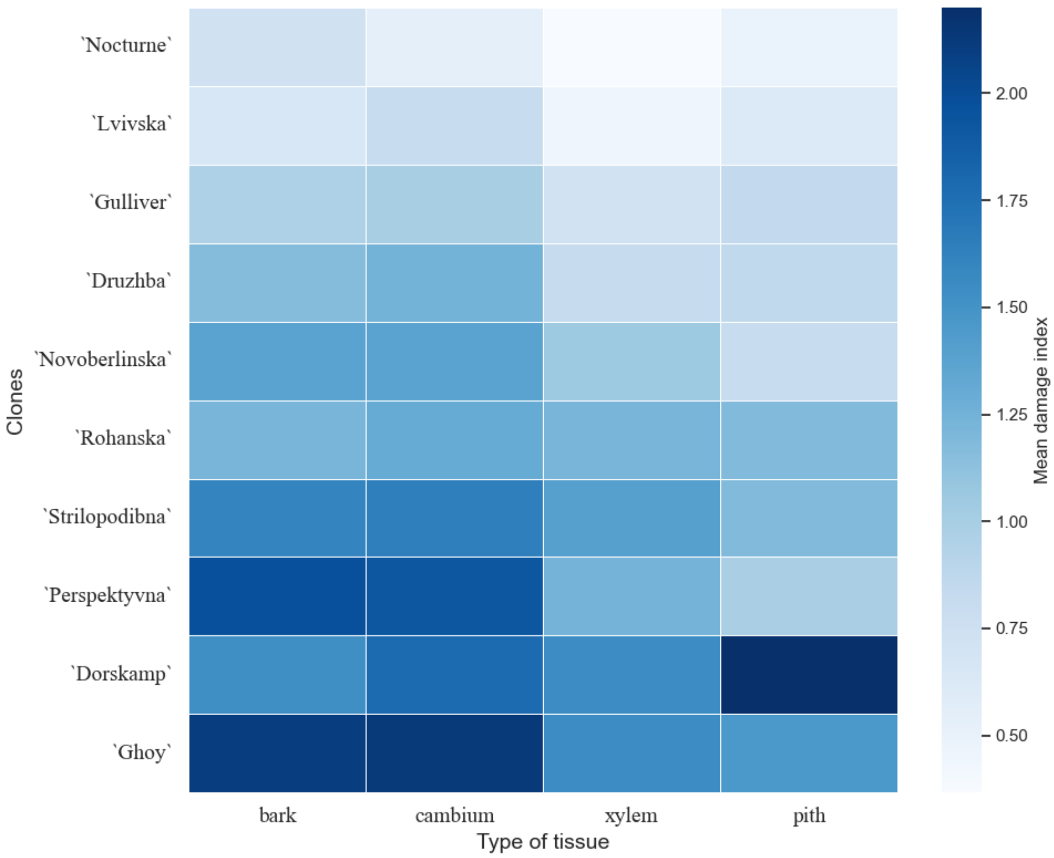

Fig. 3. Heatmap illustrating the range of tissue damage (bark, cambium, pith, and xylem) across different clones. The color intensity represents the mean damage index, with darker shades indicating higher levels of damage. Clones are arranged in ascending order based on the overall damage severity.

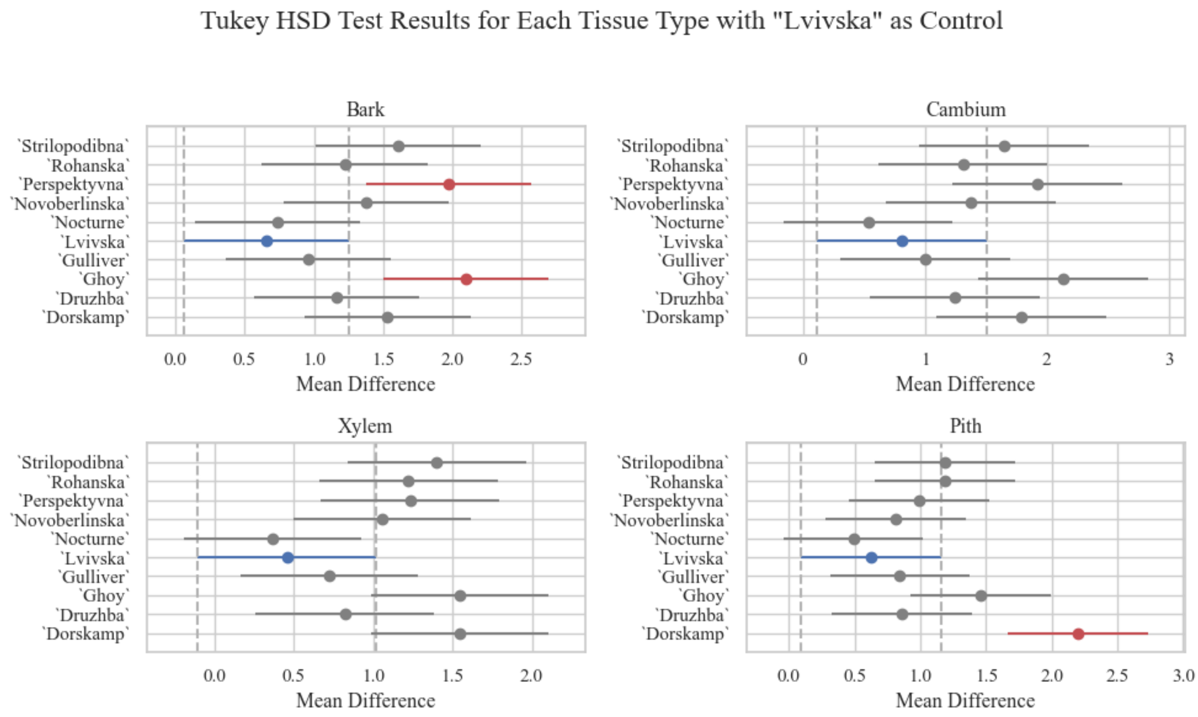

Fig. 4. Tukey HSD test results: The control clone, 'Lvivska', is highlighted in blue. Clones highlighted in red show a statistically significant difference from the control group in terms of frost resistance.

P. trichocarpa 'Nocturne' and 'Lvivska', as well as the clone 'Gulliver' (*P. deltoides*), characterized by the highest frost resistance. Cluster 2 includes clones 'Druzhba' and 'Novoberlinska', as well as 'Rohanska' (*P. simonii*), demonstrating moderate frost resistance. Cluster 3 includes clones 'Dorskamp' and 'Ghoy', showing the highest susceptibility to low temperatures both in control and at –25 °C and –30 °C. Cluster 4 includes clones 'Strilopodibna' and 'Perspektyvna', demonstrating moderate resistance at the control level and at –30 °C, while the highest susceptibility was noted at –25 °C. Clusters 3 and 4 include only hybrids of *P.* × *euramericana*.

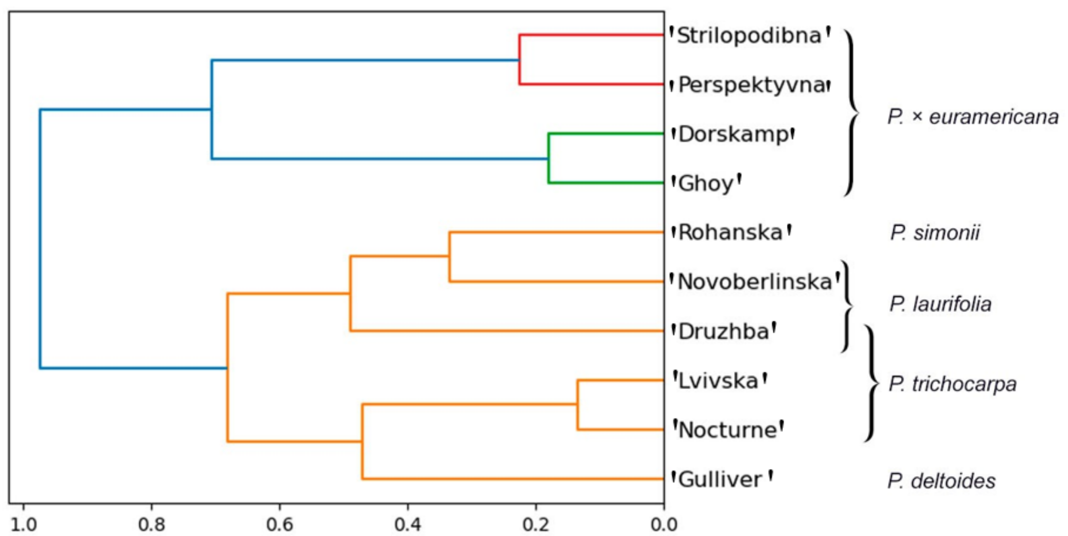

Fig. 5. Dendrogram of the 10 poplar clones clustered by frost resistance. The most resistant clones were 'Lvivska' 'Nocturne' and 'Gulliver'.

4. Discussion

Climate change including temperature and precipitation – can have a significant impact on poplars growth and survival in the future (Lazdiņa et al. 2016; Mirzaei et al. 2018; Niemczyk et al. 2019; Kovačević et al. 2020; Fang et al. 2021; Heilig et al. 2021). Clones react differently to extremely low temperatures. Frost hardiness is influenced by a range of factors, including the genetic characteristics of the trees, the physiological responses of the tree to cold, and environmental factors such as soil moisture and nutrient availability (De Antonio et al. 2021).

As the architecture of the aboveground part of the tree affects the distribution of temperature (microclimate) (Havryliuk & Kondratenko 2020), the potential frost damage to different tree structures (such as buds, shoots, and branches) will vary. In this study, an top of the one-year-old shoot in all poplar clones was found to be the most vulnerable. At the same time, the symptoms of frost on apical buds were the least developed. In all tested clones, the most marked frost damage, observed at both –25 °C and –30 °C temperatures. The lower (basal) part of the shoots was least damaged by frost may be due to more developed wood structure and thickness of the shoot. The thinner parts of the shoots have less woody tissues and, consequently less resistant to frost.

Different tissues of woody plants have different frost resistance. During the active growth phase of trees, cambium, young bark, and xylem are most sensitive to low temperatures. In mature tissues, the pith of branches dies first, followed by xylem and then outer or old cells of the bark (Havryliuk & Kondratenko 2020). Our study found that clones like 'Nocturne', 'Lvivska', and 'Gulliver' showed high frost resistance, particularly in the internal tissues (pith and xylem). In contrast, 'Dorskamp', 'Ghoy', and 'Perspektyvna' were more susceptible to damage, especially in the outer tissues (bark and cambium). Reducing freezing-induced embolism through narrower xylem vessels is an essential adaptive strategy for maintaining long-term productivity in hybrid poplar plantations (Schreiber et al. 2013).

A frozen tree may not die immediately but as a result of damage from pathogenic microorganisms, to which its resistance is greatly reduced (Cambours et al. 2006). The recovery of a frozen tree largely depends on the state of the cambium, which is the most frost-hardy tissue in winter. As long as the cambium remains intact after severe frost, the tree can often recover. However, if both the bark and cambium are damaged, the tree's chances of recovery diminish (Sperry et al. 1994).

Rapacz et al. (2015) reported that the survival of plants in natural conditions was significantly more affected by the environment compared to the survival of plants undergoing freezing tests in the laboratory. Of note is that controlled freezing tests evaluate only a plant's ability to endure short-term freezing stress. However, for winter survival, it is crucial for plants to maintain high freezing tolerance in field conditions with sudden temperature fluctuations (Gusta &Wisniewski 2013).

Cluster analysis, based on the resistance to low-temperature damage of various poplar clones, identified four clusters of potential frost resistance. *Populus deltoides* ('Gulliver') and *P. trichocarpa* ('Nocturne', 'Lvivska') were the most resistant to extremely low temperatures in a controlled environment and belonged to cluster 1. Cluster 2 included hybrids of *P. laurifolia* and *P. lasiocarpa* ('Druzhba' and 'Novoberlinska'), as well as 'Rohanska' (*P. simonii*), which demonstrate moderate resistance to frost. Clones from clusters 1 and 2 are recommended for wide-scale testing in eastern Ukraine.

Clusters 3 and 4 include only hybrids of *P.* × *euramericana* and showed the lowest resistance to frost damage. Cluster 3 includes poplar clones introduced to eastern Ukraine over the past 20 years, such as 'Dorskamp' and 'Ghoy', which have not adapted to the conditions of the region and showed the worst results. Therefore, it is not advisable to cultivate these clones in this region. This is because the physiological mechanisms that trees use to tolerate low temperatures include cold acclimation in autumn, winter dormancy, and maintaining a certain frost resistance during early spring hardening (Vitasse et al. 2014). *Populus nigra* is a taxon that needs a temperate climate and its growth is regulated by accumulated heat unit (Sykes et al. 1996; Yu et al. 2001; Pliura et al. 2014).

Cluster 4 includes clones 'Strilopodibna' and 'Perspektyvna', which are descendants of trees cultivated in Ukraine since the 1960s, demonstrating moderate resistance at the control level and at temperatures of –30 °C, with the highest damage observed at temperatures of –25 °C.

In trials conducted in Northern Europe, *P. trichocarpa* and *P. balsamifera* × *P. trichocarpa* were among the fastest-growing poplar hybrids. The hybrid between *P. deltoides* and *P. trichocarpa* had the second-highest tree height growth after the *P. maximowiczii* × *P. trichocarpa* hybrid (Pliura et al. 2014). In our study, clones 'Nocturne', 'Novoberlinska', 'Druzhba', and 'Gulliver' have the greatest potential for cultivation in eastern Ukraine for biomass production. This is confirmed by previous research on the growth indicators of these clones. The 'Druzhba' clone was characterized by significantly higher than average (2.71 g dry weight of shoots) biomass growth of 4.09 g. The biomass indicators of the 'Gulliver', 'Nocturne', and 'Novoberlinska' clones were average, at 2.94, 2.74, and 2.70 g respectively. The biomass indicators of the 'Lvivska' clone were significantly lower than average, at 1.69 g (Kutsokon 2018). Maximum productivity can be achieved by combining the high resistance and productivity of a certain clone. Therefore, despite its high frost resistance in the conditions of eastern Ukraine, the 'Lvivska' clone is not advisable for cultivation to obtain biomass.

The high productivity of *Populus* hybrids is related to the prolonged vegetation period, compared to the parental species (Yu et al. 2001). In Northern Europe, bud break of *P. trichocarpa* was the earliest among all tested poplar and aspen hybrids. *P. deltoides* and *P. nigra* exhibited the latest bud break, as well as the lowest tree height and height growth rate (Pliura et al. 2014). Plants with a late vegetative period risks of being damaged by early autumn frosts, as their shoots do not have enough time to harden off. Other mismatches between plant requirements for warmth and the ecological conditions of the region can result in additional waves of autumn growth that weaken the plants. Trees that are not acclimated to their growing location may be damaged by cold injuries (Sykes et al. 1996).

5. Conclusions

Our results revealed significant differences in frost tolerance among the studied *Populus* genotypes. Clones derived from trees cultivated in Ukraine since the 1960s are more frost resistant due to the acclimatization of their parental individuals. In eastern Ukraine, clones 'Gulliver' (native hybrid *P. deltoides*), 'Lvivska' (*P.* × *euramericana* cv. 'Regenerata' × *P. trichocarpa*), and 'Nocturne' (*P. trichocarpa* × *P. lasiocarpa*) exhibit the highest frost resistance. Hybrids of *P.* × *euramericana* ('Strilopodibna', 'Perspektyvna', 'Dorskamp', and 'Ghoy') were the most damaged by frost. Clone and freezing temperature are key determinants of tissue damage, explaining 64% of the variation in the data. Cluster analysis revealed four distinct clusters of frost resistance, highlighting that only hybrids of *P.* × *euramericana* were the most susceptible to freezing damage. These observations may aid breeders in identifying high resistance to low-temperature hybrid poplar clones, and exclude stress-sensitive genotypes.

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