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Changes in Malondialdehyde, Anthocyanin, and Hydroxycinnamic Acid Contents in Shoots and Buds of *Prunus* L. Representatives Induced by Cold Stress

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This study is devoted to the assessment of cold tolerance in selected cultivars of the genus *Prunus* L., including *P. persica*, *P. cerasifera*, and *P. domestica*, during winter dormancy based on biochemical markers associated with oxidative stress and antioxidant defense. The contents of anthocyanins, hydroxycinnamic acids, and malondialdehyde (MDA) were determined in shoots and buds of eleven cultivars, and an integrated Cold Tolerance Index (CTI) was calculated using Max-normalized biochemical parameters. The results demonstrated that buds generally accumulated higher levels of anthocyanins and hydroxycinnamic acids than shoots across all studied *Prunus* species, indicating enhanced antioxidant activity in generative organs. The highest anthocyanin contents were recorded in *P. persica* cv. Antotsianovyi, *P. domestica* cv. Oda and Stanley, while elevated hydroxycinnamic acid levels were characteristic of *P. domestica* (cv. Oda and Stanley), *P. cerasifera* cv. Kubanska Kometa, and *P. persica* cv. Antotsianovyi. In contrast, MDA content varied considerably among cultivars and species and reflected the intensity of lipid peroxidation under low-temperature stress, with generally higher values observed in *P. persica* compared to *P. cerasifera* and *P. domestica*. Cultivars of *P. domestica*, particularly Oda and Stanley, combined relatively low MDA accumulation with high CTI values, suggesting an efficient and stabilized adaptation strategy. The CTI values ranged from 42 to 72%, revealing substantial interspecific and intraspecific differentiation in cold tolerance. The highest cold tolerance was observed in *P. domestica* cultivars, whereas several *P. persica* cultiars, including Suputnyk, Lisostepovyi, and Osinnii Siurpryz, showed increased sensitivity to cold stress. Cluster analysis (UPGMA, Euclidean distance) identified distinct groups of genotypes differing in adaptive strategies across species. A significant inverse correlation between MDA content and CTI (R^2 = 0.6499) confirmed the key role of oxidative membrane damage in determining cold tolerance in *Prunus* species. Overall, the study demonstrates that cold tolerance in *Prunus* is strongly species-dependent, with *P. domestica* showing the highest adaptive potential, *P. cerasifera intermedia*te responses, and *P. persica* the greatest variability, highlighting the importance of species-level differentiation in biochemical adaptation to low-temperature stress.

Keywords: *Prunus* spp., malondialdehyde, anthocyanins, hydroxycinnamic acids

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Introduction

Low temperature is one of the most important abiotic factors limiting the growth, productivity, geographical distribution, and overwintering success of fruit crops. Representatives of the genus *Prunus* L., including plums, cherries, apricots, and related species, are particularly sensitive to winter and early spring temperature fluctuations. Exposure to freezing and chilling conditions leads to disturbances in cellular metabolism, membrane destabilization, oxidative stress, and impairment of physiological processes, ultimately affecting plant survival and yield stability (Suzuki et al., 2014; Qian et al., 2024, Shu et al., 2025). In temperate climatic regions, the ability of plants to tolerate low temperatures is therefore considered one of the key adaptive traits determining the success of cultivation and breeding programs.

Cold stress is closely associated with excessive production of reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals. Under normal physiological conditions, ROS are involved in signaling pathways; however, under stress their overaccumulation causes oxidative damage to lipids, proteins, nucleic acids, and cellular membranes (Franzoni et al., 2023; Yu et al., 2024). One of the most informative indicators of oxidative membrane damage is MDA, a secondary product of lipid peroxidation widely used as a biochemical marker of stress intensity and cellular injury (Heath and Packer, 1968). Increased MDA accumulation under low-temperature conditions has been reported in numerous plant species and is generally associated with reduced stress tolerance (Alabd et al., 2023; Liao et al., 2023; Xiong et al., 2025).

To counteract oxidative damage, plants activate complex antioxidant defense systems consisting of both enzymatic and non-enzymatic components. Among non-enzymatic antioxidants, phenolic compounds play a particularly important role in stress adaptation (Thomashow, 1999; He et al., 2024). Anthocyanins and hydroxycinnamic acids are known to possess strong antioxidant properties, including ROS scavenging, membrane stabilization, and protection of cellular structures from oxidative injury (Chalker-Scott, 1999; Gould, 2014; Li and Ahammed, 2023; Wang et al., 2024; Qian et al., 2024). In addition to their antioxidant functions, anthocyanins may contribute to photoprotection and osmotic adjustment during stress conditions. Enhanced accumulation of phenolic compounds under cold stress has been described as an important adaptive mechanism in many woody and fruit species (Christmann et al., 2006; Schulz et al., 2016; Li and Ahammed, 2023; Ninkuu et al., 2025; Yu et al., 2026).

The level of accumulation of antioxidant compounds and the intensity of lipid peroxidation may differ significantly among genotypes, reflecting variability in adaptive strategies. Some cultivars exhibit efficient stabilization of cellular metabolism and suppression of oxidative damage, whereas others respond through intensive activation of inducible antioxidant systems. Therefore, the combined analysis of MDA, anthocyanins, and hydroxycinnamic acids provides valuable information about the physiological and biochemical mechanisms underlying cold tolerance in plants (Dixon and Paiva, 1995; Solecka et al., 1999).

Despite the considerable economic importance of representatives of the genus *Prunus*, comparative studies integrating biochemical indicators of oxidative stress and antioxidant protection during winter dormancy remain limited. Furthermore, differences between vegetative and generative organs in their response to cold stress are still insufficiently understood (Dixon and Paiva, 1995; Ninkuu et al., 2025). Buds, which determine reproductive success and future productivity, are often more vulnerable to low temperatures than shoots due to their higher metabolic activity and structural sensitivity (Wisniewski et al., 2003).

In addition to their agricultural and ecological importance, representatives of the genus Phytochemistry *Prunus* are also recognized as valuable medicinal and nutraceutical plants. Various species of the genus are rich in biologically active compounds, including anthocyanins, flavonoids, hydroxycinnamic acids, tannins, vitamins, and other phenolic metabolites that exhibit pronounced antioxidant, anti-inflammatory, antimicrobial, cardioprotective, and antidiabetic properties. Fruits, leaves, bark, and seeds of many *Prunus* species have long been used in traditional medicine and are increasingly studied as promising sources of natural phytochemicals for pharmaceutical and functional food applications (Mieszczakowska-Frąc et al., 2025; Habibi et al., 2026).

Particular attention has been devoted to anthocyanin-rich cultivars due to their high antioxidant capacity and ability to neutralize reactive oxygen species. These compounds not only contribute to plant adaptation under abiotic stress conditions, including low temperatures, but also possess significant therapeutic potential for humans by reducing oxidative stress associated with cardiovascular, neurodegenerative,

and metabolic disorders. (Levon and Golubkova, 2016; Habibi et al., 2026; McCune et al., 2011; Wallace, 2011; Stacewicz-Sapuntzakis, 2013). Hydroxycinnamic acids, such as chlorogenic and caffeic acids, are likewise considered important bioactive metabolites with strong antioxidant and protective properties (Santana-Gálvez et al., 2017).

The accumulation of these secondary metabolites is often enhanced under environmental stress conditions, including cold exposure. Therefore, the study of biochemical responses to low temperatures in *Prunus* genotypes is important not only for understanding mechanisms of cold tolerance, but also for identifying cultivars with increased concentrations of valuable phenolic compounds. Such genotypes may represent promising material both for breeding cold-resistant fruit crops and for the development of functional foods and medicinal plant products with elevated antioxidant potential.

In this regard, the aim of the present study was to evaluate the cold tolerance of selected *Prunus* L. cultivars during winter dormancy based on biochemical markers of oxidative stress and antioxidant protection. Special attention was paid to the content of anthocyanins, hydroxycinnamic acids, and malondialdehyde in shoots and buds, as well as to the development of an integrated Cold Tolerance Index for comprehensive assessment of genotype adaptability to low-temperature conditions.

Material and Methodology

Collection of plant material

Plant material was collected from the collection of the Department of Fruit Plants Acclimatization at the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (NBG). Sampling was carried out during winter dormancy in February 2026, with the minimum air temperature reaching -18.2 °C. Samples for analysis were taken from the middle part of the plants, considering four orientations: north, south, west, and east. The elevation of the study site was 127 m above sea level.

Plant material studied

The study included representatives of the genus *Prunus* L. Specifically, the following cultivars were analyzed: *Prunus domestica* 'Stanley' and 'Oda'; *Prunus cerasifera* 'Kubanska Kometa' and 'Kyivska

Figure 1 Representatives of the genus *Prunus* L. from the collection of the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine. *Prunus cerasifera*: 1 – Kubanska Kometa, *Prunus domestica*: 2 – Stanley, 3 – Oda, *Prunus persica*: 4 – Lisostepovyi, 5 – Antotsianovyi, 6 – Suputnyk, 7 – Osinnii Siurpryz, 8 – Kyivska Hibrydna

Hibrydna'; and *Prunus persica* 'Antotsianovyi', 'Lisostepovyi', 'Osinnii Siurpryz', 'Pamiat Shevchenka', 'Slavutych', 'Shchedryi', and 'Suputnyk'. All cultivars are of Ukrainian origin and were developed within the breeding program of the M.M. Gryshko National Botanical Garden (Figure 1).

Meteorological data

Meteorological data used in this study were obtained from the Open-Meteo Historical Weather database, which provides access to archived weather information, including air temperature and other relevant climatic parameters for the corresponding observation periods (https://open-meteo.com).

Biochemical analyses

Biochemical analyses were carried out in the laboratory of the Department of Acclimatization of Fruit Plants. The studies were conducted on shoots and buds in a well-ventilated room without direct sunlight at a temperature of 20–25 °C. The samples were ground using an electric mill (Vector HR-200), resulting in a particle size of approximately 200 µm.

Chemicals

All chemicals and reagents were of analytical grade and were purchased from Merck (Darmstadt, Germany) and HIMLABORREACTIVE (Ukraine).

Total anthocyanin content

Anthocyanins were extracted using acidified ethanol to enhance pigment recovery and ensure structural stability. A precisely weighed portion of finely powdered plant material was extracted with 70% ethanol adjusted to pH 1.0–1.5 (acidified with hydrochloric acid) under continuous stirring to ensure complete solvent penetration and efficient extraction of pigments. The extraction was carried out at room temperature, and the samples were protected from light to prevent anthocyanin degradation.

The absorbance of the filtrate was measured spectrophotometrically at 530 nm, with correction for background absorption at a reference wavelength to account for possible interference from other co-extracted compounds. Quantification of total anthocyanins was performed using a calibration curve constructed with cyanidin-3-glucoside as an external standard. The results were expressed as milligrams of cyanidin-3-glucoside equivalents per 100 g of dry matter (mg·100^{-1} g DM) (Wrolstad, 1993).

The anthocyanin concentration, expressed as cyanidin-3-glucoside, was calculated using the following formula:

$$Cant = (D \times V \times R \times K) / (l \times m)$$

where: D – the optical density of the solution; V – the volume of the extract, ml; R – dilution ratio of a solution of 3.5 % hydrochloric acid in ethanol; l – working length of the cuvette, cm; m – weighed quantity, g; K – is the conversion factor, based on a calibrated graph

Hydroxycinnamic acid content

The plant material was extracted with a water-ethanol solution under reflux in a boiling water bath with constant stirring. The extract was filtered to remove insoluble residues. The optical density was measured at 330 nm using distilled water as a reference, corresponding to the absorption maximum of hydroxycinnamic acid derivatives (Musiienko et al., 2001).

The concentration of the target compounds was calculated from measured absorbance values using an appropriate calibration approach or a specific absorption coefficient. The results were expressed as mg·g^{-1} of dry matter.

The content of all derivatives of hydroxycinnamic acid in terms of chicory acid is calculated using the formula:

$$Cha = (D \times 2{,}500) / (M \times 782 \times (100 - W))$$

where: D – optical density of the test solution; M – mass of raw materials, g; W – loss in mass during drying of raw materials, %; 782 – specific absorption index of chicory acid at 330 nm

Malondialdehyde content

The determination of malondialdehyde was based on its reaction with thiobarbituric acid (TBA), resulting in the formation of a colored trimethine complex under high-temperature conditions in an acidic medium. This complex exhibits a characteristic absorption maximum at 532 nm (Ohkawa et al., 1979). Freshly harvested plant leaves were homogenized in 5% trichloroacetic acid (TCA) to extract lipid peroxidation products, followed by centrifugation at 12,000 × g for 10 minutes at 27°C to obtain a clear supernatant. An equal volume of the supernatant was then mixed with 0.5% TBA prepared in 20% TCA. The reaction mixture was incubated at 96°C for 30 minutes to allow complete development of the chromogenic

complex, and subsequently rapidly cooled in an ice bath to terminate the reaction. After an additional centrifugation step at 12,000 × g for 10 minutes to remove precipitated materials, the absorbance of the supernatant was measured spectrophotometrically at 532 nm, with correction for nonspecific turbidity at 600 nm. The MDA content was calculated based on the corrected absorbance values. MDA was determined in fresh material (FM) and expressed as nmol·g^{-1}.

The MDA concentration was determined using the following formula:

$$C_{MDA} = (D1 - D2) / 155 \times m$$

where: C_{MDA} – TBA-reactive products, mM·g^{-1} of fresh weight; $D1$ – optical density at 532 nm; $D2$ – optical density at 600 nm; 155 – TBA extinction coefficient, nmol·g^{-1}; m – weight of plant material, g

Cold tolerance index (CTI)

Max-normalization is usually computed as standardization relative to the feature's maximum value (Jain et al., 2005). This indicator was calculated using the formula:

$$CTI = \sum_{i=1}^{n} Wi \cdot \frac{Xi}{Xi, \max}$$

where: Xi – value of the i-th indicator; Xi, max – the maximum value of this indicator in the sample set; Wi – indicator weight (if all are equally important, then $Wi = 1/n$; N – number of indicators

If a measure is harmful when elevated (for example, MDA), it is often inverted: $X' = 1 - X/X$ max.

Statistical analysis

Statistically processed data are presented in histograms as mean values ± standard error (SE). The level of significance was set at α = 0.05. Statistical analysis was performed using TIBCO Statistica v.14.0.0.15 (x64).

Results and Discussion

To comprehensively evaluate the adaptive responses of the studied cultivars to low-temperature conditions, the contents of MDA, anthocyanins, and hydroxycinnamic acids were analyzed in shoots and buds. These biochemical parameters reflect two interconnected aspects of stress physiology: the intensity of membrane oxidative damage and the efficiency of antioxidant protection mechanisms. MDA was used as an indicator of lipid peroxidation and membrane destabilization, whereas anthocyanins and hydroxycinnamic acids were considered major non-enzymatic antioxidants involved in ROS scavenging, stabilization of cellular structures, and maintenance of redox balance.

Anthocyanin content was consistently higher in buds than in shoots across all studied genotypes, indicating

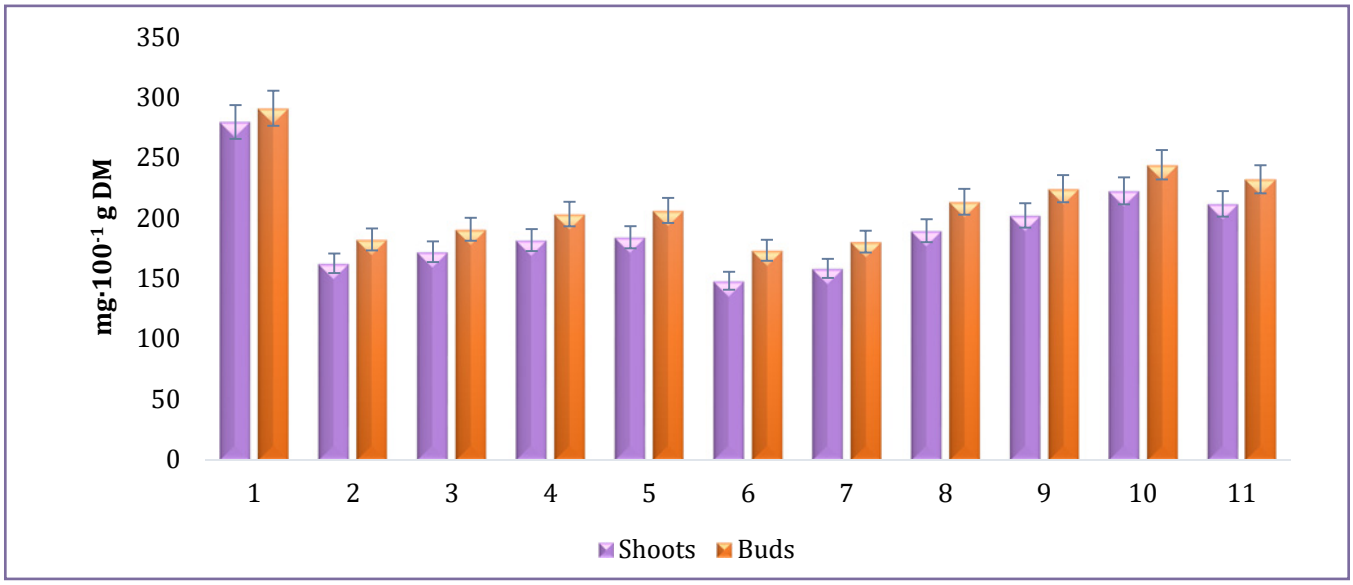

Figure 2 Anthocyanin content in shoots and buds of *Prunus* L. cultivars during winter dormancy
Prunus persica cultivars: 1 – Antotsianovyi; 2 – Lisostepovyi; 3 – Osinnii Siurpryz; 4 – Pamiat Shevchenka; 5 – Slavutych; 6 –Suputnyk; 7 – Shchedryi. *Prunus cerasifera* cultivars: 8 – Kyivska Hibrydna; 9 – Kubanska Kometa. *Prunus domestica* cultivars: 10 – Oda; 11 – Stanley

enhanced antioxidant activity in generative organs under cold stress conditions (Figure 2). The highest levels were recorded in *P. persica* cv. Antotsianovyi (shoots: 279.6 mg·100^{-1} g DM, buds: 291.0 mg·100^{-1} g DM), *P. domestica* cv. Oda (shoots: 222.6 mg·100^{-1} g DM, buds: 244.2 mg·100^{-1} g DM), and *P. domestica* cv. Stanley (shoots: 211.8 mg·100^{-1} g DM, buds: 232.2 mg·100^{-1} g DM).

The content of hydroxycinnamic acids, similarly to anthocyanins, was significantly higher in cultivars belonging to different *Prunus* species, including *P. domestica* cv. Oda (shoots: 3.84 mg·g^{-1} DM, buds: 4.22 mg·g^{-1} DM) and cv. Stanley (shoots: 3.71 mg·g^{-1} DM, buds: 4.09 mg·g^{-1} DM), *P. persica* cv. Antotsianovyi (shoots: 3.39 mg·g^{-1} DM, buds: 3.96 mg·g^{-1} DM), and *P. cerasifera* cv. Kubanska Kometa (shoots: 3.58 mg·g^{-1} DM, buds: 4.03 mg·g^{-1} DM) (Figure 3).

In all examined genotypes, hydroxycinnamic acid levels were higher in buds than in shoots, indicating enhanced antioxidant activity in generative organs under cold stress conditions.

In contrast, MDA content, reflecting lipid peroxidation intensity, showed elevated values in buds and significant variability among cultivars belonging to different *Prunus* species (Figure 4). In general, *P. persica* cultivars tended to exhibit higher MDA levels compared to *P. cerasifera* and *P. domestica*, indicating a greater degree of membrane lipid peroxidation under cold stress conditions. Notably, cultivars with high anthocyanin accumulation often demonstrated increased MDA content, suggesting a reactive stress-response strategy rather than efficient damage limitation.

Conversely, *P. domestica* cultivars Oda (shoots: 3.31 nmol·g^{-1}, buds: 3.58 nmol·g^{-1}) and Stanley (shoots: 3.42 nmol·g^{-1}, buds: 3.73 nmol·g^{-1}) combined relatively low MDA levels with high CTI values, indicating a more stable and efficient adaptive strategy to low-temperature stress. These results suggest that anthocyanin accumulation is more closely associated with stress intensity than with resistance efficiency, as higher pigment levels were often accompanied by increased lipid peroxidation (shoots: 5.12 nmol·g^{-1}, buds: 5.62 nmol·g^{-1}).

Based on the obtained meteorological data, an integrated cold tolerance index was calculated, reflecting the cumulative response of plants to low-temperature stress and providing a quantitative assessment of the adaptive potential of the studied cultivars. This approach combines environmental conditions with plant biochemical responses, providing a comprehensive framework for evaluating cold stress tolerance. Cold tolerance index is an indicator that describes the ability of a biological organism to survive and function when exposed to low temperatures (Ding et al., 2024). The cold tolerance of *Prunus* genotypes was evaluated using a composite Cold Tolerance Index (CTI) integrating key biochemical markers associated with stress protection and oxidative damage. The index was constructed based on max-normalized values

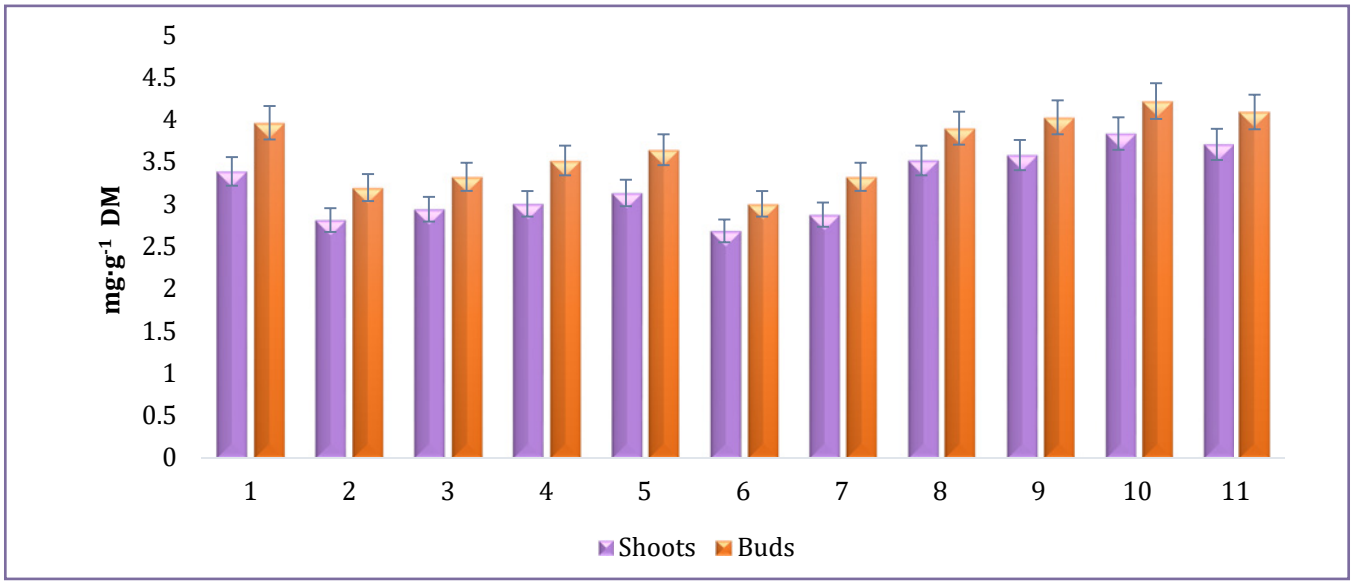

Figure 3 Content of hydroxycinnamic acids in shoots and buds of *Prunus* L. cultivars during winter dormancy
Prunus persica cultivars: 1 – Antotsianovyi; 2 – Lisostepovyi; 3 – Osinnii Siurpryz; 4 – Pamiat Shevchenka; 5 – Slavutych; 6 –Suputnyk; 7 – Shchedryi. *Prunus cerasifera* cultivars: 8 – Kyivska Hibrydna; 9 – Kubanska Kometa. *Prunus domestica* cultivars: 10 – Oda; 11 – Stanley

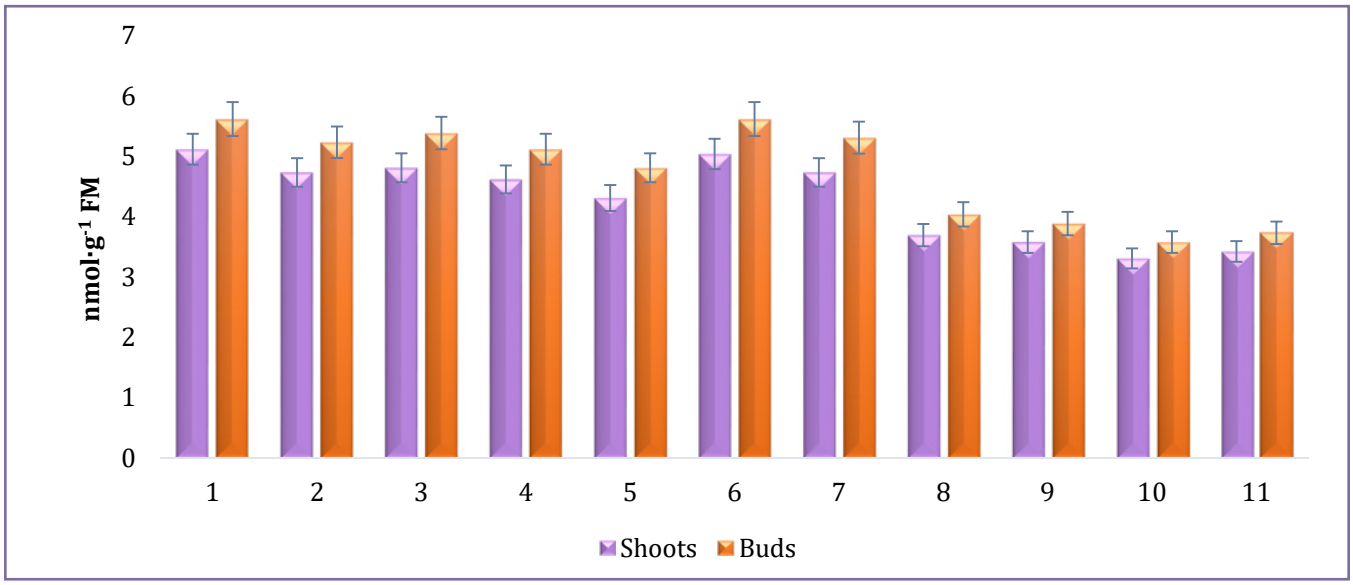

Figure 4 Malondialdehyde content in shoots and buds of *Prunus* L. cultivars during winter dormancy. *Prunus persica* cultivars: 1 – Antotsianovyi; 2 – Lisostepovyi; 3 – Osinnii Siurpryz; 4 – Pamiat Shevchenka; 5 – Slavutych; 6 –Suputnyk; 7 – Shchedryi. *Prunus cerasifera* cultivars: 8 – Kyivska Hibrydna; 9 – Kubanska Kometa. *Prunus domestica* cultivars: 10 – Oda; 11 – Stanley

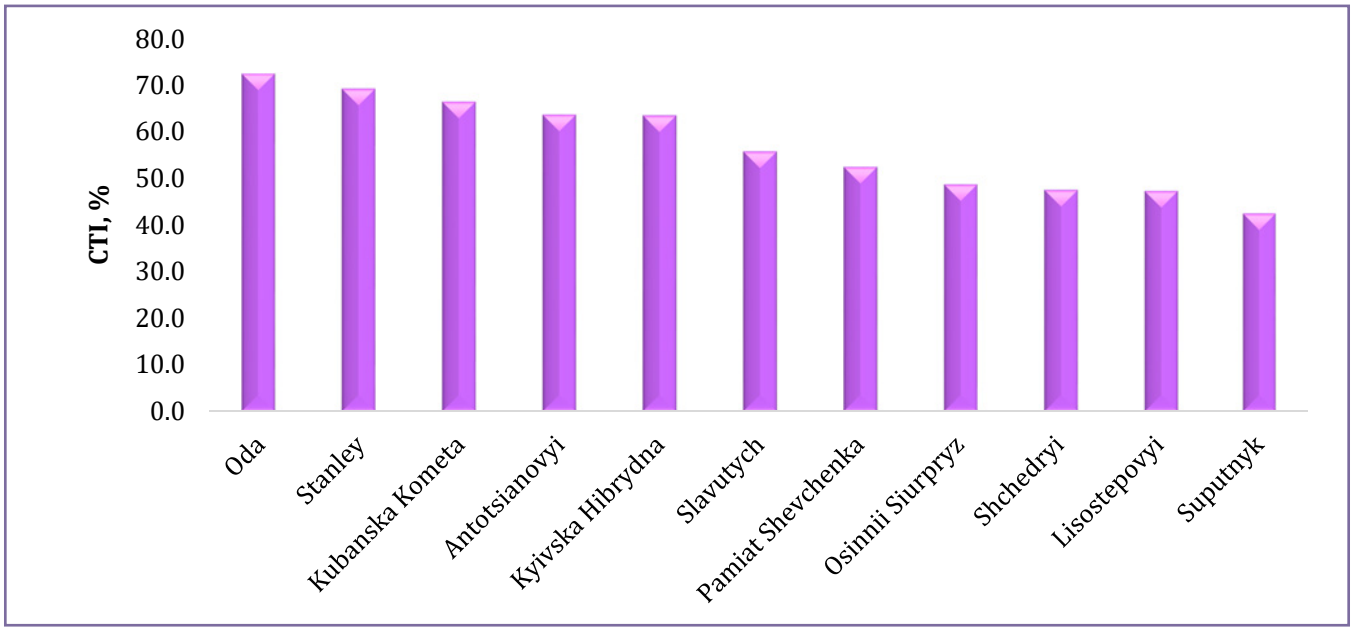

Figure 5 Distribution of cultivars of the genus *Prunus* L. by the cold tolerance index (CTI), calculated based on a set of biochemical indicators standardized by MAX normalization

of anthocyanins and hydroxycinnamic acids, and an inversely scaled MDA level as an indicator of lipid peroxidation (Nardo et al., 2005). CTI values range from 0 to 1, with higher values indicating greater cold tolerance, characterized by increased accumulation of protective phenolic compounds and reduced oxidative membrane damage.

The highest CTI values were recorded in *P. domestica* cultivars Oda (72%) and Stanley (69%), suggesting a high level of adaptability to low-temperature conditions in this species. A group of genotypes with relatively elevated cold tolerance also included *P. cerasifera* (Kubanska Kometa and Kyivska Hibrydna) together with *P. persica* cv. Antotsianovyi, which exhibited CTI values in the range of 63–67%, indicating comparable adaptive potential across different species.

Cultivars of *P. persica* such as Slavutych and Pamiat Shevchenka occupied an *intermedia*te position (52–56%), reflecting moderate cold tolerance. The lowest CTI values were observed predominantly in *P. persica* cultivars, including Osinnii Siurpryz, Shchedryi, Lisostepovyi, and particularly Suputnyk (42–48%), indicating increased sensitivity to cold stress within this species.

Overall, the results demonstrate clear species-specific patterns in cold tolerance, with *P. domestica* showing the highest adaptive capacity, *P. cerasifera* and selected *P. persica* cultivars exhibiting *intermedia*te responses, and several *P. persica* genotypes demonstrating lower resistance to low-temperature stress.

The ranking of genotypes based on CTI is consistent with previously identified biochemical patterns: higher CTI values are associated with lower MDA levels and higher contents of phenolic compounds, whereas lower CTI values reflect intensified lipid peroxidation processes and, consequently, more pronounced cellular damage (Ruelland et al., 2009; Theocharis et al., 2009). Overall, the obtained results confirm the high informativeness of the integrated CTI for the comprehensive assessment of cold tolerance. The use of Max normalization enabled the proper comparison of heterogeneous biochemical parameters and the identification of genotypes with superior cold tolerance, which are promising for breeding and introduction under cold-climate conditions.

The obtained results also indicate differences in cold tolerance between vegetative and generative organs. Buds are characterized by greater sensitivity to low temperatures than shoots, due to their higher metabolic activity and lower tissue structural stability. In this regard, cold-tolerance indicators determined for buds may be lower and reflect the potential risk of damage to generative organs during periods of winter temperature fluctuations (Arora et al., 2003; Wisniewski et al., 2003).

To identify the degree of similarity and differences among the studied species and cultivars based on a set of biochemical (anthocyanin, hydroxycinnamic acids, and MDA contents) and physiological parameters (CTI), as well as to group them according to their level of adaptation to low-temperature stress, a dendrogram was constructed (UPGMA, Euclidean distance). Euclidean distance was used as a measure of dissimilarity between objects based on a set of quantitative traits: the smaller the distance, the greater the similarity between genotypes with respect to the studied biochemical and physiological parameters. The UPGMA method enabled the sequential clustering of objects into groups based on their pairwise distances. As a result, a hierarchical structure of similarity among cultivars was obtained, allowing the visual identification of groups of genotypes with similar adaptive characteristics (Jain et al., 1999; Everitt et al., 2011).

The dendrogram revealed substantial differences in the metabolic profiles of the studied cultivars, as evidenced by the formation of two major groups that merged at a relatively high Euclidean distance (~2.8–3.2) (Figure 6).

Most *P. persica* cultivars, namely Lisostepovyi, Shchedryi, Osinnii Siurpryz, Pamiat Shevchenka, Suputnyk, and Slavutych, clustered together due to their low MDA levels, relatively stable anthocyanin and hydroxycinnamic acid contents, and high similarity of biochemical traits. The short clustering distances within this group (~0.2–1.2) indicate coordinated metabolic responses and efficient control of oxidative stress, suggesting a stable adaptive strategy aimed at minimizing cellular membrane damage under low-temperature conditions.

In contrast, cultivars belonging to different *Prunus* species, including *P. cerasifera* (Kyivska Hibrydna and Kubanska Kometa) and *P. domestica* (Oda and Stanley), formed a separate cluster characterized by elevated or more variable MDA levels together with increased accumulation of anthocyanins and hydroxycinnamic acids. The greater clustering distances within this group (~0.5–1.5) reflect higher metabolic variability and suggest a more reactive stress-response strategy associated with intensive activation of antioxidant defense mechanisms. However, the elevated MDA content also indicates a higher degree of membrane damage despite relatively high CTI values.

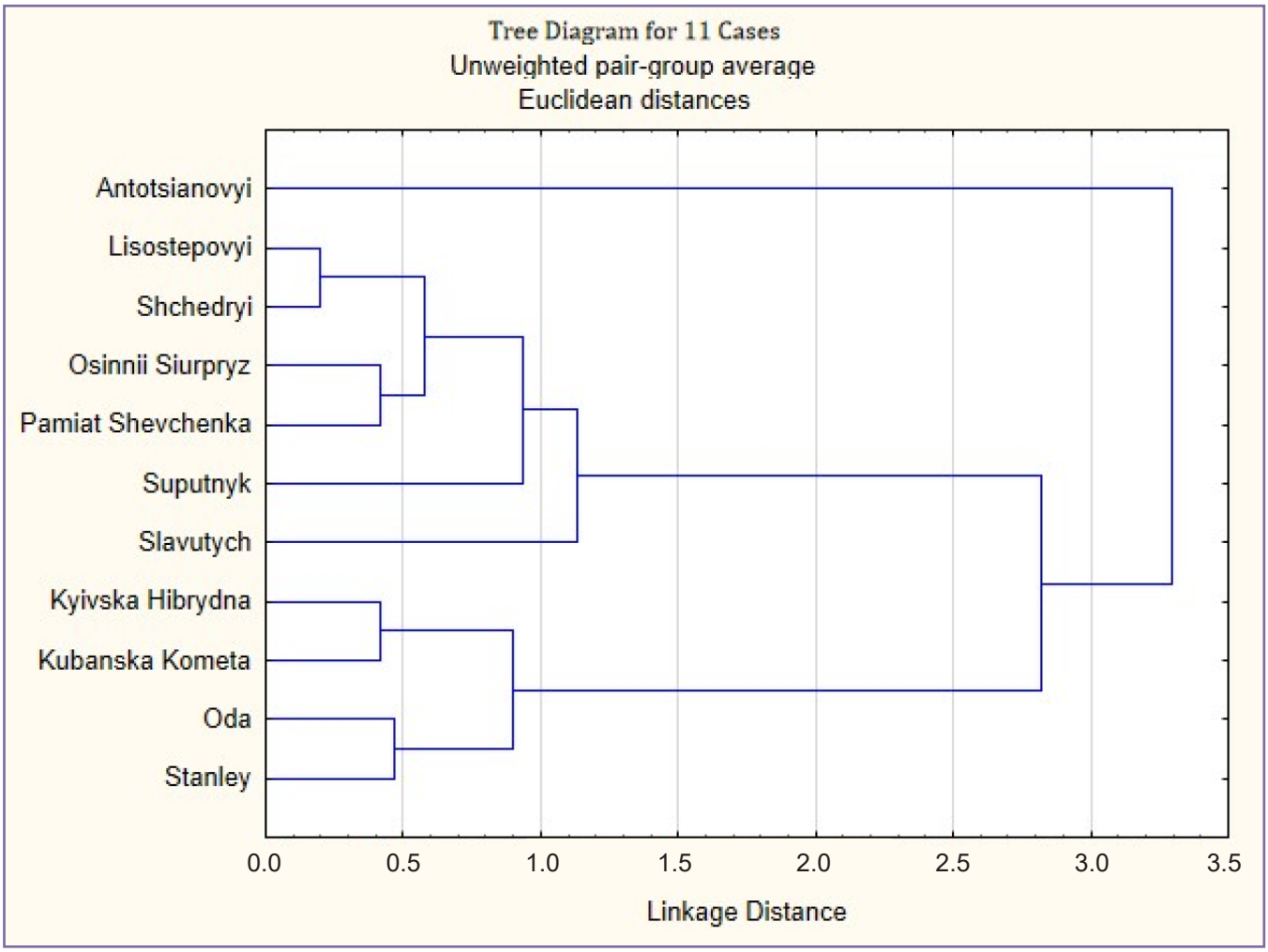

Figure 6 Integration of cold tolerance index (CTI) distribution and hierarchical clustering (UPGMA, Euclidean distance) of *Prunus* L. cultivars based on biochemical parameters (anthocyanins, hydroxycinnamic acids and MDA)

The cultivar Antotsianovyi (*P. persica*) occupied a distinct and highly distant position in the dendrogram, with the maximum linkage distance (~3.2–3.4), indicating a unique biochemical profile and adaptive mechanism. This separation is likely associated with its exceptionally high anthocyanin content and specific metabolic response to cold stress, allowing it to be considered as representing a separate adaptive strategy (Theocharis et al., 2009).

Overall, the cluster analysis confirmed the presence of species- and cultivar-specific differences in the mechanisms of adaptation to low-temperature stress among *Prunus* representatives.

A significant inverse relationship was observed between MDA content and the cold tolerance index (CTI) in the studied *Prunus* genotypes (Figure 7). Linear regression analysis revealed a pronounced negative trend described by the equation y = -12.256x + 108.97, with a coefficient of determination (R^2 = 0.6499), indicating that approximately 65% of the variability in CTI can be explained by differences in MDA levels. This result suggests that genotypes with elevated MDA content tend to exhibit lower cold tolerance, reflecting increased lipid peroxidation and membrane damage under low-temperature stress conditions.

The distribution of genotypes along the regression line further supports this relationship and also highlights species-specific tendencies. Highly cold-tolerant cultivars of *P. domestica*, such as Oda and Stanley, were characterized by relatively low MDA levels and high CTI values. In contrast, several *P. persica* cultivars, including Suputnyk, Osinnii Siurpryz, and Shchedryi, showed higher MDA accumulation accompanied by reduced CTI, indicating greater sensitivity to cold stress within this species. *P. cerasifera* cultivars, including Kubanska Kometa and Kyivska Hibrydna, occupied *intermedia*te positions, reflecting moderate cold tolerance and an *intermedia*te physiological response.

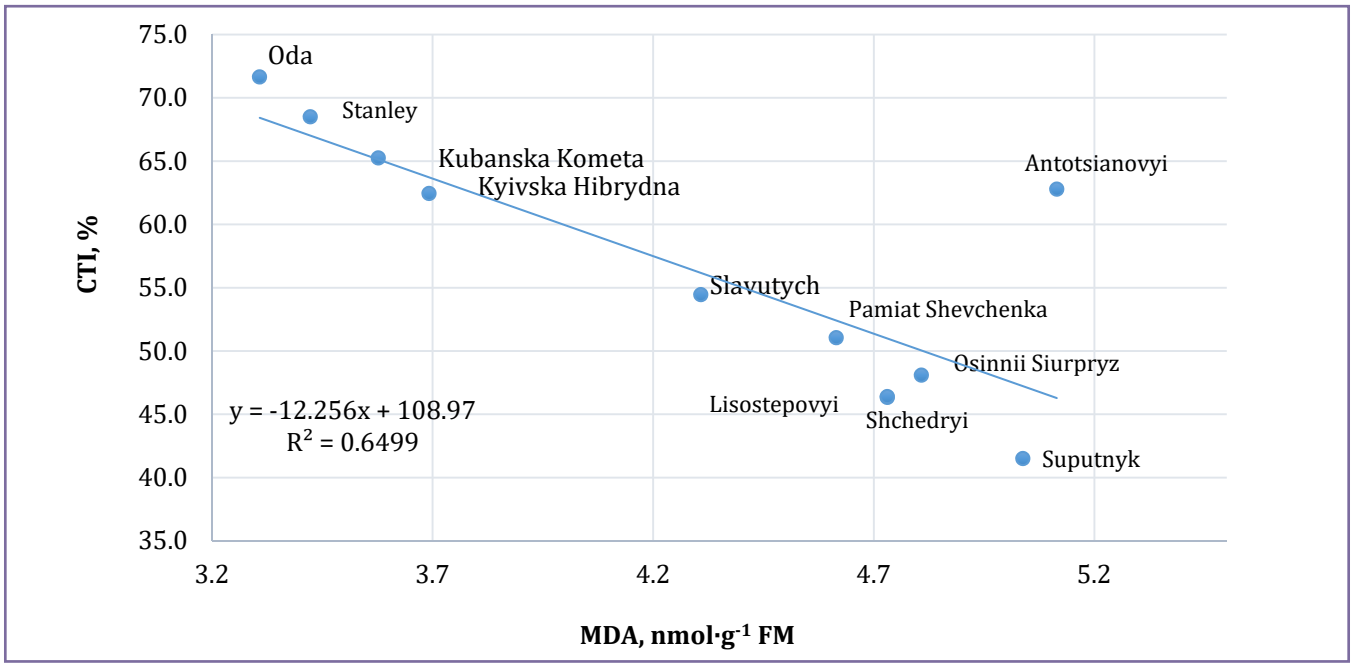

Figure 7 Correlation diagram between MDA and cold tolerance index (CTI)

Notably, *P. persica* cv. Antotsianovyi deviated from the general trend, maintaining comparatively high CTI despite elevated MDA levels, which may indicate the involvement of additional protective mechanisms, such as enhanced accumulation of phenolic antioxidants, including anthocyanins. A similar deviation was observed in *P. persica* cv. Pamiat Shevchenka, suggesting genotype-specific differences in stress-response pathways within this species (Mittler, 2002; Sharma et al., 2012).

An important aspect of plant adaptation to cold stress is the coordination between oxidative damage and antioxidant protection. Plants may adopt different strategies: either limiting ROS formation or enhancing antioxidant capacity. The cultivar-specific differences observed in this study, particularly in MDA and phenolic compounds, indicate the presence of distinct adaptive strategies (Solecka et al., 1999). This is consistent with current concepts of plant stress physiology, where both constitutive and inducible defense mechanisms contribute to stress tolerance (Ritonga and Chen, 2020; Niu et al., 2023; Zhao et al., 2024).

Furthermore, the differences between shoots and buds highlight tissue-specific responses to cold stress. Buds, which are critical for overwintering survival, often possess enhanced protective mechanisms, including higher baseline antioxidant levels or more efficient ROS detoxification systems. Similar organ-specific responses have been described in other species, where different tissues exhibit distinct metabolic adjustments under chilling stress (Zhou et al., 2018).

Conclusions

The results obtained in this study are consistent with the current understanding that cold tolerance in representatives of the genus *Prunus* is determined by the balance between reactive oxygen species production and the capacity of antioxidant defense systems. The observed differences among species, including *P. persica*, *P. cerasifera*, and *P. domestica*, indicate that cold stress responses are not only genotype dependent but also species specific. Accordingly, MDA, anthocyanins, and hydroxycinnamic acids can be considered key biochemical markers of cold stress response across these *Prunus* species. Cultivars of *P. domestica* and selected genotypes of *P. cerasifera* characterized by lower MDA accumulation and higher levels of phenolic antioxidants demonstrated more efficient protective mechanisms, including improved redox regulation and membrane stabilization. In contrast, several *P. persica* cultivars showed higher variability in MDA content and a more reactive antioxidant response, suggesting differences in the efficiency of stress mitigation strategies among species. Such genotypes, particularly those exhibiting stable biochemical profiles within and across species, may be better adapted to low-temperature environments and represent promising material for cultivation and breeding programs aimed at improving cold tolerance in *Prunus* species.

Conflict of interest

The authors have no competing interests to declare.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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