# Frost hardiness of apple generative buds during dormancy

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Abstract: The success of apple production is influenced by frost damages. Occurrence of extreme temperatures is increasing worldwide because of global warming, so the risk of frost damages is also increasing in apple orchards during dormancy and blooming time. In our work the frost hardiness of flower buds of eight apple cultivars was observed with artificial freezing tests during four subsequent dormancy periods in Hungary. The studied cultivar assortment contained two standard commercial cultivars ('Gala', 'Idared'), two scab-resistant cultivars from abroad breeding programmes ('Florina', 'Prima') and four new Hungarian multi-resistant (mainly scab-resistant) cultivars ('Artemisz', 'Cordelia', 'Hesztia', 'Rosmerta'). There were remarkable differences between cultivars and years from the aspect of frost hardiness of generative overwintering organs. At the end of hardening period, in January, the LT<sub>50</sub> values of flower buds were between -22.4 °C and -30.4 °C according to cultivar and year. LT<sub>50</sub> means the temperature causing 50 % frost damage in the flower buds of the certain cultivar in the certain time. 'Gala' and 'Florina' were the most frost hardy, while 'Prima', 'Cordelia' and 'Idared' the most sensitive to frost. Cold hardiness values of flower buds of 'Artemisz', 'Rosmerta' and 'Hesztia' cultivars were regularly between the values of two extreme groups. In winters with inappropriate weather the generative overwintering organs were unable to reach the genetically possible frost hardiness of them.

Key words: *Malus x domestica*; artificial freezing tests;  $LT_{so}$  values; dormancy; generative buds

#### Odpornost cvetnih brstov jablane na mraz med mirovanjem

Izvleček: Na uspešno pridelavo jabolk vplivajo poškodbe zaradi mraza. Pojavljanje ekstremnih temperatur se povečuje po vsem svetu zaradi globalnega segrevanja, zato se tudi povečuje tveganje za pozebe v nasadih jablan med mirovanjem ali v času cvetenja. V raziskavi je bila preučevana odpornost na mraz cvetnih brstov osmih sort jablane z umetnim zmrzovanjem v štirih zaporednih rastnih sezonah na Madžarsekm. Preučevani izbor sort je obsegal dve standardni komercialni sorti ('Gala', 'Idared'), dve na škrlup odporni sorti iz tujih žlahniteljskih programov ('Florina', 'Prima') in štiri madžarske multirezistentne sorte (v glavnem odporne na škrlup) ('Artemisz', 'Cordelia', 'Hesztia', 'Rosmerta'). Med sortami in leti so bile opazne razlike glede na mrazno odpornost njihovih prezimnih generativnih organov. Na koncu obdobja odpornosti na mraz, v januarju, so bile LT<sub>50</sub> vrednosti cvetnih brstov med -22,4 °C in -30,4 °C, odvisno od sorte in leta. LT<sub>50</sub> pomeni, da temperatura povzroči 50 % mraznih poškodb v cvetnih brstih pri določeni sorti v določenem času. 'Gala' and 'Florina' sta bili na mraz najbolj odporni medtem, ko so bile 'Prima', 'Cordelia' in 'Idared' na mraz najbolj občutljive. Odpornost cvetnih brstov na mraz je bila pri 'Artemisz, 'Rosmerta' in 'Hesztia' vedno med vrednostmi obeh prej omenjenih ekstremnih skupin. V zimah z netipičnim vremenom cvetni brsti niso bili sposobni doseči genetsko pogojene odpornosti na mraz.

Ključne besede: *Malus x domestica*; preiskusi z umetnim zmrzovanje; LT<sub>50</sub> vrednosti; dormanca; cvetni brsti

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# 1 INTRODUCTION

Apple is the most important temperate zone fruit, with high economic importance worldwide. The success of apple production is influenced by many factors, one of them is the risk of frost damages. Attention was drawn to this fact in early literature (Modlibowska, 1946; Childers, 1949; Chandler, 1954). There are growing districts, especially in the colder parts of temperate zone, where low temperatures often cause damages to the overwintering organs of apple trees during the dormancy period, hence the knowledge of the winter frost tolerance of the cultivars is especially important in those regions. For example, in the northern parts of the United States millions of apple trees died off in November 1955 and December 1964 due to severe temperature drops. Significant apple tree decay occurred in Poland in the winter of 1986/87 because of persistent frost. Low temperatures have caused significant tree destruction on several occasions in apple orchards of Canada as well (Coleman, 1992; Palmer et al., 2003; Tóth, 2013). The forecasting models of global warming predict more frequent occurrence of extreme climatic conditions, including extraordinary temperatures, which may affect the phenological processes and tolerance of our plants against frost and other environmental factors. It should be stated that frost damage is becoming more common in apple orchards (Eccel et al., 2009; Kaukoranta et al., 2010; Vitasse et al., 2018).

Frost hardiness of generative organs can be studied by field surveys, artificial freezing tests, and indirect laboratory methods (Palonen & Buszard, 1997; Lindén et al., 1999; Palmer et al., 2003). The differences between cultivars and frost tolerance at the given developmental stage could be observed with filed assessments after severe cooling. The frost hardiness of overwintering organs is changing gradually. The process of this changing could be studied by frequent freezing tests. Generative buds are the most frost-sensitive parts of apple trees in winter. Flower buds are the most frost resistant at the end of their endodormancy period, sometimes they tolerate temperatures even below -30 °C, while in the flowering period, temperature only a few degrees below freezing point may be critical. The pistils are the most frost sensitive parts of the flower buds, but other organs are often damaged in low temperatures as well (Holubowicz, 1982; Warner, 1982; Nybom, 1992; Lindén et al., 1999; Lindén, 2002; Rodrigo, 2000; Palmer et al., 2003; Tóth, 1982, 2004; 2013; Soltész, 1988; Lysiak et al., 2016; Tudela & Santibanez 2016).

There are results of frost hardiness of vegetative organs of apple cultivars measuring by field observations and artificial freezing tests as well, the vegetative organs usually survive lower temperature than generative organs (Quamme et al., 1973; Ashworth et al., 1988; Lindén et al., 1996; Palmer et al., 2003; Cline et al., 2012; Pramsohler et al., 2012; Ozherelieva & Sedov 2017).

Differential thermal analysis, electrical impedance spectroscopy, infrared spectroscopy, electrolyte leakage measurement as indirect laboratory methods can provide information about the differences between genotypes from the aspect of frost hardiness of them (Quamme, 1976, 1991; Quamme et al., 1972, 1982; Ceccardi et al., 1995, Kang et al., 1998; Pearce, 2001; Salazar-Gutiérrez et al., 2016; Wu et al., 2019; Yu & Lee 2020; Kaya et al., 2020).

In our study the frost hardiness of flower buds of eight apple cultivars was observed with artificial freezing tests during four subsequent dormancy periods in Hungary. The studied cultivar assortment contained standard commercial cultivars, Hungarian and foreign resistant cultivars as well.

### 2 MATERIALS AND METHODS

The research work was carried out in the experimental orchard of the Department of Pomology HUALS (predecessor SZIE), which is located on the outskirts of Budapest, in Soroksár. The studies were conducted during four consecutive dormancy periods, from September 2016 to April 2020. The cultivars included in the study were as follows: 'Artemisz', 'Cordelia', 'Hesztia' and 'Rosmerta', four multi-resistant (mainly scab-resistant) cultivars from the breeding programme of our Department (Tóth et al., 2012). 'Prima' and 'Florina', two scab-resistant cultivars from earlier foreign breeding programmes. 'Idared' and 'Gala', two standard commercial cultivars. Thus, a total of 8 cultivars were examined in 4 consecutive test seasons. The studies were performed in the middle of each month from September to March. The last test date for each cultivar was the onset of flowering, which fell to April. In the experimental plantation, the trees were planted with a row and tree spacing of 4 x 2 m in 2007. The growing system is slender spindle. The plantation incorporates integrated cultivation technology, with regular maintenance pruning, manual fruit thinning, nutrient replenishment, drip irrigation and integrated plant protection every year.

The artificial freezing experiments were performed in the climate chamber Rumed 3301 (Rubarth Apparate GmbH, Laatzen, Germany), according to the method and protocol developed on the Department of Pomology (Szalay et al., 2018, 2019). Four or five appropriate freezing temperatures were used at each testing date, according to the rate of dormancy. Both cooling and warming were performed at a rate of 2 °C/h and shoots were kept

at the given freezing temperature for four hours. Five branches were used for all of treatments, each of them with 20-25 flower buds. Every branch was one repetition for statistical analysis. After freezing, all branches were kept at room temperature until analysis. The flower buds were cut longitudinally, and the frost damage was determined based on the rate of discoloration of tissues (green - unharmed, brown - frost damaged) on a numerical scale from 0 % to 100 %, respectively. Based on the results of artificial freezing treatments the LT<sub>50</sub> values (mean value of frost hardiness) were determined in each sampling dates. LT<sub>50</sub> means the temperature causing 50 % frost damage in the flower buds of the certain cultivar in the certain time. The  $LT_{50}$  was calculated using a linear regression model, assuming that the section of the hardiness sigmoid curve between 20 % and 80 % can be regarded as linear (Gu, 1999). Mean and standard deviation values were calculated during the statistical analysis, and two-way analysis of variance with replicates was performed with Microsoft Excel 365 programme.

### 3 RESULTS

The frost hardiness of flower buds of observed cultivars is characterized by  $LT_{50}$  values. The results of four years experiment are demonstrated on the Figure 1. The process of the changing of frost tolerance can be divided into two periods. Until January the frost hardiness of flower buds increased gradually, it was the hardening period. After it, the generative buds lost their frost hardiness gradually, it was the dehardening part of this process. The rate of hardening and dehardening was not the same in different years, because of different climatic conditions, but the frost hardiness profile of the observed cultivars during dormancy, and the sequence of cultivars from the aspect of frost tolerance in different sampling dates, both were very similar. It was not consistent the changing of LT<sub>50</sub> values during the hardening period. After the initial fast decreasing, the changing slowed down until a certain point, after it this process was accelerated again, until the lowest value of LT<sub>50</sub>. So, the hardening period can be divided into two phases as well. The first stage took place at temperatures above freezing. The start of the second, accelerated phase was when the ambient temperature was continuously below freezing point. In the second half of the winter, dehardening also took place at different rates each year due to different climatic conditions. Based on our results, it can be stated that the survival of the flower buds of the studied apple varieties was ensured by the current frost tolerance formed during their hardening and hardening processes in the four test seasons. During the winter, even the most sensitive cultivars did not experience frost damage. There was an intensive cooling in early April of 2020 with -9 °C, this arrived at the end of the deherdening period, just before



**Figure 1:** The LT<sub>50</sub> values of the flower buds of the studied apple cultivars determined by artificial freezing tests (below) and the daily maximum and minimum ambient temperatures in the experimental orchard (above) in 2016/17 (a), 2017/18 (b), 2018/19 (c) and 2019/20 (d) test season

the blooming time of apple trees. It caused a severe frost damages in the generative organs.

The differences between the cultivars were smaller in the autumn and spring months, and higher during the three winter months (December, January and February). Therefore, differences between cultivars are evaluated based on the test results of these three months of four years (Fig. 2.).

Based on the statistical analysis, the studied varieties can be divided into three groups. The most frost sensitive group includes 'Prima', 'Cordelia' and 'Idared'. The most frost tolerant were 'Florina' and 'Gala' according to our study, while 'Artemisz', 'Rosmerta' and 'Hesztia' belonged to the group of medium frost tolerant. In the fouryear studies, the frost resistance of the flower buds of the cultivars did not reach the genetically possible maximum value in each year. This is analyzed based on the January measurement results, as this month was the most frost tolerant period in each year. At this geographical location, during the four-year study, the flower buds of the studied varieties reached the most frost-resistant values in 2018 and partly in 2017 (Fig. 3.).

Further studies are needed to determine whether these values are genetically encoded maximum values. During the study period, flower buds were least hardened in 2020. There was a difference of 1.5-2.6 °C between the  $LT_{50}$  values of the years with the best and the weakest frost resistance, depending on the cultivar. In 2019 the frost resistance of flower buds was between the extreme values.

### 4 DISCUSSION AND CONCLUSIONS

There is a certain amount of information in the literature about frost hardiness of apple cultivars. The lowest critical temperatures were determined on the basis of the frost damages of vegetative organs and the cultivars were classified into frost tolerance groups based on these results. Usually four groups were created from tender to very hardy (Forsline, 1983; Friedrich & Fischer 2000; Palmer et al., 2003; Tóth, 2013). Generative organs are more sensitive to frost than vegetative organs, so their examination is also very important. Flower buds and flowers are vital organs for fruit trees, they will produce the fruit, it is also very important to know their frost tolerance. In addition, the frost resistance of vegetative and generative organs is often not closely related (Westwood, 1993; Palonen & Buszard 1997; Palmer et al., 2003; Tóth, 2013). In many places of production, strong cooling also occurs during the flowering period, so it is worthwhile to study the frost tolerance of the cultivars during this period as well. The more advanced the flowering, the more

sensitive the flower organs (Palmer et al., 2003; Aygün & San 2005; Szalay et al., 2019).

Photoperiod and temperature are key environmental factors in cold acclimation of apple trees (Howell & Weiser 1969; Faust, 1989; Tromp, 2005; Heide & Prestrud 2005; Wu et al., 2019). During the dehardening period the temperature is the most important environmental factor affecting the frost hardiness of overwintering organs (Tromp, 2005; Quinones et al., 2020). Frost tolerance of trees is also influenced by numerous other factors, such as the cultivar, the rootstock, the cultivation system, the cropping technology, the health status of the trees, the characteristics of the geographical location (Westwood, 1993; Faust, 1989; Janick & Moore 1996; Palmer et al., 2003; Tóth, 2013). Due to all these, there are large differences in the development of frost tolerance between cultivars, production sites and years.



**Figure 2:** LT<sub>50</sub> values of flower buds of the studied apple cultivars in December, January, and February, averaged over the four study years; The columns show the mean values, the lines the standard deviation, and the letters the homogeneous groups, the different letters indicate significantly ( $p \le 0.05$ ) different values



**Figure 3:**  $LT_{50}$  values of flower buds of the studied apple cultivars in mid-January in the four study years; the values of 2018 (normal numbers) and 2020 (italics) are shown; the lines represent the standard deviation

In the present experimental work, the frost tolerance of flower buds of eight apple cultivars has been investigated by artificial freezing method for four consecutive years in our experimental plantation, in Central Hungary. The trees stand on the same rootstock and have received the same cultivation technology. Thus, we were mostly able to establish the differences between the cultivars. In addition, we were able to describe the course of the change in frost resistance as the tests were performed monthly during the winter dormancy periods. The four years offered only a limited opportunity to determine the impact of environmental factors and years. However, restricted conclusions can be drawn from the differences between the years, based on our results.

We have data on the frost tolerance of the vegetative and generative organs of the most important apple cultivars grown in Hungary based on field studies (Zatykó, 1986; Tóth, 1982, 2004, 2013; Soltész, 1988; Soltész et al., 2010; Dremák, 2011). However, resistant cultivars recently introduced into cultivation were not included in these studies. The international literature contains data about frost resistance of different apple cultivars. The lowest survival temperatures were determined with the observation of the frost hardiness of vegetative organs, trunk, branches, twigs (Ashworth et al., 1988; Lindén et al., 1996; Palmer et al., 2003; Cline et al., 2012; Pramsohler et al., 2012; Ozherelieva & Sedov 2017). For example, the lowest survival temperature of 'Gala' was -35.6 °C, in the case of 'Fuji' it was -37.7 °C, while vegetative tissues of 'Jonagold' survived just -31.2 °C. There is limited information about the frost hardiness of generative organs, but general conclusion is, the lowest survival temperature of them is higher (frost hardiness is weaker) than vegetative organs (Lindén et al., 1999; Lindén 2002; Pramsohler & Neuner 2013; Lysiak et al., 2016; Salasar-Gutiérrez et al., 2016; Tuleda & Santibanez 2016; Ozherelieva & Sedov 2017).

In our present work the frost hardiness of flower buds of eight cultivars was determined with artificial freezing tests. From the studied assortment 'Florina' and 'Gala' belonged to frost hardy category, 'Artemisz', Rosmerta' and 'Hesztia' belonged to the middle frost-tolerant category, while 'Prima' 'Cordelia' and 'Idared' represented frost sensitive cultivars.

In our previous work the frost hardiness of flowers of some apple cultivars was determined during blooming time (Szalay et al., 2019).

The frost resistance of the vegetative organs of apple trees develops in two stages in autumn, so the hardening period of them can be divided into two stages. The first stage takes place at temperatures above freezing, but the second stage requires permanently low temperatures. It has been experimentally demonstrated that in the absence of low temperatures vegetative overwintering organs cannot harden properly (Howell & Weiser 1970; Palmer et al., 2003; Wu et al., 2019). The functioning of the generative organs is similar. In our earlier work, the role of low temperature in the hardening of peach flower buds was experimentally confirmed (Szalay et al., 2010). Our present experimental results suggest this for apple cultivars as well. In the first part of frost tolerance profile of flower buds of apple cultivars a breaking point is observed, after which hardening continues at persistently low temperatures. The role of temperature in hardening is also indicated by the fact that the LT<sub>50</sub> values of the flower buds of the studied apple cultivars were different from year to year in January. In case of unfavorable weather, the genetically possible level was not reached. Dehardening of flower buds also took place at different rates in the study years, which suggests the role of temperature in this process as well. Further studies are planned to better understand the role of environmental factors in hardening and dehardening of apple overwintering organs.

It is difficult to compare our results with previous research results, as such a systematic study of the frost resistance of flower buds has not yet been performed. Research results suggest that there is no strong correlation between frost resistance of vegetative and generative organs. For this reason, it is worth examining the vegetative and generative organs separately. Frost tolerance of vegetative organs of 'Idared' is good, but the flower buds and flowers of it are very sensitive to frost during dormancy (Mittelstadt & Murawski 1975; Soltész et al., 2010; Tóth, 2013; Szalay et al., 2019). Frost sensitivity of 'Idared' flower buds is confirmed in our present work as well. Vegetative organs of 'Gala' is moderately tender (Palmer et al., 2003; Tóth, 2013), the flower buds of this cultivar have good frost tolerance during dormancy according to our experimental results.

Knowledge of frost hardiness of cultivated apple varieties in different phenological stages is important for estimation of suitability of them for growing sites. In this area of research, field studies and artificial freezing experiments together can give good results. Our experimental work provides new information about the second one.

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