Frost hardiness of flower buds of three Hungarian almond cultivars during dormancy

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Abstract: Frost hardiness of flower buds of three Hungarian almond cultivars ('Tétényi Bőtermő', 'Tétényi Kedvenc', 'Tétényi Keményhéjú') was investigated by artificial freezing tests during ten dormancy periods. LT₅₀ values were calculated after artificial freezing treatments on different temperatures. Based on the results of regular observations, the frost hardiness profile of three cultivars has been described in each dormancy period. Frost tolerance was significantly affected by year and genotype. The potential frost tolerance of cultivars in our geographical location, in the middle of Hungary, has been characterised by LT_{50} values in January 2017, as the best values of them. Flower buds of 'Tétényi Keményhéjú' were the most frost hardy, its LT₅₀ in this sampling date was -20.5 °C, 'Tétényi Bőtermő' was the most sensitive (LT₅₀: -17.6 °C), while 'Tétényi Kedvenc' showed intermediate frost hardy (LT₅₀: -19.1 °C). Nevertheless, in mild winters the cultivars were unable to reach their genetically potential maximum frost hardiness. Hungary is situated at the northern part of almond growing area, so frost tolerance of flower buds is one of the most important traits of cultivars. Based on the results of artificial freezing tests the best cultivars can be selected from the aspect of crop safety.

Key words: *Prunus dulcis*; frost tolerance; generative organs; LT_{so} values; artificial freezing tests Odpornost na mraz treh madžarskih sort mandljevca v obdobju mirovanja

Izvleček: V raziskavi je bila z umetnim zmrzovanjem preučevana odpornost na mraz cvetnih brstov treh madžarskih sort mandljevca ('Tétényi Bőtermő', 'Tétényi Kedvenc', 'Tétényi Keményhéjú') v obdobju mirovanja. Vrednosti LT₅₀ so bile izračunane po obravnavanjih z umetnim zmrzovanjem pri različnih temperaturah. Na osnovi rezultatov rednih opazovanj je bil opisan profil odpornosti na mraz treh sort za vsako obdobje mirovanja. Na toleranco na mraz sta značilno vplivala leto poskusa in genotip. Potencial tolerance na mraz vseh treh sort v geografskem območju osrednje Madžarske je bil najboljše opredeljen z vrednostmi LT₅₀ pridobljenimi januarja 2017. Cvetni brsti sorte 'Tétényi Keményhéjú' so bili najbolj odporni na mraz, njihova LT₅₀ je bila na ta vzorčni termin, -20,5 °C. Sorta 'Tétényi Bőtermő' je bila najbolj občutljiva (LT₅₀: -17,6 °C) med tem, ko je sorta 'Tétényi Kedvenc' izkazala srednjo odpornost na mraz (LT₅₀: -19,1 °C). V milih zimah preikušene sorte niso mogle doseči največjega genetskega potenciala največje odpornosti na mraz. Madžarska se nahaja na severnem robu uspevanja mandljevca, zato je odpornost cvetnih brstov na mraz ena od najpomembnejših sortnih lastnosti. Na osnovi rezultatov poskusov umetnega zmrzovanja bi lahko izbrali najboljše sorte z vidika varnosti pridelkov.

Ključne besede: Prunus dulcis; toleranca na mraz; generativni organi; LT_{50} vrednosti; preiskusi z umetnim zmrzovanjem

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1 INTRODUCTION

Frost sensitivity of cultivars is the most important limitation factor of almond production in Hungary (Kállayné, 2003, 2014). Flower buds are the most frost-sensitive overwintering organs of almond trees. They can suffer frost damages during winter and early spring because of low temperatures. Frost tolerance can be tested by various indirect and direct methods. Indirect methods, such as ion leakage observation (Werner et al., 1993; Afshari et al., 2011; Dumanoglu et al., 2019), water, starch and sugar content measurement (Zayan, 1981; Burak & Eris, 1992; Bolat, 1995), or differential thermal analysis /DTA/ (Quamme, 1974, 1978; Proebsting & Sakai, 1979; Faust, 1989; Kang et al., 1998; Kaya et al., 2018, 2019, 2020) are suitable just for estimation of frost hardiness. The direct methods are those used to examine the actual frost damage of plant parts after they have been exposed to low temperatures. This can be a natural frost damage survey or an evaluation of the results of artificial freezing experiments (Tromp, 2005).

There are data of frost hardiness of flower buds of related species of almond based on field studies - such as peach (Szabó & Nyéki, 1988, 1991; Nyéki & Szabó, 1989; Szabó et al., 1998; Szalay, 2001; Szabó, 2002; Miranda et al., 2005; Szalay et al., 2010) and apricot (Szabó & Nyéki, 1991; Szalay, 2001; Miranda et al., 2005). Results of artificial frost treatments of peach (Proebsting, 1970; Proebsting & Mills, 1978; Szalay, 2001), and apricot (Pedryc et al., 1999; Szalay, 2001; Szalay et al., 2006), are available as well. However, only a few studies have addressed the frost resistance of almonds, and the information published is primarily about the spring frost resistance of flowers. Viti et al. (1994) examined frost sensitivity of almond flowers at different phenological stages during blooming time. Based on their experiences, cultivars with late flowering time had higher frost resistance, even if their flowers were in advanced phenological stages. A similar study was published by Snyder and Conell (1996) on the frost tolerance of flowers and fruitlets of Californian almond cultivars. Pink flower buds of the varieties Sonora and Price were less sensitive, they suffered only 30 % frost damage at -5° C, while another seven varieties had higher frost damage. In the case of these two varieties, the open flowers were also more frost tolerant: while 100 % flowers damaged at -3 °C frost of other varieties, critical T was -4.5 and -5.5 °C in a case of Sonora and Price cultivars. Likewise, the differences between several varieties and between various flowering-phenological stages were investigated by Sepahvand et al. (2014). Frost tolerance of almond cultivars and hybrids were tested in different phenological stages during blooming time by field observation and laboratory methods in Iran. There were big differences between genotypes and sampling dates from the aspect of frost hardiness (Imani et al., 2012). In Spain 12 commercial almond cultivars were observed, and the tolerance of flowers to frosts was evaluated by chlorophyll fluorescence after artificial freezing (Kodad et al., 2010). Miranda et al. (2005) examined cultivars 'Marcona' and 'Ferragnes' by artificial freezing during the ecodormancy period. The critical temperature for frost tolerance of flower buds was -16.3 °C. These studies do not track the frost resistance of flower buds during the whole dormant period, but they give only a snapshot of frost tolerance.

Late flowering and frost hardiness are important breeding aims, because almond may be affected by frost due to its early flowering even in subtropical climates (Daneshvar & Sardabi, 2006; Dicenta et al., 2011; García-Gusano et al., 2011; Imani & Mahamadkhani, 2011; Imani et al., 2011, 2012; Moheb et al., 2018). The results of physiological and genetic research can be of great help in this work. Karimi et al. (2016) identified small RNAs that play a role in frost tolerance of reproductive organs in almond. Hosseinpour et al. (2017) identified a cold-shock protein in a frost tolerant genotype which plays role in frost resistance.

In the present study, frost hardiness of the flower buds of three Hungarian almond cultivars were investigated for 10 years (selected between 2004 and 2019) by regular artificial freezing tests during dormancy periods with the aim of determination of frost hardiness profile of them. In our article, the results of this study are presented.

2 MATERIALS AND METHODS

Samples were taken from the cultivar collection of the Department of Pomology, Institute of Horticultural Science, HUALS, Budapest. Hungarian cultivars, 'Tétényi Bőtermő', 'Tétényi Kedvenc', and 'Tétényi Keményhéjú' were examined. Six trees of each cultivar were included into examinations. Almond trees, grafted on almond seedling rootstocks were planted in the experimental orchard in 1992, at a row and tree spacing of 6 x 4 meters. The growing system is free vase. Integrated cultivation technology is taking place in the plantation without irrigation.

Investigations were carried out in the dormancy period of the following years: 2004/05, 2005/06, 2006/07, 2007/08, 2010/11, 2013/14, 2014/15, 2015/16, 2016/17, 2018/19. Experimental work began in early September each year and continued until next spring. 7-9 low temperature-treatments were performed each winter. The last test has been done just before flowering. The experiments were performed in a Rumed 3301 (Rubarth Apparate GmbH, Laatzen, Germany) climate chamber, using a method previously developed by the department (Szalay et al., 2010, 2016, 2017). Each time, 3 or 4 freezing temperatures were applied with a difference of 2 or 3 °C. In order to determine the LT_{50} values (the temperature at which 50 % of the flower buds were damaged) the treatment temperatures were chosen that all cultivars should get frost damage below as well as above 50 %. In the chamber initial room temperature was reduced by 2 °C/h and the samples were kept at the desired freezing temperature for 4 h, after which the temperature was raised by 2 °C/h. After 12 hours at room temperature, the percentage of frost damage was determined by cutting the flower buds in half lengthwise and observing the discoloration of the inner tissues. Five twigs from each cultivar per treatments were put into the climate chamber, where one twig with 30-40 flower buds was considered as a replication for the statistical analysis. Based on the experimental results, the LT₅₀ values of each cultivar were determined by linear regression, assuming a linear relationship between the treatment temperature and the percentage of frost damage in the range of 20 % and 80 % (Gu, 1999). The mean and standard deviation of five replications were calculated. Based on the calculated values, the flower bud frost hardiness profile of each cultivar was outlined between 1st of September and 1st of April for each year, characterized by LT₅₀ values. Frost hardiness profile of the observed cultivars in averaged of 10 years was determined as well. Due to different sampling times in different years, LT₅₀ values were calculated by interpolation from adjacent data in the middle of the months. During the experiments daily minimum and maximum temperatures in the almond orchard were recorded by a local automatic meteorological station. The statistical analysis was performed with Microsoft software, Excel 365 programme. Normality of the error term was proven subsequently by Komogorov Simrnov or Shapiro-Wilks `test (p > 0.05). Pair wise comparisons were run by Tukey's post hoc test. For determining year and genotype effects the ANOVA method was applied.

3 RESULTS

The frost hardiness profiles which show changes in frost resistance of the studied cultivars throughout the dormancy period were determined based on the LT_{50} values of the flower buds. Data for three highlighted winters and ten-years average are shown in Figures 1-4. The profiles can be divided into two parts. The first part is the hardening, when the frost hardiness of flower buds gradually increased, lasted until December or January, depending on the year. The second part is the dehardening, when the flower buds have gradually lost their frost tolerance. There were significant differences between the years in the change of frost hardiness due to different weather conditions.

Data for the dormancy period 2006/2007 are shown in Figure 1, which was the mildest winter during the studied period. There were nine sampling times during



Figure 1: Daily maximum and minimum ambient temperatures, and LT_{50} values of flower buds of three almond cultivars observed based on artificial freezing tests in 2006/07 winter

this season. At the beginning of September, the LT₅₀ values of the flower buds of the examined cultivars were between -2.2 °C and -4.4 °C. Then, until the second half of December, the frost tolerance of the flower buds has been increased. The highest one was measured on 22 December, when the LT₅₀ value of 'Tétényi Bőtermő' was -14.9 °C, while -16.0 °C for 'Tétényi Kedvenc', and -17.9 °C for 'Tétényi Keményhéjú' were detected. In the second half of winter, the frost resistance of flower buds decreased rapidly because of high temperatures. Flowering was very early this year, in early March. The final sampling date was just before blooming time, when the LT₅₀ values were between -3.2 °C and -5.1 °C. During this winter there was no natural frost in the orchard, but during the flowering period, low temperatures caused minor damages.

Figure 2 shows the results of the 2013/14 winter. During this season, when the weather was moderate, 8 sampling dates were applied. In early September, the frost tolerance of flower buds was similar to the year presented earlier. Later, frost tolerance increased until mid-December and the differences between cultivars were more pronounced at this time. At the sampling date of 15 December, the LT_{50} of flower buds of the examined cultivars were between -16 °C and -18.6 °C. The most frost hardy was 'Tétényi Keményhéjú', while 'Tétényi Bőtermő' was the most sensitive. In the second half of winter, the frost resistance of flower buds decreased gradually. Cold weather at the end of January and early February caused natural frost damages, however not all flowers were damaged, so we could continue our studies. The flowering

time in 2014 started on 10 March. Based on the results of the climate chamber tests at this time, the LT_{50} values were between -2 °C and -3 °C.

The third dormancy period, the results of which are presented in detail, was in 2018/19. It was the coldest of the winters studied. Eight sampling dates were applied (Figure 3). At the first sampling date, in early September, the frost resistance values of flower buds were similar to the other two years introduced earlier, the LT_{50} values ranged between -2.9 °C and -4.8 °C. Then, frost resistance values as well as differences between the cultivars were increased. This winter, the highest cold hardiness values were measured in mid-January, followed by a slow gradual decline in frost tolerance. On 15 January, the LT₅₀ value of the flower buds was -17.6 °C for 'Tétényi Bőtermő, -19.1 °C for 'Tétényi Kedvenc', and -20.5 °C for 'Tétényi Keményhéjú'. At the beginning of January, due to the low temperature, there were a natural frost damages in our experimental orchard, but it did not endanger our investigations. In the second half of winter, due to the persistently low temperatures, the decrease in frost tolerance of flower buds was slower than in the other two years, and the flowering started late, after 20 March. Just before flowering, according to the results of the climate chamber studies, the LT_{50} values were between -2 °C and -3.2 °C.

Based on the results of ten years, the average flower bud cold hardiness profile, calculated as a 10-year LT_{50} average, was determined for the studied cultivars. These values show the expected frost resistance of these cul-



Figure 2: Daily maximum and minimum ambient temperatures, and LT_{50} values of flower buds of three almond cultivars observed based on artificial freezing tests in 2013/14 winter



Figure 3: Daily maximum and minimum ambient temperature, and LT_{50} values of flower buds of three almond cultivars observed based on artificial freezing tests in 2016/17 winter



Figure 4: Averages of maximum and minimum daily temperature, and averages of LT₅₀ values of flower buds of three almond cultivars observed during ten-years winter dormancy

tivars in our geographical location in a common year. Because of technical reason the sampling dates were not the same days in different years, the characteristic points of frost hardiness profiles (in the middle of each month) were calculated by interpolation (Figure 4). In the middle of September average LT_{50} values varied between -5.4

°C and -7.3 °C and parallel with decreasing temperatures frost tolerance of flower buds were increased, first faster, then slower. In the middle of December LT₅₀ values were -15.3 °C (\pm 1.83 °C) for 'Tétényi Bőtermő', -16.4 °C (\pm 1.91 °C) for 'Tétényi Kedvenc', and -17.8 °C (\pm 2.18 °C) for 'Tétényi Keményhéjú' in average of the years. It means

significant difference between 'Tétényi Bőtermő' and 'Tétényi Keményhéjú' (p > 0.05), but no significant difference between 'Tétényi Bőtermő' and 'Tétényi Kedvenc', and no significant difference between 'Tétényi Kedvenc' and 'Tétényi Keményhéjú'.

By the middle of March average LT $_{50}$ values have decreased to -2.7 °C, -3.5 °C, and -4.5 °C respectively.

The frost hardiness profile of the examined cultivars, which was characterized by the LT₅₀ values of flower buds, was different each year. This was due to differences in environmental factors, especially temperature. In all ten years the daily maximum and minimum temperatures showed great daily fluctuations in our experimental station, and the differences between years were also remarkable. The flower buds of the observed cultivars did not reach the genetically programmed maximum frost tolerance each year. The best frost tolerance was expressed in the coldest winter (2016/17), when the daily minimum temperatures dropped below zero after 1 November, and except for a few milder periods, it remained there until the end of February (Figure 3). The daily minimum temperatures stayed below -5 °C for long periods, and even temperatures below -10 °C frequently occurred. In that winter the best frost hardiness was measured in January. The situation was quite similar in the winter of 2015/16 and 2018/19, but in all of other winters the hardening period lasted earlier, in December, and during January the decreasing of frost tolerance was detected. The genetically potential maximum frost hardiness of flower buds of studied cultivars in our geographical location, and the expected values under different weather conditions were calculated based on the best LT_{50} values of certain years (Figure 5). The statistical analysis shows significant differences between years from this aspect. If the autumn temperatures are decreasing gradually, and sub-zero temperatures are lasting, then slow increasing of temperature is detected, and there are no great fluctuations, LT_{50} of flower buds can be -17.5 °C for 'Tétényi Bőtermő', -19 °C for 'Tétényi Kedvenc', and -20.5 °C for 'Tétényi Keményhéjú' in the middle of winter. But in extremely mild winters, with temperature fluctuations, just LT_{50} values between -14.5 °C and -16 °C can be expected in these cultivars under our geographical location.

4 DISCUSSION

Almond production is limited by ecological conditions in Hungary. Winter and spring frosts mean the biggest risks. Unfortunately, the Hungarian variety descriptions do not address the issue of frost resistance (Brózik, 1998; Brózik et al., 2003; Apostol, 2013). There is little data on the frost tolerance of almond cultivars in the international literature as well. Some research works on almond have been dealing with frost resistance of flowers in different phenological stages or fruitlets during spring (Viti et al., 1994; Snyder & Conell, 1996; Kodad et al., 2010; Sepahvand et al., 2014), others have observed the



Figure 5: LT₅₀ values of flower buds of the studied almond cultivars in the middle of winter of different years (2004-2019); The columns show the mean values, the lines the standard deviation, and the letters the homogeneous groups, the different letters indicate statistically significant ($p \le 0.05$) different values

frost hardiness of overwintering organs during dormancy (Szalay & Fonai, 2002; Miranda et al., 2005). The experimental results are difficult to compare because other cultivars, and different years were studied in different production sites. As general conclusion, however, it was shown that there are big differences between genotypes, and the ecological conditions have significant effect on the frost resistance.

The present paper is the first report about changes in frost hardiness of flower buds of Hungarian almond cultivars during the whole dormancy period. Summarising the results of ten years, frost tolerance of cultivars has varied over the years. In all years studied 'Tétényi Bőtermő' proved to be the most sensitive and 'Tétényi Keményhéjú' was the most tolerant, value of 'Tétényi Kedvenc' could be positioned in between them. In the first half of winter cold hardiness of overwintering organs developed progressively and reached their maximum in December, or, in some cases in January. Then their frost tolerance decreased until spring. Frost hardiness profile of the cultivars has been characterised by the LT₅₀ values of flower buds, calculated based on artificial freezing tests. Differences in frost resistance of cultivars were less representative in September and around flowering, however, the most considerable differences were detected in December and January, by the time the maximum frost tolerance developed.

In each year, fluctuation of winter temperatures were observed. Hardening and dehardening processes of almond flower buds were largely affected by weather conditions, especially temperature. Due to differences among years, we can conclude that the more years are studied, the most accurate results can be achieved. On the basis of a ten-year experiment, we made similar conclusions like in the case of apricot and peach, where Szalay (2001) and Szalay et al. (2010, 2016) found out that a gradual decrease in temperature at the first part of winter and later permanent cold is required for developing frost hardiness of flower buds. If any of these factors are missing, the genetic potential of frost resistance of overwintering organs cannot be realised. Among the interpreted years in this paper, the mildest winter resulted the worst frost hardiness of almond, whereas in the coldest season the best frost tolerance profile could be achieved. For describing correlation between changing in temperature and frost tolerance application of statistical analyses are limited. It could be that plant physiological processes are controlled by the inner temperature of plants that is always different from outside temperatures. The other reason is that not only the temperature, but other abiotic factors (light conditions, precipitation, photoperiod, etc.) have effect on plant physiology, therefore on cold hardiness of overwintering organs. Nevertheless, climate change results often mild and fluctuating winter temperatures, which are not conducive to hardening processes. The expected average frost hardiness of a cultivar can be determined as an average of LT_{50} values of different years. In our case, based on 10-years observation, it is -15.3 °C for 'Tétényi Bőtermő', -16.4 °C for 'Tétényi Kedvenc', and -17.8 °C for 'Tétényi Keményhéjú'. The highest genetic potential of frost resistance has been determined, but due to the increasingly mild climate resulting from global warming, this will be less and less achieved by the cultivars. Therefore, it is very important to consider cold hardiness of the selected cultivars and the climate conditions of the growing site when designing an almond orchard.

5 CONCLUSIONS

Based on our results it is not recommended to establish an almond orchard in growing sites where winter temperatures regularly drop below -18 °C. From practical point of view it is important to have adequate information on the frost hardiness of almond cultivars that should be included into cultivar descriptions, our work hopefully could contribute to this aim. We can conclude that the growing site and the cultivar must be chosen very carefully when we want to establish an economically functioning plantation from almonds. Such a recommendation is an agreement with several publications dealing with different fruit species (Mohácsy & Porpáczy, 1951; Pejovics, 1976; Brózik et al., 2003; Kállayné, 2003, 2014; Di Lena et al., 2017).

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