The Thesis of the Ph.D. dissertation

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PHENOLOGICAL CHARACTERIZATION, EVALUATION OF CHILLING REQUIREMENT AND FROST HARDINESS OF ALMOND (*Prunus dulcis* Mill)

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1. SCIENTIFIC BACKGROUND AND AIMS

The primary gene centre of cultivated almond (*Prunus dulcis* Mill.) is in Asia Minor, mostly in arid, subtropical climates, where winters are mild. From here it is widespread and has long been cultivated in the temperate zone as well. However, due to its origin, one must count regularly with winter and spring frost damage in temperate zone countries, which greatly endangers crop safety. The sensitive organs are the flower buds that go through a special development from leaf fall until flowering.

The frost sensitivity of almond flower buds depends on the length of its dormancy period. Cultivars with short endodormancy period can suffer winter and spring frost much easily. Late flowering almond cultivars have long dormancy period and can escape spring frosts. Endodormancy is a period from autumn to mid-winter when flower buds are less sensitive to cold temperatures, however, bud development is continuous. The length of endodormancy has been determined by the chilling requirement of a certain cultivar. During the dormancy period, chill accumulation allows the gradual transition from flower bud endodormancy to flower bud ecodormancy, where subsequent heat accumulation controls flower bud development. When an almond tree gets into ecodormancy, the flower buds are more sensitive to frost and after having the appropriate amount of heat, they are ready to flower. In temperate climates, cultivars with fast flower bud development are highly exposed to the danger of winter frost. The reason for it is that the faster the flower bud development is the earlier the blooming date is and the more likely they are exposed to winter and spring frost (Hajnal et al., 2013; Szalay and Németh, 2010). In order to successfully cultivate temperate fruit such as almonds, it is important to understand the flower bud development rate, including the transition from endodormancy to

ecodormancy and finally to flower. Unfortunately, there is limited information available regarding almond bud development during winter related to their climatic adaptability. Also, there is a lack of a standard method for accurate identification of dormancy release, which makes it difficult to know if a given cultivar is adapted to a specific region based on its rate of phenological development or quantification of chilling and heat requirements for proper flowering.

According to the literature, little is known about climatic adaptability of the Hungarian commercial almond cultivars or accessions. HUALS Érd Elvira major hosts a gene bank collection together with popular Hungarian almond cultivars that represents a wide variability in flowering time. Therefore we decided to use it as a basis in order to analyse the Hungarian genetic resources and some Spanish cultivars known to have late flowering time in order to assess their climatic adaptability potential. Therefore we set our objectives as follows:

1. Describing the flower bud developmental process of almond cultivars, and then to find out the differences among almond cultivars and years in the speed of flower bud development that refers to their climatic adaptability
2. Determining the end of endodormancy breaking date using three biological methods. Selecting the right biological method that indicates end of endodormancy and
3. Describing differences among almond cultivars regarding their chilling and heat requirement that refers to their climatic adaptability
4. Modelling the changing of frost hardiness of almond flower buds during dormancy and assessing the potential best frost tolerance of them
5. Finding the correlation between chilling requirement and winter frost hardiness of almond accessions

6. Screening the frost susceptibility of flowers during blossom development together with observation of blooming time

2. MATERIALS AND METHODS

Plant material

Plant material was obtained from the genebank collection of the Fruit Research centre of Hungarian University of Agriculture and Life Sciences (HUALS), Érd Elvira. The experimental orchard was planted in 1996. The spacing is 7 x 3 meters, the orchard has no irrigation, and the trees are on GF-677 rootstock. Our collection includes Hungarian landraces and cultivars (old and novel). Among twenty-five accessions used five ('Budatétényi 70', 'Tétényi keményhéjú', 'Tétényi rekord', 'Tétényi bőtermő' and 'Tétényi kedvenc') are commercial almond cultivars widely grown in Hungary. The remaining twenty are landrace selections around the hills of Bakony collected in the 1960's.

Microsporogenesis studies

Flower bud development studies were conducted over three years in 2019/20, 2020/21 and 2021/22. Three twigs with one year old laterals were collected weekly from each accession every year. In the laboratory ten flower buds per accession were selected randomly. The anthers were removed by a tweezer, stained with carminic acetic acid and squash preparations were made for microscopic studies. The microspore development stage (archesporium, string, pollen mother cells, tetrad cells, microspores, pollen cells) of each microspore was recorded. The proportion of each stage was calculated by accessions and sampling dates. On the basis of weekly data we estimated when 50% of the stages occurred and this calendar date was regarded as the transmission date from one stage to another. An accession having at least 50% of their

flower buds in string stage regarded as reaching the end of endodormancy.

To evaluate the similarity of the cultivars based on their developmental rates of microsporogenesis, hierarchical cluster analysis (with squared Euclidean distance and Ward's agglomeration method) as well as K-means clustering were performed.

Pistil length measurements

Pistils of the ten flower buds per accession that were used in microsporogenesis studies were examined. The length of pistil was recorded on the microscope slide equipped with stage micrometer (Carl Zeiss, Germany), with the accuracy of 0.1 mm. The resumption of pistil growth after being constant was considered as endodormancy release.

Method of forcing

Another set of three sample twigs with 40–50 flower buds of each accession was collected along with the microsporogenesis and pistil method study. They were transferred to the laboratory and immediately placed in one-liter containers with 0.5l of water and forced to flower with a natural photoperiod reflected through the window at room temperature. After 10 days, the number of open flowers was counted, and the percentage of open flowers was calculated to the total number of flower buds. The date when accessions had 50% of open flowers was regarded as the end of endodormancy

The dates of endodormancy determined by the three methods (microsporogenesis, pistil length measurements and forcing twings) were compared using two-way MANOVA with factors 'year' and 'accession'. The statistical analysis was performed using R statistical program version 2.1 (R.CoreTeam, 2021).

Method of chilling and heat estimation

The beginning of endodormancy was considered when a regular chilling accumulation occurred that was indicated by natural leaf fall. The end to the endodormancy date was determined using the microsporogenesis method. Hourly temperature data were recorded by the meteorological station located at the study area and used for the calculation of chilling and heat requirements. The accumulated chilling was estimated as chilling unit using the Utah model (Richardson et al., 1974) and as chilling hour number using a chilling hour model (hours below <7.2˚C, (Weinberger, 1950). Heat requirements were calculated during the period between the dormancy breaking date and the full flowering date according to (Richardson et al., 1974) as growing degree hours (GDHs) by subtracting the base (b) temperature of 4.5 °C from the hourly temperature in degrees Celsius.

For the chilling unit, chilling hour number and the growing degree hour's requirements, the accessions were compared using one-way MANOVA for each year to detect significant differences ($p \leq 0.05$) between the mean values of the accessions of each year. Pairwise comparisons were run by Duncan's post hoc test. The year effect on the accumulated chill unit, chill hour and heat unit was compared separately. Correlation coefficients between chilling/heat requirement and flowering time were determined as Pearson correlation coefficients. The statistical analysis was performed using IMB SPSS25 statistical program.

Methods of frost hardiness study

Plant materials were performed as described at point 2.1. This study examined only 20 almond accessions. Investigations were carried out in the dormant period of the following years: 2016/17, 2017/18, 2018/19,

2019/20 and 2021/22. The experiment could not be carried out in the winter of 2020/21 due to technical reasons. In each dormancy season, the samples were collected 7 times, except the last winter, when there were six sampling dates.The experiments were performed in a Rumed 3301 (Rubarth Apparate GmbH, Laatzen, Germany) climate chamber, in the laboratory of Pomology Department, Hungarian University of Agriculture and Life Sciences. Each time, 4 or 5 freezing temperatures were applied with a difference of 2 degrees Celsius. In order to determine the LT50 values, the treatment temperatures were chosen that all accessions 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 scored by cutting the flower buds in half lengthwise and observing the discoloration of the tissues. Five twigs from each accession per treatments were put into the climate chamber where one twig with 40–60 flower buds was considered as a replication for the statistical analysis. Based on the experimental results, the LT50 values were determined by linear regression. Assuming the linear relationship between the treatment temperature and the percentage of frost damage in the range of 20% and 80%.

Based on the calculated values, the flower bud freezing tolerance profile of each accession was outlined during dormancy characterized by LT50 values. The potential frost resistance of the observed accessions was determined by variance analysis. For determining year and accessions effect the ANOVA method was applied using SPSS software. Finally, different homogeneous groups were performed based the on the best frost tolerance (LT50) value of the five tested years. Daily minimum and

maximum temperatures in the almond orchard were recorded by a local automatic meteorological station.

3. RESULTS AND DISCUSSIONS

The process of microsporogenesis

The results showed that flower buds of almond cultivars underwent the classical developmental stages of microsporogenesis as described in Materials and Methods.

In 2019/20, the development of the archesporial tissue ranged between 27 and 64 days from the 1st of November. The string stage, which was marked as the beginning to the microsporogenesis process lasted 18 to 23 days. The transition periods of the pollen mother cells (PMC) and tetrads were short; the anthers remained in the pollen mother cells stage from 6 to 10 days and likewise in the tetrads from 8 to 11 days depending on the almond cultivars. The microspore stage began around 30–45 days after the start of the microsporogenesis process. The transition of the microspore stage lasted between 37 and 44 days. The end of microsporogenesis (ecodormancy) was between March 9 and April 5. These indicated clearly that the process of microsporogenesis began around 90 to 100 days before flowering depending on cultivars. The cultivars differed in the developmental rate of microsporogenesis in particular in showing an important variation in the amount of time taken from the archesporium stage to differentiate into the string stage of microsporogenesis. At later stages of microsporogenesis, the transition periods became shorter, and the variation increased during the whole process of microsporogenesis.

In 2020/21, the period of the development of archesporial tissue to produce string cells was in most cultivars shorter compared to the first

year, in particular, in the early flowering types, such as accession ‘1/7 where the development of the archesporium stage was noted after 15 days from the establishment of dormancy. However, in the case of the latest two cultivars ‘Vairo’ and Constanti’, it remained almost the same. The string stage lasted 9 to 36 days, while the transition periods of pollen mother cells to tetrads and then tetrads to microspores lasted 6 to 13 and 8 to 9 days respectively. This means that the microspore stage began around 20–60 days after the start of the microsporogenesis process. The period of the development of microspores cells to produce pollen grain was relatively longer for most cultivars compared to the previous season. This stage lasted between 29 and 83 days. Consequently, the end of microsporogenesis was extended by 5 to 22 days as the start to blooming date was between March 22 and April 14.

In 2021/22, the speed of microsporogenesis was comparable to that of 2020/21. The development of the archesporial tissue ranged between 14 and 65 days during this year. However, the transition periods of the string stage were quite short for all the cultivars as they lasted between 5 and 8 days only. The transition periods of pollen mother cells and from tetrads to microspores lasted 4 to 8 and 8 to 9 days respectively. This explains that the microspore stage began around 17–24 days after the start of the microsporogenesis process. But the development of microspore cells to produce full pollen grains was much slower in this year compared to both the other years as it lasted between 50 and 90 days depending on cultivars. The pollen grains were noticed 19 to 20 days before the end of microsporogenesis as blooming started between March 21 and April 9. Similar to 2020/21, microsporogenesis began about 90 to 130 days before flowering this year.

Cultivars were classified based on developmental rates of microsporogenesis of all years studied and the dendrogram generated by hierarchical cluster analysis with Ward method is presented in Figure 1.

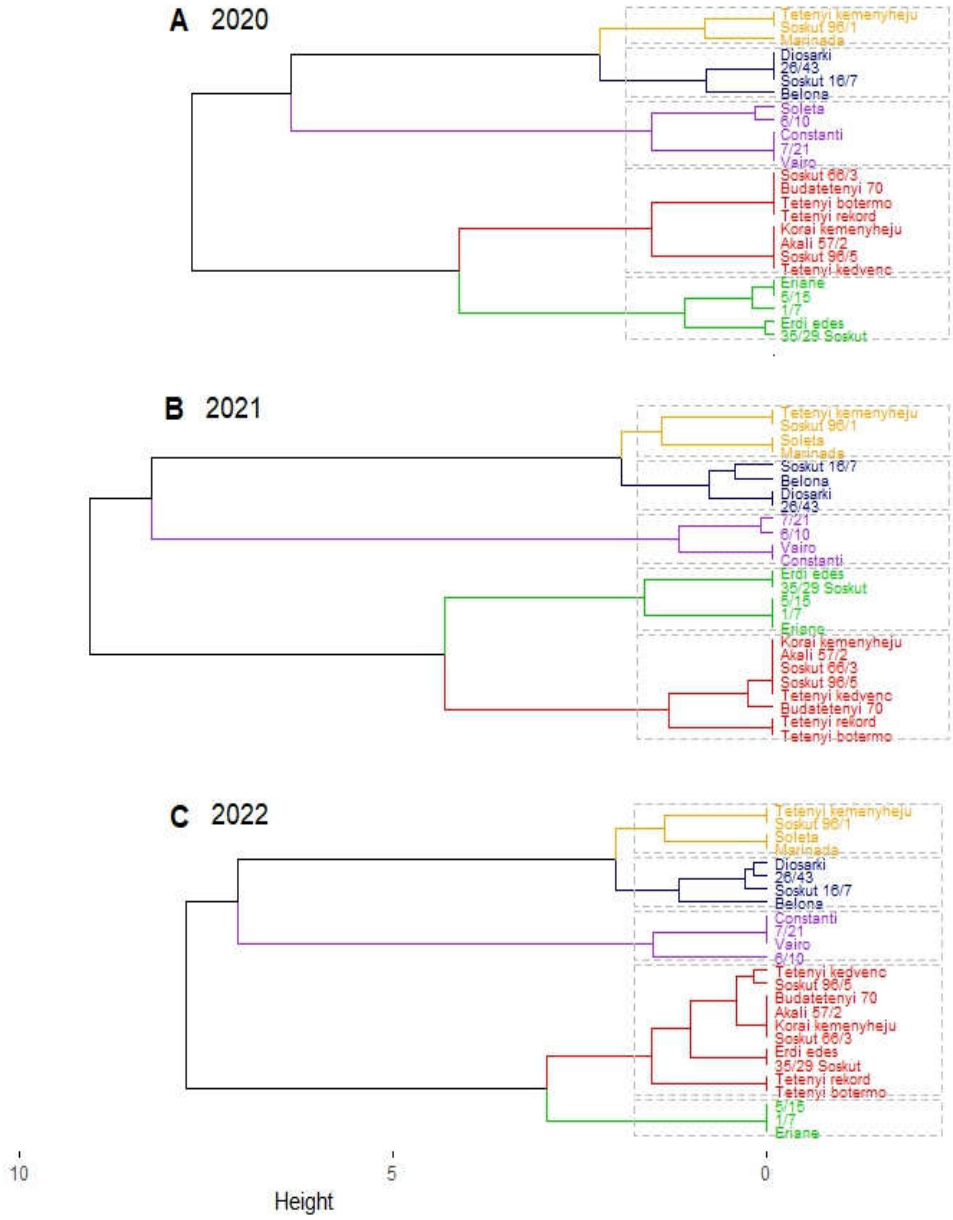

Figure 1. Dendrogram obtained by analyzing the developmental rate of microsporogenesis of almond cultivars

Pistil length measurements

At the beginning of the endodormancy phase pistil growth was arrested, the increment was not apparent, with an average length of 1 mm pistil growth resumed when archesporium tissues in the anthers of the flower buds differentiated into the string stage which was considered as a transition from endodormancy to the ecodormancy phase, first at a very slow increment followed by a few days of highly concentrated growth prior to blooming. This indicated that accumulation certain amount of cold required by a cultivar is a prerequisite for pistil growth resumption.

The accessions had considerable variations in their pistil growth rate particularly between the early and late types. The growth increment rate after resumption was rapid of the latest ones than those of the earliest.

Flower bud development under the forcing conditions

The developmental rates of bud shoots exposed to forcing conditions were clearly affected by accessions and yearly climatic conditions. The flower bud development overlapped into ten groups with slight differences of overlapping each year.

Flower buds of forced shoots began to present open flowers with the appearance of tetrads and presented around 50% open flowers with the appearance of microspore stage.

Connection between temperature conditions and flower bud development results

In the first analysis the effect of the year and cultivars were studied including all data on microsporogenesis, pistil length and forcing. The overall MANOVA resulted in significant cultivar and year effect (Wilk's lambda =0.006 and 0.023, respectively, both with $p<0.001$). The follow-

up univariate ANOVA revealed highly significant cultivar and year effect for all the three methods (cultivar: F (24; 48)>28.8; year: F (2; 48)>13.71, all with $p<0.001$).

Results of chilling and heat requirement calculation

Table 2 presents the calculated chilling requirements of the cultivars by the two methods (Utah model and chilling hours (CH) model) and heat requirements calculated according to the Utah model in each seasons analysed. Based on the data calculated, the almond cultivars presented here showed enormous diversity of chilling and heat requirements ranging from 285 CU /174 CH -893 CU/ 1092 CH and 3284-4857 GDH respectively. Cultivars showed variability in their cold and heat requirements between seasons as well. The two models were also compared as a source of variation for the estimation of chilling requirements and the differences between the two models were not found statistically significant ($p>0.056$).

Figure 2 shows the distribution of almond cultivars based on their chilling units and growing degree hours requirements. Cultivars with low chilling and heat requirements occupy the lower left section of the figure. While those with a high degree of both requirements occupy the top right top section. The cultivars 'Tétényi keményhéjú' and 'Sóskút 96/1' with high chill and medium heat requirements are in the right bottom section.

Table 1: Chilling and heat requirements of almonds in (2019/20, 2020/2021 and 2021/2022)

Cultivars	Chill units Utah (CU)	Chilling hours (CH) (<7.2°C) model	Heat requirements (GDH) b=4.5^{0}C
1/7	285^{a}	174^{a}	344^{a}
Eriane	285^{a}	174^{a}	344^{a}
5/15	285^{a}	174^{a}	344^{a}
35/29 Sóskút	378^{ab}	257^{ab}	341^{a}

Érdi édes	378^{ab}	257^{ab}	341^{a}
Korai keményhéjú	410^{ab}	353^{bc}	352^{ab}
Akali 57/2	410^{ab}	353^{bc}	360^{ab}
Sóskút 96/5	410^{ab}	353^{bc}	370^{ab}
Tétényi kedvenc	410^{ab}	353^{bc}	3571^{ab}
Sóskút 66/3	421^{ab}	393^{bc}	3525^{ab}
Budatétényi 70	426^{ab}	407^{bc}	3600^{ab}
Tétényi bőtermő	453^{bc}	464^{cd}	3775ab
Tétényi rekord	453^{bc}	464^{cd}	3562^{ab}
Belona	564^{cd}	601de	3833^{ab}
Sóskút 16/7	603^{d}	700^{ef}	4075^{ab}
26/43	626^{bd}	725^{ef}	3845^{ab}
Diósárki	626^{bd}	725^{ef}	3916^{abc}
Sóskút 96/1	681de	790^{fg}	3284^{a}
Tétényi keményhéjú	681de	790^{fg}	3284^{a}
Marinada	769^{ef}	827^{fg}	4056^{abc}
Soleta	786^{f}	890^{gh}	4150^{bc}
6/10	823.0^{f}	983^{h}	4517^{c}
7/21	865.0^{f}	1058^{h}	4821^{c}
Vairo	893^{f}	1092^{h}	4857^{c}
Constanti	893.0^{f}	1092 ^{h}	4821^{c}

Variables represent the mean of 3 replications. Superscript lower letters indicate significant difference along the column, according to one -way MANOVA followed by Duncan's post hoc test ($P \leq 0.05$). base temperature

When we make attempt to compare our results with other authors), it becomes clear that it is not easy to discuss them due to different methods used (Razavi et al., 2011) and climatic conditions (Aron, 1975; Bartolini et al., 2006). The first difference is in the models used for chilling calculations. The results of early studies on almond chilling ranged from 266-996 CU (Egea et al., 2003) Egea et al. (2003), 167-638 CU (Prudencio et al., 2018) and 270-1100 CU (Guillamón et al., 2022), which is most similar to the results we obtained in Hungary (285-893). This comparison suggests that the climate itself does not affect the chilling requirement of a given cultivar. However, all three studies calculated higher heat requirements (5942-7577, 6279-8571 and 6038-7892, respectively) compared to our GDH results (3284-4857). The difference might be in the determination

of dormancy break: while both studies used *in vitro* forcing techniques, we used microsporogenesis studies. The analysis of Alonso et al. (2005 and 2010) resulted in different chilling range from ours, in spite of the fact that their climate is continental in Zaragoza. They obtained 400-600 CU by Utah model, even though they analysed 44 cultivars for seven years and GDH ranged from 5500 to 9300. However, the endodormancy break was calculated according to a mathematical model based on phenology and meteorological data. If we put together chilling and heat requirement data (Figure 2), we notice that chilling and heat requirement usually go hand in hand. Eriane, 15/5 and 1/7 not surprisingly have extremely low chill and heat requirement, forecasting their early flowering time and fast flower bud development that has been already approved. The Hungarian commercial cultivars have low or medium chilling and low heat requirements, expect Tétényi keményhéjú having somewhat higher chilling values. All late flowering Spanish almond cultivars have high chill requirement; however, their heat requirement differs. Together with the Hungarian 7/21 and 6/10 accession, Vairo and Constatnti have outstanding chilling and heat requirements. These findings are in accordance to their speed of flower bud development.

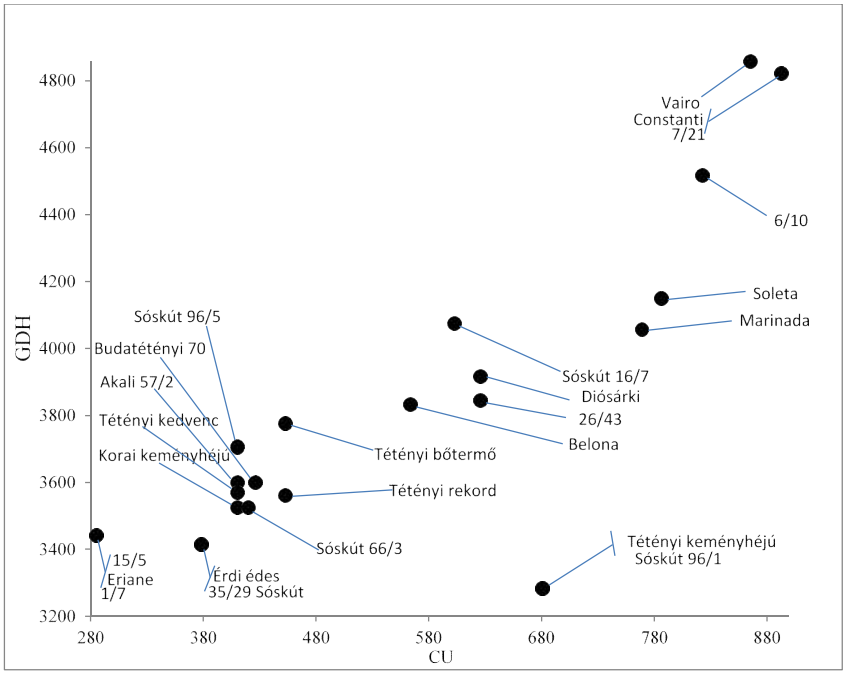

Figure 2. Comparison of chilling (CU) and heat requirement (GDH) of almond cultivars analysed.

Pstil growth and *in vitro* forcing results are rather connected with heat accumulation than chilling. If forcing temperature is not sufficient, cultivars with high CU and GDH demand will flower later (e.g. Soleta and Belona) as compared to accessions having high CU but low GDH (e.g. Tétényi keményhéjú and Sóskút 96/1).

The effect of cultivars and years on chilling and heat requirement

MANOVA revealed significant differences between cultivars in chill units, chill hours, and heat requirements. The year effect resulted in a significant effect on almonds' heat requirements, but the year effect for the accumulated amount of chill units and chill hour number was insignificant). The correlation between chill / heat requirement and

flowering time is strong, based on our statistical results. The Pearson correlation coefficient between chilling requirements and flowering time was 0.915, while between heat requirements and flowering time it was 0.948. *These figures indicate that the flowering date was influenced by heat requirements rather than chilling requirements, although the difference is small.* The correlations between chilling and heat requirements were also statistically significant with a correlation coefficient of r 0.813.

Frost hardiness of almond cultivars

The frost hardiness profiles of the observed cultivars during dormancy were similar. In all five years studied the frost tolerance of flower buds increased gradually in the first half of winter (hardening period), and by increasing outdoor temperature in the second half of winter they gradually lost their frost tolerance (dehardening period).

The statistical analysis distinguished three homogeneous groups depending on the LT50 value of the five test seasons that can be labelled as frost-tolerant, medium-frost-tolerant and frost-sensitive cultivars within the studied cultivar range. ‘Sóskút 96/5’, ‘Akali 57/2’, ‘Eriane’ and ‘Érdi édes’ cultivars form the frost-sensitive group. ‘Budatétényi 70’, ‘6/10’, ‘Sóskút 96/1’, ‘Sóskút 66/3’, ‘Tétényi kedvenc’, ‘Korai keményhéjú’ and 'Diósárki' belong to the group with medium frost resistance. ‘Tétényi keményhéjú’ alone forms the frost tolerant group. ‘35/29 Sóskút’, ‘Tétényi rekord’, ‘Tétényi bőtermő’ and ‘1/7’ form a transition between the frost-sensitive and the medium-frost tolerant groups, while ‘26/43’, 7/21’, ‘5/15’ and ‘Sóskút 16/7’ belong to the transition type between the medium-frost tolerant and frost tolerant groups (Figure 3).

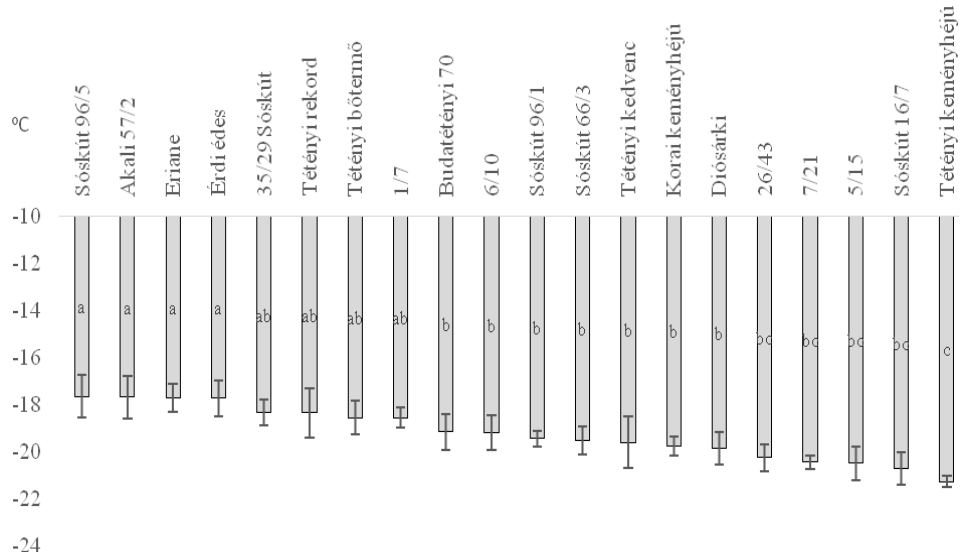

Figure 3. Average LT50 values of flower buds of the studied almond cultivars based on the results of artificial freezing

Correlation between frost tolerance and chilling of cultivars

The results of the study showed that there was a correlation between the chilling requirements and the frost hardiness of the cultivars. However, there was a weak correlation between the two variables. It was found that the correlation was not linear. According to cubic regression analysis, chilling requirements and frost hardiness were correlated with a correlation coefficient of R^2 =0.39.

4. CONCLUSIONS AND RECOMMENDATIONS

The flower bud development of the accessions showed differences in their total length and in each developmental stage, especially in the length of archesporium and microspore stage. The accessions ‘1/7’, ‘Eriane’ and ‘5/15’ appeared to have the shortest development, while ‘7/21’, ‘6/10’, ‘Constanti’ and ‘Vairo’ had the longest flower bud

development in all three years studied. ‘Tétényi bőtermő’, ‘Tétényi kedvenc’ and ‘Tétényi rekord’ had medium length flower bud development, while ‘Tétényi keményhéjú’ had short or medium. Among Spanish cultivars, the flower bud development of ‘Marinada’ and ‘Soleta’ was more affected by yearly weather conditions.

As seen in previous chapters, there is not an agreed method that is used by different authors in order to calculate chilling and heat requirement of almond cultivars. This results in discrepancies and incomparable calculations that makes decision difficult regarding adaptation of a cultivar to different climates.

Among the three biological methods studied, the microsporogenesis method proved to be the most accurate in forecasting endodormancy break. Regarding pistil growth, in early flowering cultivars the resumption of pistil growth appeared at the full string stage of the flower buds, while in late flowering cultivars the pistil appeared to resume growth at the moment when the separate pollen mother cells and tetrads in the anthers of the flower buds were distinguishable. The growth of the pistil is more related to weather temperature conditions, therefore not suitable for indicating endodormancy break.

In vitro forcing of shoots resulted in contradictory data. Cultivars reaching the microspore stage early varied in their forcing results indicating that they differ in their heat requirement.

Flower bud development can be examined accurately by using the microsporogenesis method to better understand the transitional changes occurring throughout the different phases of flower bud development, from bud formation in the summer to flowering in the following spring.

The start of microsporogenesis process (meiosis) can be useful in determining the endodormancy release of almonds, subsequently

determining their climatic adaptability However more work both at outdoor and indoor is required to clearly understand if start of microsporogenesis process is a function of only due to chilling accumulation or not.

Almond accessions showed considerable differences in their chilling and heat requirements. As compared with other authors' values, we can conclude that it is not the climate that determines the chilling and heat requirement of a given almond cultivar, it is rather controlled by genetic factors.

The flowering time was correlated with heat requirement stronger than with chilling, but as there was only a slight difference among the strength of correlation, we can state that both factors affect strongly the flowering time.

Our results are in accordance with those described in flower bud development chapter. The accessions 'Vairo', 'Constanti', 7/21 and 6/10 showed high chilling and heat requirements having late blooming time. They are at risk for growing in warm areas with problems of insufficient chilling. But in cold areas exposed to spring frost, these cultivars have great values of chilling and heat requirements and are the best choice as parents in a breeding program for late blooming to avoid spring frost. On the other hand, the chill and heat requirement of '1/7', 'Eriane' and '5/15' are easily satisfied, therefore they are exposed to winter and spring frosts.

From the aspect of safe yield, the frost hardiness of flower buds is an important trait of cultivars. Despite the fact that it is a frost-sensitive species, little data can be found in literature on the actual frost tolerance of almond cultivars in different phenological stages. Significant differences were detected among the accessions and the years. From the 20 almond accessions 'Sóskút 96/5' was the most sensitive and 'Tétényi keményhéjú' was the most frost hardy. Among frost tolerant accessions here we mention 26/43 (a

candidate cultivar for the national cultivar register list), ‘7/21’, ‘5/15’ and ‘16/7’. The other Tétényi cultivars appeared to be sensitive or mid sensitive.

When comparing accessions regarding flower bud development, chilling requirement and frost resistance of buds (winter and spring), we may think that accessions with fast flower bud development are early flowering and more frost sensitive than those with slow development, more chilling- and heat requirement. It was indeed the case in ‘Eriane’ accessions. However, the accession ‘5/15’ in spite of its low chilling and heat requirement and fast flower bud development showed winter frost tolerance.

The correlation between chilling and frost hardiness of the flower buds in winter was not strong according to our statistical analysis. If we remove cultivars with extremities, a linear regression probably can support better this connection.

5. NEW SCIENTIFIC RESULTS

1.This study presents new phenological description about the flower bud development of 25 unstudied almond accessions from the beginning of paradormancy to the end of ecodormancy.

2.This study proved that the analysis of microsporogenesis is the most accurate method in determining the break of endodormancy of almond.

3. This study presents new data about the chilling and heat requirements of the Budatétnyi 70, Tétényi bőtermő, Tétényi keményhéjú, Tétényi record and Tétényi kedvenc Hungarian almond cultivars calculated by Utah and growing degree models

4. It was discovered that the correlation of flowering time with the heat requirement is stronger than with chilling

5. By means of these studies the winter frost hardiness of almond flower buds of 20 unstudied accessions have been firstly characterized during dormancy by in vitro method.

6.These studies proved that the most frost resistant Hungarian commercial almond cultivar is the Tétényi keményhéjú, followed by the 6/43, 7/21, 5/15, 16/7 accessions.

PUBLICATIONS RELATED TO THE SUBJECT OF THE PH.D THESIS

IF journal articles:

Keleta, T.B., Békefi, Zs., Bakos, J.L., Örsi, D., Szalay, L. (2023).. Frost hardiness of almond flower buds during dormancy. Acta Biologica Szegediensis 66: 170-179. (IF: 0.56, Q3)

Szalay, L., Bakos, J.L., Tosaki, A., Keleta, T.B., Froemel-Hajnal, V., Karsai, I. (2021). A 15-year-long assessment of the cold hardiness of apricot flower buds and flowers during the blooming period. Scientia Horticulturae 290: 110520 (IF: 5.10, Q1)

Szalay, L., Keleta, T.B., Bakos, J. L., Békefi, Zs. (2022). Frost hardiness of flower buds of three Hungarian almond cultivars during dormancy. Acta Agriculturae Slovenica 118: 1–9. (IF: 0.207; Q4)

Peer rewied journal articles:

Szalay, L., Keleta, T.B., Békefi, Zs. (2021). Frost tolerance of flower buds and flowers of almond cultivars on the field. Kertgazdaság 53: 1-13.

Keleta, T.B., Szalay, L., Békefi, Zs. (2022). Hazai és külföldi mandulafajták virágzási ideje. (Flowering time of domestic and foreign almond varieties) Kertgazdaság 54: 3-13.

Conference proceedings:

Keleta, T.B., Szalay, L., Békefi, Zs. (2020). Chilling and heat requirement of almond genetic resources. In: Fodor, M., Bodor-Pesti, P., Deák, T. (szerk.) SZIEntific Meeting for Young Researchers 2020: ITT Ifjú Tehetségek Találkozója 2020. Szent István University, Budapest, 2021. p. 218-226. p.*

Keleta, T.B., Szalay, L., Békefi, Zs. (2021). Determination of endodormancy and ecodormancy breaking date of almond genetic resources by biological methods. In: Fodor, M., Bodor-Pesti, P., Deák, T. (szerk.) Proceedings of János Lippay – Imre Ormos –Károly Vas (LOV) Scientific Meeting. MATE, Buda Campus, Budapest, 2022. 419-430. p*

REFERENCES:

Alonso, J.M., Ansón, J.M., Espiau, M.T., Socias i Company, R., 2005. Determination of endodormancy break in almond flower buds by a correlation model using the average temperature of different day intervals and its application to the Estimation of chill and heat requirements and blooming date. J. Am. Soc. Hortic. Sci. 130, 308–318.

Alonso, J.M., Socias I Company, R., 2010. Chill and heat requirements for blooming of the cita almond cultivars. Acta Hortic. 814, 215–220.

Aron, R.H., 1975. Chilling as a factor in crop location with particular reference to deciduous orchards in California. Oregon State University.

Bartolini, S., Viti, R., Laghezali, M., Olmez, H.A., 2006. Xylem vessel differentiation and microsporogenesis evolution in ´canino´ cultivar growing in three different climatic areas: Italy, Morocco and Turkey. Acta Hortic. 701 I,

Egea, J., Ortega, E., Martínez-Gómez, P., Dicenta, F., 2003. Chilling and heat requirements of almond cultivars for flowering. Environ. Exp. Bot. 50, 79–85.

Guillamón, J.G., Egea, J., Mañas, F., Egea, J.A., Dicenta, F., 2022. Risk of extreme early frosts in almond. Horticulturae 8, 1–7.

Hajnal, V., Omid, Z., Ladányi, M., Tóth, M., Szalay, L., 2013. Microsporogenesis of apricot cultivars in hungary. Not. Bot. Horti Agrobot. Cluj-Napoca 41, 434–439. https://doi.org/10.15835/nbha4129162

Prudencio, A.S., Martínez-Gómez, P., Dicenta, F., 2018. Estimation of chilling and heat requirements of early, extra-late and ultra-late almond cultivars during cold and warm seasons. Acta Hortic. 1229, 115–120.

Razavi, F., Hajilou, J., Tabatabaei, S.J., Dadpour, M., 2011. Comparison of chilling and heat requirement in some peach and apricot cultivars. Res. Plant Biol. 1, 40–47.

Richardson, E., Seleey, S., Walker, D., 1974. A model for estimating the completion of rest for “Redhaven” and “Elberta” peach trees. HortScience 9, 331–332.

Szalay, L., Németh, S., 2010. Phenological processes of dormancy in apricot genotypes in the central part of the Carpathian Basin, in: Acta Horticulturae. pp. 251–256.

Weinberger, J.H., 1950. Chilling requirements of peach varieties. Proceedings. Am. Soc. Hortic. Sci. 56, 122–8.