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

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1. INTRODUCTION

Almond (*Prunus dulcis* Mill.) is one of the most common nut bearing fruit species produced worldwide (Ahmed and Vermna, 2009; Vishal, 2021). The United States is the world's largest producer of almonds, production in 2021 reached 2,189,040 tons (in shell basis). In this country, California is one of the biggest areas of almond production. In the world, Spain becomes the second largest producer followed by Australia. Morocco is the biggest almond producer in Africa (FAOSTAT, 2023). Almond has been produced for its kernel which is full of nutrients, cholesterol-free, and has lots of valuable essential oils and proteins. In addition to being low in saturated fat, they also contain a number of protective nutrients (Singh et al., 2022).

The primary gene centre of cultivated almonds 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 resistance of the vegetative and generative organs of almond cultivars has been studied by several methods in different places and significant differences were found between the cultivars.

The frost sensitivity of almond flower buds also 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. This issue is particularly relevant for the successful cultivation of almonds as they display a wide range of flowering time and chilling requirements (Bassi et al., 2006; Čolić et al., 2016; Miranda et al., 2005; Prudencio et al., 2018a).

Thus, successful almond production requires cultivars that are adapted to the growing environment, before new cultivars are introduced into cultivation, it is a necessary precaution to seriously test their climatic suitability for a given location before any practical establishment steps (Vargas et al., 2008). If we know the chilling and heat requirement of a cultivar together with the length of endodormancy and the frost resistance of the flower buds, we can safely advise growing sites for them.

The production of frost tolerant and late flowering cultivars is an important breeding aim, because almond even in subtropical places can suffer frost damage due to its early flowering time (Daneshvar and Sardabi, 2006; Dicenta et al., 2011; García-Gusano et al., 2011; Imani et al., 2011).

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 accessions, and then to find out the differences among almond accessions 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 accessions 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. LITERATURE REVIEW

2.1 Origin and history of almond cultivation

Almond (*Prunus dulcis* Mill.) is an ancient species of fruit trees that originated in the Mediterranean region and among the first fruit that were domesticated in history (Gradziel, 2008; Ladizinsky, 1999). In terms of botany, almonds belong to the family *Rosaceae*, together with typical fruit species such as cherries, plums and peach. In contrast to these fruits, where the fruit flesh is consumed, the edible part of an almond tree is its seed – the kernel. Therefore, from a horticultural point of view, almonds are classified as nuts (Gradziel, 2011). The gene center for almonds is believed to be in central and southwest Asia (Barreca et al., 2020; Gradziel, 2011, 2008) and from there it spread along the shores of the Mediterranean in Northern Africa and Southern Europe by Egyptians, Greeks and Romans (Ahmed and Vermna, 2009).

Ancient traders valued almonds because of their ease of propagation and the nature of non-perishable food. A variety of wild almond species were traded and consumed by early human communities with each species having its own unique qualities, morphology, and geographic location. Early trade routes of emerging civilizations led to the dissemination of this genetically diverse commodity. In prehistoric Asia, North Africa, and Europe, almonds were an extremely valuable commodity due to their wide spread availability and easy transportability (Gradziel, 2011).

2.2 Climatic requirements of almonds

2.2.1 Temperature requirements

It is most ideal for almonds to grow in a Mediterranean climate with hot summers (30-35°C) and mild, cool winters (Ahmed and Vermna, 2009; Kumar et al., 2023). Frost is a problem in almost all growing areas of the world, particularly in blooming. Blossoms before petals opening are known to withstand cold up to -2.2°C, but blossoms at the petal fall stage are killed at -0.5°C to -1.1°C. The blossoms can often withstand temperature from -2.2°C to -3.3°C for a short time but if low temperature continue for many hours they get damage (Ahmed and Vermna, 2009). Temperatures between 15 and 30°C are optimal for their growth (Kumar et al., 2023).

2.2.2 Water and light requirements

Almonds with long and deep root systems are drought tolerant. However, severe water stress can have both current and next year's effects on production. The time of irrigation and the amount of water to be applied depends on both weather conditions and on soil types (Doll, 2017). The critical time for almond water requirements varies depending on the growth stage. Almonds are most sensitive to water shortages when growth begins in spring i.e. at flowering (Ahmed and Vermna, 2009). They require 300-400 mm of rainfall per year (Kumar et al., 2023). Almonds require full solar radiation for the proper process of photosynthesis. Plants receive light based on the intensity and duration of photosynthetic active radiation (Alonso, 2017).

2.2.3 Soil requirements

Deep loamy soils with excellent drainage of excess water are ideal for almond growing but can be grown in average soils supplemented with farmyard manure (FYM) and irrigation is ensured. Trees do not thrive well in heavy clay or poorly drained soils (Ahmed and Vermna, 2009; Kumar et al., 2023).

2.3 Almond production in the world

Presently, almonds are produced throughout the world in regions with a hot, arid climate similar to the Mediterranean (Gradziel, 2008). There are many varieties of almonds that can be found around the world. There are about 30 varieties of almonds available in California which is the main almond producer, with 13 major varieties accounting for more than 98% of production (Almond Board of California, 2020). California's almond cultivars were developed largely from almond seedlings. Cross-pollination with Nonpareil was performed in the California region using cultivars such as Merced, Price, Carmel and Fritz. Other later blooming varieties, such as Thompson and Livingston, were also combined with Texas. At present, the most cultivated almonds in the USA are 'Nonpareil', 'Carmel', 'California types', and 'Mission'. 'Due to the high shelling percentage of kernels, 'Nonpareil' remains the dominant commercial cultivar. After cracking, the thin shell protect damage of kernels (Gradziel, 2017). In the second-largest producer of almonds in the world, hardy shells and late blooming are the most common characteristics. Among the most popular almond varieties in Spain are 'Marcona', 'Largueta', 'Comuna', 'Guara', and 'Ferragnes'. A large number of almond orchards are not irrigated, which

results in very low productivity (Gradziel, 2011; Mahhou and Dennis, 1992). Table 1 below shows 10 of the top almond-producing countries ranked by FAOSTAT (2023).

Table 1: Top countries in almond production

S.NO.	Country	Production in tons (in shell)
1	United states	2,189,040
2	Spain	365,210
3	Australia	285,605
4	Turkey	178,000
5	Morocco	169,255
6	Iran	163,568
7	Syria	87,768
8	Tunisia	75,000
9	Italy	71,620
10	Algeria	55,448

Source: FAOSTAT (2023)

The average world yield of almond orchards (in shell) is 1t/ha; however, it greatly depends on the growing system used. For instance, in UAE (United Arab Emirates) the yield can reach 27.3t/ha and in USA 41.15t/ha (FAOSTAT, 2023). Production and productivity can be increased manyfold if high- yielding varieties and intensive technologies are suited to the region of production (Ahmed and Vermna, 2009; Vishal, 2021). Almond production in Hungary is severely limited due to problems of winter frost. A total of 190 tons of dried fruit in shells are produced annually by Hungary from its commercial almond orchards which cover a total area of 390 ha. There are some Hungarian almond varieties adapted to Hungarian climate conditions for growers (FAOSTAT, 2023).

2.4 Phases of dormancy in temperate zone fruit species

The growth cycle of *Prunus* species such as almonds can be divided into three chronological phases: paradormancy, endodormancy and ecodormancy (Campoy et al., 2011; Lang, 1987; Prudencio et al., 2020; Tromp, 2005). Paradormancy refers to the state of dormancy where growth inhibition is caused by a specific biochemical signal available within the plant stem

outside the affected structure or bud, such as apical dominance (Lang, 1987; Tromp, 1996). In the early season, trees stop growing and form terminal buds. These terminal buds and later lateral buds born on short shoots and spurs cease growth in the current season. Buds can be forced into growth by cultural practices including pruning, defoliation, irrigation after a dry period, or heavy nitrogen application. Buds gradually move from the paradormancy to the endodormancy phase (Tromp, 2005).

2.5 Winter dormancy in temperate fruits

Temperate fruit tree species such as almonds enter a dormancy stage when temperatures drop in autumn. This is in order to survive under unfavorable environmental conditions (Campoy et al., 2011; Fadón et al., 2020a, 2018; Herrera et al., 2022; Prudencio et al., 2020; Rohde and Bhalerao, 2007). Although temperate fruit trees remain alive throughout the winter season, they do not produce any visible growth as an adaptation to the cold. It is called winter dormancy when a plant structure temporarily stops growing even under favourable conditions (Tromp, 2005). The plants remain physiologically active as activity inside the buds does not stop (Fadón et al., 2018; Tromp, 2005). However, the transport of water and solutes is still interrupted both at the whole plant level as well as at the cellular level inside the meristems (Fadón et al., 2020a). Thus, dormancy of flower buds is a survival mechanism that inhibits growth until suitable weather conditions come for flowering (Prudencio et al., 2020). Genetics and the environment play a role in the release of dormancy (Alonso and Socias I Company, 2010; Julian et al., 2009; Szalay et al., 2019, 2018), with chilling and heat requirements playing a communal role in determining dormancy development of reproductive buds and the time of flowering (Luedeling, 2012; Prudencio et al., 2018a). Besides the temperature effect, environmental signals like water stress, light quality and photoperiod play a role in the establishment of dormancy (Allona et al., 2008). However, cold temperatures are one of the most significant environmental factors that control dormancy (Fadón et al., 2020a).

Endodormancy is regulated by an environmental or endogenous signal within the affected bud alone, such as chilling and photoperiodic responses (Fadón et al., 2020b; Lang, 1987; Tromp, 2005). During this phase, the meristems are inactive and remain protected within the buds. Even when environmental conditions are favorable, it prevents new buds from developing (Campoy et al., 2011; Lang, 1987). The transition to endodormancy can be advanced by low temperatures. Once buds enter the endodormancy phase, they do not react to any growth stimulation as long as

endodormancy is unbroken (Campoy et al., 2011; Lang, 1987). Growth resumption may delay severely or does not occur at all if the required cold is not met. Especially in tropical or subtropical climates, it is a problem (Tromp, 2005). In late October or early November, almonds enter endodormancy (Guillamón et al., 2022; Prudencio et al., 2018a). However, varieties vary in the time and depth of endodormancy peak (Egea et al., 2003).

When the chilling requirement of a fruit cultivar has been fulfilled, it enters ecodormancy. During this phase of dormancy, growth is prevented by one or more unfavourable environmental factors, like low temperatures in early spring (Fadón et al., 2020b; Tromp, 2005). After having adequate amount of heat, the plants start blooming.

2.6 Reproductive buds of almond and their development during dormancy

The reproductive buds of almonds are usually located on single spurs or long shoots. Usually, two flower buds embrace a vegetative bud in one nodus. Almond flower buds are predominantly borne on spurs, have higher quality flowers and tend to open early compared to long shoots. A single spur of almond can contain one to several flower buds (Lamp et al., 2001). Flower bud size, shape and colour are cultivar traits showing high variability. Flowers are perfect and pentamerous, with five sepals, five petals, a variable number of stamens and a single pistil (Socias I Company et al., 2017).

The process of flower initiation is the transformation of vegetative buds into flowers. Almond start to flower in early spring but floral bud establishment occurs the previous summer (Socias I Company et al., 2017). Flower buds develop slowly during the winter months, progressing from one stage to the next at certain stages of development (Hajnal et al., 2013; Julian et al., 2009; Szalay et al., 2019). The transition period between phenological stages is not immediate but rather gradual (Szalay et al., 2019). The speed of flower bud development is high during paradormancy, and then it slows down during endodormancy, during which period there is no visible change and it accelerates again at the end of winter , some weeks before blooming (Socias I Company et al., 2017; Szalay, 2006). Chill accumulation during the dormancy period allows the gradual change from flower bud endodormancy to flower bud ecodormancy where flower bud development is controlled by subsequent heat accumulation (Bartolini et al., 2006b; Egea et al., 2003; Hajnal et al., 2013; Sánchez-Pérez et al., 2014; Szalay et al., 2019). When flower initiation occurs in the meristem, the reproductive buds begin to develop into various floral organs (Socias I Company et al., 2017; Szalay, 2006). As described by Lamp et al. (2001),

almond flower buds develop in eight stages including a pre- reproductive stage in which the apical meristem is in a vegetative state and producing bud scales (Figure 1A). Transition to the reproductive stage, the increase in meristem size at the shoot apex (Figure 1B), after which the apex broadens and thickens, forming an elongated broad dome. Bract primordia are produced on the apex periphery by the dome indicating the flower initiation stage (Figure 1C). The sepal initiation stage is marked by the sequential initiation of five sepals at the terminal apex (Figure 2A and B), which indicates the beginning of organogenesis, while petal primordia emerge within the calyx during the petal initiation stage (Figure 2C). The initiation of multiple stamen primordia within the corolla is regarded as a stage of stamen initiation (Figure 2D). Pre-carpel initiation indicates the stage characterized by the growth and development of the calyx, corolla and stamen bases forming the hypanthium, while the floral apex becomes concave. During the final carpel initiation stage, there is a visible carpel at the center of the apex (Figure 3).

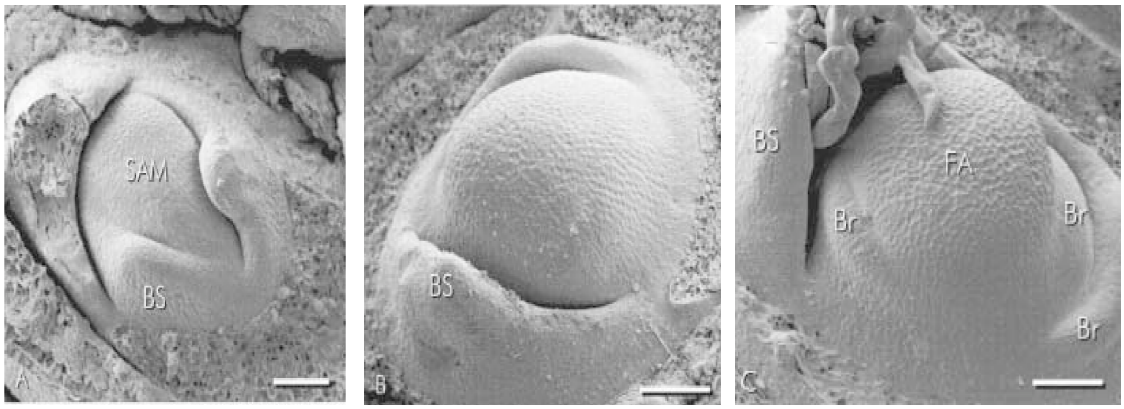


Figure 1. Almond bud apices showing stages of development through floral initiation. (A) Pre-reproductive stage, (B) Transition to the reproductive stage and (C) flower initiation stage the floral apex and three bracts are formed. Br=bract, BS =bud scale, FA = floral apex. SAM = shoot apical meristem. Source Lamp et al. (2001).

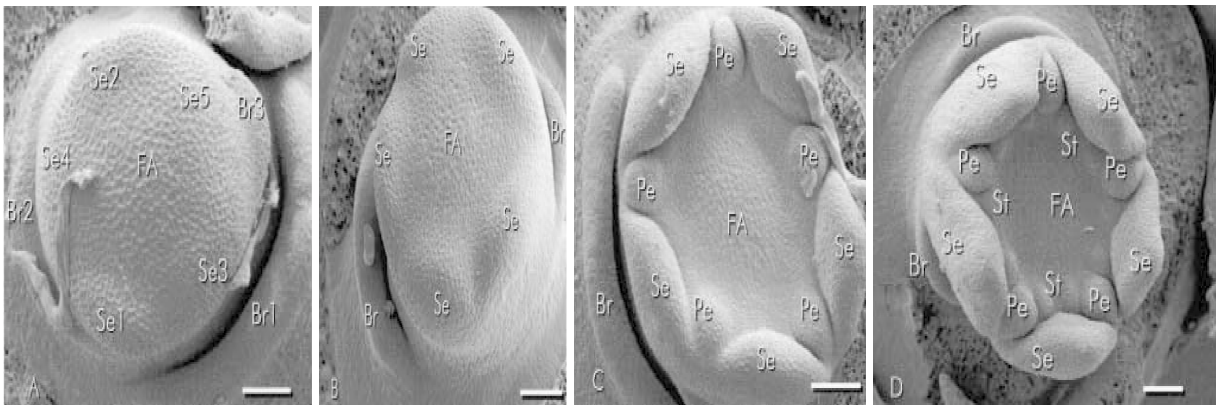


Figure 2. Almond bud apices showing initiation of floral organs through stamen initiation (A) bract and sepal initiation stage at the floral apex with three bracts (Br1-Br3. Five sepals begin at the floral apex and are subtended by bracts (B) Primordia of the petals alternate with those of the sepals. Stamen initiation (D). Note that Br=bract, FA= floral apex, pe =petal, se =sepal, st=stamen. Source Lamp et al. (2001).

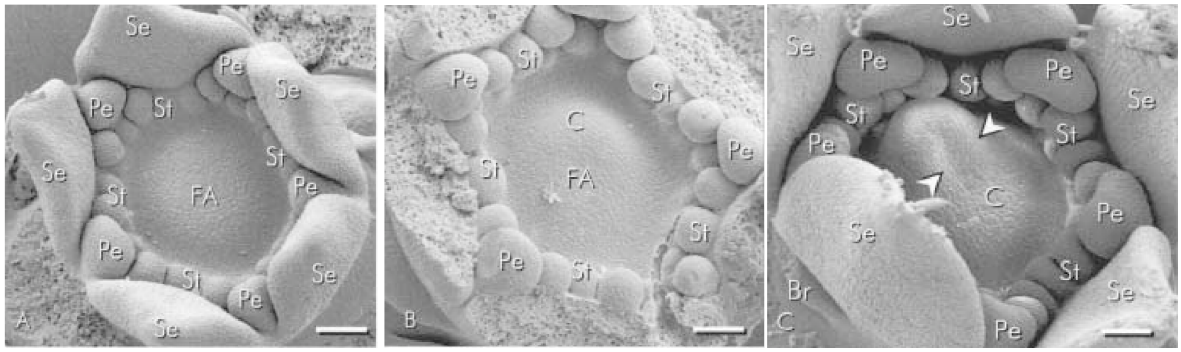


Figure 3. Almond bud apices showing initiation of carpel. The floral apex has yet to differentiate a carpel primordium from stamen primordial (A). At the floral apex, a terminal carpel primordium emerges (B). The terminal carpel primordium consumes the entire floral apex as it differentiates. An arrowhead indicates the margin of a carpel (C). Source Lamp et al. (2001).

Flower bud development can be carefully studied during the dormancy period by several biological methods and microsporogenesis study is one of the biological methods that suitable for the characterization of dormant flower bud development (Szalay et al., 2019). It has been reported that the speed of microsporogenesis varies between cultivars and yearly climatic conditions (Bartolini et al., 2006a, 2006b; Hajnal et al., 2013). Early cultivars are quick to initiate microsporogenesis (Hajnal et al., 2013). Those cultivars that develop flower buds quickly are highly susceptible to winter frost damage. The earlier a flower bud develops, the earlier its blooming date will be and the more likely they are to be exposed to spring frost (Hajnal et al.,

2013; Szalay and Németh, 2010). Researchers studied the microsporogenesis development of apricots (Andreini et al., 2012; Bartolini et al., 2006a, 2006b; Hajnal et al., 2013; Németh, 2012; Scalabrelli et al., 1991; Szalay et al., 2019, 2006; Szalay and Németh, 2010; Viti and Monteleone, 1991) and sweet cherries (Fadón et al., 2019). In the early winter, the archesporium tissue was observed in the anthers of all the studied cultivars and at a later stage of microsporogenesis of the ecodormancy phase the string, pollen mother cell, pollen of tetrads, microspores and pollen grains stages were distinguished. As reported by different authors (Fadón et al., 2018; Hajnal et al., 2013; Scalabrelli et al., 1991; Szalay et al., 2019), accumulated chill units influence the microsporogenesis process of flower buds. Moreover, in apricot Viti et al. (2010) observed that the higher the chilling requirement the longer the microsporogenesis stage of endodormancy lasted. Bartolini et al. (2006b) reported a positive correlation between meiosis onset and flowering. Different locations require different amounts of chili units for the development of flower bud xylem differentiation and microsporogenesis. Some authors (Fadón et al., 2018; Szalay, 2006) reported that flower buds develop slowly during early winter, arrest development during endodormancy and resume growth during ecodormancy (Figure 4). Similarly, Fadón et al. (2018) reported that sweet cherry flower buds remain physiologically active while accumulating starch during endodormancy, reaching a maximum at chilling fulfillment, while starch was lost during ecodormancy before bud break. In addition, the study of apricot Szalay and Németh (2010), showed intense growth to a length of about 1mm during paradormancy, and constant growth during endodormancy. But during ecodormancy, growth started again, first at a very slow rate, then rapidly accelerated as flowering approached. In a similar study, Fadón et al. (2018) found qualitative and quantitative changes in sweet cherry pistils as it moves from endodormancy to ecodormancy.

Figure 4. Flower bud development during autumn, stage 2-4 and arrest development during endodormancy, stage 4 and resume growth after fulfillment of chilling and heat requirements, stage 5 and 6 (Fadón et al. (2018).

In apricot cultivars (Viti and Monteleone, 1991) observed differences in their speed of microsporogenesis that the late blooming cultivars form tetrads of microsporogenesis late than the early type. However, as with the formation of young pollen grains, the authors observed less variability among the cultivars. Besides, (Scalabrelli et al., 1991) observed flower bud development using fresh and dry bud weight before and after forcing and microsporogenesis method in outdoor chilled apricot cultivars. The results showed that the mild outdoor temperatures had a negative influence on the bud weight and microsporogenesis development as these buds went for long periods without any weight increment and pollen grains development than those artificially chilled.

Changes in frost tolerance in relation to the developmental rate of floral buds were determined by studying their microsporogenesis (Szalay et al., 2006). These authors reported that apricot cultivars with short endodormancy or quick floral bud development had the weakest winter hardiness. Selection of genotypes with slow phenological development and high chilling unit requirement of more than 1000 hours is a possible way of avoiding spring frost damage in cold regions (Szalay et al., 2006). Some authors believed that flower bud development is largely genetically determined (Lamp et al., 2001; Szalay, 2006), but it is also influenced by environmental factors (Szalay, 2006).

2.7 Chilling and heat requirements for breaking dormancy and flowering of almond

Fruit trees that are temperature-sensitive such as almond require accumulation of winter chill during endodormancy to break dormancy, and of heat during ecodormancy to produce flowers (Benmoussa et al., 2017; Fadón et al., 2020b; Luedeling et al., 2013a, 2013b). This is also explained by Prudencio et al., 2018a for almond cultivars (Figure 5). Inadequate fulfillment of the chilling requirements of any cultivar in any growing area, particularly in the tropics and subtropics causes negative consequences for their adaptations (Campoy et al., 2011). For instance, growing a high chill cultivar in the tropical areas may lead to delayed and/or abnormal flowering. When a tree with low chilling is planted to continental regions, the flowers will open too early and will suffer from spring frost damages (Alonso and Socias I Company, 2010; Benmoussa et al., 2017). On the other hand, early- flowering almonds are a wise choice for growing in warm areas with insufficient chilling problems (Prudencio et al., 2018b).

Hence, knowledge of the cold and heat requirements of any cultivar is essential in identifying the right cultivars to be planted in their appropriate areas (Alonso and Socias I Company, 2010; Bassi et al., 2006; Campoy et al., 2019; Fadón et al., 2020b), especially to avoid frost risks and to optimize cross-pollination for self-unfruitful cultivars within the same orchard (Alonso and Socias I Company, 2010). Also, knowledge of chilling requirements has significant importance for timing the application of dormancy-breaking synthetic chemicals (Gao et al., 2012).

The chilling and heat requirements of only few almond cultivars were studied (Alonso and Socias I Company, 2010; Benmoussa et al., 2017; Egea et al., 2003; Prudencio et al., 2020, 2018a, 2018b) and the cultivars showed remarkable diversity. Alonso et al. (2005) were among first calculating chilling and heat requirement of almond cultivars after a long break in such studies. The authors developed a model based on the significance of correlation coefficients between the temperatures during dormancy and the date of full bloom. When the correlation turned from positive to negative, and it was significant, that date was regarded as transition into ecodormancy. This work was performed under continental conditions, the experimental orchard is located in the valley of the river Ebro, near Zaragoza. They concluded that among 44 almond cultivars some showed high chilling requirement and low heat requirement, others behave in the opposite way. According to their results the CU values ranged between 300 and 500, whereas GDH values ranged from 5500 and 9300. As there were more considerable differences among cultivars in their heat requirement, it was concluded that flowering time depends on the amount of heat accumulated rather than chilling. Some cultivars originated from cold winter regions such as Yaltinskij, Primorskij and Miagkoskorlupij from Ukraine had medium-high CU and high GDH requirements. In their later work, Alonso and , Socias i Company (2010) calculated the CU and GDH values of some late blooming Spanish cultivars with their method described above. Chilling and heat requirement of ten almond cultivars were studied *in vitro* by Egea et al. (2003), for only one year, shoots were collected during dormancy season and kept at room temperature, bud phenology - open flowers were recorded. They concluded that chilling rather than heat requirement is determining the flowering time, as opposed to Alonso et al. 2005.

Benmoussa et al. (2017) analyzed 30 years meteorological and phenological dataset in order to find association between flowering time and chilling / heat requirements among Tunisian conditions. They found this correlation to be discontinuous in all cultivars. They explain it with other findings: Jiménez et al. (2010) suggest that the DAM6 gene described by peach (Fan et al.,

2010) may cause gene repression after dormancy release. Regarding different chilling models, the Utah model regularly resulted in negative GDH values concluding that this model is not suitable to warm climates. They highlighted that in tropical and Mediterranean areas it is important to use the appropriate chilling model that is in their case is the Dynamic model that still needs to be modified. Warming periods during dormancy delayed blooming, whereas such periods in ecodormancy resulted in earlier bloom. Prudencio et al. (2018a) studied chilling and heat requirement of three Spanish almond cultivars representing very early, late- and extra late flowering times (Desmayo Largueta, Penta and Tardona, respectively). The experiment was performed similarly as described by Egea et al. (2003) – by observing flower openings on *in vitro* shoots. Utah model and dynamic model were used for calculating chilling requirement. Their results confirmed the statement of Egea et al. (2003) that chilling plays a major role in flowering time. Recently in Spain, (Guillamón et al., 2022) reported chill requirements of 270 CU, 426 CU, 558 CU, 880 CU,1100 CU and heat requirements of 6038, 6681, 7466, 7181 and 7892 GDH for extra-early Desmayo Largueta, early Marcona, Ferragnès, late Penta and Tardona almond cultivars respectively. Furthermore, (Prudencio et al., 2018a, 2018b) determined the chilling and heat requirements in almonds and noted that flowering occurs only after these requirements are met, with high chilling requirements as a desirable trait of cultivars to avoid frost. Table 2 summarizes the main conditions and results of almond chilling and heat requirement studies.

Table 2. Summary of almond chilling and heat requirement studies and their conditions from various authors

Authors	Chilling model type	Chilling results	Heat model results (GDH)	Cultivars, years	Method for determining endodormancy release
Egea et al. 2003	Utah	266-996 CU	5942-7577	- 10 cultivars with different flowering time. - one year study	in vitro forcing
Alonso et al. 2005	Utah	400-600	5500-9300	- 44 cultivars from different climates - 7 years	model
Benmoussa et al. 2017	Dynamic	3,4-15,5 CP 6,7-22,6 CP	3962-8873 2894-10.504	- 12 local Tunisian, 25 international - 30 years	calculation from flowering time
Prudencio et al. 2018a	Utah Dynamic	167-638 21-56 CP	6279-8571	- 3 cultivars with different flowering time. - three-year study	based on in vitro forcing
Guillamón et al. 2022	Utah	270-1100	6038-7892	- from extra early to extra late Spanish - one year study	based on in vitro forcing

According to Benmoussa et al., 2017; Egea et al., 2003; Prudencio et al., 2018a flowering time in almonds is a function of chilling requirements, with heat requirements adding less effect. These results are also verified in other stone fruits such as apricot (Ruiz et al., 2007), nectarine and peach (Maulión et al., 2014). Conversely, some authors state that in almond (Alonso et al., 2005; Guo et al., 2014); in apricot and peach (Razavi et al., 2011) it was shown that heat accumulation drove of blooming time rather than chilling, with late blooming genotypes demanding greater heat requirements.

Adaptation of *Prunus* tree species including almond to new climatic conditions depends on their ability for endodormancy breaking and subsequent flower development (Dicenta et al., 2005; Martínez-Gómez et al., 2017). Chilling unit and the growing degree hour's requirements of cultivar determines its adaptation to specific ecological conditions (Bassi et al., 2006; Herrera et

al., 2022; Julian et al., 2009). However, lack of standard method to establish the end of endodormancy makes it difficult to know if a given cultivar is adapted to specific region based on its chilling unit and heat requirements (Herrera et al., 2022).

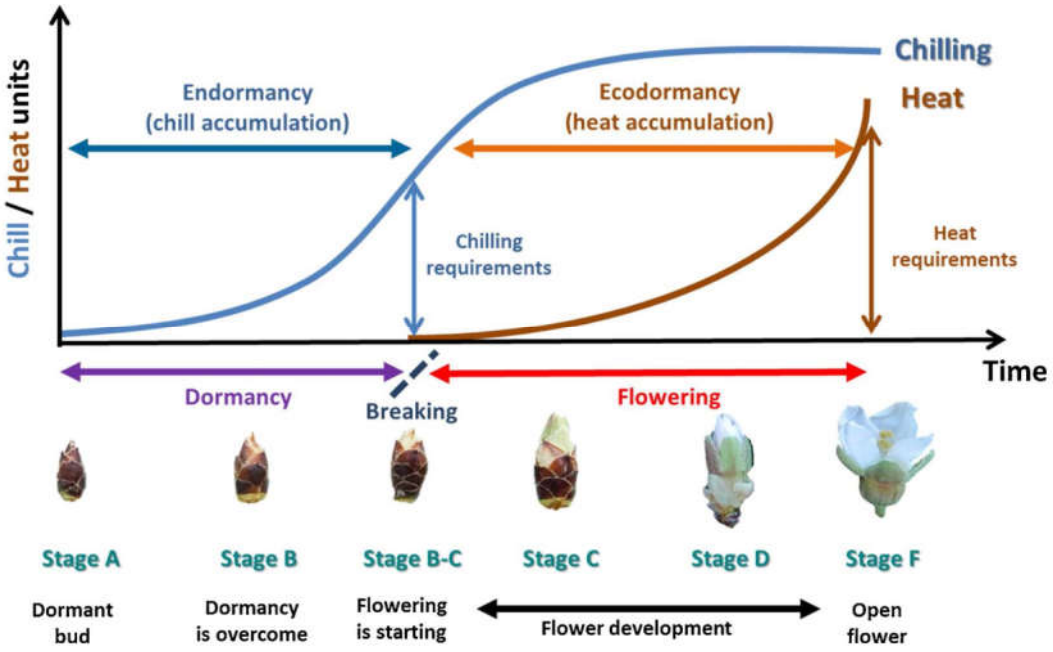


Figure 5. Dormancy breaking of reproductive buds and flowering in almond. Source: Prudencio et al. (2018a)

2.8 The chill hour and the Utah Models

Scientists have developed several temperatures based chilling models for the quantification of chilling requirements of fruit tress during dormancy. Among them, the Chilling Hours model (Weinberger, 1950), and the Utah chilling unit model (Richardson et al., 1974) are most widely used in Horticulture (Luedeling and Brown, 2011). The Chilling Hours model is an old model, however, due to its simplicity, it is the most widely used up to date. This model quantifies the chilling hour's requirements (CH) by counting all hours with temperatures below 7.2 °C as equally capable for winter chill contribution to complete dormancy (Weinberger, 1950).

The Utah model depends on the sum of chill units (CU) which establishes a different chilling contribution for different ranges of winter temperatures. In this model chilling units can be accumulated by the plant, they can also be lost or canceled out by warm temperatures. A range of winter temperatures between 2.5–9.1°C is optimum for dormancy completion, which contributes one chill unit at every rate of an hour. The ranges of temperatures between 1.5–2.4 °C and

between 9.2–12.4°C contribute less (0.5) to the chill unit accumulation. Winter temperatures below 1.4°C and between 12.5–15.9°C do not contribute to chilling accumulation and temperatures above 16°C have a negative effect on chill unit accumulation during dormancy season. The Utah model could be a more suitable option for the calculation of chilling requirements in cold winter climates. Winter chill totals were negative in many subtropical regions, where it did not appear to be helpful (Luedeling and Brown, 2011).

With regard to heat requirements calculation, the concept originates from the growing degree hours model (Richardson et al., 1974), which is the most widely used across the world compared with other forcing models (Anderson et al., 1986) which quantifies the heat requirements by using parameters for base, optimum, and critical temperature thresholds for grow. The Growing Degree Hour (GDH) model estimated the accumulated heat, between a base temperature of 4.5°C and an upper limit of 25°C and it is used to predict when certain growth and development phases will occur after dormancy has been released (Richardson et al., 1974).

2.9 Determination of endodormancy release to estimate chilling and heat requirements

To determine the chilling requirement of a given cultivar, it is helpful to know the beginning and the end period of endodormancy (Campoy et al., 2011). While the beginning of endodormancy and the end of ecodormancy has visible, observable outer signs (leaf fall and blooming, respectively), the end of edodormancy is an inner process that is hard to observe. knowledge of biological markers linked to dormant conditions remains scarce, making it difficult to establish the end of endodormancy (Herrera et al., 2022). Dormancy release has been determined by both statistical correlation (Alonso, et al., 2005) and partial least squares (PLS) regression (Luedeling et al., 2013a). Such a correlation was not observed regarding cultivars with a longer endodormancy period. The results of both types of approaches are not directly comparable under different conditions (Fadón et al., 2018). Herrera et al. (2022) compared three methods for indirectly estimating dormancy release by forcing shoots and two statistical approaches that related seasonal temperatures and blooming dates. All three methods estimated different dates for endodormancy end.

In early studies changing in flower bud weight during dormancy was used as an indicator. For instance, Brown and Abi-Fadel, 1953 tried to determine the end of endodormancy based on flower bud weight changes between controlled conditions and in vivo. The first chilling and heat

requirements in almond were performed by Tabuenca (1972) based on bud weight method of some Spanish almond cultivars and clones.

In apricot (Guerriero et al., 2006) compared fresh and dry weight changes and the development of phenological stages of buds after forcing. Bud weight was not sufficient to provide an indication of endodormancy release and chill units calculated based on bud weight were not consistent with the phenological stages of buds.

Later attempts were made by using *in vitro* forcing of bud shoots. In almonds (Egea et al., 2003), studied the phenological stages of flower buds *in vitro* in only one season in order to identify the end of endodormancy. The authors observed a change in the development of phenological stages after forcing with increasing cold accumulation. The order of dormancy release in controlled conditions matched that of flower openings in the field. However, data were not consistent and the climatic conditions of the field might play a negative influence on the development of buds as reported in pear by (Sugiura et al., 2002).

Maneethon et al.(2007) reported that flower bud development under forcing depends on accumulated chilling units in winter. For most of the almond genotypes, flower bud opening appeared to reach 50% and above when the meiotic cell division had already been completed in the anthers of the flower buds.

According to different authors (Andreini et al., 2012; Bartolini et al., 2006a, 2006b; Herrera et al., 2022; Socias I Company et al., 2017) dormancy release of *Prunus* species is indicated by microsporogenesis and marked by the appearance of tetrads or male meiotic division. It has also been demonstrated in the literature (Fadón et al., 2019; Hajnal et al., 2013; Julian et al., 2009; Szalay et al., 2019, 2000b; Szalay and Németh, 2010) that the development of the string stage was marked as a potential biomarker for flower bud dormancy release using the microsporogenesis method. Bartolini et al. (2006b) calculated the endodormancy breaking date by comparing flower bud weight before and after forcing and microsporogenesis. For cultivars with high chilling requirements, artificial warm temperatures failed to determine the endodormancy breaking date. The appearance of pollen tetrads of low-chilling-required cultivars were significantly correlated with the endodormancy breaking date based on bud weight.

In almond, apricot, peach (Szalay et al., 2006, Szalay and Németh, 2010) the dormancy status of the flower buds was determined by the changing characteristics of the pistil growth rate. This trend has also been observed in sweet cherry (Fadón et al., 2018), where a slow development of the pistil has been noted during paradormancy, while development continues until

endodormancy, whereas development is suspended during endodormancy and resumed during ecodormancy.

2.10 Almond flowering time

There is a wide range of flowering times among all fruit and nut species (Alonso and Socias I Company, 2010; Čolić et al., 2016; Prudencio et al., 2018a), which gives a high possibility for growing them in wide environments where they are almost capable of performing well by regulating their climatic requirements (Bassi et al., 2006; Čolić et al., 2016; Prudencio et al., 2018a). Almond is one of the earliest temperate stone fruit trees to flower in spring (Alonso and Socias I Company, 2010). Flowering time is a trait of particular interest in almond as it strongly determines its adaptation to specific climatic conditions (Connell et al., 2018; Sánchez-Pérez et al., 2014). Flowering time in almonds depends on the interaction between chilling and heat requirements (Sánchez-Pérez et al., 2014), which plays an essential role in the successful adaptation to various ecological conditions (Martínez-Gómez et al., 2017), particularly in preventing freezing damage (Kodad et al., 2010). The flowering time of almond is also important for the selection of cultivars for new orchards, as cross-pollination and nut set are dependent on the synchronous pollination of self-unfruitful almond cultivars (Alonso et al., 2005; Connell et al., 2018). Early flowering almond cultivars are accompanied by less favourable weather, particularly late spring frost and cultivars have to be adapted to avoid late spring frost by late flowering (Vargas et al., 2008).

In the Mediterranean basin, breeding aims primarily at obtaining self-compatible and late-flowering cultivars to increase fruit sets and avoid spring frost damage (Dicenta et al., 2016; Socias I Company et al., 2010).

A low temperature during the ecodormancy period can extend the flowering period of cultivars since slower heat accumulation delays flowering by delaying flower bud development. While warm temperatures can have the opposite effect (Alonso et al., 2005; Lamp et al., 2001).

In almonds (Čolić et al., 2016) and (Connell et al., 2018) studied blooming time in Serbian and Californian conditions, respectively, they reported that flowering time was significantly influenced by genotypes and temperatures. Connell et al., 2018) compared blooming time of 34 almond cultivars to Nonpareil in California, and the authors found that blooming time is a cultivar trait that varies depending on yearly climatic conditions, and bloom duration is influenced by genetic and weather conditions.

Di Lena et al. (2017) were testing the impact of climate change on almond blooming and spring frost occurrences for six decades, in south-center Italy (Mediterranean climate) using the model created by Alonso et al. (2005). They concluded that climate warming resulted in milder springs, however, as a counterbalance, also in advanced blooming, making the chance of spring frosts higher.

In a study (Daneshvar and Sardabi, 2006) flowering time data were recorded of 60 almond genotypes for 3 years in Iranian conditions (cold winters), local cultivars. It was found that there was a 21-day difference between the early and late blooming almond genotype.

2.11 Frost formations

In temperate regions, frost – temperatures below zero Celsius - often occur from autumn to winter, and sometimes even during early spring (Rossi et al., 2002). Some authors (Leoni et al., 2017) described the process of frost formation as dynamic and depends on various environmental factors.

Frost can be classified as either advection frost or radiation frost. Normally, advection frosts occur in cold and windy weather. To replace warm air, cold air blows into an area. Radiation frost is caused by radiant energy loss from the atmosphere, soil, and plants on clear, windless nights. A late frost in early spring is usually a radiation frost (Song et al., 2021). Frost appears in late autumn and early spring when the temperature drops below 0℃ due to radiation cooling. During this time, the surface temperature of the plant body falls below 0℃. In the plant body, water between each cell is frozen into tiny ice crystals. The ice crystals grow gradually as they condensate the water inside the plant cells. As water permeates outwards and solidifies the protoplast colloid, crops wither and die within hours from dehydration caused by ice-crystal interaction (Lu et al., 2019; Snyder and De Melo-Abreu, 2005). Intracellular ice formation causes mechanical disruption of the protoplasmic structure (Snyder and De Melo-Abreu, 2005).

The occurrence of frost on a clear, windless night is common in the cold season. The occurrence of frost can be delayed or prevented at night if there are clouds in the sky since they weaken a large portion of the long-wave radiation emitted by the soil and vegetation. Additionally, wind speed affects frost development. When there is a breeze, air passes slowly over a cold surface, cooling it. The movement of vapor facilitates the formation of frost. However, in a high wind speed, the contact time between the cold surface and the air is too short. As a result, it hinders the formation of frost (Song et al., 2021).

Plants that are tender lack the ability to resist intracellular freezing and are sensitive to temperatures as low as -5°C. Plants that are moderately hardy can accumulate sufficient solutes to resist freezing injury down to -10°C primarily by reducing dehydration damage. By focusing on the cells, hardy plants are able to avoid intracellular freezing and damage (Snyder and De Melo-Abreu, 2005).

2.12 Frost hardiness and frost damages

2.12.1 Frost hardiness

Temperature is the most important environmental factor affecting the frost hardiness of overwintering organs during the dehardening period (Heide and Prestrud, 2005; Tromp, 2005; Wu et al., 2019). The frost tolerance of trees can also be affected by numerous other factors, such as the cultivar, the rootstock, the cultivation system, the cropping technology, the health status of the trees, and the geographical location (Tromp, 2005). Due to all these, there are large differences in the development of frost tolerance between cultivars, production sites and years.

Early literature sources draw attention to the frost sensitivity of almonds and their close relatives, peach and apricot (Bereczki, 1882; Childers, 1949; Mohácsy and Magyar, 1936; Wood, 1947). The frost resistance of the vegetative and generative organs of almond cultivars has been studied by several methods in different places. Significant differences were found between the cultivars (Afshari et al., 2011; Imani et al., 2012; Imani and Mahamadkhani, 2011; Kodad et al., 2010; Kodad and Socias I Company, 2004; Moheb et al., 2018). Peach is the close relative to almond. The susceptibility of peach cultivars to frost has also been studied and significant differences have been found between cultivars (Hatch and Walker, 1969; Miranda et al., 2005; Nyeki and Szabo, 1989; Okie, 1998; Szabò, 1992; Szabó et al., 1998; Szalay et al., 2010; Szymajda and Zurawicz, 2016).

Flower buds are the most frost-sensitive overwintering organs of almond. Changing of cold hardiness of overwintering organs can be most accurately determined by artificial freezing tests.
A study by Viti et al. (1994) examined the frost sensitivity of almond flowers at various phenological stages during flowering. Based on their experiences, cultivars with late flowering time had higher frost resistance, even if their flowers were in advanced phenological stages.
Snyder and Conell (1996) published a similar study on Californian almond cultivars' frost tolerance. Pink flower buds of the varieties ‘Sonora’ and ‘Price’ were less sensitive, they suffered only 30% frost damage at –5°C, while the other seven varieties had higher frost damage. In the

case of these two varieties, the open flowers were also more frost tolerant: while 100% flowers were damaged at –3°C frost of other varieties, it was –4.5 -–5.5°C in the case of 'Sonora' and 'Price'. Likewise, the differences between several varieties and between various flowering-phenological stages were investigated by (Sepahvand et al., 2014). In Spain 12 commercial almond cultivars was observed, and the tolerance to frosts of flowers was evaluation by chlorophyll fluorescence after artificial freezing (Kodad et al., 2010). The frost tolerance of different overwintering organs can be studied in several ways. Indirect laboratory methods can be used to infer the development of frost tolerance of genotypes. By measuring ion efflux, chlorophyll fluorescence assay, and determining the antioxidant capacity of plant organs, a large number of samples can be tested, which provides breeders with useful information during selection (Afshari et al., 2011; Kodad et al., 2010; Moheb et al., 2018). However, these studies do not track the frost resistance of flower buds during the whole dormant period; they give only a snapshot of spring frost tolerance. Miranda et al. (2005) examined two almond cultivars by artificial freezing ('Marcona' and 'Ferragnes') during the ecodormancy period. The critical temperature for frost tolerance of flower buds was -16.3°C.

2.12.2 Frost damages

Agricultural production is adversely affected by severe frost, which often results in crop freeze injury, low crop yield, and reduction in fruit quality (Song et al., 2021). An injury caused by frost is called frost damage. Plants are exposed frost damage not only due to the frost itself but also due to their freezing tolerance (Ambroise et al., 2020). In cold regions, frost is one of the greatest threats to the cultivation of almonds (Di Lena et al., 2017; Guillamón et al., 2022; Imani and Mahamadkhani, 2011; Rodrigo, 2000; Tromp, 2005). Particularly, spring frost damage, which is closely related to bud phenology, is an extremely significant factor in productivity (Campoy et al., 2011; Thomas and Hayman, 2018). It is considered the most important abiotic factor determining the distribution of most almond cultivars to regions with risks of spring frosts (Guillamón et al., 2022; Kodad et al., 2010; Vishal, 2021).

The most severe damage to deciduous fruit trees including almonds happens in buds, flowers, and developing fruits after dormancy, and losses caused by frost during bloom are usually more severe than those caused by low winter temperatures (Rodrigo, 2000). The risk of frost damage decreases as the spring season progresses. This is because temperatures are more favorable to fruit sets and later- blooming almond varieties are desirable adaptation traits in areas

where frost is a problem (Alonso and Socias I Company, 2010; Benmoussa et al., 2017; Guillamón et al., 2022; Prudencio et al., 2018a, 2018b).Temperatures and exposure time can affect the severity of damage at this time(Song et al., 2021).A few hours at temperatures below -1 or -2°C can cause serious damage and even ruin production for the year(Vitra et al., 2017). A drop in temperature below 0°C within 48 hours affects agricultural production. And if the temperature drops below -3°C within 24 hours, it seriously impacts agriculture (Song et al., 2021). However, different genotypes have different critical temperatures that damage overwintering organs (Afshari and Parvane, 2013; Imani et al., 2012; Imani and Mahamadkhani, 2011; Kodad et al., 2010; Szalay et al., 2016).

In Iran, Imani et al. (2012) evaluated the frost resistance of 'Ferrangness', 'Tuono' 'K-9-7', and 'K-16-25' almond genotypes at bloom under field and laboratory conditions. The results indicated that the severity of frost damage was influenced by temperature, variety, and stages of development, in that the laboratory test showed flower buds suffered a more severe frost damage rate at flowering at-3.2°C (100,100, 58, and 45% for Ferrangness, Tuono, K-9-7 and K-16-25) compared with the ballon stage at-6.4°C (100%,100%, 85% and 58% for Ferrangness, Tuono K-9-7 and K-16-25 respectively). A study was also conducted by (Imani and Mahamadkhani, 2011) on the resistance of almond cultivars to late frost at the flowering time under field conditions. The frost damage rate at -4°C at anthesis for 'Ferrangness', 'K-9-7', 'Rabie', and 'K-9-20' was 100% 50% 100%.and 0% respectively. At the popcorn stage, the 'Ferrangness' was damaged 25% and 'K-9-7' was not damaged at the same temperature.

A study was carried out by Moheb et al. (2018) to evaluate the susceptibility of almond genotypes to artificial freezing. The results indicated that susceptibilities were influenced by genotypes and temperature regimes. Chlorophyll fluorescence has been used to estimate the frost tolerance of almond cultivars stressed to different low temperatures (Kodad et al., 2010) and frost tolerance of almond flowers has shown the presence of a high genotypic variability in response to different frost stress.

2.13 Frost protection methods

Some countries have begun to use multiple frost prevention methods, such as traditional smoke, cover, spray, chemical fuel frost prevention, wind machines, and sprinklers to prevent frost damage (Song et al., 2021). These methods require a great deal of energy and are physically based. They must be done on the day or night of the frost event. Frost damage can also be

reduced by biological (avoidance and resistance) and ecological methods. Biological methods include inducing resistance without altering the genetic makeup of the plant; selecting species for phenological timing; and improving plant genetics. While ecological protection methods can include site selection for cropping improvement. Some ecological methods involve site selection for cropping, controlling nutritional status, soil management, cover cropping, and others (De Melo-Abreu, 2018; Snyder and De Melo-Abreu, 2005).

Research has also begun into the detection of genes responsible for the frost resistance of almonds, so we know more and more about the genetic background of frost tolerance in each variety (Alisoltani et al., 2016, 2015; Mousavi et al., 2014).

2.14 Physiology of hardening and dehardening of flower buds

As mentioned earlier, in cold regions, the geographical distribution of plant species is determined by the ability to tolerate low freezing temperatures as low temperature is the primary limiting factor for successful cultivation (Tromp, 2005). Cold temperature tolerance can be influenced by factors such as ice formation, water content, sugar content, starch content, and the nutritional status of the pistil (Rodrigo, 2000). However, genotype is the most influential factor (Szalay et al., 2017). Cold hardness in fruit trees avoids the occurrence of injuries typically caused by freezing temperatures. To distinguish the tolerance level of cultivars to low temperatures, it is very essential to know the optimum temperature for frost treatment at the different stages of bud development (Pedryc et al., 1999).

Hardening is a metabolic process in which carbohydrates and others are assimilated. During the hardening process in winter, flower buds of temperate trees undergo a series of developmental stages (Tromp, 2005). Over time, generative organ trees' frost hardiness also changes (Imani et al., 2012; Szalay et al., 2010; Tromp, 2005). During this period, the frost hardiness of overwintering organs gradually increases and then gradually decreases with the shift from rest to physiologically active growth (Lindén, 2002; Szalay et al., 2016, 2010; Tromp, 2005).

According to many studies, frost resistance is mainly determined by flower buds development. The flower buds of temperate fruit trees attain maximum hardiness when they are fully dormant (Imani et al., 2012; Miranda et al., 2005; Szalay et al., 2010; Tromp, 2005) and can even survive to the extent of -20 °C to -30 °C (Tromp, 2005). But as they begin to swell and expand into blossoms, they become more susceptible to freeze injury (Imani et al., 2012; Miranda et al., 2005; Szalay et al., 2010). The tolerance level of almond cultivars to low artificial

temperatures was studied at different phenological stages (Afshari and Parvane, 2013). The authors reported that buds were most hardy when they were fully dormant. As they began to swell and blossom, the frost hardiness of cultivars decreased. In almonds (Masip et al., 2018) assessed the cultivar tolerance level to low artificial temperatures at fruit set stages. The results obtained showed significant differences regarding susceptibility to frost temperatures in the studied cultivars.

The closely related peach to almond (Szalay et al., 2018), studied the cold hardiness of flowers at different phenological stages. The authors explained that cold hardiness decreased as blooming progressed and differences between the cultivars gradually decreased. These results are also verified in other Prunus species such as apricot (Szalay et al., 2016), plum (Szalay et al., 2017), and sweet cherry (Salazar-Gutiérrez et al., 2014). A study carried out in artificial freezing by the same worker on peach (Szalay et al., 2010), apricot (Szalay et al., 2017, 2016), plum (Szalay et al., 2017) and sweet cherry (Salazar-Gutiérrez et al., 2014) indicated frost hardiness of flower buds can be gained and lost as a function of time and temperature. The effect of time and cultivars decreased before full dormancy and during the ecodormancy stages. Buds were most susceptible to frost during the transitional periods of hardening and de-hardening. Equally on peach (Szalay et al., 2010), apricot (Szalay and Németh, 2010) reported that warm conditions increase flower vulnerability while low temperatures decrease it.

3. MATERIALS AND METHODS

3.1 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. Here the yearly mean temperature is 9.9-10 °C, the mean temperature of the growing season is 16.7-16.9 °C. The number of sunny hours per year is 1950 h, and the yearly precipitation is 550-570 mm. The soil is tsernozyom with 5% total lime content and 2.3-2.5 % humus (Ambrózy and Kozma, 1990).

The experimental orchard was planted in 1996. The spacing is 7 x 3 meters and the trees are on GF-677 rootstock. The orchard has no irrigation, the space between rows are covered by lawn. The canopy of the trees has a free style open structure with 3-4 limbs. During maintenance pruning, shoot and branch thinning is carried out every 2-3 years. 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. The list of accessions used in our experiment, their origin and their main characters are in Table 3. Two-four trees of each of all observed almond accessions were available for research work.

Table 3: Almond cultivars and accessions analyzed in our experiments

Name	**origin**
1/7	Hungarian genebank accession, presumably collected around Balaton-felvidék
26/43	Hungarian genebank accession, a candidate for national cultivar list, place of collection is unknown
35/29 Sóskút	Hungarian genebank accession, presumably collected around Balaton-felvidék
5/15	Hungarian genebank accession, presumably collected around Balaton-felvidék
6/10	Hungarian genebank accession, presumably collected around Balaton-felvidék
7/21	Hungarian genebank accession, presumably collected around Balaton-felvidék
Akali 57/2	Hungarian genebank accession, presumably collected around Balaton-felvidék
Diósárki	Hungarian genebank accession, presumably collected around Balaton-felvidék

Continued

Name	Origin
Érdi édes	Hungarian genebank accession, presumably collected around Balaton-felvidék
Eriane	origin is unknown, presumably a former French cultivar
Korai keményhéjú	Hungarian genebank accession, presumably collected around Balaton-felvidék
Sóskút 16/7	Hungarian genebank accession, presumably collected around Balaton-felvidék
Sóskút 66/3	Hungarian genebank accession, presumably collected around Balaton-felvidék
Sóskút 96/1	Hungarian genebank accession, presumably collected around Balaton-felvidék
Sóskút 96/5	Hungarian genebank accession, presumably collected around Balaton-felvidék
Budatétényi 70	Hungarian commercial cultivar, selected from a seedling population of unknown origin
Tétényi bőtermő	Hungarian commercial cultivar, selected from a seedling population of unknown origin
Tétényi kedvenc	Hungarian commercial cultivar, selected from a seedling population of unknown origin
Tétényi keményhéjú	Hungarian commercial cultivar, selected from a seedling population of unknown origin
Tétényi rekord	Hungarian commercial cultivar, selected from a seedling population of unknown origin
Belona	bred in IRTA Spain, parents: Blanquerna x Genco
Constanti	bred in IRTA Spain, parents: FGFD2 x open pollination
Marinada	bred in IRTA Spain, parents: Lauranne x Glorieta
Soleta	bred in IRTA Spain, parents: Blanquerna x Genco
Vairo	bred in IRTA Spain, parents: 4-665 x Lauranne

3.2 Flower bud development studies

Flower bud development studies were conducted over three years in 2019/20, 2020/21 and 2021/22.

3.2.1 Microsporogenesis studies

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 (Bartolini et al., 2006b; Hajnal et al., 2013; Julian et al., 2009; Németh, 2012; Szalay et al., 2006).

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.

Main Stages of microsporogenesis: Following the endodormancy establishment, the archesporium tissues appeared to gradually differentiate into a pollen string. After some time, the pollen mother cells (PMC) completely detached from each other prior to reduction process. When the reduction process occurred in pollen mother cells, the tetrads of four haploid cells were formed. At the end of the reduction process, the freely moving microspores were visible. Figure 6 shows the main stages of microsporogenesis.

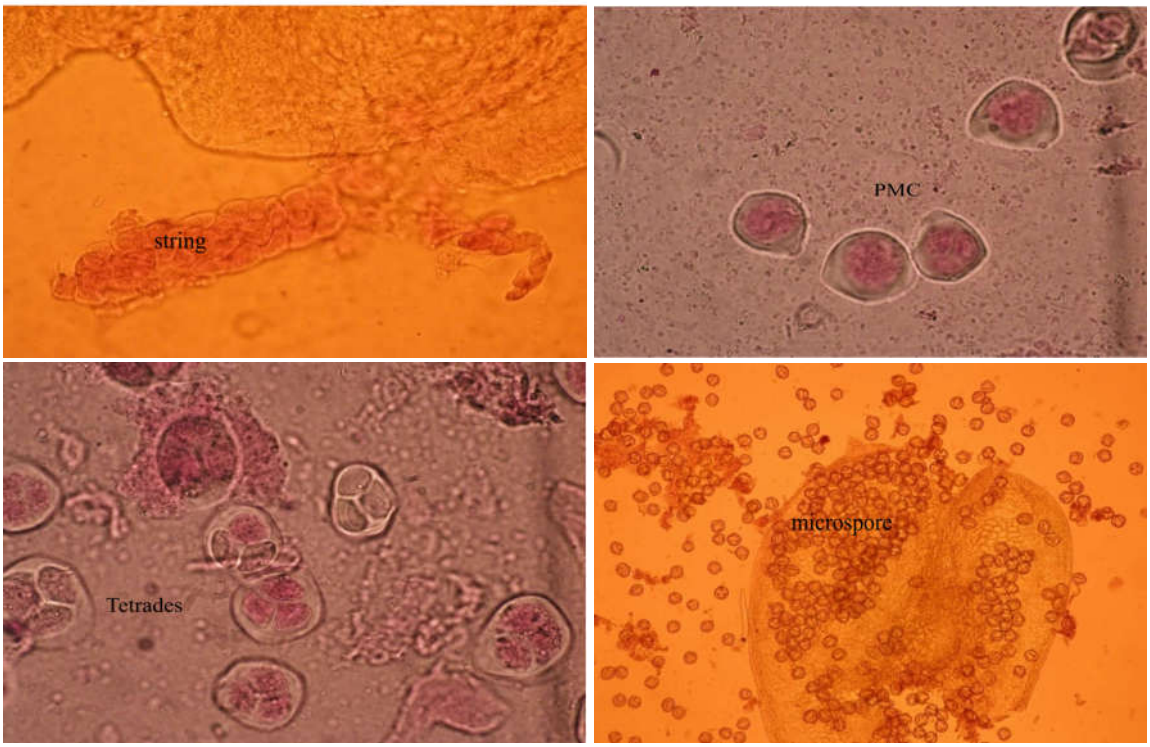


Figure 6. Important stages of microsporogenesis

3.2.2 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.

3.2.3 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. This was done every year between the periods mentioned in the microsporogenesis study method. One twig with 40–50 flower buds was taken as one replication. The samples were collected from different directions of one or two trees of the same accession. The collected samples of twings were transferred to the laboratory and immediately placed with their basal ends cut in one-litre 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 phenological developmental stage of each flower bud was assessed and the results were compared. The date when accessions had 50% of open flowers was regarded as the end of endodormancy.

3.2.4 Analysing the results regarding the date of endodormancy release

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’. Normality of the variables was checked with Shapiro-Wilk’s test (p>0.25). Homogeneity of variances was accepted by Levene’s equality test (p>0.05), with the except of pistil method in which case the homogeneity of variances was slightly violated. MANOVA was followed by univariate two-way ANOVA with Bonferroni’s correction. Finally, pair-wise comparisons were performed by Games-Howell’s post hoc test in case of pistil method and by Tukey’s post hoc test in other cases. The statistical analysis was performed using R statistical program version 2.1 (R.CoreTeam, 2021).

3.3 Blooming dates

Almond blooming dates were determined visually using the BBCH (Biologische Bundesantalt Bundessortenamt and chemische industrie) phenology scale of growth stage

identification key for stone fruits (Meier et al. 1994). Observations began at swollen buds and continued through bloom and petal fall in all three years. Two-four trees of each available cultivar were observed in all the three years. The beginning of the blooming date was recorded as when approximately 10% of the floral buds were open (BBCH61), the full blooming date as when at least 50% of the floral buds reached the full bloom stage with first the petal falling (BBCH65) and when all petals fallen (BBCH69). The length of blooming was determined based on the dates of the beginning and end of blooming. Hierarchical cluster analysis with squared Euclidean distance and Ward's agglomeration method were performed to classify cultivars based on the time to the beginning of flowering.

3.4 Method of chilling and heat estimation

Plant materials and sampling were performed as described at point 2.1. The beginning of endodormancy was considered when a regular chilling accumulation occurred that was indicated by natural leaf fall. It was 1st November in all three years. The end to the endodormancy date was determined using the microsporogenesis method for this study. 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.

The homogeneity of variances was accepted by Levene's equality test (p>0.05). The normality of the variables of chilling unit, chill hour number and growing degree hours was violated with Shapiro-Wilk's and Kolmogorov-Smirnov test (p>0.05). However, skewness and Kurtosis values indicate that it was not seriously violated. 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≤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.

3.5 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. Between September and February there was observation in the middle of every month. Occasionally, for technical reasons, this was done in the first or second half of the month. The last sampling day was directly before blooming in March. 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 temperature at which 50% of the flower buds were damaged) 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. Normality of the variables was checked by Skewness and Kurtosis value. Homogeneity of variances was accepted by Levene's equality test (p>0.05). At different sampling dates the year and accessions effect were examined separately. 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 (Figure 19).

3.6 Spring frost studies

To measure the freezing resistance of almond accessions at blooming time samples of twings with flower buds at the closed sepals, first pink, balloon, the start of bloom, full bloom, and end of bloom were collected for each accession at different dates. For each accession, the collected twings were subjected to artificial freezing temperatures. To determine the LT50 for each stage of each accession, we used 4 or 5 freezing temperatures. Three twigs from each accession per treatment were put into the climate chamber where one twig with 40–60 flower buds was considered as a replication for statistical analysis. The homogeneity of variance was accepted by Levene's equality test ($p>0.05$). The normality of the variables was checked by Skewness and Kurtosis values. The LT50 values were analysed using two-way ANOVA with factors 'cultivar' and 'flowering stage'. Pairwise comparisons were run by Tukey's post hoc test. The statistical analysis was performed using the IMB SPSS25 statistical program.

4. RESULTS AND DISCUSSIONS

4.1 Flower bud development

4.1.1 The process of microsporogenesis

The results showed that flower buds of almond accessions 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 (Figure 7). The string stage, which was marked as the beginning to the microsporogenesis process lasted 18 to 23 days. The transition from endodormancy to ecodormancy phase was also marked at this stage. The transition periods of the pollen mother cell (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 accessions. 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 accessions. The accessions 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.

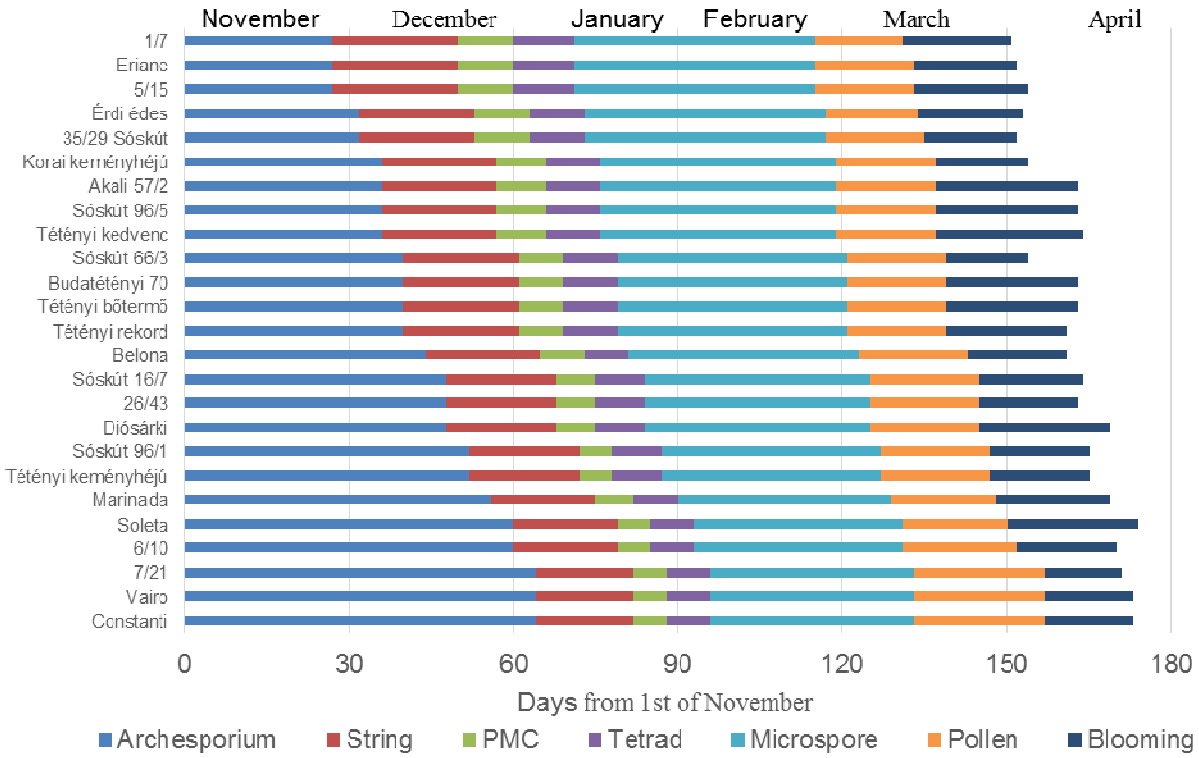


Figure 7. Phenological stages of microsporogenesis and blooming of almond accessions, 2019/20

In 2020/21, the period of the development of archesporial tissue to produce string cells was in most accessions shorter compared to the first year mainly 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 (Figure 8). 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 cells was relatively longer for most accessions 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.

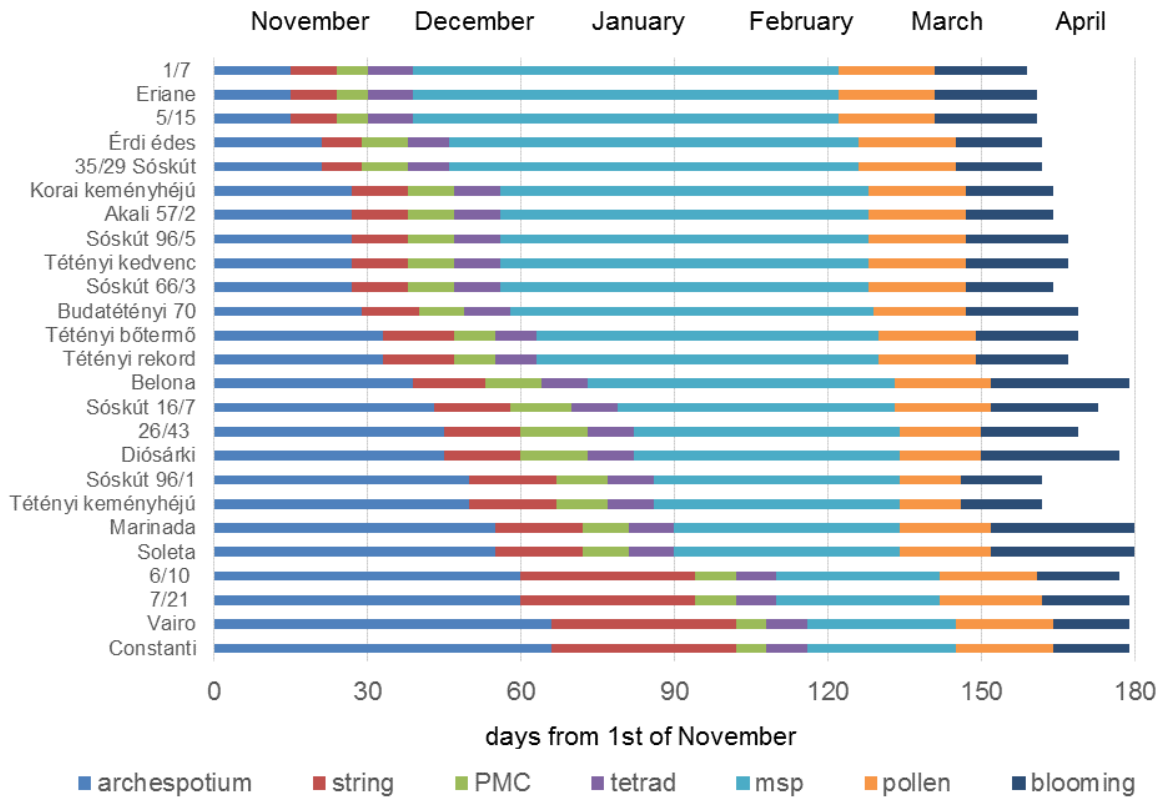


Figure 8. Phenological stages of microsporogenesis and blooming of almond accessions, 2020/21

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 (Figure 9). However, the transition periods of the string stage were quite short for all the accessions 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 cells was much slower in this year compared to both the other years as it lasted between 50 and 90 days depending on accessions. 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.

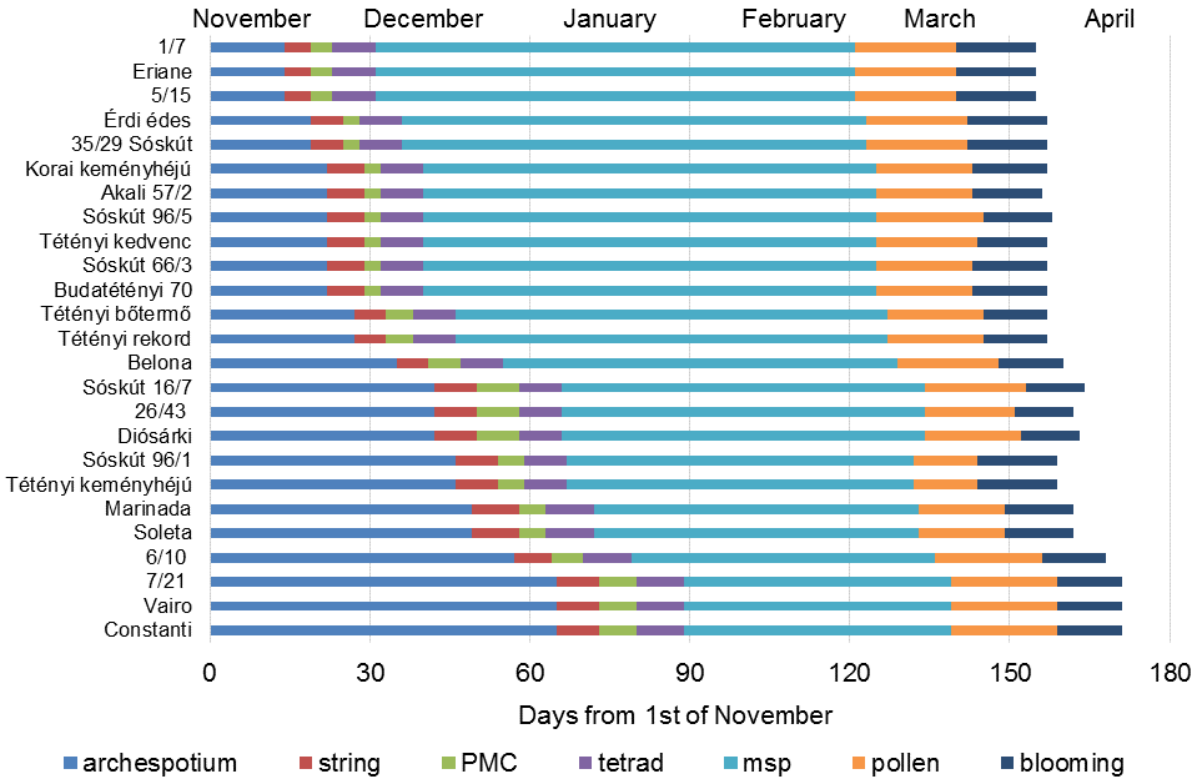


Figure 9. Phenological stages of microsporogenesis and blooming of almond accessions, 2021/22

As a summary, in all three years studied the accessions differed mainly in the length of archesporium and microspore stage. In 2020 we can see more differences in the length of archesporium stage as well. When we take only one year, some accessions had shorter archesporium period with longer string /microspore stage or vice versa, thus, by the time of pollen development the flower formation of the most accessions took approximately the same time. However, as the end of endodormancy is indicated by the appearance of string stage, they differed in their chilling requirement. Late flowering accessions (at the bottom of the diagrams) were less affected by the weather conditions as they showed similar flower development in each year.

In 2019 the extremely warm autumn resulted in delayed flower development, the archesporium period was longer, especially in early flowering accessions. The most differences among accessions in their flower bud development could be observed in 2020 when daily average temperatures remained above zero until the beginning of January.

Among all the studied accessions, ‘1/7’, ‘Eriane’ and ‘5/15’ had the shortest period of archesporial stage in the three studied years. The development of archesporial tissues of these

accessions took 27, 15 and 14 days and the development of microspore stage through tetrads took 71, 39 and 31 days in all the three during 2020, 2021 and 2022, respectively. The accession ‘1/7’ came to the end of microsporogenesis process on 9th of April, two days earlier than ‘Eriane’ and ‘5/15’ in 2020. However, in 2021 and 2022 all the three accessions had their end of microsporogenesis (ecodormancy) on the same day, i.e., on the 22nd or the 21st of March, respectively. Accessions ‘7/21’, ‘Constanti’ and ‘Vairo’ had the longest archesporium stage (64, 66 and 65days in all) during 2020, 2021 and 2022, respectively. The development of pollen mother cells to produce the microspores through tetrads took 96,116 and 89 days in that order. In the flower buds, the final form of pollen grain was noticed 19–24 days before blooming; the blooming started on the 5th, 14th and 9th of April in 2020, 2021 and 2022 respectively.

From the statistics point of view, the accessions showed significant difference ($p<0.001$) for their total length and in each developmental stage of microsporogenesis. Accordingly, accessions 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 10, where five main considerable groups and ten subgroups can be observed for each year. In Figure 10, the green group contains the accessions with the shortest microsporogenesis period (extremely short), while the purple consists of accessions with the longest (extremely long). The red group can be called as ‘short’ (microsporogenesis), while the orange and navy groups are of the ‘medium’ and ‘long’ groups, respectively. We can see that ‘1/7’, ‘Eriane’ and ‘5/15’ were grouped into the same ‘extremely short group in all the three years while ‘35/29 Sóskút’ and ‘Érdi édes’ were classified into that group in 2020 and 2021 but into the ‘short’ group in 2022 which shows the potential climate sensitivity of these two accessions. Note that the K-means clustering with 4 groups revealed almost the same clustering output as the introduced hierarchical one with the only difference that ‘35/29 Sóskút’ and ‘Érdi édes’ were classified into the ‘extremely short’ group in 2022, too. ‘Korai keményhéjú’, ‘Akali 57/2’, ‘Sóskút 96/5’, ‘Tétényi kedvenc’, ‘Sóskút 66/3’, ‘Budatétényi 70’, ‘Tétényi bőtermő’ and ‘Tétényi rekord’ were grouped as having short microsporogenesis in all the three years.

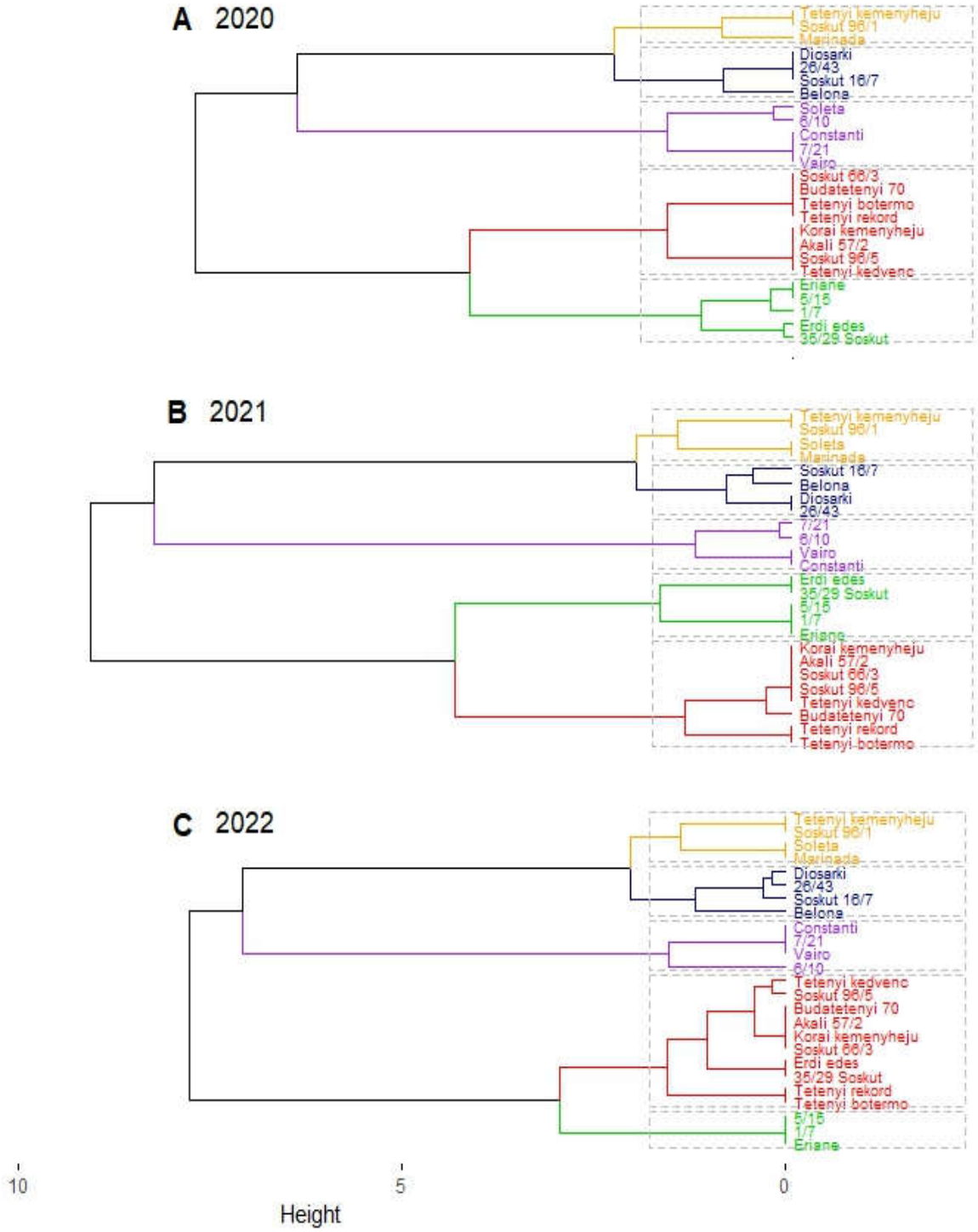


Figure 10. Dendrogram obtained by analyzing the developmental rate of microsporogenesis of almond accessions

'Belona', 'Sóskút 16/7', '26/43', 'Diósárki' are belonging to the medium group while 'Sóskút 96/1', 'Tétényi keményhéjú' and 'Marinada' are the 'long' accessions. 'Soleta' was classified as

medium accession in 2021 and 2022 while as ‘extremely long’ in 2020. In this difference K-means and hierarchical clustering agreed which refers to the climate sensitivity of accession ‘Soleta’. Together with accessions ‘7/21’, ‘Constanti’ and ‘Vairo’, ‘6/10’ was also classified in the ‘extremely long’ group in all the three years by both classification methods.

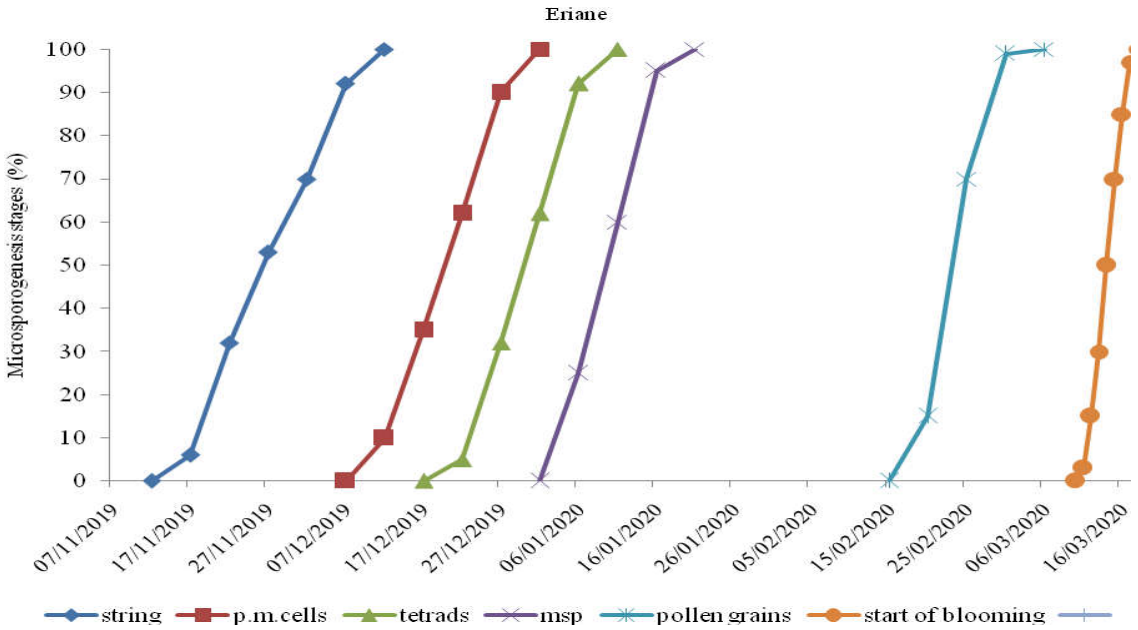
For each year, the proportion or occurrence of development as the percentage of all phenological stages of microsporogenesis was estimated. In Figure 11, one accession from the shortest and longest microsporogenesis groups is presented graphically.

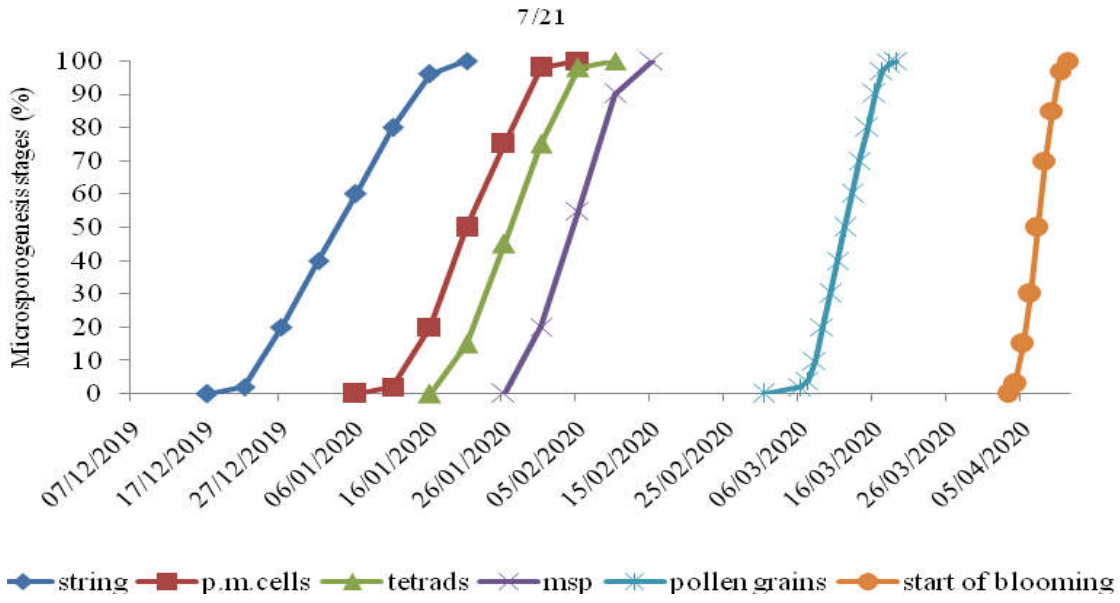
In 2019/20, the archesporium tissue of the earliest accession of ‘Eriane’ began to differentiate after the 17th of November. About 10 days later, the transition date (50% of string) to the string stage occurred on the 27th of November. The transition dates to the tetrad and microspore stages were recorded as the 30th of December and the 10th of January in that order. The final form of pollen grain was seen in the flower buds 18 days before blooming, as blooming began on the 12th of March. With the latest accessions, represented by ‘7/21’ the differentiation of archesporium tissue began right after the 17th of December. Transitions into the string and tetrad stages were estimated to occur on the 3rd and 27th of January respectively. The date of transition to microspores was observed on the 4th of February.

The archesporium tissue of flower buds ‘Eriane’ began to differentiate after the 6th of November in 2020/21. As a result, the date of transition to the string stage has been estimated to be the 16th of November. Tetrad and microspore transitions were recorded on December 1st and 11th, respectively. In the case of ‘7/21’, differentiation of archesporium tissue began after the 16th December. The transition to the string stage occurred on the 6th of January. Transitions to tetrads and microspores occurred on the 17th and 25th of January, respectively.

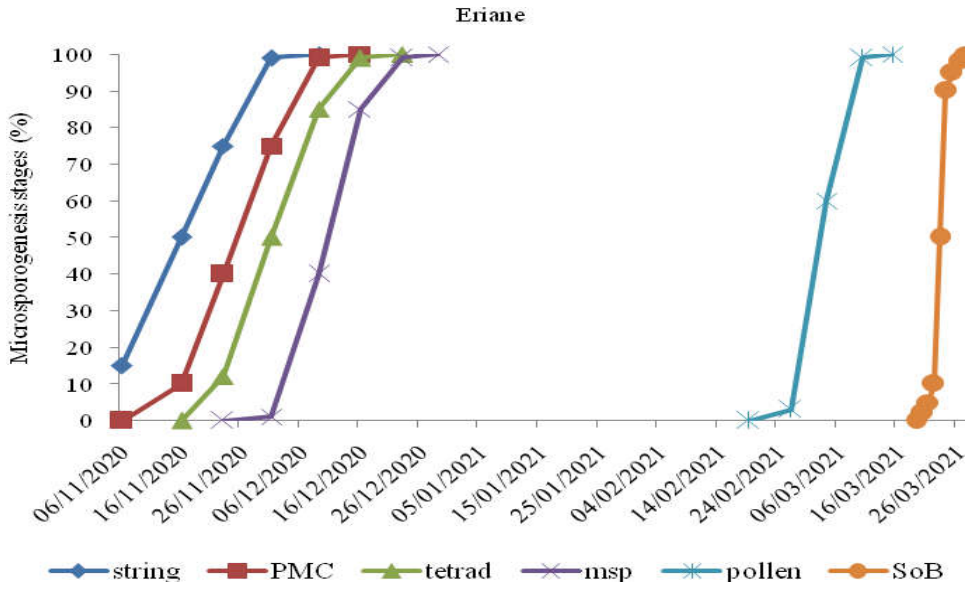
In 2021/22, the differentiation of archesporium tissue of flower buds of ‘Eriane’ started right after the 7th of November. The date of transition to the string stage was estimated as the 15th of November. Transition dates to the tetrad and microspore stage were recorded on the 24th of November and the 2nd of December, respectively. For ‘7/21’, the differentiation of archesporium tissue began right after the 21st of December. The date of transition to the string stage was estimated as the 5th of January. The date of transition to tetrads was observed on the 17th of February. Tetrads developed to full microspore stage eight days later, i.e. on the 25th of February.

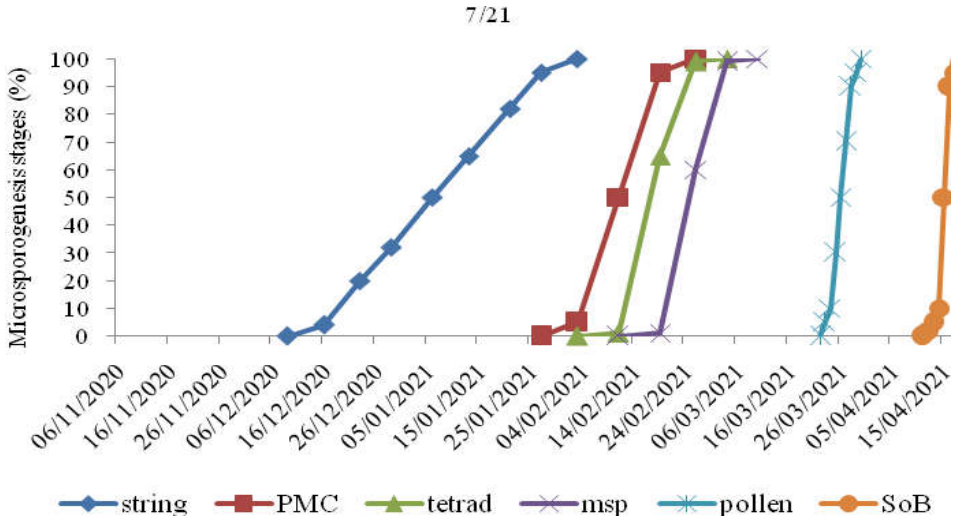
(a)


Eriane
Microsporogenesis stages (%)
100
90
80
70
60
50
40
30
20
10
0
07/11/2019
17/11/2019
27/11/2019
07/12/2019
17/12/2019
27/12/2019
06/01/2020
16/01/2020
26/01/2020
05/02/2020
15/02/2020
25/02/2020
06/03/2020
16/03/2020
string
p.m.cells
tetrads
msp
pollen grains
start of blooming


7/21
Microsporogenesis stages (%)
100
90
80
70
60
50
40
30
20
10
0
07/12/2019
17/12/2019
27/12/2019
06/01/2020
16/01/2020
26/01/2020
05/02/2020
15/02/2020
25/02/2020
06/03/2020
16/03/2020
26/03/2020
05/04/2020
string
p.m.cells
tetrads
msp
pollen grains
start of blooming

(b)


Eriane
Microsporogenesis stages (%)
100
90
80
70
60
50
40
30
20
10
0
06/11/2020
16/11/2020
26/11/2020
06/12/2020
16/12/2020
26/12/2020
05/01/2021
15/01/2021
25/01/2021
04/02/2021
14/02/2021
24/02/2021
06/03/2021
16/03/2021
26/03/2021
string
PMC
tetrad
msp
pollen
SoB


7/21
Microsporogenesis stages (%)
100
90
80
70
60
50
40
30
20
10
0
06/11/2020
16/11/2020
26/11/2020
06/12/2020
16/12/2020
26/12/2020
05/01/2021
15/01/2021
25/01/2021
04/02/2021
14/02/2021
24/02/2021
06/03/2021
16/03/2021
26/03/2021
05/04/2021
15/04/2021
string
PMC
tetrad
msp
pollen
SoB

(c)

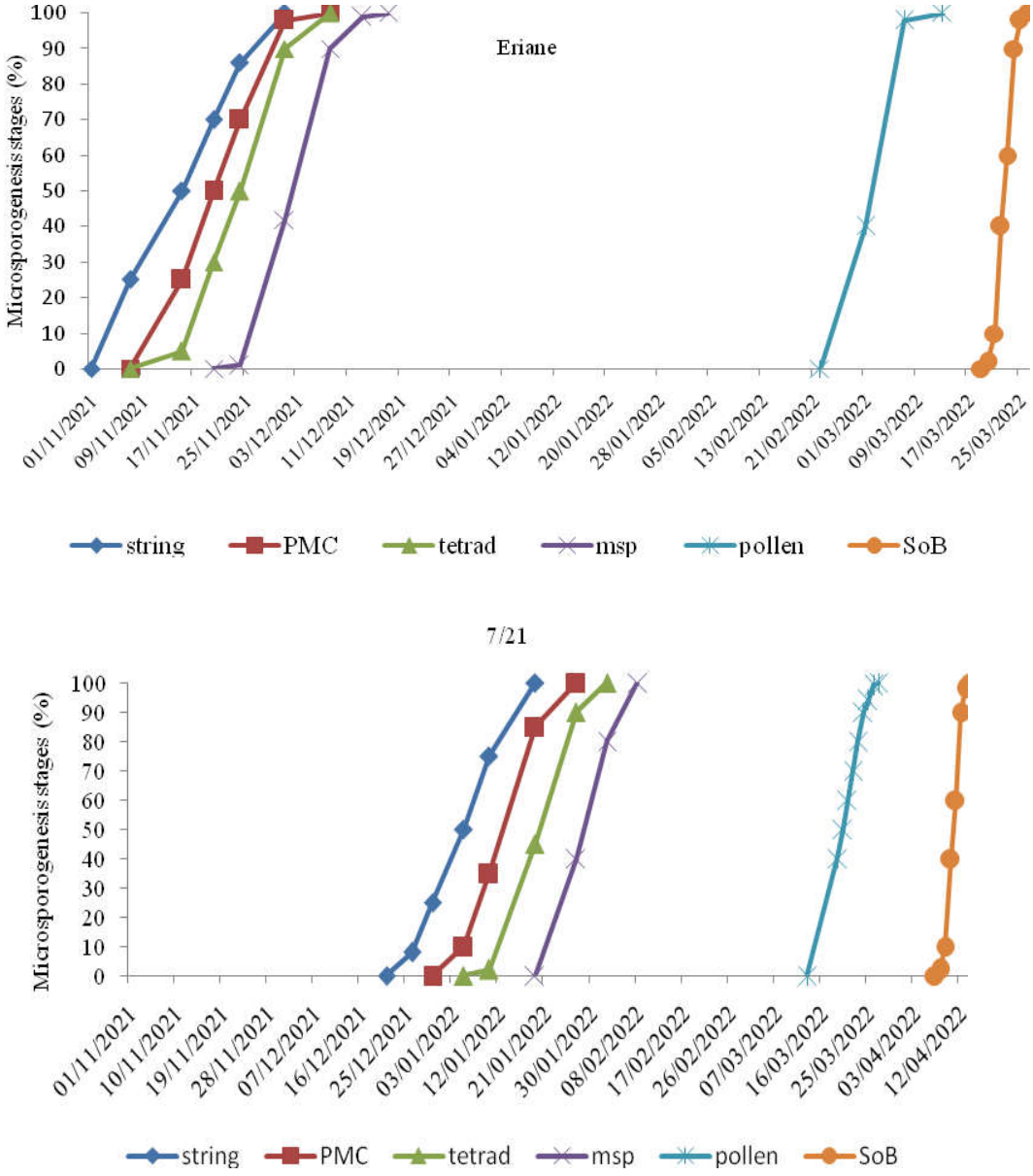


Figure 11. Occurrence of the phenological stages of microsporogenesis in the flower bud of almond accessions 'Eriane' and '7/21' in 2019/20(a), 2020/21(b) and 2021/22(c)

4.1.2 Pistil length measurements

Before the plants entered the endodormancy phase, the pistil growth was continuous and consistent (data not shown). Here we present the pistil growth of almond accessions from the start of endodormancy (Figure 12). 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

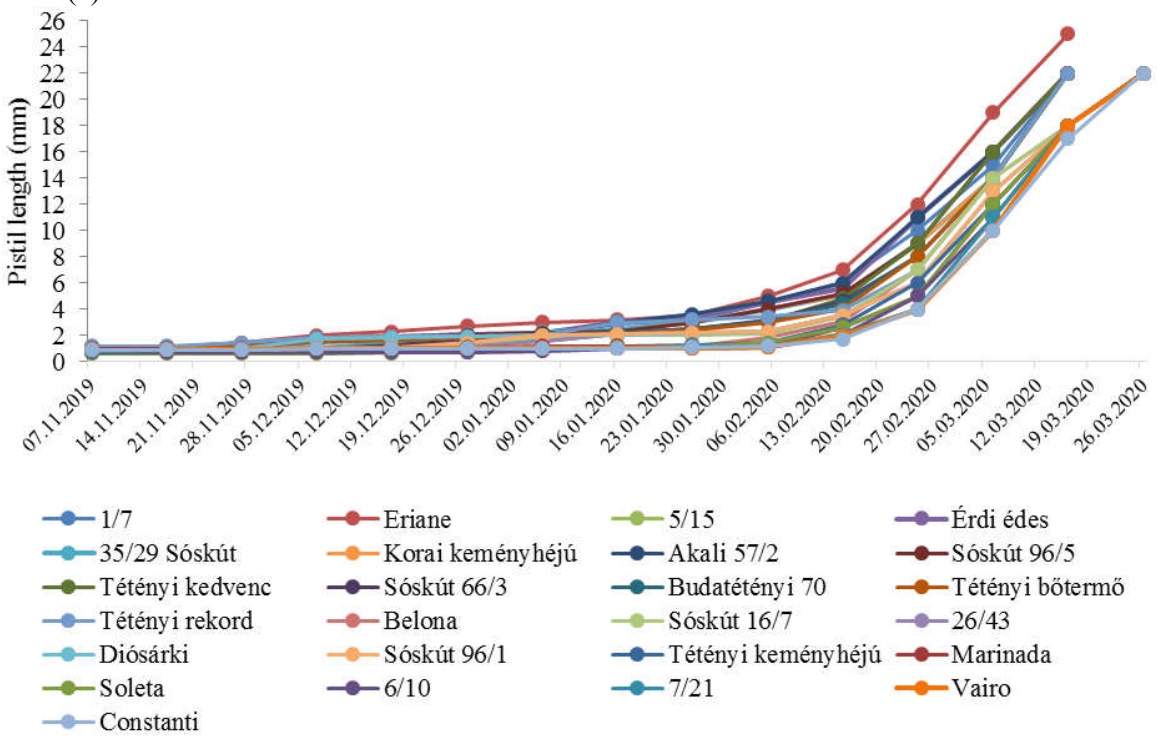
resumed when archesporium tissues in the anthers of the flower buds differentiated into the string stage which was considered as a transition from endodormancy to the ecodormancy phase, first at a very slow increment followed by a few days of highly concentrated growth prior to blooming. This indicated 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.

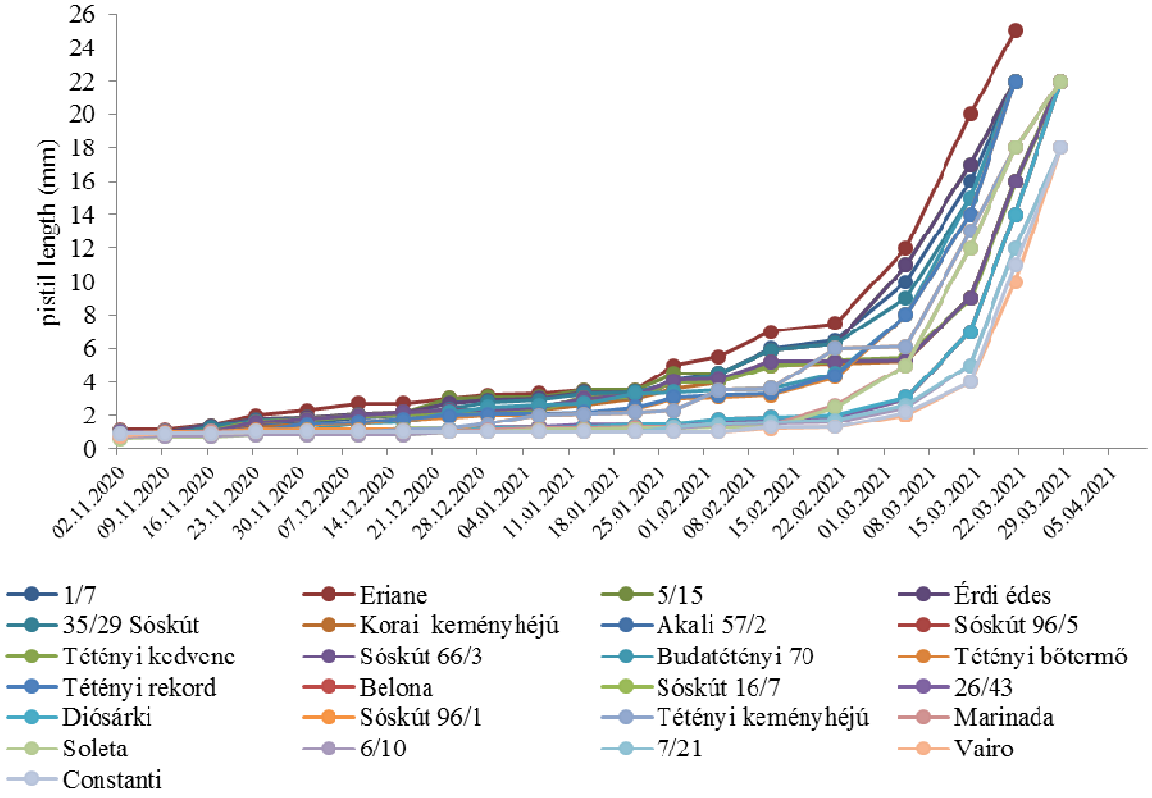
In 2019/20, the almonds presented pronounced variation in their pistil growth rate from the end of November onwards (Figure 12a). On the 27th of November for 'Eriane' and '5/15,' after a period of steady 1mm length, the rate of growth appeared to increase dramatically to 1.4 mm, after the necessary amount of chill had been accumulated. Also, the pistil length of '1/7' increased slightly at this time although the visible increment was recorded on the 7th of November.' This change in pistil growth characteristics was seen after the 16th of January for the latest ones such as '7/21', 'Vairo' and 'Constanti'.

In the years 2020/21 and 2021/22, the resumption of pistil growth of those earliest ones occurred on the 16th and 24th of November respectively. In the case of the latest ones, pistil growth was arrested until the 19th of January in 2020/21 (Figure 12b). While in 2021/22 their growth was arrested further up to the beginning of February (Figure 12c). By that time, the earliest accessions were already with pistil lengths between 4.5 mm and 5.5 mm. In that pistil growth stage, the microspores were distinguishable in the anthers of those earliest ones. Of those early flowering accessions, the resumption of pistil growth appeared at the moment when in the anthers of the flower buds, the full string stage was distinguishable. While of those latest ones, 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.

(a)


Pistil length (mm)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
07.11.2019
14.11.2019
21.11.2019
28.11.2019
05.12.2019
12.12.2019
19.12.2019
26.12.2019
02.01.2020
09.01.2020
16.01.2020
23.01.2020
30.01.2020
06.02.2020
13.02.2020
20.02.2020
27.02.2020
05.03.2020
12.03.2020
19.03.2020
26.03.2020
1/7
Eriane
5/15
Érdi édes
35/29 Sóskút
Korai keményhéjú
Akali 57/2
Sóskút 96/5
Tétényi kedvenc
Sóskút 66/3
Budatétényi 70
Tétényi bőtermő
Tétényi rekord
Belona
Sóskút 16/7
26/43
Diósárki
Sóskút 96/1
Tétényi keményhéjú
Marinada
Soleta
6/10
7/21
Vairo
Constanti

(b)


pistil length (mm)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
02.11.2020
09.11.2020
16.11.2020
23.11.2020
30.11.2020
07.12.2020
14.12.2020
21.12.2020
28.12.2020
04.01.2021
11.01.2021
18.01.2021
25.01.2021
01.02.2021
08.02.2021
15.02.2021
22.02.2021
01.03.2021
08.03.2021
15.03.2021
22.03.2021
29.03.2021
05.04.2021
1/7
Eriane
5/15
Érdi édes
35/29 Sóskút
Korai keményhéjú
Akali 57/2
Sóskút 96/5
Tétényi kedvenc
Sóskút 66/3
Budatétényi 70
Tétényi bőtermő
Tétényi rekord
Belona
Sóskút 16/7
26/43
Diósárki
Sóskút 96/1
Tétényi keményhéjú
Marinada
Soleta
6/10
7/21
Vairo
Constanti

(c)

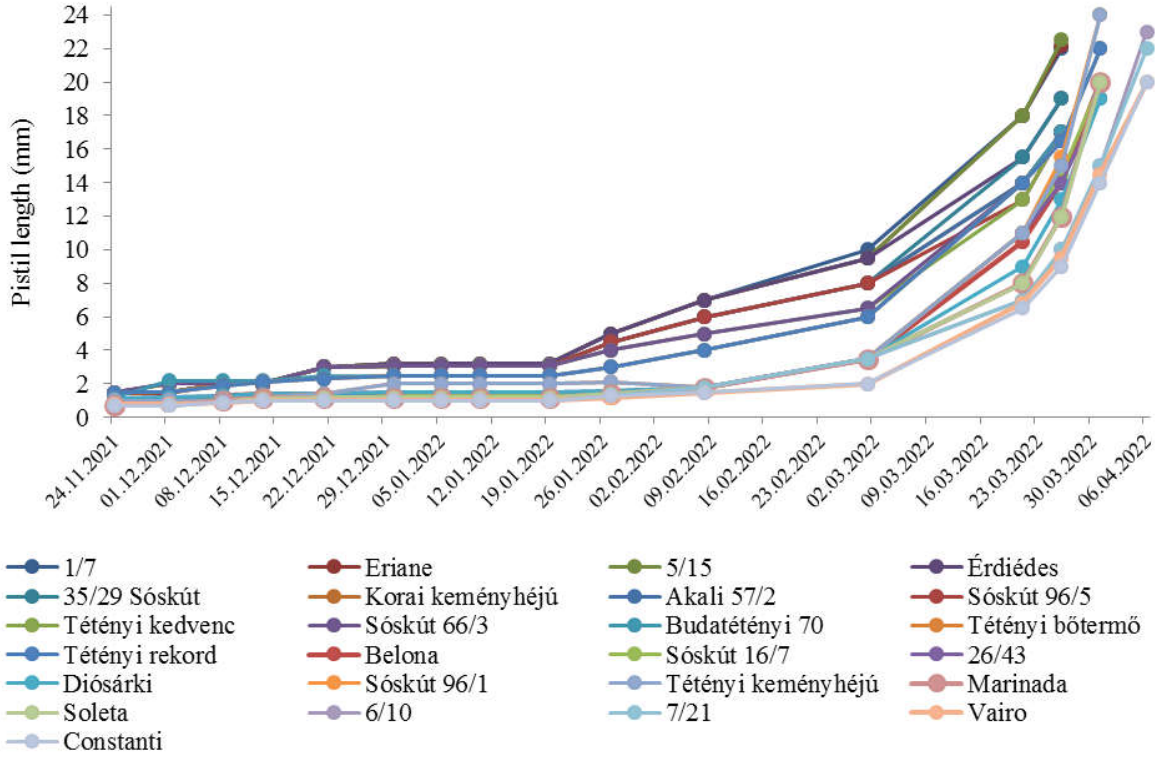


Figure 12. Changes in pistil length of almond accessions during winter of 2019/20 (a) 2020/21 (b) and 2021/22 (c)

4.1.3 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 (Figure 13).

In the year 2019/20, flower buds of the earliest accessions ‘Eriane’, ‘1/7’ and ‘5/15’ started to present open flowers in the first week of November (Figure 13a). The developmental rates were gradual. On the 17th of November, about 22% of the buds presented open flowers. Twenty days later, on the 7th of December, the amount reached 50% and 100% on the 16th of January. By that time, they reached 50% of open flowers and in the anthers of the flowers, 90% of string was distinguishable. Flower buds opened 100% following the appearance of microspore stage and pistil growth was already resumed.

In the case of the latest accessions ‘7/21’, ‘Vairo’ and ‘Constanti’, flower bud opening started after the 6th of January. In the anthers of the flower buds, separate pollen mother cells of

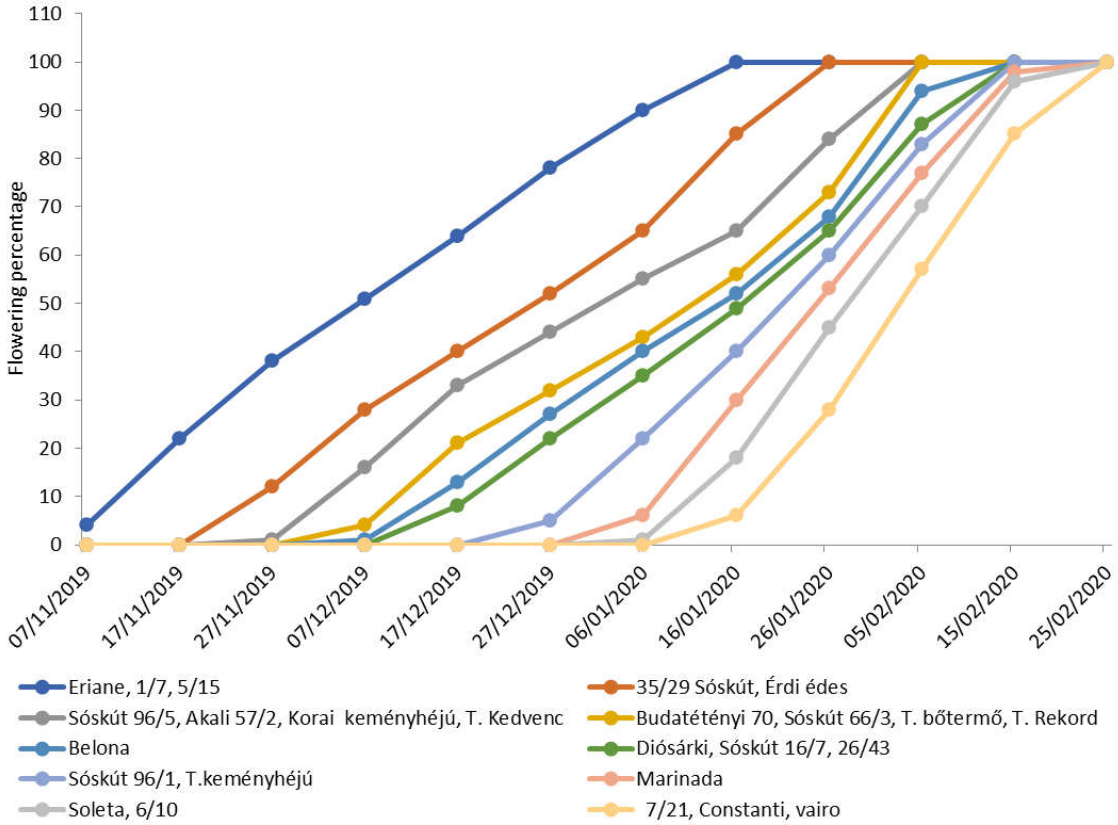
microsporogenesis stages were distinguishable at this moment. Once started, of flower bud opening increased at higher rates as indicated by steep slopes of the sigmoid lines. Of those latest ones, the proportion of opened flowers reached the half in the first week of February and 100% toward the end of the month. This was thirty days before flowering under field conditions. During this year, the flower buds of forced shoots for most of the studied accessions presented around 50% open flowers with the appearance of tetrads and microspore stage.

In the year 2020/21, flower buds of the earliest accessions began to present open flowers after the 1st of December and fifteen days later about 50% of the buds presented open flowers (Figure 13b). The flower bud opening began with the appearance of tetrads and in the anthers of the flower buds the end of the reduction process was already indicated by the occurrence of microspore stage at the time when about 50% of the buds presented open flowers.

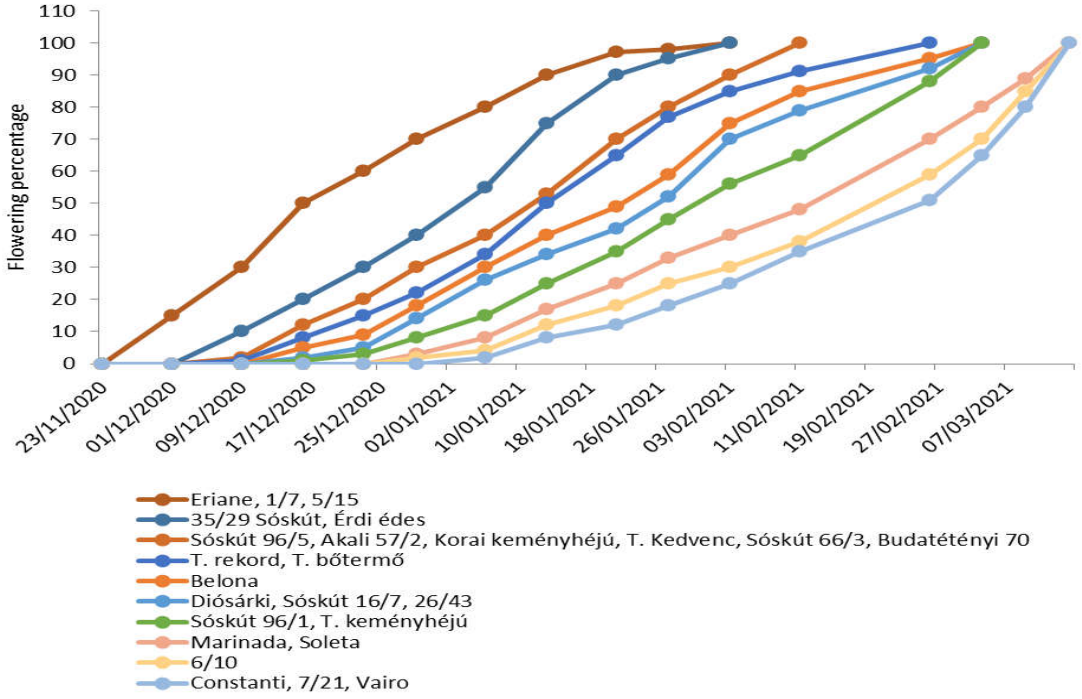
For the case of the latest ones '7/21', 'Vairo' and 'Constanti', flower bud opening began after the 6th of January with the string stage formation. Around fifty days later on the 26th of February, the proportion of opened flowers reached more than half when in the anthers of the flower buds the microspores and the first change of pistil length increment rates were noticeable. 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 similar to that of 2019/20. However, with some accessions flower bud opening started with the transition to string stage and presented more than half open flowers with development of tetrads.

In the year 2021/22, the development of flower buds under the forcing started a bit earlier than in 2020/21 but similar to that of 2019/20. The rate of flower bud opening was quicker in this year than in the years 2019/2020 and 2020/21 (Figure 13c). On the 15th of November, about 25% of the buds of the earliest accessions 'Eriane', '1/7' and '5/15'presented open flowers when the archesporium tissue in the anthers of flowers differentiated to the string stage. Nine days later, the flower buds presented 50% open flowers with the formation of tetrads. By that date, the flower buds of these accessions presented around 38% and 1% open flowers in 2020 and 2021 respectively. This is probably due to differences in environmental factors observed in the three years influenced the response of buds to forcing temperatures and flowering date in spring. For most of the studied accessions, the flower bud opening started with the formation of string stage and reached around 50% open with the appearance of separate pollen mother cells and tetrads during this year. This is probably due to differences in environmental factors, especially temperature.

(a)


Flowering percentage
110
100
90
80
70
60
50
40
30
20
10
0
07/11/2019
17/11/2019
27/11/2019
07/12/2019
17/12/2019
27/12/2019
06/01/2020
16/01/2020
26/01/2020
05/02/2020
15/02/2020
25/02/2020
Eriane, 1/7, 5/15
35/29 Sóskút, Érdi édes
Sóskút 96/5, Akali 57/2, Korai keményhéjú, T. Kedvenc
Budatétényi 70, Sóskút 66/3, T. bőtermő, T. Rekord
Belona
Diósárki, Sóskút 16/7, 26/43
Sóskút 96/1, T.keményhéjú
Marinada
Soleta, 6/10
7/21, Constanti, vairo

(b)


Flowering percentage
110
100
90
80
70
60
50
40
30
20
10
0
23/11/2020
01/12/2020
09/12/2020
17/12/2020
25/12/2020
02/01/2021
10/01/2021
18/01/2021
26/01/2021
03/02/2021
11/02/2021
19/02/2021
27/02/2021
07/03/2021
Eriane, 1/7, 5/15
35/29 Sóskút, Érdi édes
Sóskút 96/5, Akali 57/2, Korai keményhéjú, T. Kedvenc, Sóskút 66/3, Budatétényi 70
T. rekord, T. bőtermő
Belona
Diósárki, Sóskút 16/7, 26/43
Sóskút 96/1, T. keményhéjú
Marinada, Soleta
6/10
Constanti, 7/21, Vairo

(c)

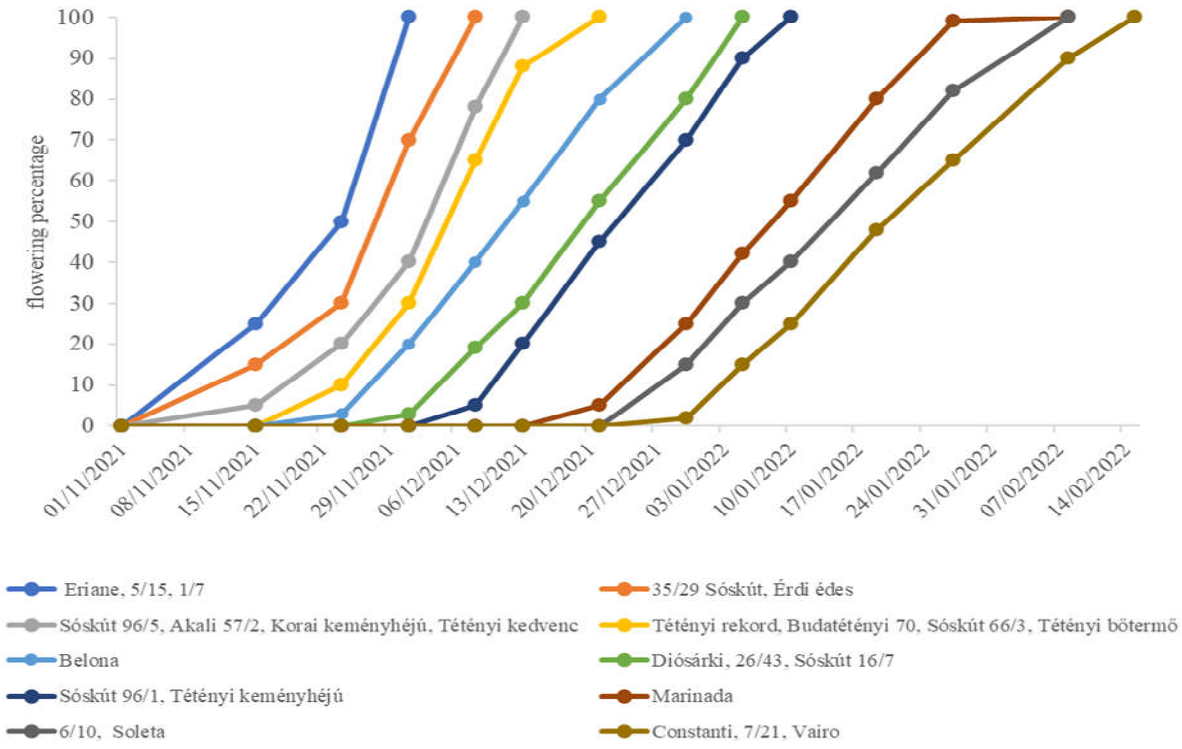


Figure 13. Flower bud development under forcing conditions of almond accessions in 2020/21 in 2019/20 (a), 2020/21 (b) and 2021/22 (c)

4.1.4 Connection between temperature conditions and flower bud development results

In the first analysis the effect of the year and accessions were studied including all data on microsporogenesis, pistil length and forcing. The overall MANOVA resulted in significant accessions 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 (accessions: F (24; 48)>28.8; year: F (2; 48)>13.71, all with $p<0.001$). Post hoc test results show that all three methods were able to differentiate the accessions, however, showed different resolutions and slightly different classifications of the accessions (Table 4). The most groups (eight) were found among microsporogenesis studies, whereas three groups formed by pistil length and two forcing method. They all agree that accessions '1/7', 'Eriane' and '5/15' are significantly earlier than all the others. However, based on pistil method, accessions '35/29 Sóskút', 'Érdi édes' 'Akali 57/2'and 'Tétényi bőtermő' are also appear among the earliest. Similarly, according to pistil length measurements, the accessions '6/10' 'Soleta', '7/21', 'Constanti', and 'Vairo' are significantly later. But based on the other two methods, accessions

‘7/21’ ‘Constanti’ and ‘Vairo’ are the significantly later ones. As for the year comparisons, post hoc tests agreed that year 2021/22 resulted in the earliest development based on all the three methods. However, while in case of microsporogenesis method, the development was significantly earlier in this year than in 2020/21 and in year 2019/2020 it was significantly later than both of the other two years, the order was different in case of forcing method: the significantly latest year was 2020/21. In case of pistil method, years 2021/22 and 2020/21 did not differ significantly, while 2019/2020 resulted in significantly later development.

Table 4: Length of endodormancy based on microsporogenesis, pistil and forcing methods.

accessions	endodormancy Length (days) for 2019/20, 2020/21, 2021/22		
	Microsporogenesis method	Pistil method	Forcing method
5/15	18±6.66^{a}	21±5.69^{a}	35±11.06^{a}
1/7	18±6.66^{a}	25±10.59^{a}	35±11.06^{a}
Eriane	18±6.66^{a}	21±5.59^{a}	35±11.06^{a}
35/29 Sóskút	24±6.43^{ab}	28±16.09^{a}	48±18.36^{ab}
Érdi édes	24±6.43^{ab}	21±5.69^{a}	48±18.36^{ab}
Akali 57/2	28 ±6.03^{abc}	25±4.93^{a}	54±20.42 ^{ab}
Korai keményhéjú	28 ±6.03^{abc}	30±13.58^{ab}	54±20.42^{ab}
Sóskút 96/5	28 ±6.03^{abc}	25±4.93^{a}	54±20.42 ^{ab}
Tétényi kedvenc	28±6.03^{abc}	33±20.31^{ab}	54±20.42^{ab}
Sóskút 66/3	29 ±8.74^{abcd}	29±14.57^{ab}	59 ±20.50^{ab}
Budatétényi 70	30 ±88.54^{abcd}	30±4.04^{ab}	59±20.50 ^{ab}
Tétényi bőtermő	33 ±6.03^{abcde}	28±4.36^{a}	59 ±21.08^{ab}
Tétényi rekord	33 ±6.00^{abcde}	37±18.34^{ab}	59±21.08 ^{ab}
Belona	39±4.51 ^{bcdef}	53±14.64^{ab}	67±22.75 ^{ab}
Sóskút 16/7	45±2.89 ^{cdefg}	53±14.73^{ab}	71±19.70 ^{ab}
26/43	45±2.52 ^{defg}	52±12.70^{ab}	71 ±21.17^{ab}
Diósárki	45±2.52 ^{defg}	37±5.00^{ab}	71 ±19.70^{ab}
Sóskút 96/1	46 ±2.65^{efgh}	54±3.46^{abc}	72±16.74 ^{ab}
Tétényi keményhéjú	46 ±2.65^{efgh}	64±19.29^{bcd}	72 ±17.35^{ab}
Marinada	53±3.46 ^{fgh}	89±9.29^{cd}	85 ±16.50^{ab}
Soleta	54±5.03 ^{fgh}	93±6.56^{d}	88±13.50 ^{ab}
6/10	58±3.03 ^{gh}	93±6.56^{d}	91±18.68 ^{ab}
7/21	63±2.52^{h}	91±7.23^{d}	97±19.01^{b}
Constanti	65±1.16^{h}	96±8.51^{d}	97±19.22^{b}
Vairo	65±1.16^{h}	97±5.69^{d}	97±19.22^{b}

Variables represent the mean of replications ± their corresponding standard deviation. Means having same letter(s) in a column are not significantly different according to two -way ANOVA followed by Tukey’s post hoc test ($P \leq 0.05$).

Daily average temperatures in the almond orchard were recorded by a local automatic meteorological station in the three studied years (Figure 14 and Appendix 2).

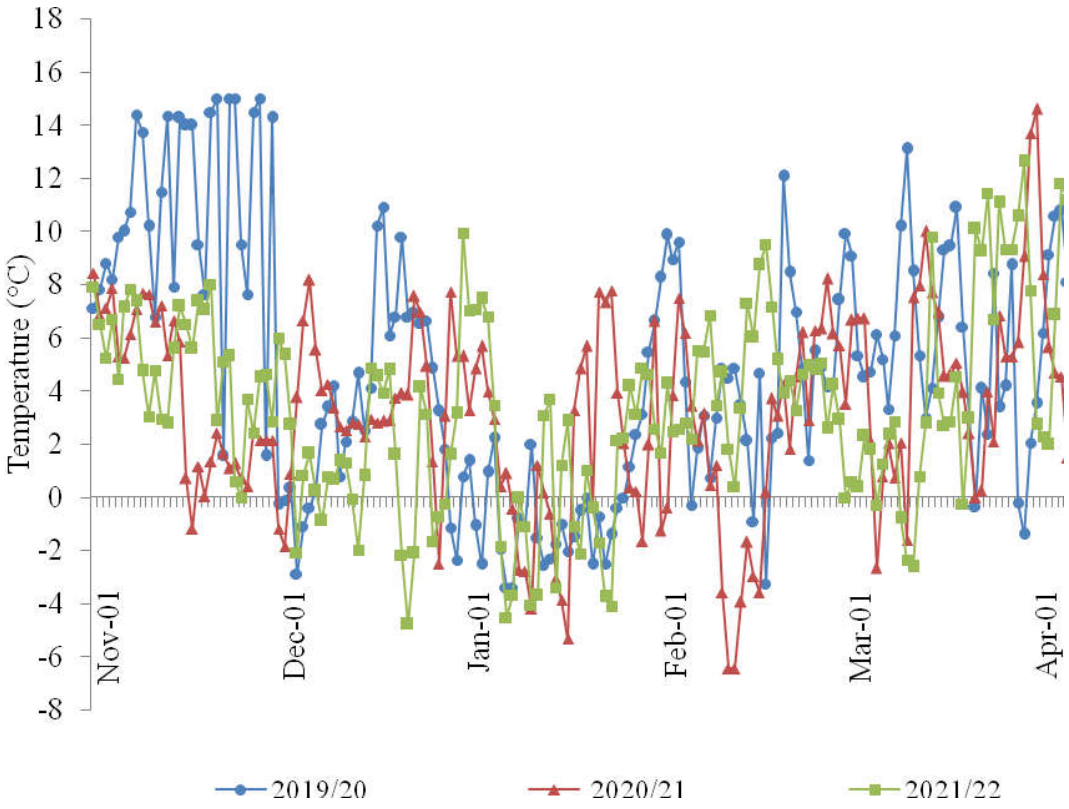


Figure 14. Daily average temperatures observed in the studied seasons of 2019/20, 2020/21 and 2021/22.

According to our results, anthers of flower buds remained at the same stage of development during endodormancy, characterized by the appearance of archesporium tissues, with a pistil having no visible growth. At winter dormancy release, archesporium tissues of anther differentiated into pollen mother cells and pistil growth rate accelerated. Similar anther development process has been also reported in other *Prunus* species (Fadón et al., 2019, 2018; Szalay, 2006). In apricot many studies agree (Andreini et al., 2012; Hajnal et al., 2013; Herrera et al., 2022; Julian et al., 2009; Szalay and Németh, 2010; Wu et al., 2019) that the finished differentiation of archesporium tissues of flower buds has been related to the chilling fulfilment and with endodormancy release, an advanced microsporogenesis process was observed with the appearance of tetrads and pollen grain. The result of this work is in accordance with the reports of the authors.

The speed of flower bud development differed depending upon almond cultivars but the dates and transition periods to each stage of microsporogenesis for a specific cultivar altered to some degree depending on yearly local climatic conditions, particularly the temperatures. The climate sensitivity of almonds is not the same. In previous studies (Lamp et al., 2001) variations in time of flower bud development of almonds occurred among locations, years, within and among almond cultivars. The same result has been revealed in apricot (Bartolini et al., 2006b; Hajnal et al., 2013; Szalay et al., 2019). Our results show that low temperatures during the endodormancy period had an important microsporogenesis process advancing effect, whereas extended periods of low temperatures during the microsporogenesis process of ecodormancy phase had delaying effect for development of pollen grains and consequently, on bloom dates of almonds. Similar results have been reported by (Citadin et al., 2001; Harrington et al., 2010; Scalabrelli and Couvillon, 1985). The reason is that cold temperatures during ecodormancy delay flower bud development as longer time is required for the buds to accumulate their heat requirements. But heat accumulation may take place much faster toward early springs and can speed up the change in flower bud development (Alonso et al., 2005; Lamp et al., 2001; Szalay and Németh, 2010). On the other hand, the mild winter during the ecodormancy of the microsporogenesis process accelerated the flower bud developmental rate and decreased frost hardiness.

Flower bud developmental rate emphasizing special attention on determining endodormancy release has been the focus of great interest in this field of research for the quantification of chilling units and the growing degree hour's needed of a given cultivar (Szalay et al., 2019).

4.2 Flowering time of the almond cultivars.

The flowering time of the tested accessions is shown in Figure 15. The accessions have been arranged in the order of their flowering time in 2020. In the other two years, the sequence of flowering time of the accessions was different. The flowering time of the following accessions was variable and unstable: 'Belona', 'Budatétényi 70', 'Tétényi keményhéjú', 'Sóskút 16/7', 'Sóskút 66/3', 'Sóskút 96/1', 'Sóskút 96/5'. The flowering time of other accessions was much more stable. During the three-year period, 2020 was the earliest year of flowering, while the other two years were about a week and a 12 later. And the latest flowering was observed in 2021.

Nevertheless, some accessions had differences of less than a week. The earliest-blooming cultivars had the biggest difference between years.

In 2020, the flowering of the earliest accession began on March 10, while flowers first opened on the latest accession 26 days later, on April 5. Depending on the accession, flowering lasted from 14 to 27 days.

In 2021, the earliest one bloomed on March 22 and the latest on April 14, a 23 difference of days. The length of the flowering time was between 15 and 28 days, depending on the accession.

In 2022, we observed the opening of the first flowers on the earliest accession on March 21, and on the latest 19 days later, on April 9. The length of the flower opening varied between 11 and 15 days. A negative correlation is observed between flowering time and length of blooming. The later an accession starts blooming in a given year, the shorter it lasts. There are accessions with a prolonged flowering time among the 25 investigated accessions. These are: 'Akali 57/2', 'Belona', 'Budatétenyi 70', 'Diósárki', 'Marinada', 'Soleta', 'Sóskút 96/5', 'Tétényi bőtermő', 'Tétényi kedvenc'. According to the three-year observations for the beginning to flowering times, the tested accessions were classified and the dendrogram generated by hierarchical cluster analysis with Ward method is presented in Figure 16, where five groups can be observed. In Figure 16, the accessions belonging to group 1 indicate early accessions while those accessions in group 2 indicate medium. The accessions in group 3 can be called middle accessions while the accessions in group 4 and group 5 are of the mid-late and late accession groups respectively. In our experiment, five of the 10 varieties listed in the National Register of Cultivars were examined; these are the main cultivars in Hungary today. All them ('Tétényi kedvenc' 'Budatétenyi 70', 'Tétényi bőtermő', 'Tétényi rekord' and 'Tétényi keményhéjú') are included in the group of medium flowering time.

Almond is one of the earliest flowering fruit species, so spring frosts strongly threaten their crop safety. Its cultivars are self-fertile, and during the flowering period the environmental conditions are not always suitable for the work of insects, ensuring adequate pollen transfer. Flowering time is an important factor in choosing promising cultivars. The flowering of almond trees can start earlier in January in Mediterranean climates (Egea et al., 2003; Lamp et al., 2001; Martínez-Gómez et al., 2017). According to the result of this research work, flowering times differ depending on cultivars as well as yearly climatic conditions. These results have also been reported in almonds (Alonso et al., 2005; Connell et al., 2018; Lamp et al., 2001; Prudencio et al.,

2018b; Thomas and Hayman, 2018). and other *Prunus* species (Alburquerque et al., 2008; Szalay et al., 2000a).

As has been observed previously on almond cultivars (Benmoussa et al., 2017; Connell et al., 2018) high temperatures during the chilling phase delayed the flowering date of the studied almond cultivars. But high temperatures during the ecodormancy phase had the reverse effect. The duration of the flowering period was also inversely related to the commencement of the flowering date and warm temperatures. Of these latest cultivars, the duration of flowering was somewhat shorter when compared with those of the early bloomers. This may be because the higher temperatures in spring coincided with late flowering, favoring the flower development and shortened the flowering period for them. This observed flowering characteristic of almonds agrees with the previous report of apricot cultivars (Szalay et al., 2000a). Almond flowering time is an essential agronomic characteristic for synchronizing flowering time to a given climatic condition in specific production areas, particularly late flowering in cold climates is an advantage as a mechanism for frost escapes (Kodad et al., 2010; Rodrigo, 2000). From the point of view of increasing crop security, it would be of great importance if we could grow cultivars that bloom later in areas with problems of spring frost. We have found these types of cultivars in the study area. ‘Marinada’, ‘Soleta’, ‘Vario’ and ‘Constanti’ get a lot of attention due to their mid-late or late flowering time.

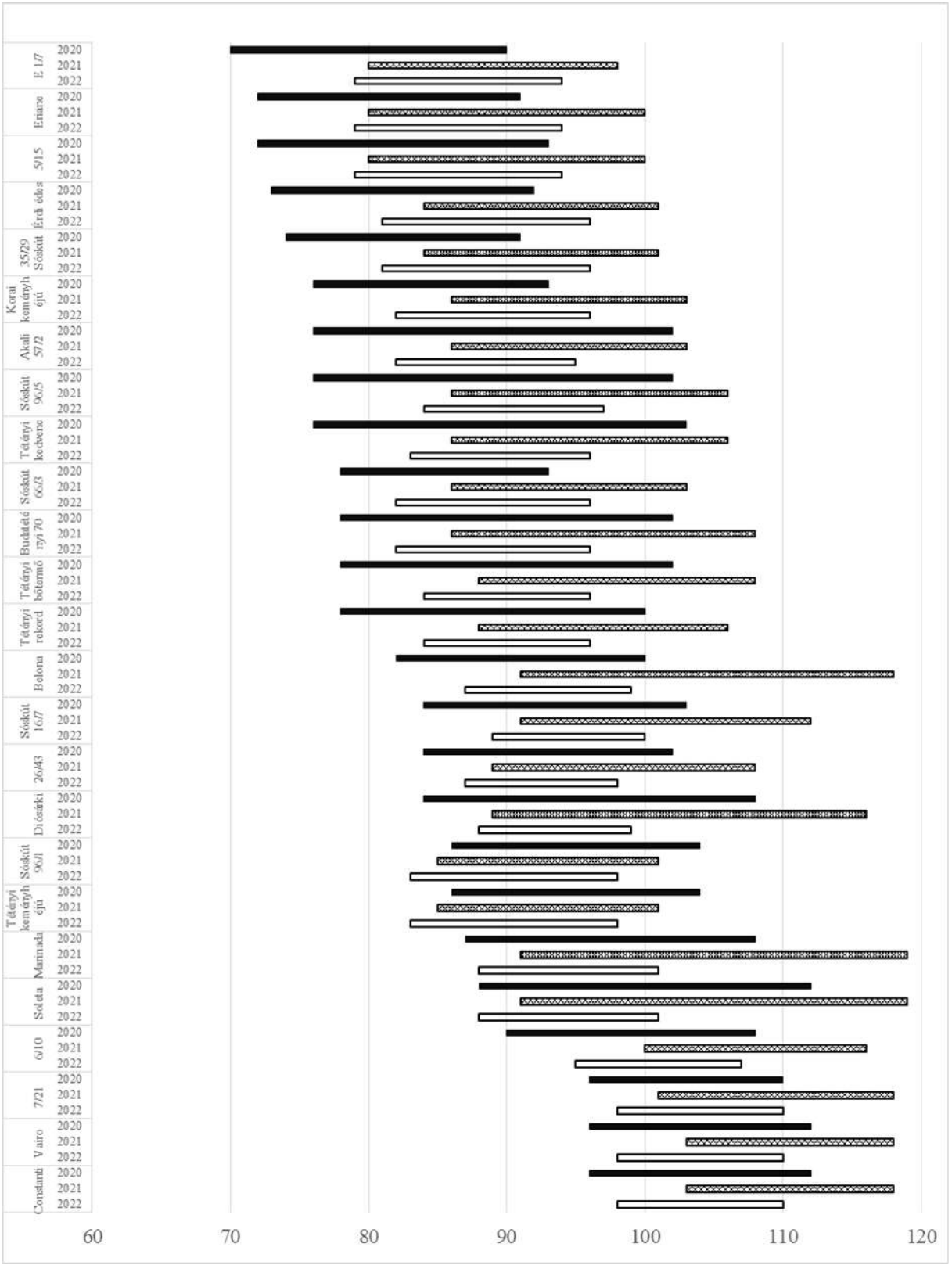


Figure 15. The flowering time of the investigated almond accessions in the three years between 2020-2022

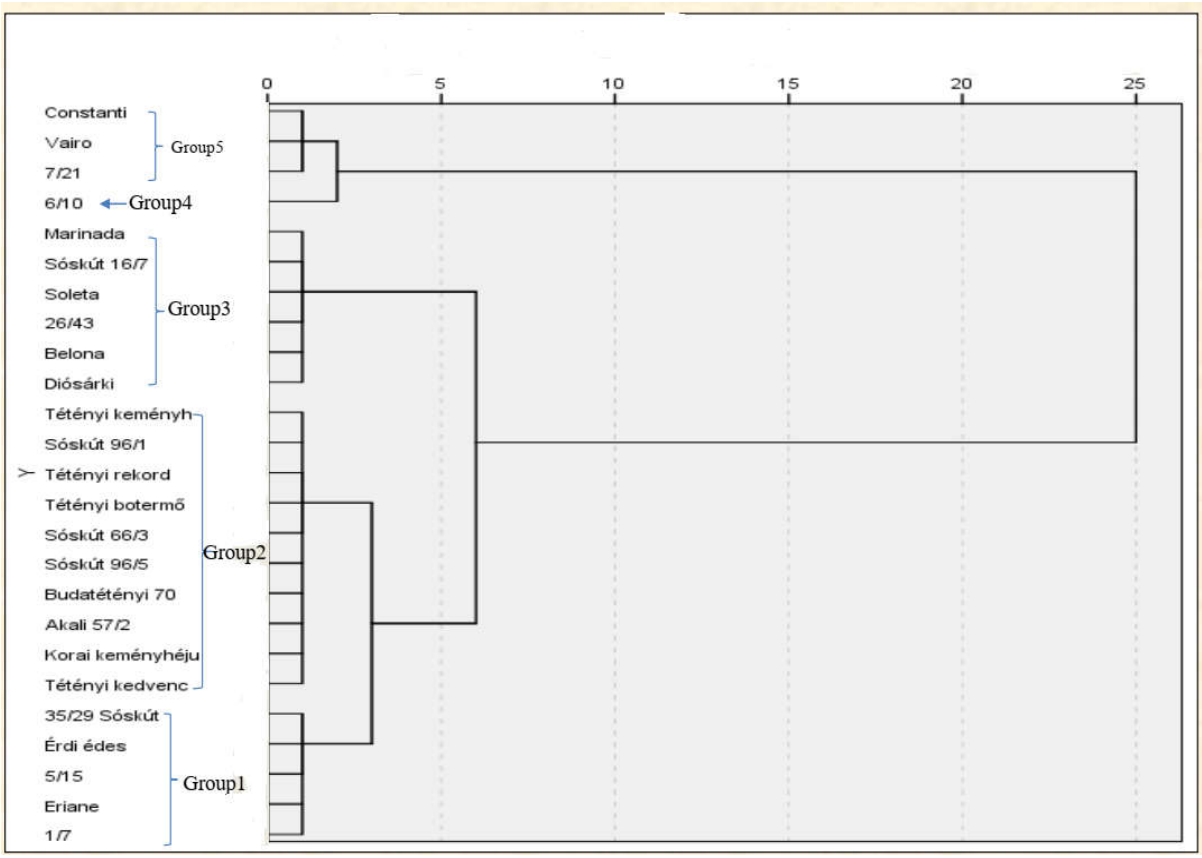


Figure 16. Group of almond accessions according to their flowering time

4.3 Evaluation of chilling and heat requirements

4.3.1 Results of chilling and heat requirement calculation

Table 5 presents the calculated chilling requirements of the accessions by the two methods (Utah model and chilling hours (CH) model) and heat requirements calculated according to the Utah model in each seasons analysed. The end of endodormancy date we used in our calculation was the one we obtained by microsporogenesis studies. Based on the data calculated, the almond accessions 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. Accessions 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). Numerically the chill hour <7.2˚C model (CH) had slightly higher values of the chill hour number than the Utah model. The Utah model showed more homogenous results between years

particularly with these late accessions breaking dormancy after December. There were some accessions that showed similar values between the three years studied, such as Marinada and 26/43 estimated with the Utah model. However, as Utah model makes finer differences among temperature ranges, as well as it has been recommended and used among continental conditions, we prefer using it in the future.

Figure 17 shows the distribution of almond accessions based on their chilling units and growing degree hours requirements. Accessions 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 5: Chilling and heat requirements of almonds in (2019/20, 2020/2021 and 2021/2022)

Accessions	Year	Endo-dormancy break	Chill units Utah (CU)		Chilling hours (CH)(<7.2°C) model		Eco-dormancy break (F50)	Heat requirements (GDH) b=4.5^{0}C	
			Value	Mean	Value	Mean		Value	Mean
1/7	2019/20	27-Nov	397	285^{a}	182	174^{a}	18-Mar	3916	3442^{a}
	2020/21	16-Nov	232		152		28-Mar	2841	
	2021/22	15-Nov	226		187		24-Mar	3569	
Eriane	2019/20	27-Nov	397	285^{a}	182	174^{a}	18-Mar	3916	3442^{a}
	2020/21	16-Nov	232		152		28-Mar	2841	
	2021/22	15-Nov	226		187		24-Mar	3569	
5/15	2019/20	27-Nov	397	285^{a}	182	174^{a}	18-Mar	3916	3442^{a}
	2020/21	16-Nov	232		152		28-Mar	2841	
	2021/22	15-Nov	226		187		24-Mar	3569	
Érdi édes	2019/20	02-Dec	485	378^{ab}	269	257^{ab}	20 Mar	3977	3416^{a}
	2020/21	22-Nov	327		250		30-Mar	2830	
	2021/22	20-Nov	321		251		25-Mar	3440	
35/29 Sóskút	2019/20	02-Dec	485	378^{ab}	269	257^{ab}	20 Mar	3977	3416^{a}
	2020/21	22-Nov	327		250		30-Mar	2830	
	2021/22	20-Nov	321		251		25-Mar	3440	
Korai keményhéjú	2019/20	05-Dec	491	410^{ab}	341	353^{bc}	20-Mar	3977	3525^{ab}
	2020/21	28-Nov	368		378		31-Mar	3026	
	2021/22	23-Nov	370		340		27-Mar	3571	
Akali 57/2	2019/20	05-Dec	491	410^{ab}	341	353^{bc}	22-Mar	4203	3600^{ab}
	2020/21	28-Nov	368		378		31-Mar	3027	
	2021/22	23-Nov	370		340		27-Mar	3571	
Sóskút 96/5	2019/20	05-Dec	491	410^{ab}	341	353^{bc}	22-Mar	3431	3706^{ab}
	2020/21	28-Nov	368		378		02-Apr	3977	
	2021/22	23-Nov	370		340		28-Mar	3710.0	

Continued

Accessions	Year	Endo-dormancy break	Chill units Utah (CU)		Chilling hours (CH)(<7.2°C) model		Eco-dormancy break(F50)	Heat requirements (GDH) b=4.5^{0}C	
			value	mean	value	mean		value	mean
Tétényi kedvenc	2019/20	05-Dec	491		341		20-Mar	3026	
	2020/21	28-Nov	368	410^{ab}	378	353^{bc}	31-Mar	3977	3571^{ab}
	2021/22	23-Nov	370		340		28-Mar	3710	
Sóskút 66/3	2019/20	10Dec	527		461		20-Mar	3026	
	2020/21	28-Nov	368	421^{ab}	378	393^{bc}	31-Mar	3977	3525^{ab}
	2021/22	23-Nov	370		340		27-Mar	3571	
Budatétényi 70	2019/20	10Dec	527		461		22-Mar	4203	
	2020/21	30-Nov	382	426^{ab}	420	407^{bc}	31-Mar	3027	3600^{ab}
	2021/22	23-Nov	370		340		27-Mar	3571	
Tétényi bőtermő	2019/20	10Dec	527		461		22-Mar	4209	
	2020/21	4-Dec	389	453^{bc}	508	464^{cd}	02-Apr	3432	3775^{ab}
	2021/22	28-Nov	443		422		28-Mar	3683	
Tétényi rekord	2019/20	10Dec	527		461		20-Mar	3026	
	2020/21	4-Dec	389	453^{bc}	508	464^{cd}	31-Apr	3977	3562^{ab}
	2021/22	28-Nov	443		422		28-Mar	3683	
Belona	2019/20	15Dec	608		581		28-Mar	4442	
	2020/21	10-Dec	518	564^{cd}	623	601de	03-Apr	3474	3833^{ab}
	2021/22	6-Dec	567		600		31-Mar	3584	
Sóskút 16/7	2019/20	18Dec	647		631		28-Mar	4255	
	2020/21	14-Dec	583	603^{d}	699	700^{ef}	07-Apr	3705	4075^{abc}
	2021/22	13-Dec	580		766		05-Apr	4265	
26/43	2019/20	18Dec	647		631		28-Mar	4255	
	2020/21	16-Dec	650	626^{bd}	780	725^{ef}	02-Apr	3248	3845^{ab}
	2021/22	13-Dec	580		766		31-Mar	4034	
Diósárki	2019/20	18Dec	647		631		03-Apr	4255	
	2020/21	16-Dec	650	626^{bd}	780	725^{ef}	26-Mar	3248	3916^{abc}
	2021/22	13-Dec	580		766		03-Apr	4246	
Sóskút 96/1	2019/20	22Dec	717		664		26-Mar	3638	
	2020/21	21-Dec	717	681de	847	790^{fg}	30-Mar	2663	3284^{a}
	2021/22	17Dec	610		860		28-Mar	3550	
Tétényi keményhéjú	2019/20	22Dec	717		664		26-Mar	3638	
	2020/21	21-Dec	717	681de	847	790^{fg}	30-Mar	2663	3284^{a}
	2021/22	17Dec	610		860		28-Mar	3550	

Continued

Accessions	year	Endo-dormancy break	Chill units Utah (CU)		Chilling hours (CH)(<7.2°C) model		Eco-dormancy break(F50)	Heat requirements (GDH) b=4.5^{0}C	
			value	mean	value	mean		value	mean
	2019/20	26-Dec	813		719		02-Apr	4007	
Marinada	2020/21	26-Dec	817	769^{ef}	933	827^{fg}	11-Apr	3840	4056^{abc}
	2021/22	20-Dec	677		830		05-Apr	4320	
	2019/20	30-Dec	867		813		5-Apr	4289	4150^{abc}
Soleta	2020/21	26-Dec	817	786^{ef}	933	890^{gh}	11-Apr	3840	
	2021/22	20-Dec	675		924		05-Apr	4320	
	2019/20	30Dec	867		813		6-Apr	4443	
6/10	2020/21	31-Dec	877	823^{f}	1015	983^{h}	15-Apr	4034	4517^{bc}
	2021/22	28-Dec	726		1120		13-Apr	5075	
	2019/20	03-Jan	890		909		11-Apr	5318	
7/21	2020/21	31-Dec	877	865^{f}	1015	1058^{h}	21-Apr	4411	4821^{c}
	2021/22	05-Jan	828		1250		13-Apr	4733	
	2019/20	03-Jan	890		909		12-Apr	5479	
Vairo	2020/21	05-Jan	961	893^{f}	1116	1092^{h}	21-Apr	4359	4857^{c}
	2021/22	05-Jan	828		1250		13-Apr	4733	
	2019/20	03-Jan	890		909		11-Apr	5318	
Constanti	2020/21	05-Jan	961	893^{f}	1116	1092^{h}	21-Apr	4411	4821^{c}
	2021/22	05-Jan	828		1250		13-Apr	4733	
	Differences between models	CU ^{a} CH ^{a}	552.6			577.9			3819.0
	Differences between years			2019/20^{a} 2020/21^{a} 2021/22^{a}		a a a			b^{b} ^{a} ^{b}

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≤0.05). b=base temperature

When we make attempt to compare our results with other authors (see details in Table 2 , in Literature review), 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., 2006b).

The first difference is in the models used for chilling calculations. Benmoussa et al. (2017) performed a long-term analysis, however, they were not able to use Utah model, only the dynamic model in the Mediterranean-subtropical region in Tunisia, thus we are not able to compare our data with them. Three analyses were performed in southeastern Spain, using Utah model. Egea et al. (2003) analysed cultivars with different flowering time and origin, even some having Ukrainian ancestors (Primorskij in the cultivar Marta and S5133 selection). They state that temperatures between 2.5 and 12.4ºC were predominant and temperature rarely was below zero in the year of their experiment. Prudencio et al. (2018) analysed an early, a late and an extra late cultivar regarding chilling and heat requirement. Guillamón et al. (2022) calculated chilling and heat requirement of some Spanish cultivars, but that year the winter was frosty because of Filomena storm appeared in the region. The above-mentioned author's chilling data range (Egea et al. 2003: 266-996 CU, Prudencio et al. 2018: 167-638 and Guillamón et al. 2022: 270-1100) is the 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.

Among the accessions studied, only two were mentioned in earlier studies. Alonso et al. (2010) assigned 340 CU and 2872 GDH to cultivar Soleta (later corrected to 7872 GDH by Socias i Company et al. 2015), 353 CU and 7741 CU to Belona. According to our data, Soleta has on average 780 CU and 4150 GDH, Belona has 564 CU and 3833 GDH. The differences again can be attributed to the different endodormancy breaking calculation methods.

Previous studies on almonds (Alonso and Socias I Company, 2010; Benmoussa et al., 2017), sweet cherry (Alburquerque et al., 2008), and peaches (Pawasut et al., 2004) indicated that different cultivars required different amount of chilling and heat. They report that even cultivars with similar chilling requirements differ in heat requirements, with the latest blooming cultivars having the highest chilling and heat requirements. Our results clearly support the authors' definition of the issue. Blooming of the studied cultivars took place once their chilling and heat requirements have been satisfied. According to our results the latest blooming cultivars had high chilling requirements; cultivars with low chilling requirements released the endodormancy period earlier and showed earlier flowering dates. This current result defined that differences in heat requirements had a stronger influence on the blooming date than did chilling requirements. Our findings are in line with Alonso et al. (2005) who reported that the heat requirements had a greater influence on flowering time than the chilling requirements ranging between 400 and 600 chill units in the cold area. However, these authors did not measure the breaking of dormancy by collecting branches in the field, their results are based on a model designed from long term meteorological and phenological observations for 30 years. On the contrary, (Benmoussa et al., 2017; Egea et al., 2003; Gaeta et al., 2018; Prudencio et al., 2018a) in almonds, (Ruiz et al., 2007) in apricots (Maulión et al., 2014) in nectarines and peaches reported chilling has a more substantial on blooming date than heat requirements.

For cultivars with less chilling requirement, extended cold temperatures post-endodormancy lengthened ecodormancy in general. These cultivars began flowering soon after the onset of warm temperatures. Regarding cultivars with high chilling requirements, it was observed that a short period of cold exposure post the endodormancy stage led to an increment in heat requirements. This led to a reduction in the ecodormancy period. The same result was observed by Alonso et al., 2005; Aslamarz et al., 2009; Citadin et al., 2001; Harrington et al., 2010; Lamp et al., 2001; Razavi et al., 2011; Scalabrelli and Couvillon, 1985. In temperate climates cultivars having low chilling requirements are exposed to winter frosts. According to Egea et al., 2003, one of the main objectives in breeding programs for almond is late flowering to avoid winter frost, which can be accomplished by increasing either the chill or post-chill heat requirement of the crop (Alonso et al., 2005; Guo et al., 2014). In our case, ‘1/7’, ‘Eriane’, and ‘5/15’are most affected. These cultivars flower early and are therefore at risk of spring frosts.

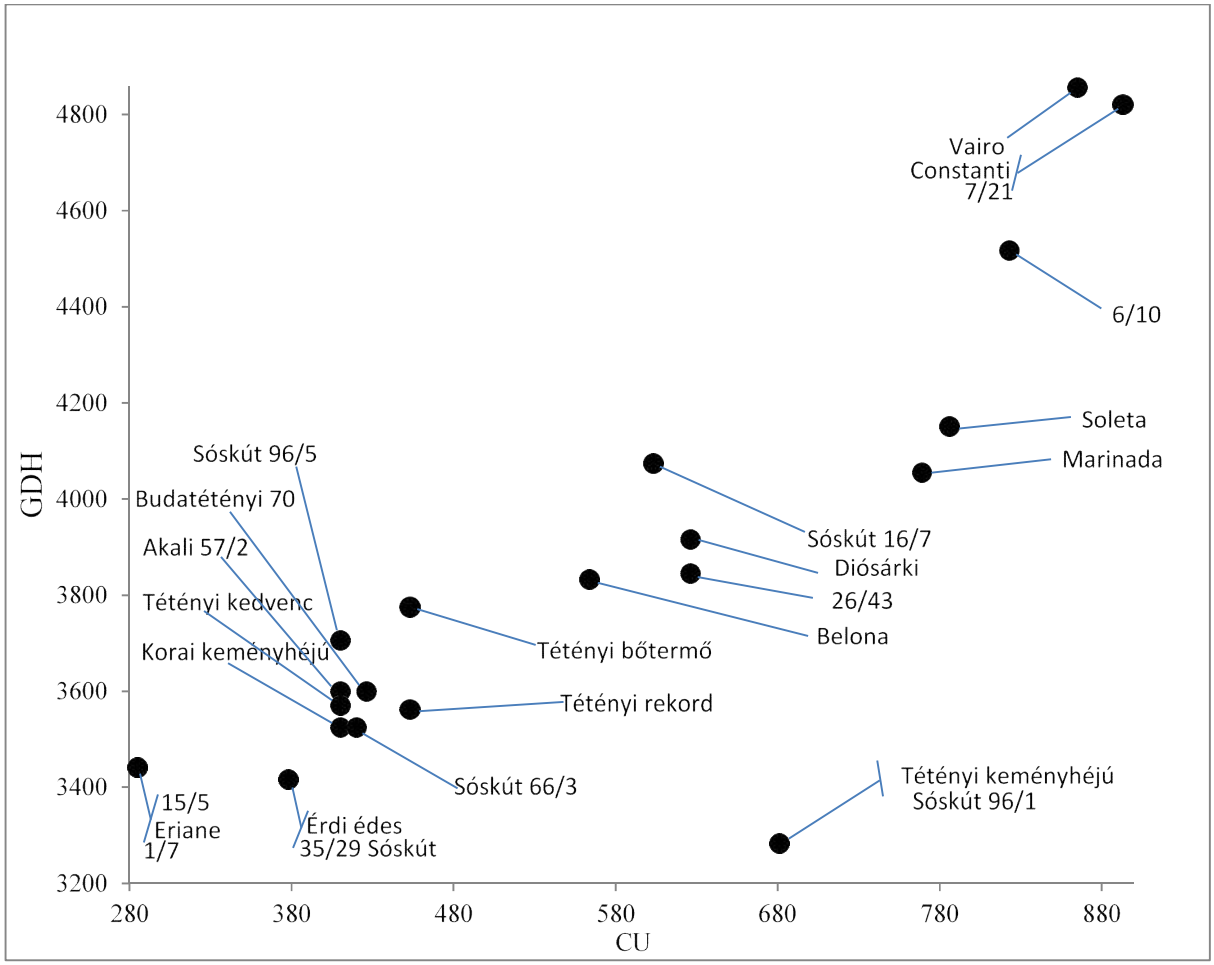


Figure 17. Comparison of chilling (CU) and heat requirement (GDH) of almond accessions analysed.

If we put together chilling and heat requirement data (Figure 17), 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.

Here we would like to give some feedback regarding the three biological methods used in chapter2, 4.1.1, 4.1.2, and 4.1.3. In our opinion, pistil growth and *in vitro* forcing results are

rather connected with heat accumulation than chilling. For instance, cultivars having the same chilling requirement, but different heat requirement does not behave the same way during forcing. 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).

4.3.2 The effect of cultivars and years on chilling and heat requirement

The effects of accessions on accumulated chill and heat differences were compared. MANOVA revealed significant differences between accessions in chill units, chill hours, and heat requirements (Wilk`s Lambda=0.017, $p<0.01$). The year effect resulted in a significant effect on almonds' heat requirements ($p<0.05$), where the accumulated heat in 2020/21 was significantly different than in the other two years. But the year effect for the accumulated amount of chill units and chill hour number was insignificant ($p>0.05$). Duncan's post hoc test distinguished accessions into different homogenous groups depending on their chilling and heat requirements. The output agreed that accessions '1/7', 'Eriane' and '5/15' were significantly earlier than the latest ones to break endodormancy between November 16 and November 27 after the accumulation of 285 CU/174 CH. Those accessions were also the first to break ecodormancy between March 18 and March 28 after the accumulation of 3442 GDH. Following those accessions, 'Érdi édes' and '35/29 Sóskút' entered to endodormancy and ecodormancy after accumulating 3374 CU/257CH and 3488 GDH, respectively. In the Utah model, the chill and heat values recorded by '1/7', 'Eriane' and '5/15' were not significantly different from 'Érdi édes', '35/29 Sóskút', 'Korai felálló keményhéjú', 'Akali 57/2', 'Sóskút 96/5', 'Tétényi kedvenc', 'Sóskút 66/3' and 'Budatétényi 70'. But in the case of the chill hour model, their chill hour value was found to be significantly different from 'Érdi édes' and '35/29 Sóskút'. The accessions 'Vairo' and 'Constanti', '7/211' and '6/10', broke endodormancy significantly later between December 28 and 5, after an accumulation of 893/1092, 893/1092, 865/1058 and 823 CU/983 CH respectively. Consequently, these accessions completed the ecodormancy between 6 and 21 April significantly later than the earliest ones after accumulations of 4821, 4857, 4821, 451 GDH in that order. The almond accessions 'Vairo' and 'Constanti' had the same chilling requirements. However, their heat requirements were not exactly the same. The latest accessions had the highest chill accumulation and their ecodormancy period was short. In the Utah model, the estimated chill and heat requirements for cultivars 'Vairo', 'Constanti', '7/21', and '6/10'

were not different from ‘Soleta’ and ‘Marinada’. But in the chill hour model, the value (827 CH) recorded by Marinada was found to be significantly different from theirs.

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. Accessions having either low chilling and/or low heat requirement also bloom early and are exposed to spring frosts. In our experiment, chilling correlated well with heat requirements. However, some cultivars such as ‘Sóskút 96/1’ and ‘Tétényi keményhéjú’ had high chilling requirements, but their heat requirement was among the lowest. Among Hungarian commercial cultivars, ‘Tétényi kedvenc’ had the lowest chilling requirement, followed by ‘Tétényi bőtermő’, ‘Tétényi rekord’, and ‘Tétényi keményhéjú’, respectively. Regarding heat requirements, their order differed to a certain extent. The cultivars of Spanish origin (‘Belona’, ‘Soleta’, ‘Constanti’, ‘Vairo’ and ‘Marinada’) had higher chilling requirements and their flowering time was also later than that of Hungarian commercial cultivars.

4.3.3 Length of endodormancy

The length of endodormancy and ecodormancy period of each accession determined based on microsporogenesis method is presented in Table 6. For the earliest accessions such as ‘1/7’, ‘Eriane’, and ‘5/15’, the endodormancy period terminated after average of 18 days.

The endodormancy release date for those accessions in 2019/20 was November 27. While in 2020/21/and 2021/22 it happened about 10 days earlier, on the 16th and 15th of November, respectively. In the same way, the end of ecodormancy period of those cultivars happened significantly earlier with average length of 82 Julian days from first of January.

However, accessions such as ‘6/10’ and ‘7/21’, ‘as well as ‘Vairo’ and ‘Constanti’ finished endodormancy after 58, 63 64, and 64 days respectively. In terms of ‘7/21’, ‘Vairo’, and ‘Constanti’, ecodormancy ended after 114 days, while 6/10 finished with averages of 102. Those late ones reached the endodormancy release on a similar date in all three years. The cultivars ‘7/21’, ‘Vairo’, and ‘Constanti’ reached the end dormancy release between 3 and 5 January in all three years. December marks the end of the endo dormancy period for the rest of the cultivars.

Cultivars which were early to break endodormancy were early to break the ecodormancy period too. Regarding genotypes with long endodormancy it was observed that short period of chilling exposure post of endodormancy stage led to a reduction of ecodormancy period. The same result was observed by (Alonso et al., 2005; Aslamarz et al., 2009; Citadin et al., 2001; Harrington et al., 2010; Lamp et al., 2001; Razavi et al., 2011; Scalabrelli and Couvillon, 1985).

Table 6. Estimated length of endodormancy and ecodormancy period depending on microsporogenesis method of almonds in 2019/20, 2020/21 and 2021/22.

Accessions	Year	Endo-dormancy break	*Length of endodormancy (*Days from 1st November*)*		Eco-dormancy break (F50)	Length of Eco-dormancy (Days from 1st January)	
			Value	Mean		Value	Mean
1/7	2019/20	27-Nov	26	18	18-Mar	78	82
	2020/21	16-Nov	15		28-Mar	87	
	2021/22	15-Nov	14		24-Mar	82	
Eriane	2019/20	27-Nov	26	18	18-Mar	78	82
	2020/21	16-Nov	15		28-Mar	87	
	2021/22	15-Nov	14		24-Mar	82	
5/15	2019/20	27-Nov	26	18	18-Mar	78	82
	2020/21	16-Nov	15		28-Mar	87	
	2021/22	15-Nov	14		24-Mar	82	
Érdi édes	2019/20	02-Dec	31	24	20 Mar	80	84
	2020/21	22-Nov	21		30-Mar	88	
	2021/22	20-Nov	19		25-Mar	83	
35/29 Sóskút	2019/20	02-Dec	31	24	20 Mar	80	84
	2020/21	22-Nov	21		30-Mar	88	
	2021/22	20-Nov	19		25-Mar	83	
Korai keményhéjú	2019/20	05-Dec	34	28	20-Mar	80	85
	2020/21	28-Nov	27		31-Mar	90	
	2021/22	23-Nov	22		27-Mar	85	
Akali 57/2	2019/20	05-Dec	34	28	22-Mar	82	86
	2020/21	28-Nov	27		31-Mar	90	
	2021/22	23-Nov	22		27-Mar	85	
Sóskút 96/5	2019/20	05-Dec	34	28	22-Mar	82	87
	2020/21	28-Nov	27		02-Apr	92	
	2021/22	23-Nov	22		28-Mar	86	

Continued

Accessions	Year	Endo-dormancy break	*Length of endodormancy* (Days from 1st November)		Eco-dormancy break (F50	Length of Eco-dormancy (Days from 1st January)	
			Value	Mean		Value	Mean
Tétényi kedvenc	2019/20	05-Dec	34	28	20-Mar	80	85
	2020/21	28-Nov	27		31-Mar	90	
	2021/22	23-Nov	22		28-Mar	86	
Sóskút 66/3	2019/20	10Dec	39	29	20-Mar	80	85
	2020/21	28-Nov	27		31-Mar	90	
	2021/22	23-Nov	22		27-Mar	85	
Budatétényi 70	2019/20	10Dec	39	30	22-Mar	82	86
	2020/21	30-Nov	29		31-Mar	90	
	2021/22	23-Nov	22		27-Mar	85	
Tétényi bőtermő	2019/20	10Dec	39	33	22-Mar	82	87
	2020/21	4-Dec	33		02-Apr	92	
	2021/22	28-Nov	27		28-Mar	86	
Tétényi rekord	2019/20	10Dec	39	33	20-Mar	80	85
	2020/21	4-Dec	33		31-Apr	90	
	2021/22	28-Nov	27		28-Mar	86	
Belona	2019/20	15Dec	44	39	28-Mar	88	90
	2020/21	10-Dec	39		03-Apr	94	
	2021/22	6-Dec	35		31-Mar	89	
Sóskút 16/7	2019/20	18Dec	48	45	28-Mar	88	93
	2020/21	14-Dec	42		07-Apr	97	
	2021/22	13-Dec			05-Apr	94	
26/43	2019/20	18Dec	47	45	28-Mar	88	91
	2020/21	16-Dec	45		02-Apr	92	
	2021/22	13-Dec	42		31-Mar	94	
Diósárki	2019/20	18Dec	47	45	03-Apr	91	92
	2020/21	16-Dec	45		26-Mar	93	
	2021/22	13-Dec	42		03-Apr	92	
Sóskút 96/1	2019/20	22Dec	51	46	26-Mar	93	89
	2020/21	21-Dec	50		30-Mar	89	
	2021/22	17Dec	36		28-Mar	86	
Tétényi keményhéjú	2019/20	22Dec	51	46	26-Mar	89	89
	2020/21	21-Dec	50		30-Mar	86	
	2021/22	17Dec	36		28-Mar	93	

Continued

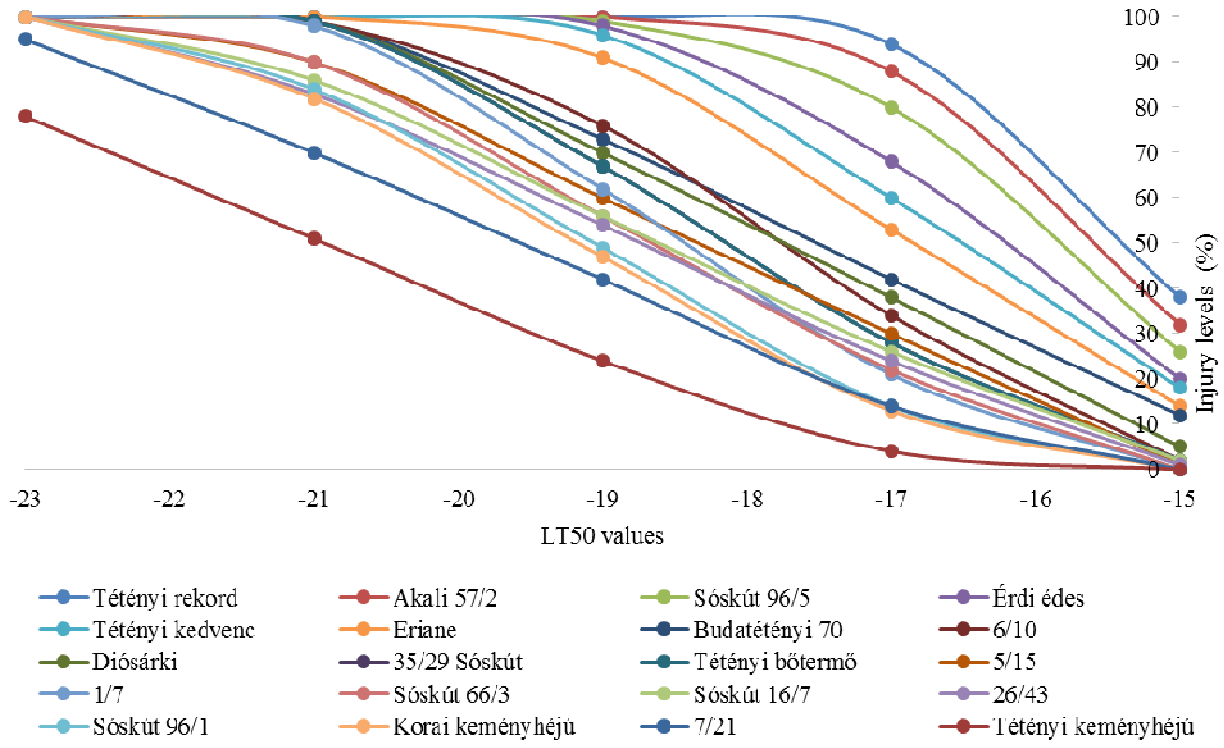
Accessions	Year	Endo-dormancy break	*Length of endodormancy (*Days from 1st November)		Eco-dormancy break (F50)	Length of Eco-dormancy (Days from 1st January)	
			Value	Mean		Value	Mean
	2019/20	26-Dec	55		02-Apr	92	
Marinada	2020/21	26-Dec	55	53	11-Apr	101	96
	2021/22	20-Dec	49		05-Apr	94	
	2019/20	30-Dec	59		5-Apr	96	
Soleta	2020/21	26-Dec	55	54	11-Apr	101	97
	2021/22	20-Dec	49		05-Apr	94	
	2019/20	30Dec	59		6-Apr	97	
6/10	2020/21	31-Dec	60	58	15-Apr	105	102
	2021/22	28-Dec	57		13-Apr	103	
	2019/20	03-Jan	63		11-Apr	102	
7/21	2020/21	31-Dec	60	63	21-Apr	111	114
	2021/22	05-Jan	65		13-Apr	129	
	2019/20	03-Jan	63		12-Apr	103	
Vairo	2020/21	05-Jan	66	65	21-Apr	111	114
	2021/22	05-Jan	65		13-Apr	129	
	2019/20	03-Jan	63		11-Apr	102	
Constanti	2020/21	05-Jan	66	65	21-Apr	111	114
	2021/22	05-Jan			13-Apr	129	

4.4 Frost hardiness of almond cultivars

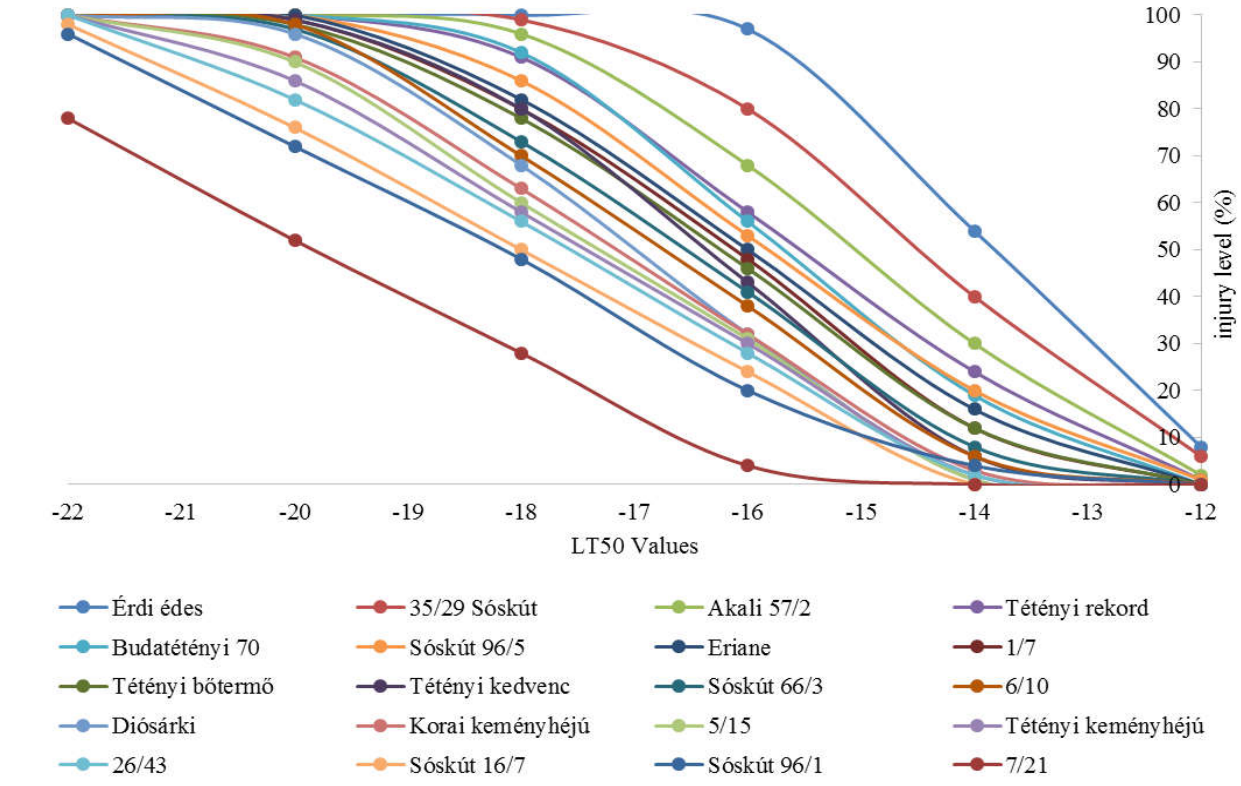
The LT50 was calculated from the sensitivity curve of each almond accession which was established from the frost damage values at the different freeze treatments. The process is represented in Figure 18 from samples collected in 2020, on the 6th of January, 2nd of December, 12th of February, and 6th of March. The results showed that the degree of frost damage was affected by almond accessions, sampling time, and freezing temperatures. During the early and late winter, flower buds were damaged at temperatures much higher than during the middle of winter. During January, it was extremely low. In this month, the range of frost damage caused by the treatment of -15°C was between 0% and 38%, while at -17°C it was between 4-95%. However, the damages for most of the accessions were less than 50%, which resulted in considerable ranges of freeze damage differences in each cultivar. Temperatures between -21°C and -23°C had the strongest effect on frost damage since the values for most flower buds were

100%. In December, these values were produced at temperatures between -17°C and -19°C. During February, the range of frost damage caused by the treatment of -11°C was between 0% and 31%, while at -13°C it was between 2-90%, which caused wide ranges of freeze damage differences. In March, the range of frost damage produced by the treatment of -5°C was between 0% and 50%, while at -7°C it was between 0% and 100%, which produced considerable ranges of freeze damage differences. The damage caused by the -11°C treatment, however, was 100% for the majority of the almonds. Temperate zone fruit trees are most frost hardy in the endodormancy period, during which they can survive temperatures as low as -20 °C to 30 °C (Tromp, 2005). The present study fully supports Tromp's (2005) conclusion that flower buds of almonds are more winter hardy in January than in March and February, since, in the current example, damage at -19°C in January was between 24-100%, but in February it was between 70-100%. However, the damage for most of the accessions was 100%. In March, most of the flower buds suffered 100% damage at a freezing temperature of -11°C.

(a)



(b)


100
90
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70
60
50
40
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10
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injury level (%)
-22
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-20
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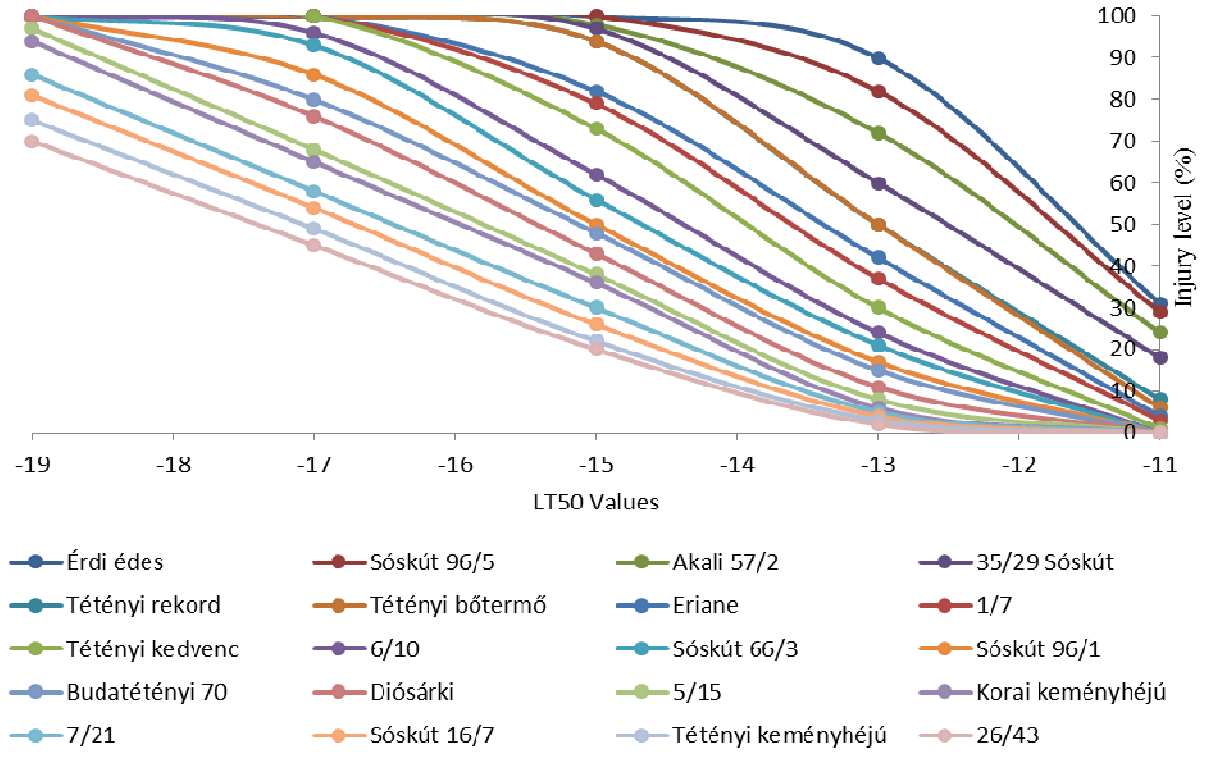
-16
-15
-14
-13
-12
LT50 Values
Érdi édes
35/29 Sóskút
Akali 57/2
Tétényi rekord
Budatétényi 70
Sóskút 96/5
Eriane
1/7
Tétényi bőtermő
Tétényi kedvenc
Sóskút 66/3
6/10
Diósárki
Korai keményhéjú
5/15
Tétényi keményhéjú
26/43
Sóskút 16/7
Sóskút 96/1
7/21

(c)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Injury level (%)
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LT50 Values
Érdi édes
Sóskút 96/5
Akali 57/2
35/29 Sóskút
Tétényi rekord
Tétényi bőtermő
Eriane
1/7
Tétényi kedvenc
6/10
Sóskút 66/3
Sóskút 96/1
Budatétényi 70
Diósárki
5/15
Korai keményhéjú
7/21
Sóskút 16/7
Tétényi keményhéjú
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(d)

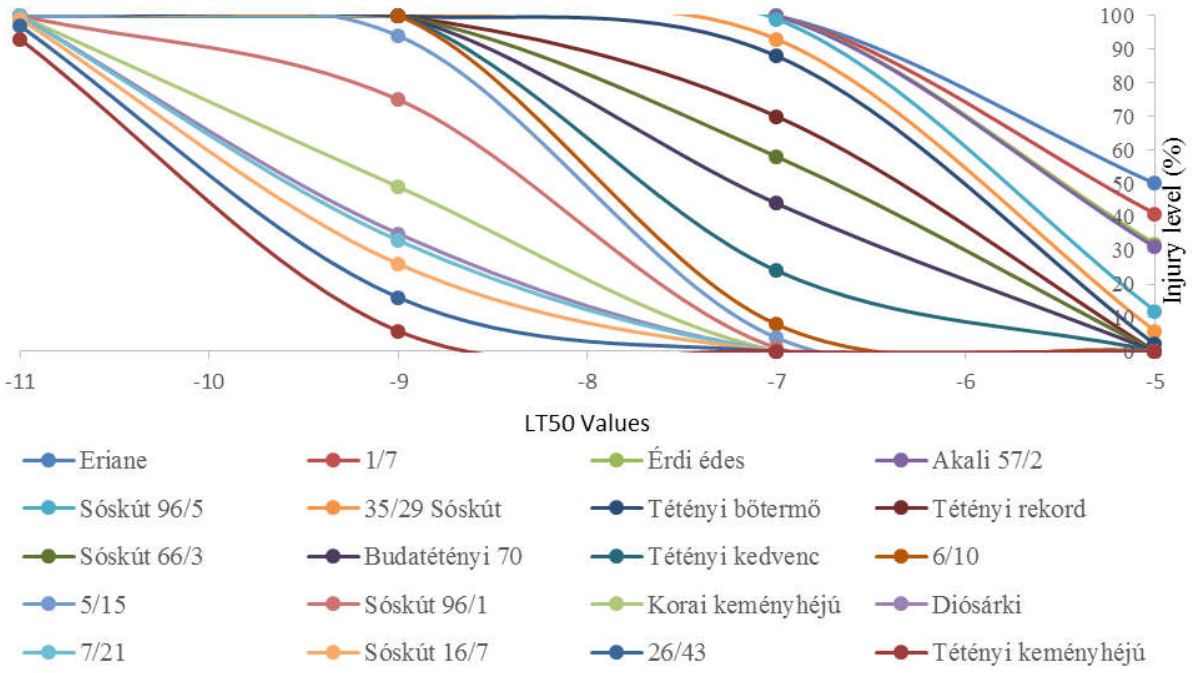
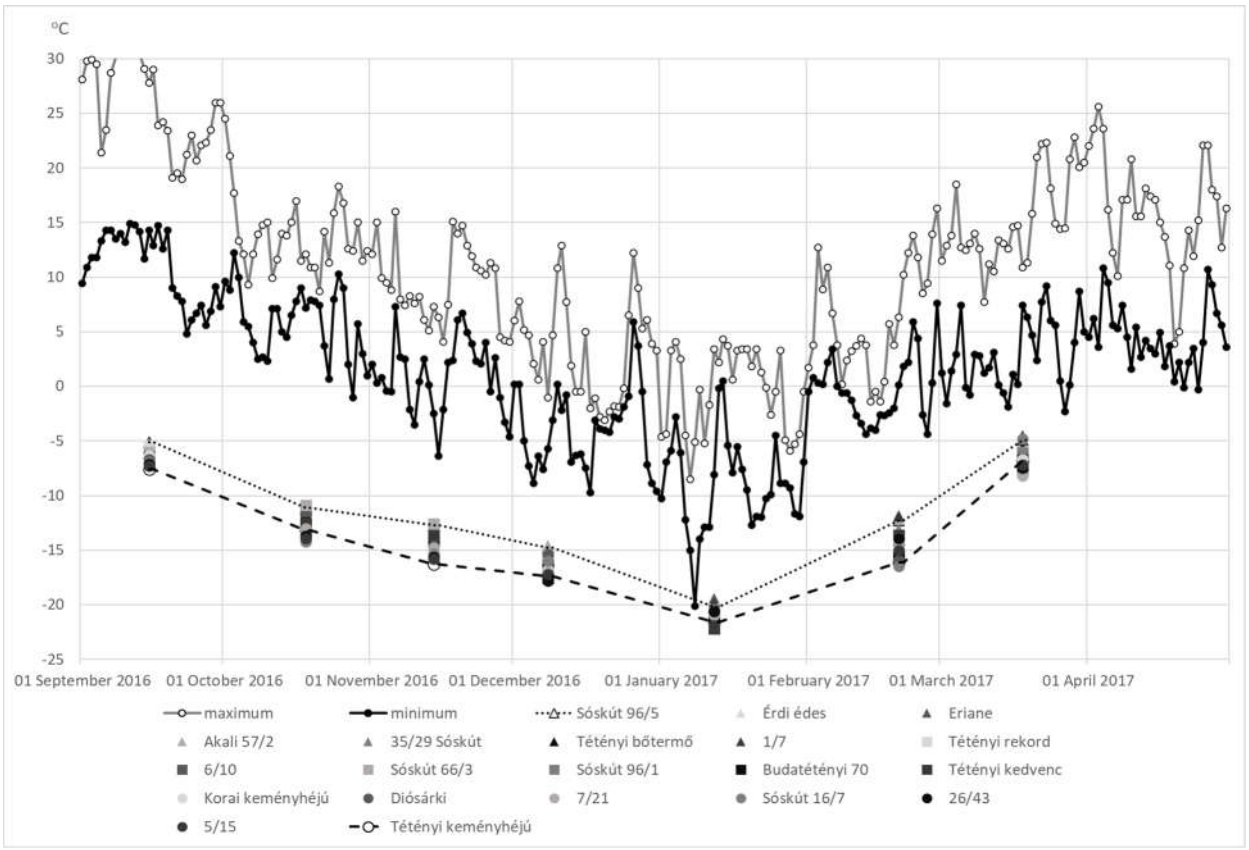


Figure 18. Frost damage to flower buds of almond accessions in artificial freezing tests in January (a), December (b), February (c) and March (d) of 2020.

The frost hardiness of flower buds of observed almond accessions is characterized by LT50 values. The changing of the LT50 values (main frost hardiness values) is shown in Figure 19, as well as presented in Appendix 3.

(a)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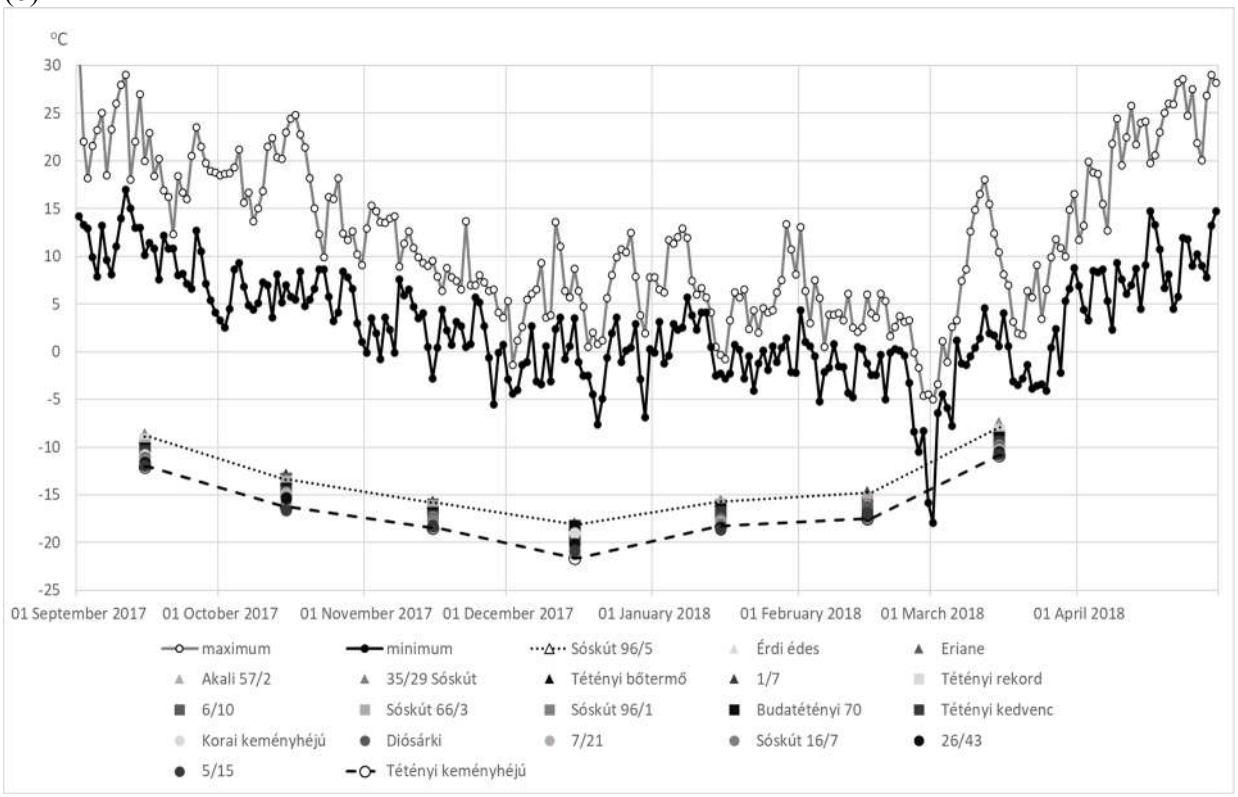
01 October 2016
01 November 2016
01 December 2016
01 January 2017
01 February 2017
01 March 2017
01 April 2017
maximum
minimum
Sóskút 96/5
Érdi édes
Eriane
Akali 57/2
35/29 Sóskút
Tétényi bőtermő
1/7
Tétényi rekord
6/10
Sóskút 66/3
Sóskút 96/1
Budatétényi 70
Tétényi kedvenc
Korai keményhéjú
Diósárki
7/21
Sóskút 16/7
26/43
5/15
Tétényi keményhéjú

(b)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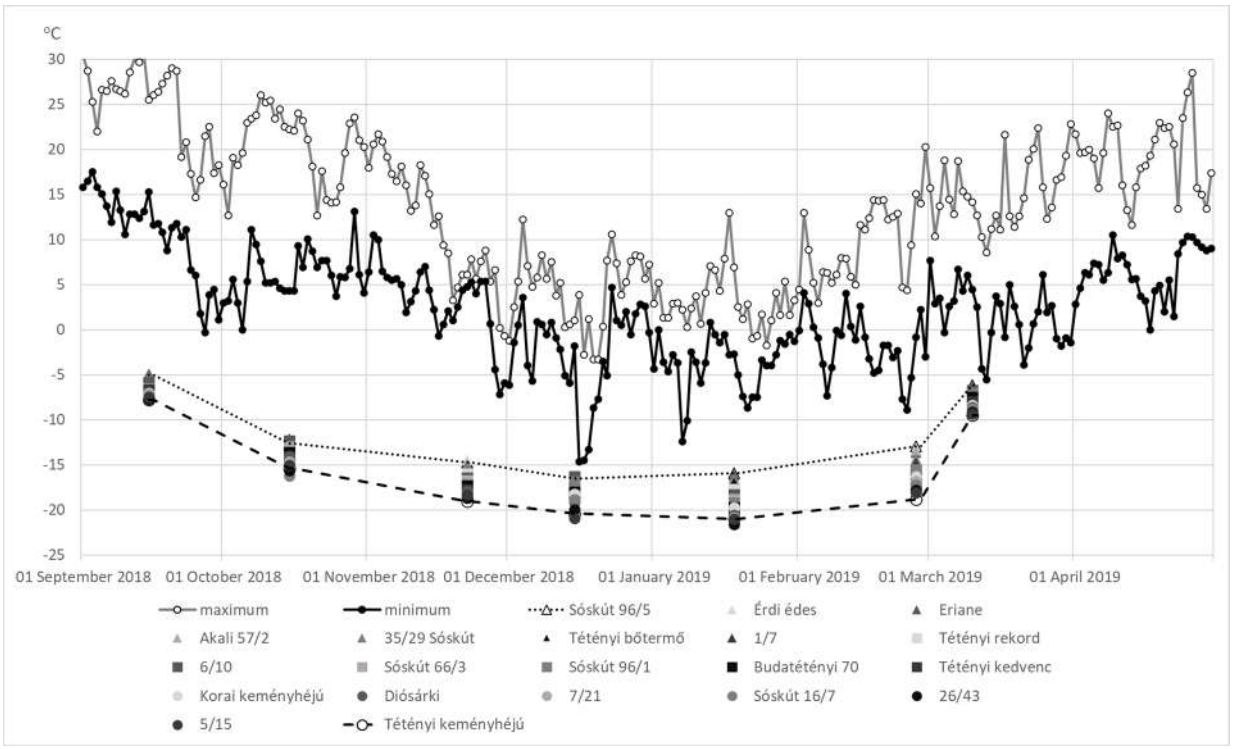
01 March 2018
01 April 2018
maximum
minimum
Sóskút 96/5
Érdi édes
Eriane
Akali 57/2
35/29 Sóskút
Tétényi bőtermő
1/7
Tétényi rekord
6/10
Sóskút 66/3
Sóskút 96/1
Budatétényi 70
Tétényi kedvenc
Korai keményhéjú
Diósárki
7/21
Sóskút 16/7
26/43
5/15
Tétényi keményhéjú

(c)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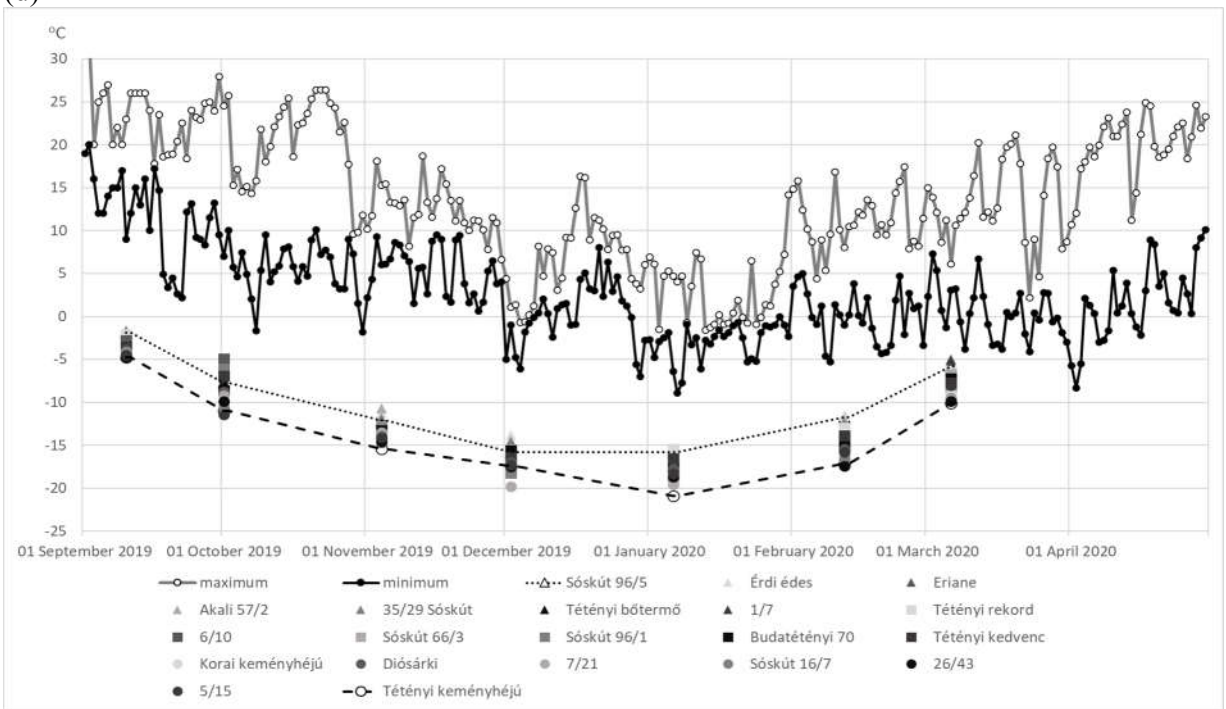
01 October 2018
01 November 2018
01 December 2018
01 January 2019
01 February 2019
01 March 2019
01 April 2019
maximum
minimum
Sóskút 96/5
Érdi édes
Eriane
Akali 57/2
35/29 Sóskút
Tétényi bőtermő
1/7
Tétényi rekord
6/10
Sóskút 66/3
Sóskút 96/1
Budatétényi 70
Tétényi kedvenc
Korai keményhéjú
Diósárki
7/21
Sóskút 16/7
26/43
5/15
Tétényi keményhéjú

(d)


°C
30
25
20
15
10
5
0
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01 September 2019
01 October 2019
01 November 2019
01 December 2019
01 January 2020
01 February 2020
01 March 2020
01 April 2020
maximum
minimum
Sóskút 96/5
Érdi édes
Eriane
Akali 57/2
35/29 Sóskút
Tétényi bőtermő
1/7
Tétényi rekord
6/10
Sóskút 66/3
Sóskút 96/1
Budatétényi 70
Tétényi kedvenc
Korai keményhéjú
Diósárki
7/21
Sóskút 16/7
26/43
5/15
Tétényi keményhéjú

(e)

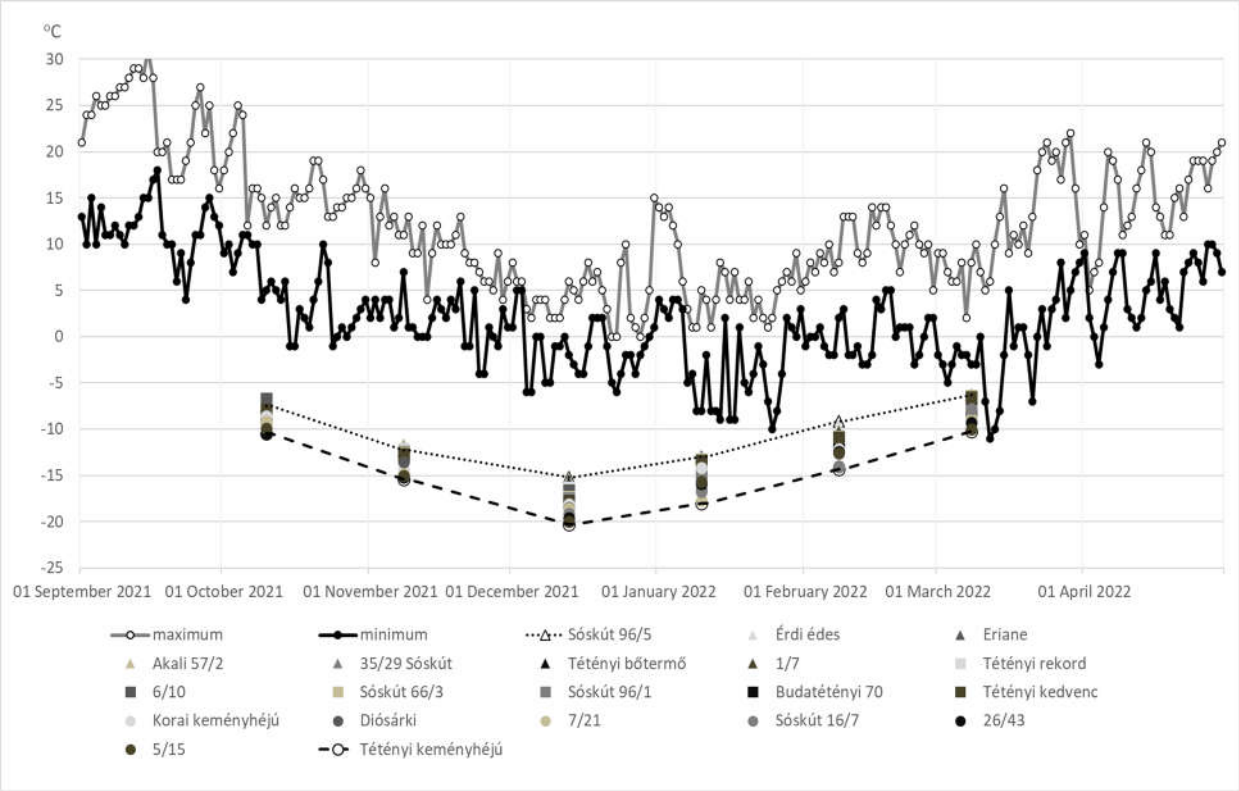


Figure 19. Daily maximum and minimum ambient temperatures, and LT50 values of flower buds of the observed almond accessions based on artificial freezing tests in winter of 2016/17 (a), 2017/18 (b), 2018/19 (c), 2019/20 (d) and 2021/22 (e**).**

The frost tolerance profile of the examined accessions was different every year. This is probably due to differences in environmental factors, especially temperature. In all observed years the daily maximum and minimum temperature values showed great daily fluctuations, and the differences between years are also remarkable. 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).

In the winter of 2017/18 and 20021/22, the best frost tolerance values were measured in December, while in the other dormancy seasons; the flower buds reached their best frost tolerance in January. It was not consistent the changing of LT50 values during the hardening periods. 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 LT50. 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. Based on the results of the five years, the flower buds of the accessions did not achieve their genetically programmed maximum frost tolerance every year. On the Figure 20, the best frost tolerance (LT50) values of the flower buds of the studied accessions in the given dormancy period are demonstrated. In our experimental station during the five-year study, the flower buds of the studied accessions reached the most frost-resistant values in 2016/17 winter (Figure 20). Further studies are needed to determine whether these values are genetically encoded maximum values. During the study period, flower buds were least hardened in 2019/20 and 2021/22 test season according to accession.

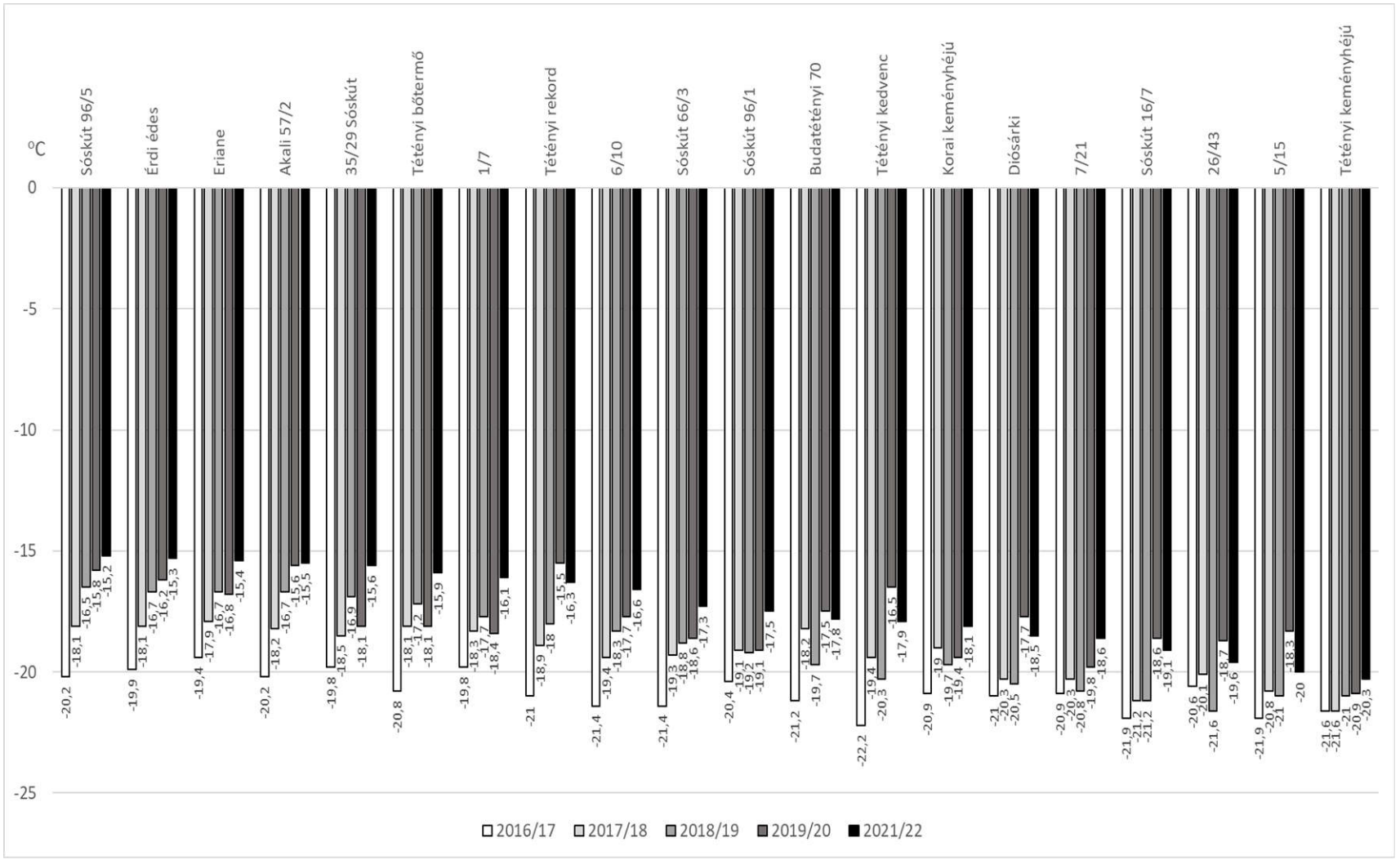


Figure 20. The LT50 values of the flower buds of the studied almonds according to years studied

During the dehardening period the changing of frost hardiness of flower buds was very different year by year because of different climatic conditions. Due to rapidly rising temperatures, dehardening was very rapid in some periods, such as January, February, and March of 2017 (Figure 19a), and March of 2019 (Figure 19c). The slow rise in temperature resulted in slow dehardening, for example in January and February of 2018 (Figure 19b), 2019 (Figure 19c) and 2020 (Figure 19d). Recurrent strong cools caused frost damage to flower buds in early March 2018 at the end of the dehardening period (Figure 19b), and in 2020 during the flowering period (Figure 19d). There was a drastically low temperature after our observations, during the flowering period in 2022, and it caused severe frost damages in the orchard. An asynchrony was observed between the change in ambient temperature and the frost tolerance profile of flower buds, especially in the last two study periods, when the decrease in frost hardiness was faster than the warming of the plantation (Figure 19c, 19d).

The sequence of accessions from the aspect of frost tolerance in different sampling dates was not the same (Figure 19). Based on all of data the ‘Sóskút 96/5’ was the most sensitive and the ‘Tétényi keményhéjú’ was the most hardy in general, that is why the values of these two cultivars are demonstrated with lines on figures, but there were sampling dates when other cultivars represented extreme values.

Differences between accessions are analysed based on the best frost hardiness values of them achieved in the different test seasons. The univariate ANOVA revealed highly significant accession and year effect for the LT50 value (accession: $F(19; 200)>41.96$; year: $F(4; 200)>209.99$, both with $p<0.001$). ‘Tétényi keményhéj’ had the highest LT50 value in 2018/19, 2019/20, and 2021/22. However, it was not found to be significantly different from the values obtained by the cultivars of ‘7/21’, ‘Sóskút 16/7’,’26/43’, and ‘5/15’, ‘Diósárki’, ‘Tétényi kedvenc’ in 2018/19, from values obtained by ‘7/21’, ‘Korai keményhéjú’ in 2019/20 and from values obtained by cultivars of ‘7/21’, ‘5/15’, ‘Diósárki’ , ‘Korai keményhéjú’ ‘Tétényi kedvenc’, ‘Budatétényi 70’, ‘Sóskút 96/1’, and ‘Sóskút 66/3’ in 2021/22.

In 2016/17, both the accessions ‘Sóskút 16/7’ and ‘5/15’ had the highest LT50 value but it was not found to be statistically different from those of ‘Tétényi keményhéjú’, ‘26/43’, ‘Diósárki’, ‘Tétényi kedvenc’, ‘Budatétényi 70’, ‘Sóskút 66/3’, ‘6/10’ and ‘Tétényi rekord’. In 2017/18, the cultivar ‘26/43’ had the highest value. The value recorded by this accession in that

year was not significantly different from the values achieved by ‘5/15’, ‘26/43’, ‘Sóskút 16/7’, ‘Tétényi kkeményhéjú’, ‘7/21’, ‘Tétényi kedvenc’ and ‘6/10’.

We analyzed the almond accessions’ LT50 values in order to differentiate them according to their frost tolerance (Figure 21). 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 accessions within the studied accession range. ‘Sóskút 96/5’, ‘Akali 57/2’, ‘Eriane’ and ‘Érdi édes’ accessions 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 21).

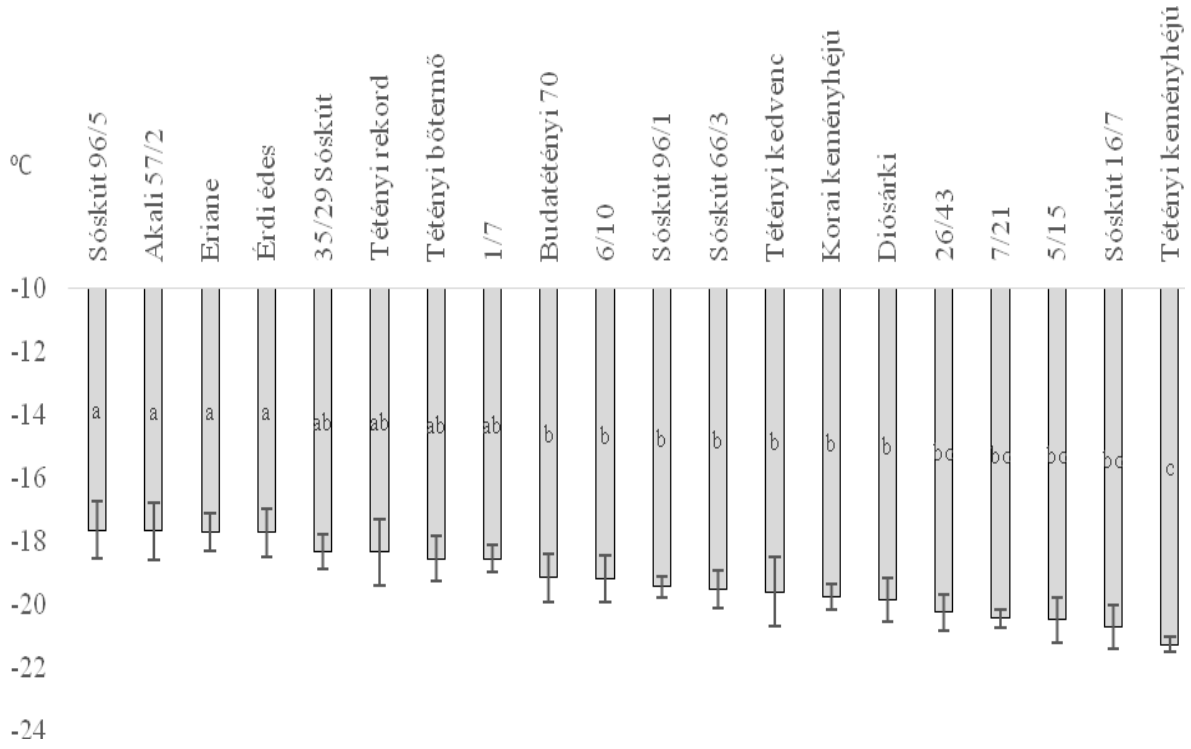


Figure 21. Average LT50 values of flower buds of the studied almond accessions based on the results of artificial freezing tests in December and January of the five test seasons (the best frost hardiness values has been calculated within a certain test season. The columns show the mean values, the lines the standard deviation, and the letters the homogeneous groups, the different letters indicate significantly (P≤0.05) different values

4.5 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 accessions, as shown in Figure 22. 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.

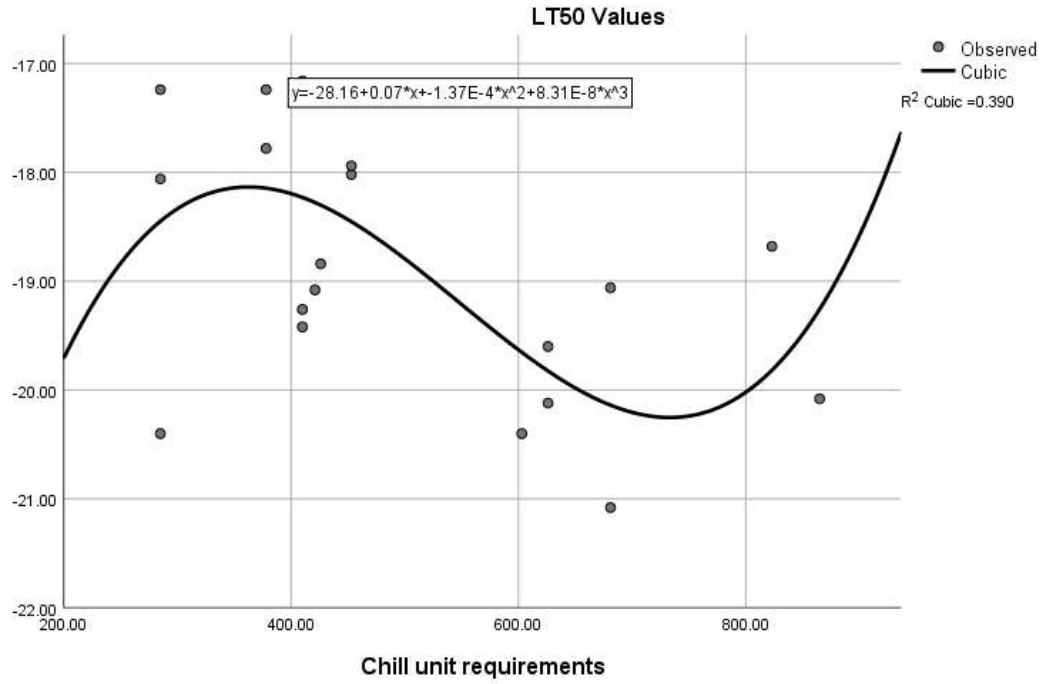


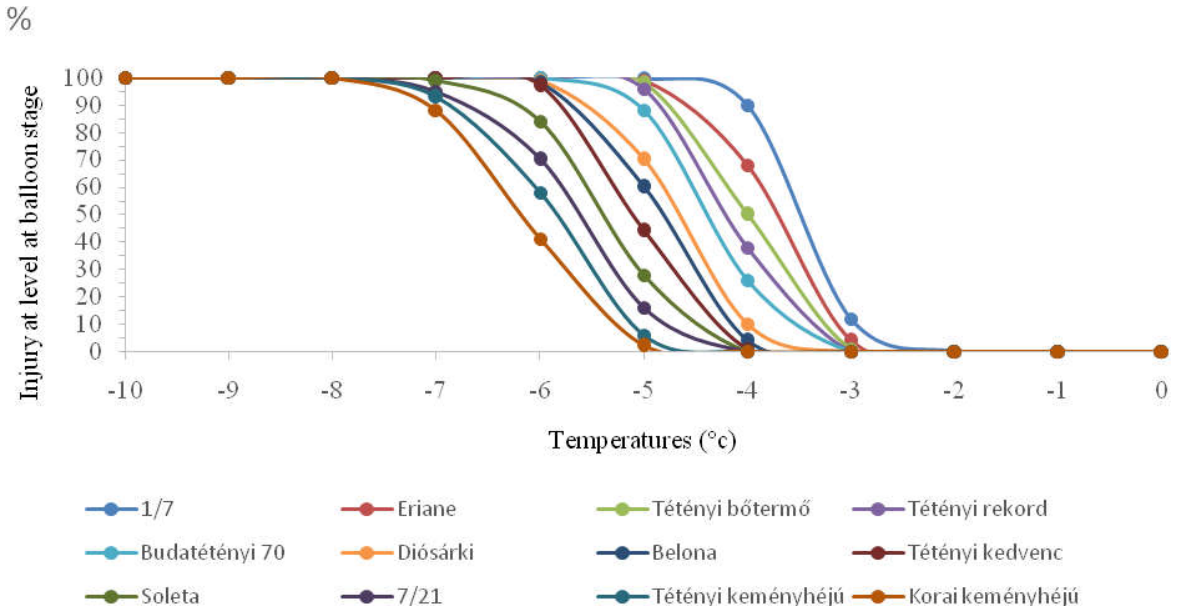
Figure 22. Correlations between chilling requirements and frost hardiness

4.6 Susceptibility of almonds to cold temperatures at blossom development

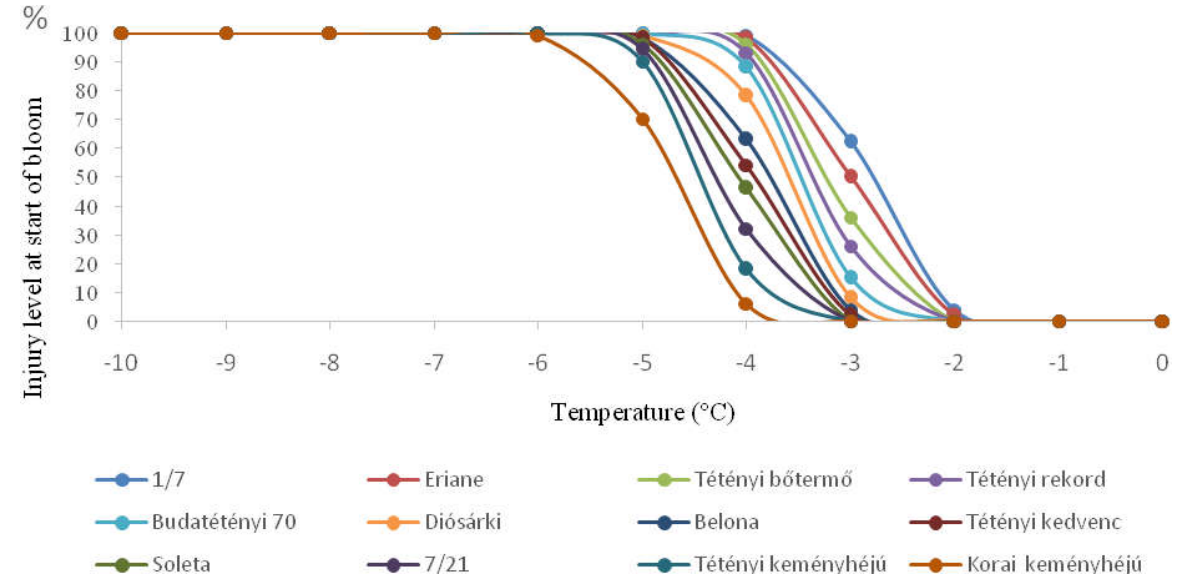
The frost injury percentage of almonds at different phenological stages was determined from 12 accessions. As illustrated in Figure 23, the frost damage of the almond flowers was different before and during blooming, and at the end of blooming. The accessions had shown considerable variations in their frost tolerance level at the same phenological development stage too. At the balloon stage, the freezing temperature was not a problem for all cultivars until -2°C. Frost damage to flower buds began as temperatures dropped and developmental stages increased. At the balloon stage, the damage rate at -3°C for '1/7' and 'Eriane' was only 12% and 4% respectively. Flower buds suffered a much higher frost damage rate at the end of bloom as both were damaged 100% at the same temperature. At the balloon stage the cultivars 'Soleta', '7/21',

'Korai keményhéjú', and 'Tétényi keményhéjú' did not damage up to a temperature of -4°C, whereas at the end of bloom all were damaged 100% at the same temperature.

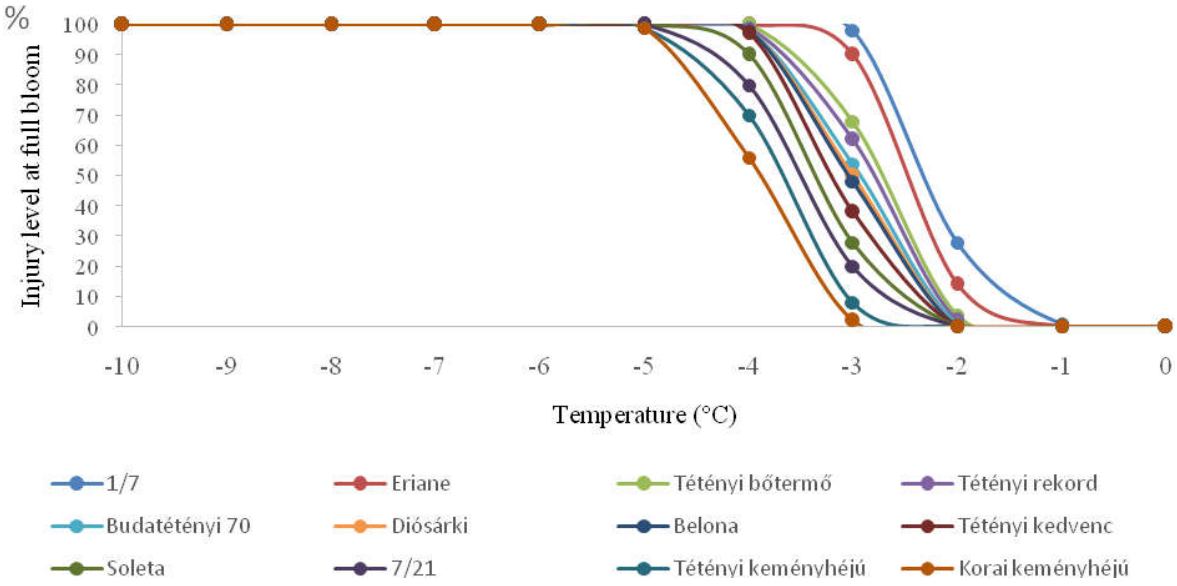
(a)



(b)



(c)



(d)

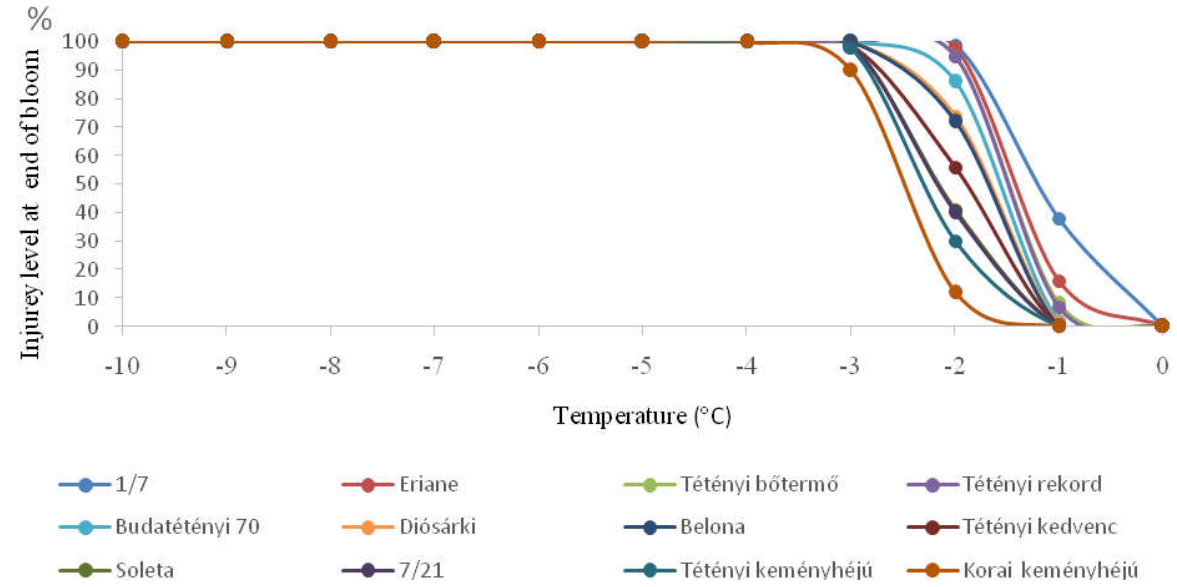


Figure 23. Susceptibility to frost damage of almond accessions, at balloon (a) start bloom (b), full boom(c) and end of bloom (d) in 2020

In Figure 24, the LT 50 values of each accession were determined at different stages of development, and the values decreased with increasing phenological development stage. At the first pink stage, the LT50 values of the flowers dropped to -3.8 to -7.5°C. But variation in the LT50 of the flower buds increased and the difference among the flowers of the cultivar decreased

from post-bloom onwards. At the first pink stage, the difference between 'Korai keményhéjú', and 1/7 was -3.7°C but the difference dropped to -1.3°C at the end of bloom. Thus, the phenological stage appears to be critical concerning the rate of frost. The analysis of variance (two-way ANOVA) revealed highly significant accession and development stage effects of the flower buds (accessions F (11, 144) = 530.659, developmental stage (5,144) = 5537.622, all with $p<0.001$. The interaction between the accession and development was also found to be significant, F (55, 144) = 14.727, all with $p<0.001$. The potential frost resistance of the cultivars was compared for all the developmental stages separately by running Tukey's post hoc test and at different stages of flower bud development different homogenous groups were distinguished (Table 7). Post hoc tests confirm that 'Korai keményhéjú' followed by 'Tétényi keményhéjú' had the highest values of all the other accessions, while '1/7' and 'Eriane' had the lowest values for all the developmental stages. The highest LT50 values recorded by 'Korai keményhéjú' at the pink stage (-6.2°C), the start of bloom (-4.7°C), and full bloom (-3.9°C) were not significantly different from the values of Tétényi keményhéjú. On the other hand, the lowest LT50 values recorded by '1/7' at the pink stage (-3.8°C), balloon stage (-3.5°C), the start of bloom (-4.7°C), and full bloom were not significantly different from the values recorded by 'Eriane'.

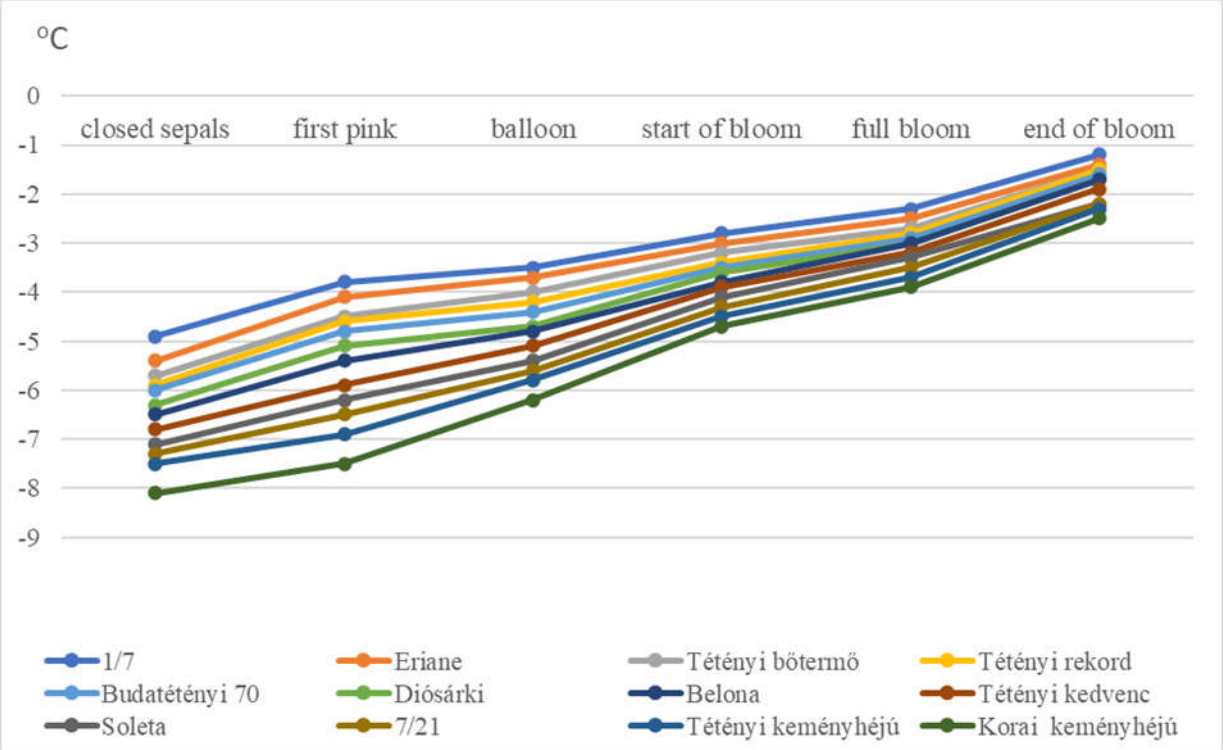


Figure 24. LT50 values of almond accessions in different phenological stages based on the results of artificial freezing tests during 2019/2020.

Table 7. LT50 values (°C) and homogenous groups of almonds at flowering stages in 2020

Accessions	Developmental stages of the flower buds. closed sepals	First pink	Balloon	start of bloom	Full bloom	end of bloom
1/7	-4.9± 0.10^{Ai}	-3.8± 0.10^{Bi}	-3.5±0.10^{Ch}	-2.8±0.10^{Di}	-2.3±0.01^{Ei}	-1.2± 0.08 ^{Fg}
Eriane	-5.4± 0.10^{Ah}	-4.1± 0.10^{Bhi}	-3.7± 0.10^{Cgh}	-3.0± 0.25^{Dhi}	-2.5± 0.06^{Ehi}	-1.4± 0.01^{Ff}
Tétényi bőtermő	-5.7± 0.10^{Agh}	-4.5± 0.20^{Bgh}	-4.0±0.10^{Cfg}	-3.2± 0.10^{Dghi}	-2.7±0.01^{Egh}	-1.5±0.02^{Fef}
Tétényi rekord	-5.9± 0.10^{Afg}	-4.6± 0.30^{Bg}	-4.2±0.10^{Bf}	-3.4± 0.20C^{fgh}	-2.8±0.02^{Dfg}	-1.5±0.03^{Eef}
Budatétényi 70	-6.0± 0.10 ^{Afg}	-4.8±0.10 ^{Bfg}	-4.4± 0.10^{Cef}	-3.5± 0.20^{Defg}	-2.9±0.03 ^{Efg}	-1.6±0.01 ^{Fde}
Diósárki	-6.3± 0.26^{Aef}	-5.1±0.10 ^{Bef}	-4.7± 0.20 ^{Bde}	-3.6± 0.27^{Cefg}	-3.0±0.26 ^{Def}	-1.7± 0.10^{Ed}
Belona	-6.5± 0.10^{Ade}	-5.4± 0.20^{Be}	-4.8± 0.10^{Cde}	-3.8± 0.10^{Ddef}	-3.0± 0.02^{Eef}	-1.7± 0.05^{Fd}
Tétényi kedvenc	-6.8± 0.10^{Acd}	-5.9± 0.10^{Bd}	-5.1±0.10^{Ccd}	-3.9± 0.05^{Dcde}	-3.2±0.04^{Ede}	-1.9±0.06^{Fc}
Soleta	-7.1± 0.26^{Abc}	-6.2± 0.20^{Bcd}	-5.4±0.20^{Cbc}	-4.1± 0.05^{Dbcd}	-3.3±0.05^{Ecd}	-2.2±0.08^{Fb}
7/21	-7.3± 0.20^{Ab}	-6.5± 0.20^{Bbc}	-5.6±0.30^{Cb}	-4.3± 0.05^{Dabc}	-3.5±0.06^{Ebc}	-2.2±0.07^{Fb}
Tétényi keményhéjú	-7.5± 0.20^{Ab}	-6.9± 0.00^{Bb}	-5.8±0.10^{Cab}	-4.5±0.11^{Dab}	-3.7±0.06^{Eab}	-2.3±0.01^{Fb}
Korai keményhéjú	-8.1± 0.10^{Aa}	-7.5± 0.20^{Ba}	-6.2±0.20^{Ca}	-4.7±0.10^{Da}	-3.9±0.07^{aE}	-2.5±0.01F^{a}

Variables represent the mean of 3 replications ± their corresponding standard deviation.
Superscript uppercase letters indicate statistically significant differences along the row.
Superscript lower letters indicate significant difference along the column, according to two -way ANOVA followed by Tukey's post hoc test (P≤0.05).

Among the overwintering organs, flower buds have been shown to be the most sensitive to frost. Frost tolerance studies in different cultivar collections all showed large differences between genotypes from the aspect of frost hardiness of them (Imani et al., 2012, 2011; Imani and Mahamadkhani, 2011; Rodrigo, 2000). During spring, some almond studies have addressed frost resistance of flowers or fruitlets in different phenological stages. These authors example (Kodad et al., 2010; Snyder and Conell, 1996) described differences among cultivars and certain phenological stages of flowers regarding their spring frost tolerance.

In the present experimental work, the frost tolerance of flower buds of different origin almond cultivars has been investigated by artificial freezing method for five 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 five 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.

The frost resistance of overwintering organs of temperate zone trees develops in two stages in autumn, so the hardening period of them can be divided into two stages. It is experimentally proven in apple vegetative organs (Howell and Weiser, 1970). 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 different vegetative and generative overwintering organs cannot harden properly in the case of several temperate zone fruit species (Palmer et al., 2003; Szalay et al., 2010; Wu et al., 2019). Our present experimental results suggest this for almond cultivars as well referring to the flower buds. In the first part of the frost tolerance profile of flower buds of studied 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 lowest LT50 values of the flower buds of the studied almond cultivars were different from year to year. 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 the hardening and dehardening of almond flower buds, and to determine the genetically fixed best frost hardiness of genotypes. Climate change results in frequent mild winter temperatures that are not favourable in hardening processes and has impact on the phenological processes of almond genotypes as well, similarly to other fruit trees (Benmoussa et al., 2017; Di Lena et al., 2017; Eccel et al., 2009; Egea et al., 2003; El Yaacoubi et al., 2019; Kaukoranta et al., 2010; Lamp et al., 2001; Vitasse et al., 2018).
It is difficult to compare our results with previous research results, as such a systematic study of the frost resistance of flower buds of almond genotypes has not yet been performed.
In this variety range, the best frost tolerance values for flower buds in a certain dormancy period were between -15.2 and -22.2 degrees Celsius, depending on the genotype and year. ‘Sóskút 96/5’ was the most sensitive, and ‘Tétényi keményhéjú’ was the most frost hardy.

An asynchrony was observed between the change in ambient temperature and the frost tolerance profile of flower buds, the decrease in frost hardiness was faster than the warming of the plantation. It caused severe frost damages sometimes.

The severity of spring frost damage is affected by temperatures, the phenological stage of flower buds, and the ability of genotypes to tolerate low temperatures (Afshari and Parvane, 2013; Imani et al., 2012; Imani and Mahamadkhani, 2011; Tromp, 2005). Almond genotypes are most susceptible to freezing injury after petals fall compared with other flower buds' developmental stages. The probable reason could be that cells do not fully develop cell walls to protect against different environmental stresses (Afshari and Parvane, 2013). This was defined for almond flower buds by the current work too. The outcome of the artificial freezing indicated that the early flowering genotypes were suffering from a much higher frost damage rate during the flowering period. Late blooming is an effective trait of a cultivar to escape spring frost (Alonso et al., 2005; Imani and Mahamadkhani, 2011; Lamp et al., 2001). From the result, late flowering and highly frost resistant cultivar could be the most effective mechanism of late spring frost avoidance. Korai keményhéjú, one of the medium flowering, was proved to be a promising cultivar to areas exposed to high spring frost damage due to its high spring frost resistance.

In Hungary, almond growing is limited by ecological conditions, the most risks are winter and spring frosts. When planning an orchard it is important to harmonize cold hardiness of the selected cultivars and growing site conditions. Based on our results it is not recommended to establish an almond orchard in growing sites where winter temperatures regularly drop below –18°C. As a conclusion, from practical point of view it is important to have adequate information on the cold hardiness of almond cultivars that should be included into cultivar descriptions, our work hopefully could contribute to this aim. So we consider our test results to be important, as in the pomological text books and variety descriptions the frost tolerance of the varieties we examined either is not mentioned at all or we can only find very incomplete data about them. To accurately describe varieties, determining their frost tolerance is very important, especially for a problematic species such as almonds. This is of substantial scientific and practical importance. The frost tolerance of the varieties can be determined by several years of research. Field frost damage recordings and artificial freezing experiments together provide adequate results.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

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 developmental rate mainly determined by genetic factors and significantly affected by yearly climatic conditions can play a key role in determining the climatic adaptability of a specific cultivar to a specific area. 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. The efficiency of almond production is greatly influenced by the frost hardiness of the cultivated varieties. Determining this requires several years of research. 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.

6. 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 ʻBudatétnyi 70ʼ, ʻTétényi bőtermőʼ, ʻTétényi keményhéjúʼ, ʻTétényi rekordʼ and ʻTétényi kedvencʼ Hungarian almond accessions 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 ʻTétényi keményhéjúʼ, followed by the ʻ6/43ʼ, ʻ7/21ʼ, ʻ5/15ʼ and ʻ16/7ʼ accessions.

7. SUMMARY

A native of the Mediterranean region, almonds are considered one of the oldest tree nuts. A close relative of peaches, they probably descend from the same ancestral species in central and southwest Asia. By the time of the Egyptians, Greeks, and Romans, it had spread across Africa and Europe along the Mediterranean shore. The United States is the world's largest producer of almonds, with 2,370,021 tons produced last year. California is one of the biggest producers of almonds in this country. Spain becomes the second largest producer in the world, followed by Australia. In Africa, Morocco is the largest producer of almonds.

Flowering time is a trait of great interest in almond as it strongly determines the spring events of flowering Almond start to flower at the end of the winter but floral bud establishment occurs the previous summer, enters dormancy during the winter and resumes growth prior to flowering.

Microsporogenesis, pistil growth and forcing of flower buds were investigated in 25 almond cultivars to determine their climatic adaptations. The string stage, pollen mother cell stage, tetrad stage, microspore stage and pollen stage were distinguished in the process of Microsporogenesis. There were differences in microsporogenesis speed due to variety and year effects. In all three studies, accessions differed mainly in the archesporium length. Among all the studied cultivars, ‘1/7’, ‘Eriane’, and ‘5/15’ had the shortest archesporial stage period in the three studied years. During 2020, 2021, and 2022, the archesporial tissues of these cultivars developed in 27, 15, and 14 days. During 2020, 2021, and 2022, cultivars '7/21', 'Constanti' and 'Vairo' had the longest archesporium stages (64, 66, and 65 days respectively). Cultivars with long archesporial stages had later flowering dates than cultivars with short archesporial stages.

Pistil growth behaves differently during paradormancy, endodormancy, and ecodoranancy. There is intensive growth during paradormancy, growth stops during endodormancy, and growth resumes during ecodormancy, first at a very slow pace, followed by a few days of highly concentrated growth before blooming. Of those early flowering cultivars, the resumption of pistil growth appeared at the moment when in the anthers of the flower buds, the full string stage was distinguishable. While of those latest ones, 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 developmental rates of bud shoots exposed to forcing conditions were affected by cultivars and yearly climatic conditions. Development rates were gradual. Flower bud opening under forcing occurred much later than the developmental process using both microsporogenesis and pistil methods. In some cultivars, flower bud opening occurs after the string stage. However, for some late cultivars in some years, it appeared very late with tetrads and microspores. After endodormancy, extended chilling periods prolonged ecodormancy and may negatively interfere with flower buds opening. While mild temperatures occurred in some years in January and February after dormancy release accelerated flower bud opening under forced conditions.

The end of endodormancy in the flower buds was recorded between November 15th and January 5th using microsporogenesis, between November 16th November and February 8th using the pistil method and between November 24th and February 27th using the forcing method depending on cultivars and years. Generally, cultivars with a fast flower bud development are highly exposed to the danger of winter frost. Because the faster the flower bud development the earlier is the blooming date and the more likely they expose to winter and spring frost.

It is important to know the flowering time of almond varieties from several perspectives. Among the fruit species grown in the world, the almond is one of the earliest to flower, thus spring frosts threaten its crop safety. Later blooming varieties avoid frost damage. Some almond varieties are not self-fertile, we can only expect a good crop when planted together with varieties that bloom in the same time. In terms of increased crop security, growing varieties that bloom later would be of great importance. We examined 25 varieties and found varieties with earlier and later flowering times.

Large differences were observed in the variation of chilling and unit heat requirements along with the evaluated almond cultivars. The cultivars showed a range of chilling unit requirements between 285 CU/174 CH and 893 CU/1092 CH for breaking dormancy. The heat requirements for flowering ranged between 3284 and 4857 GDH. Winter dormancy was caused by the interaction between chilling and unit heat requirements. Chill and heat unit requirements varied in different years to some degree. If the temperature is milder, the chilling unit accumulation is slower and the end of endodormancy is delayed. However, during ecodormancy the flower bud development is accelerated due to the faster accumulation of heat units and blooms occur earlier. Cultivars with low chill requirements or heat requirements bloom earlier and are highly exposed to spring frosts. Of the studied almond cultivars, those with long winter dormancy such as ‘7/21’, ‘Vairo’ and 'Constanti' are at risk of growing in very warm areas where

short chilling periods are problematic. These cultivars, however, can be seen as fundamental to avoiding late spring frost damage to flowers and thus ensuring stable yields in cold areas. Thus, knowledge of the cold and heat requirements of a given cultivar is important as it determines its adaptation to specific ecological conditions. Information on the chilling and heat requirements for different almond cultivars is important to select cultivars with similar chilling and heat requirements in order to obtain maximum overlap of the blooming period when establishing a new orchard, thereby optimizing cross-pollination.

Almonds are one of the most frost-sensitive fruit species. Cultivars showed significant frost resistance differences. 'Tétényi Keményhéjú' proved to be the most tolerant and 'Sóskút 96/5' was the most sensitive. Cultivation success depends on cultivar frost tolerance. Frost tolerance of cultivars has varied over the years. Frost tolerance changed constantly during the winter months. In all five years studied the frost tolerance of flower buds increased gradually in the first half of winter with increasing outdoor temperatures. It reached its maximum in December, or in January. As the temperature increased in the second half of winter, they gradually lost their frost tolerance. Frost resistance differences between cultivars were less in September and around flowering. However, the most pronounced differences were detected in December and January, when maximum frost tolerance developed. The expected average frost hardiness of a cultivar can be determined as an average of LT50 values from different years. In our case, it is -17.16°C for 'Sóskút 96/5' and -21.08°C for 'Tétényi keményhéjú'. Cultivars that showed better frost tolerance during the dormant period also had less damage during the flowering period. 'Korai keményhéjú' was the most frost hardy followed by the 'Tétényi keményhéjú' with LT50 values of -2.3 and -2.5°C respectively. '1/7' and 'Eriane' with values of -1.2 and -1.4°C were found frost sensitive at blooming but only out of the selected 12 cultivars.

APPENDIX 1 - REFERENCES

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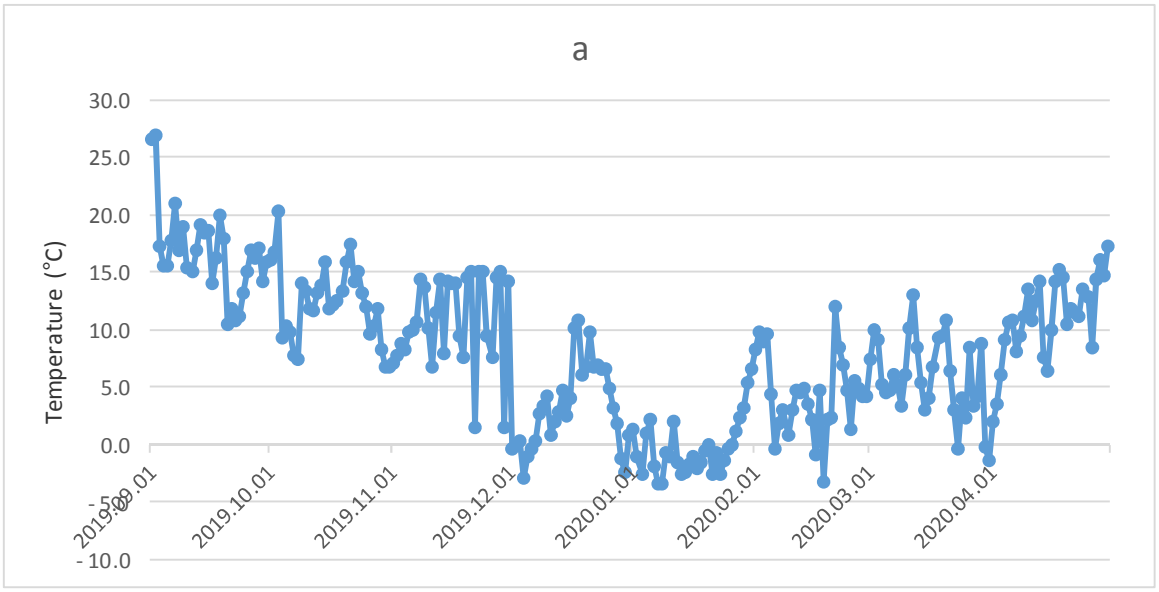
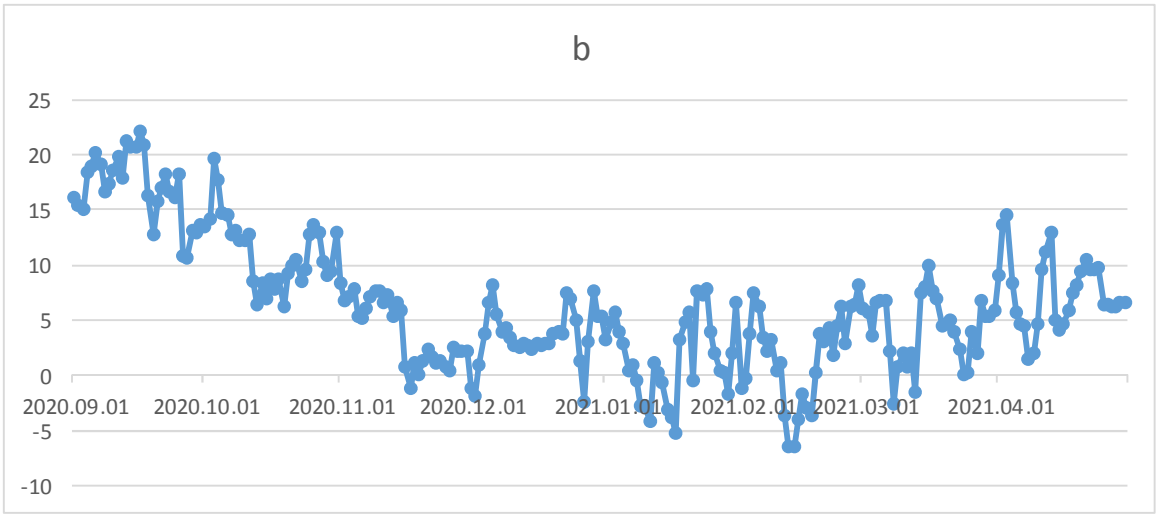
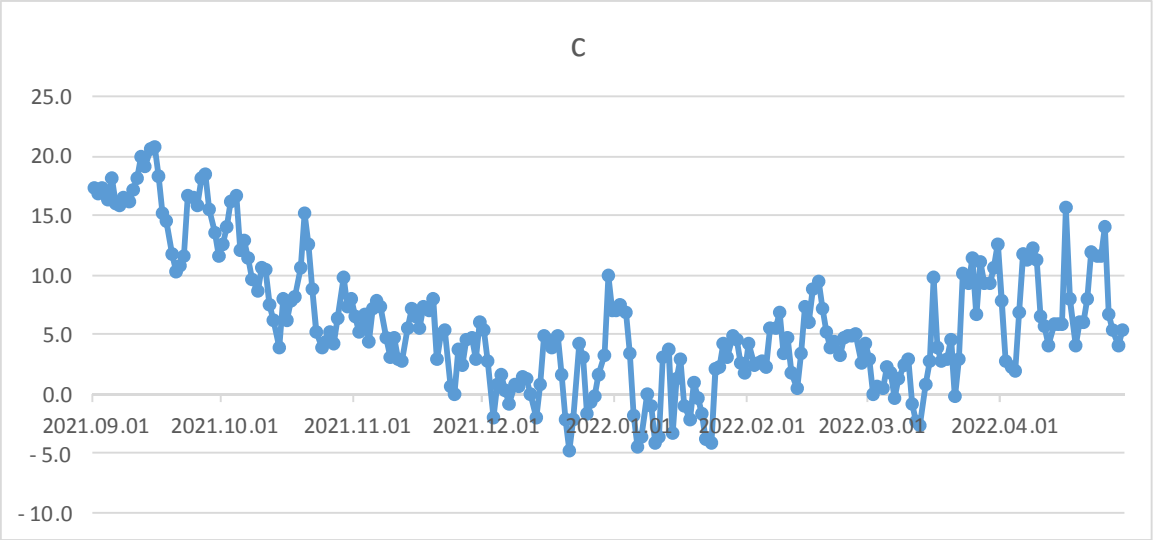
APPENDIX 2 – Daily average teperatures in 2019/20 (a), 2020/21 (b) and 2021/22 (c)



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APPENDIX 3 – ADDITIONAL TABLES

cultivars	Year					Mean
	2016/17	2017/18	2018/19	2019/20	2021/22	Mean
Sóskút 96/5	-20.2^{defA}	-18.1^{deAB}	-16.5^{hB}	-15.8^{ghB}	-15.2^{fB}	-17.16
Érdi édes	-19.9^{efA}	-18.1^{deAB}	-16.7^{ghiAB}	-16.2^{fghB}	-15.3^{efB}	-17.24
Eriane	-19.4^{fA}	-17.9^{eAB}	-16.7^{ghiAB}	-16.8^{defghAB}	-15.4^{defB}	-17.24
Akali 57/2	-20.2^{defA}	-18.2^{deB}	-16.7^{ghiC}	-15.6^{ghCD}	-15.5^{defD}	-17.24
35/29 Sóskút	-19.8^{efA}	-18.5^{deAB}	-16.9^{ghiBC}	-18.1^{bcdefB}	-15.6^{DdefC}	-17.78
Tétényi bőtermő	-20.8^{bcdeA}	-18.1^{deB}	-17.2^{ghiBC}	-18.1^{bcdefB}	-15.9^{cdefC}	-18.02
1/7	-19.8^{efA}	-18.3^{deAB}	-17.7^{fghiBC}	-18.4^{bcdeAB}	-16.1^{cdefC}	-18.06
Tétényi rekord	-21.0^{abcdeA}	-18.9^{cdeB}	-18.0^{efghiB}	-15.5^{hC}	-16.3^{cdefC}	-17.94
6/10	-21.4^{abcdA}	-19.4^{abcdeB}	-18.3^{efghBC}	-17.7^{bcdefCD}	-16.6^{bcdefD}	-18.68
Sóskút 66/3	-21.4^{abcdA}	-19.3^{bcdeB}	-18.8^{defgB}	-18.6^{bcdeB}	-17.3^{abcdeC}	-19.08
Sóskút 96/1	-20.4^{cdefA}	-19.1^{bcdeAB}	-19.2^{cdefAB}	-19.1^{abcAB}	-17.5^{abcdeB}	-19.06
Budatétényi 70	-21.2^{abcdA}	-18.2^{deBC}	-19.7^{bcdeAB}	-17.5^{cdefghC}	-17.6^{abcdeC}	-18.84
Tétényi kedvenc	-22.2^{aA}	-19.4^{abcdeBC}	-20.3^{abcdB}	-16.5^{efghD}	-17.9^{abcdeCD}	-19.26
Korai keményhéjú	-20.9^{bcdeA}	-19.0^{bcdeBC}	-19.4^{bcdeAB}	-19.4^{abcBC}	-18.1^{abcdeC}	-19.42
Diósárki	-21.0^{abcdeA}	-20.3^{abcdAB}	-20.5^{abcdA}	-17.7^{bcdefC}	-18.5^{abcdeBC}	-19.6
7/21	20.9^{bcdeA}	-20.3^{abcdA}	-20.8^{abcA}	-19.8^{abAB}	-18.6^{abcdB}	-20.08
Sóskút 16/7	-21.9^{abA}	-21.2^{abA}	21.2^{abA}	-18.6^{bcdeB}	-19.1^{defB}	-20.4
26/43	-20.6^{cdefAB}	-20.1^{abcdeB}	-21.6^{aA}	-18.7^{bcdC}	-19.6^{abBC}	-20.12
5/15	-21.9^{abA}	-20.8^{abcAB}	-21.0^{abAB}	-18.3^{bcdefC}	-20.0^{aB}	-20.4
Tétényi keményhéjú	-21.6^{abcA}	-21.6^{aA}	-21.0^{abA}	-20.9^{aA}	-20.3^{aA}	-21.08
Mean	-20.8A	-19.2B	-18.9B	-17.9C	-17.3D	

Variables represent the mean of LT50 values. Superscript uppercase letters indicate statistically significant differences along the row. Superscript lower letters indicate significant difference along the column, according to two -way ANOVA followed by Tukey's post hoc test (P≤0.05).

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