

## Differentiation of mixed flower buds in some traditional pear varieties in the region of Skopje

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**Abstract:** The results in the present paper are part of the scientific research project “Autochthonous genetic resources of pears in the Republic of Macedonia”. Flower bud differentiation was examined in five traditional pear varieties (‘Carigradsko Avche’, ‘Letna Kajkushka’, ‘Vodenka’, ‘Zimska Kajkushka’ and ‘Zimnica’) in the region of Skopje. In the region of Skopje, the differentiation of mixed flower buds in the traditional pear varieties starts in summer. The primordia of calyx and corolla are formed in the period from the second half of October to early November. In midNovember, stamen primordia are formed, while in late November the primordia of carpels are formed. In winter months, the archesporial tissue develops and microsporogenesis occurs. In the period from January to March, microgametogenesis and pollen grain development take place. The earliest differentiation of flower buds (approximately 7–10 days) and the earliest blooming (about 3-4 days) are typical of the variety ‘Carigradsko Avche’, compared to the latest varieties ‘Zimska Kajkushka’ and ‘Zimnica’.

**Keywords:** differentiation, mixed flower buds, pear, traditional varieties.

## Introduction

During its life, the flower bud passes through the so-called small, incomplete annual cycle, including the processes of transition from vegetative bud to the generative stage. It is part of the great cycle of ontogenesis. In some periods, under certain environmental conditions, in the armpit of the vegetative buds, meristem tissues transform into the generative stage. From formation until dying, the meristem goes through a complex life path or through three periods of vegetation. In the first year of vegetation, the bud occurs; in the second year the vegetative bud transforms into the generative stage; and in the third year blooming, fertilisation and ripening of fruits occur (Kuperman, 1968; Vitkovskii, 1984; Romanovskaya and Baksiene, 2007).

The final stage of the ontogenesis cycle involves the formation and differentiation of flower buds. Organogenesis in Angiospermae (*Magnoliophyta*) (small cycle of ontogeneses) occurs in 12 phases, under following a specific order (Kuperman, 1968; Vitkovski, 1984; Isaeva (cit. according to Luchic *et al.*, 1995)). Differentiation occurs in 4 phases: induction (occurring in the leaves after their emergence), initiation (physiological and chemical changes in the bud); differentiation and development of flower organs in the bud (internal invisible changes called microphenophases – I to VIII) and the development of flower organs outside buds (visible changes – phenophases IX to XII).

Seasonal rhythm and the place and time of flower bud formation are specific to each plant species and it happens under natural cycle rules (Ansimov *et al.*, 2003). Many factors affect the differentiation of flower buds, such as genotype and climatic conditions (Kuperman, 1968; Per'fileva, 1970; Mihailov and Topchiiski, 1975; Marafon *et al.*, 2010; Selamovska, 2007; Selamovska, 2013), cultivation method (Bertelsen, 2005); nutrition (Qinazhi and Bingzhen, 2000; Ito *et al.*, 2002), age of plant (Luchic *et al.*, 1995; Reynolds *et al.*, 2005), length of day (Selamovska, 2007), altitude, latitude (Darrow, 1966; Vitkovskii, 1984; Pirlak *et al.*, 2002), type of planting material (Selamovska, 2007; Selamovska, 2013a) etc.

Basically, the factors that lead to the differentiation of flower buds are divided into exogenous and endogenous. The theories that attempt to answer this question are divided into four groups: genetic, morphological-anatomical, ecological and chemical (Luchic *et al.*, 1995; Selamovska, 2007; Sestras *et al.*, 2008). According to Qinazhi and Bingzhen (2000), the beginning of morphological changes of apical meristem in the bud is correlated with the increased concentration of amino acids in the leaves.

The pear is a fruit species which forms mixed buds with differentiated rudiments of flowers on the top of the growth cone. This type of mixed buds is also present in apple, medlar, and winter pear "Oskorusha", walnut, hazel,

pomegranate and citrus. In pear, at first, terminal bud starts to differentiate, followed by lateral buds (Luchic *et al.*, 1995; Sestras *et al.*, 2008).

Knowledge of the period of flower bud differentiation has great practical significance, because it is important for appropriate selection of varieties for suitable climatic conditions and regions, as well as for the implementation of suitable cultural and tree management practices.

### Material and methods

The investigations were conducted in the region of Skopje. Skopje valley is situated at 41°57'N and 21°28'S. Samples of traditional pear varieties (Selamovska, 2013b) were found at the foot of Skopska Crna Gora Mount, near Skopje. According to Filipovski *et al.* (1996), the region of Skopje belongs to the continental - Mediterranean - climate – vegetation and soil area, which combines the impact of East - Mediterranean and continental climates. Moderate cold and humid winters, as well as hot and dry summers are typical of this region.

The average annual air temperature is 12.0°C. This region has low average minimum temperatures and many frosty days. It features a small amount of annual rainfall ranging from 460 mm to 583 mm (on average 507 mm). Aridity index by De Martonne in average amounts is 22.6 and it is particularly pronounced in July, August and September. According to all climate data, it is a dry climate area. Most forests in this climate area are destroyed and turned into farmland and pastures. Due to dry climatic conditions, typical cinnamon forest soils dominate. The relief in the research area is hilly (with slope 7–12°), with a natural pasture at the edge of a deciduous forest. The dominant soil type is red soil, fifth class.

The data about climatic factors were collected from the National Hydrometeorological Service of the Republic of Macedonia, Zajcev Rid Meteorological Station, and are presented in Table 1.

Table 1. Climatic factors

Parameters	2009						2010					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Average monthly temperatures (°C)	1.0	3.3	7.1	13.4	18.0	21.0	2.4	4.4	8.0	13.5	17.8	21.4
Monthly sum of temperatures above 10°C	10.6	31.0	41.8	391.7	549.3	631.3	10.5	13.9	161.3	395.9	552.8	641.9
Monthly sum of rainfalls (mm)	102	110	75	83	93	126	48	102	90	93	51	42

Individual trees are found on the slopes of the mountain, at altitudes of 650 to 800 meters. Trees exist in conditions without irrigation, fertilisation and chemical protection. All old specimens are aged 100 to 150 years, as testified by locals. Individual trees provide good yield. In this paper, 5 traditional pear varieties (*Pyrus communis* L.) were studied:

- ‘Carigradsko Avche’ – a summer variety that in the region of Skopje starts to ripen in early July, during the Orthodox holiday of St. Peter.
- ‘Letna Kajkushka’ – a summer variety that ripens in the last third of August, Skopje region.
- ‘Vodenka’ – an autumn variety that in the region of Skopje ripens in early September.
- ‘Zimnica’ – a winter variety whose fruits are harvested at the beginning of November and can be stored until March or even April to ripen and get ready for consumption.
- ‘Zimska Kajkushka’ – a winter variety whose name is linked with the Orthodox holiday of Christmas Eve. The fruits are harvested in late October or even November and kept for 2-3 months to ripen and become ready for consumption during the winter months.

The differentiation of flower buds was monitored every 15 days, starting from 15 July 2008 until April 2009. The microphenophases of flower bud development were examined, starting from the third stage (beginning of visible bud differentiation) to the end of the sixth stage (pollen grain development) and blooming. They were determined according to the pattern provided by Romanovskaja (2003) and Luchic *et al.* (1995). Flower buds were fixed in a fixing reagent (96% alcohol: chloroform: acetic acid = 6:3:1) and analysed under a binocular light microscope.

## Results and discussion

According to the survey, the first visible sign of the transformation from the vegetative bud into the generative stage (IIIb substage) in the analysed pear varieties can be seen in July. According to Reynoldst *et al.* (2005), initiation of flower buds in the pear varieties ‘Forelle’ and ‘Rosemarie’ starts a little earlier in June. The earliest morphological change in the vegetative cone is observed after the completion of differentiation of cover petals and after spreading of the meristematic top (Menzel, 2003; Luchic *et al.*, 1995).

At the end of August and beginning of September, inflorescence axis formation starts (IV', IV'') (Figure 1). The differentiation of inflorescence in pear mixed buds is different, depending on the order of branching of the inflorescence axis (Menzel, 2003; Luchic *et al.*, 1995). In the second half of October, inflorescence is developed and the varieties begin to form flower organs (V stage) (Fig 2). In the second half of October until early November, the

primordia of calyx are defined (Vb), and corolla petals are well developed (Vc). Stamen primordia (Vd) are developed in the second half of November, and carpel primordia are developed at the end of November (Ve).

*Figure 1. Start of inflorescence formation*



*Figure 2. Developed inflorescence*



During winter, the growth and development of flower organs are completed in the buds (Vf). In early December, histological changes of differentiation take place in the archesporial tissue (VIa). During the winter months, in late December and early January, microsporogenesis occurs (VIb). In the period from January to March, pollen grains mature and develop (VIc, VI d). Thus, the anthers mature and have changed greenish colour receiving red to purple – final red colour when fully ripe (Kocsisne *et al.*, 2011). The stamens grow in height and

later in March, the stigma is developed. This confirms that male organs are developed at first, followed by female organs, indicating that microsporogenesis occurs at first, and then macrosporogenesis follows. The phenophases of blooming and fertilisation occur during April (IX stage).

The quantity and quality of these processes are different and depend on genetic, ecological, cultural and tree management properties as well as on other factors. Considering the fact that the differentiation of varieties is followed in the same region, the early differentiation of flower buds is influenced by genotype. The earliest flower buds are differentiated in 'Carigradsko Avche'. 'Letna Kajkushka' and 'Vodenka' are next, and the latest differentiation of flower buds is characteristic of 'Zimska Kajkushka' and 'Zimnica'. The differentiation of flower buds in 'Letna Kajkushka' and 'Vodenka' occurs almost simultaneously. There is no difference in days between 'Zimska Kajkushka' and 'Zimnica' pears. According to Menzel (2000) and Perfil'eva (1970), the difference in the start of differentiation of flower buds between the earliest and latest varieties of the pear *Pyrus ussuriensis* is 5–10 days.

According to the average time of blooming, the analysed pear varieties in the region of Skopje started to bloom in the first third of April (10th of April). 'Carigradsko Avche' blooms the earliest (from 8th to 9th April), followed by 'Vodenka' (from 10th to 11th April) and 3–4 days later (from 12th to 13th April) by 'Letna Kajkushka', 'Zimska Kajkushka' and 'Zimnica'.

Selamovska *et al.* (2013a) researched blooming of the same pear varieties in different regions. In the region of Resen, 'Vodenka' and 'Zimska Kajkushka' blossom 15 to 20 days later compared to Skopje. The latest blooming is characteristic of the regions of Berovo and Pehchevo at the beginning of May. Reasons for earlier blooming of varieties in the Skopje region compared with other regions are climatic conditions, higher temperature and lower rainfalls (Selamovska and Nikolic, 2012; Selamovska *et al.*, 2012; Selamovska *et al.*, 2013a; Selamovska *et al.*, 2013b).

In 2010, the differentiation of flower buds in the studied pear varieties occurred 7–10 days earlier than in 2009. The reasons for this were higher average monthly air temperatures (0.6 °C), a higher monthly sum of air temperatures, above 10°C (by 120.6), and a smaller sum of rainfalls (by 163mm) from January to the beginning of flower bud differentiation in July. In warmer and more arid conditions, organogenesis and microsporogenesis take place earlier (Selamovska and Nikolic, 2012; Selamovska *et al.*, 2012; Selamovska *et al.*, 2013a; Selamovska *et al.*, 2013b).

## Conclusions

The first visible sign of the transformation from the vegetative bud into the generative stage of the analysed pear varieties can be seen in the summer period, in July. The earliest flower bud differentiation is characteristic of 'Carigradsko

Avche', followed by 'Vodenka', 'Letna Kajkushka', 'Zimska Kajkushka' and 'Zimnica'. Differentiation of flower buds in 'Letna Kajkushka' and 'Vodenka' occurs simultaneously as in 'Zimska Kajkushka' and 'Zimnica'. The differentiation of flower organs lasts until early December.. During the winter months of December and January, the archesporial tissue is developed and microsporogenesis occurs. In the period from January to March, microgametogenesis and pollen grain development take place.

In the region of Skopje, the examined pear varieties bloom in the first third of April (10<sup>th</sup> April). The earliest blooming variety is 'Carigradsko Avche' (8th and 9th April), and the latest blooming variety is 'Zimska Kajkushka' and 'Zimnica' that bloom 3-4 days later (12<sup>th</sup> and 13<sup>th</sup> April).

Early differentiation is positively correlated with higher temperatures and negatively correlated with rainfalls.

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