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Phenological Stages, Pollen Production Level, Pollen Viability and *in vitro* Germination Capability of Some Sour Cherry Cultivars

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Abstract: The objective of this study was to determine the phenological stages and viability, germination capability of pollen grains *in vitro* and fertility level of *Érdi bõtermõ*, *Debreceni bõtermõ*, *Kántorjánosi*, *Újfehértói fürtös*, *Éva*, *Petri*, *Oblacsinszka*, *Pándy279* and *Csengõdi* sour cherry (*Prunus avium* L.) cultivars grown in Hungary. It was determined that active pollen levels significantly varied between 12.4 and 93.7% in TTC (2, 3, 5-triphenyl-tetrazolium-chloride) tests. Anther of *Kántorjánosi* showed the highest (148) number of pollens, whereas the lowest number (27 was obtained from *Oblacsinszka*. The highest germination was obtained from *Oblacsinszka* by 64.5% and the lowest obtained by *Pándy 279* on 28.8% after 48 h. In orchard trials the mean fruit set of self-pollination were 6.3% with max. 18.6% in the case of *Oblacsinszka* and min. 0% in the case of *Pandy279*, while in open pollination trials it was 15.2% max. 28.5% in *Oblacsinszka* and min. of 7.5% in *Pándy279*.

Key words: Pollen viability, flower biology artificial, self pollination

INTRODUCTION

Determining of the phenological stages and viability, germination capability of pollen grains *in vitro* and fertility level of new cultivars are very important for breeders and growers. The majority of commercially grown sour cherry cultivars are self-fertile and for the purpose of finding adequate polleniser cultivar for auto-incompatible cultivar, have to consider not only the number of anthers per flower, but also the quantity of pollen per anther and the germination viability of pollen grains. According to Thompson (2004), first requirement for economical fruit production is the availability of an adequate source of viable and compatible pollen.

The maximum fruit set by self fertilisation may be more than 50% in sour cherry (Kozma *et al*., 2003). Nyéki *et al*. (1999) reported that high pollen production and pollen viability are important for fertilization. The environmental factors and genetic characteristics are effective in the case of cultivation of fruit trees; an abundant crop of cherries is dependent upon a successful completion of a sequence of reproductive events. Failure or deficiency of any aspect of this process can result in a crop reduction or total loss. According to Nyéki *et al*. (2008) the three selected species examined prove the tendency that self-fertile varieties produce about 2.5 times more pollen than the auto-incompatible ones. The partially self-fertile varieties produced 1,688, i.e., more than the auto-incompatible cultivars (Nyéki *et al*., 2002). Some sour cherry cultivars under favorable condition approach the self-fertile types, whereas under unfavorable conditions they are almost self-incompatible. The types of Pándy sour cherries are auto-incompatible (Kozma *et al*., 2003). Pollen performance includes pollen produced in a flower, pollen morphological homogeneity, pollen germination, pollen tube growth and pollen competition; it is an important component of fertilization success in fruit trees (Thompson, 2004; Hedly *et al*., 2004). Applying enough pollen does not guarantee a good fruit set unless the pollen used is viable with a high germination percentage. The use of selected pollen with a high degree of viability will ensure a better fruit set and consequently an acceptable yield (Bots and Mariani, 2005).

The literature on pollen viability that appeared until 1974 has been reviewed by Stanley and Linskens (1974). The accumulated data indicated that pollen viability was influenced by relative humidity, cold and hot temperature, atmospheric composition and oxygen pressure. Elevated CO_2 levels protect pollen viability against heat stress during development (Aloni *et al*., 2001).

According to Bots and Mariani (2005), pollen grains of many species may be sensitive to excessive dehydration caused by low relative humidity, but adaptations to prevent such dehydration are species

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specific. In addition, drought stress during pollen development in the anther can also strongly affect pollen viability.

Pollen viability could be assessed by different methods like staining with nuclear or non-vital dyes or *in vitro* germination tests (Parfitt and Ganeshan, 1989; Heslop-Harrison and Shivanna, 1984; Voyiatsiz and Paraskevopoulou, 2002).

Therefore, knowledge of pollen viability and germination are the most important properties in cherry tree fertilization and selection of suitable polliniser while the orchard is being established (Brown *et al*., 1996; Nyéki *et al*., 2008). Also, important research materials for morphological, physiological and the way it is affected by environmental factors may help predicting hybrid formation.

The objectives of this study were to investigate the viability and germination rate of pollen collected from some pollen donor sour cherry cultivars that will be used for the cultivar combinations program in the future. Additionally, the weather conditions of last year and its effect on pollen production level. For this purpose, pollen viability, pollen production, pollen germination rate were assessed under *in vitro* and orchard conditions.

MATERIALS AND METHODS

The study was carried out on a sour cherry collection at the fruit Research and Extension Center for Fruit Growing, Újfehértó, located in the northeastern part of Hungary. The latitude is 38°10′, the longitude is 30°37′ and the altitude is 1,050 m. The mean annual temperature is 9.5°C and the annual rainfall over 50 years was 583 mm. In general, the weather conditions during flowering time of sour cherry were partially rainy and the pollination period occurs in rainy weather, with the exception of May 7, which it was sunny. Winds are predominantly blowing from the east-northeast.

Pollen collection and viability: At the full bloom time of sour cherry (April 2008) some late balloon staged (not opened) flowers were collected from the *Érdi bõtermõ*, *Debreceni bõtermõ*, *Kántorjánosi*, *Újfehértói fürtös*, *Éva*, *Petri*, *Pándy279*, *Oblacsinszka* and *Csengõdi* sour cherry cultivars. The flowers were transferred to the laboratory immediately. Anthers were removed and placed into dark-colored bottles to promote dehiscence at room temperature. In the stain tests, pollen viability was estimated by using TTC (2, 3, 5-triphenyl-tetrazolium-chloride) 1% stains. Pollens were scattered onto TTC solutions and stained pollens were counted after 2 h. To determine pollen viability, pollens of each cultivar were observed on three slides under a light microscope (x120 magnifications). The stained pollens were considered as viable in these tests.

The pollen productions of flowers: The pollen number of each individual flower of sour cherry cultivars was calculated over 7 years' observations (1973-1974, 1988-1991 and 2008). Pollens from each individual flower of sour cherry cultivars were calculated following the method described by Cruden (1977). Fifteen mature undehisced anthers were washed in distillated water, mounted in 1:1 solution of 1% acetocarmine and 50% glycerine and shook for 2 min. Each anther was gently squashed to release the pollen. Five slides of samples were observed under a microscope and the total number was counted. This number was multiplied with the total number of anthers to calculate pollen production per flower.

Pollen germination: For this reason, pollen grains were sowed in the medium containing 0.5% agar + 15% sucrose + 5 ppm boric acid medium and incubated at the constant temperature of 25°C. The percentage of pollen germination was determined after a 24 h incubation period. The pollen tube growth was evaluated in the laboratory and 6 slides of samples were observed, using a Ziss microscope.

Statistical analysis: The percentage data were first transformed to arcsine square root transformation and an analysis of variance was performed. The differences among means were analyzed using the Duncan's multiple range test at $p<0.05$ significance.

RESULTS AND DISCUSSION

Phenological stages: The bud burst appeared during the second week of March. The balloon stage started in the first half of April. The flowering period took approximately 8-19 days. The genotypes have different flowering times: *Érdi bõtermõ* flowers in 13-April, which was the early blooming cultivar, while the *Kántorjánosi* and *Petri* flowers in 20-April which were the late blooming cultivars. This is related to the different chilling requirements needed to flower that are lower for *Érdi bõtermõ* than for *Kántorjánosi* and *Petri*. The fruits set were completed in the last week of April. Fruits turned to red color in the first and second week of June. Harvest time was at the end of June except for *Érdi bõtermõ* and *Oblacsinszka* cultivars which were harvested 10 days earlier (Table 1). The earliest cultivars were *Érdi bõtermõ*

Table 1: Phenological stages of sour cherry cultivars under climatic conditions of Újfehértó, Hungary (2008)

Cultivars	Bud burst	Balloon stage	First anthesis	Full bloom	Post bloom	Fruit set	First red fruits	Harvest date
Érdi bõtermõ	5-Mar.	8-Apr.	8-Apr.	13-Apr.	23-Apr.	25-Apr.	27-May	20-June
Debreceni bõtermõ	11-Mar.	12-Apr.	13-Apr.	15-Apr.	25-Apr.	26-Apr.	2-June	30-June
Kántorjánosi	11-Mar.	12-Apr.	13-Apr.	20-Apr.	29-Apr.	30-Apr.	2-June	30-June
Újfehértói fürtös	12-Mar.	13-Apr.	14-Apr.	18-Apr.	26-Apr.	27-Apr.	2-June	30-June
Éva	10-Mar.	11-Apr.	12-Apr.	16-Apr.	25-Apr.	27-Apr.	2-June	30-June
Petri	12-Mar.	13-Apr.	14-Apr.	20-Apr.	27-Apr.	26-Apr.	2-June	30-July
Oblacsinszka	10-Mar.	10-Apr.	12-Apr.	18-Apr.	25-Apr.	27-Apr.	27-May	18-June
Pándy 272	12-Mar.	11-Apr.	13-Apr.	18-Apr.	26-Apr.	29-Apr.	2-June	26-June
	13-Mar.	13-Apr.	15-Apr.	20-Apr.	26-Apr.	29-Apr.	2-June	18-June

Table 2: The characteristic of flowers of sour cherry cultivars, Hungary (2008)

Cultivars	Average No. of anthers/flower	Pollen No./anther	Pollen No./flower
Érdi bõtermõ	30.0	32.2d	966.0d
Debreceni bõtermõ	32.0	29.0d	928.0d
Kántorjánosi	31.0	148.4a	5716.4a
Újfehértói fürtös	31.0	21.4de	663.4de
Éva	32.0	27.2de	870.4d
Petri	29.0	26.8de	777.2de
Oblacsinszka	34.0	118.2b	4018.8b
Pándy 279	28.0	17.2e	481.6e
Csengõdi	31.1	50.7c	1735.9c

Values within a row followed by different letter(s) are significantly different ($p<0.05$)

and *Oblacsinszka* while *Kántorjánosi* cultivar was the latest. The phenological dates can change with temperature. Nyeki *et al*. (2002) studied three main phenological stages in different sour cherry cultivars: the duration between bud burst and beginning of flowering was 32-33 days, the flowering period was 10-11 days and duration between the end of flowering and fruit ripening was 63-66 days. With respect to weather conditions, flowering time can show alteration. An early flowering cultivar as *Érdi bõtermõ* cannot be recommended as a pollinator for the latest cultivar and it might not be able to pollinate other late blooming cultivars.

Pollen production: Anther of *Kántorjánosi* cultivar showed the highest (148) number of pollens, whereas the lowest numbers (27) were obtained from *Oblacsinszka* and *Debreceni bõtermõ* (29) cultivars (Table 2). Similar observations were made by Nyéki *et al*. (2008) as for the weak pollen production of Pándy clones. Redalen (1984) scored 35 sour cherry varieties as for their pollen production. According to Nyéki *et al.* (2008) sour cherry cultivars could be grouped on the basis of their pollen production per anther: 1. Small (0-600); 2. Intermediate (600-1,200); 3. Large (>1,200).

In this experiment, all the examined cultivars belonged to the first group. The pollen production of *Pándy 279* is very low. Substantial differences were produced by different seasons regarding the means of cultivars. The results of over 6 years of observations (1973-1974, 1988-1991) of 20 sour cherry cultivars shows that very low number of pollen grains (345-865) were found in the 5 Pándy meggy clones, whereas most (1976 per anther) were typical for the variety Meteor.

Table 3: The quantity of pollen per anther of 20 sour cherry cultivars (1973-1974, 1988-1991)

1973	1974	1988	1989	1990	1991	Mean*
1282	1468	1030	661	1411	518	1245

Substantial differences were produced by different seasons regarding the means of varieties. In 1991, the number was 518, whereas in 1974 it was 1,468 (Table 3).

Pollen germination tests: The pollen viability values and germination rates of sour cherry cultivars are given in Table 4. According to Davarynejad *et al*. (1993) high pollen viability and germinating as well as pollen production capacity are important to decide on a suitable pollinator, since not all pollen grains germinated on stigma can reach to the ovule. On the basis of TTC examinations, pollen viability values vary between 12.4 of *Pandy 279* and 93% *Oblacsinszka*. Tosun and Koyuncu (2007) studied sour cherry pollens using TTC and reported that pollen viability ratios reached 80 to 93%.

The highest germination was obtained from *Oblacsinszka* (64.59%) while the lowest was from *Petri* (29.12%) after 24 h of incubation (Table 4). Results of incubation duration experiments were similar to the findings of Tosun and Koyuncu (2007), who reported that the pollen germination rate of sour cherry cultivar after 24 h were between 57-67%.

With regard to meteorological characteristics of 2007, it can be seen that the precipitation in the time induction and differentiation of flowers was very low. It could be reduce the number of pollen grains per anther on some cultivars. For example in Újfehértó, precipitation from June 21, 2007 to July 4 was only 20 mm and from July 6 to August 1 was only 15.1 mm (data not shown), which was

Table 4: The number of pollen grains per anther in sour cherry cultivars of different fertility relations

Cultivars	Fertility	Pollen No. in an anther (%)	Pollen viability (%)	Pollen germination	Fruit set after self-pollination (%)
Érdi bőtermő	Self-fertile	32.20	23.6b	30.23b	13.40
Debreceni bõtermõ	Self-fertile	29.00	19.62bc	32.65b	10.60
Kántorjánosi	Partially self-fertile	148.40	91.35a	51.89a	25.70
Újfehértói fürtös	Self-fertile	21.40	33.76b	31.43b	38.80
Éva	Partially self-fertile	27.20	21.46bc	32.65b	9.80
Petri	Self-fertile	26.80	15.64bc	29.12b	15.60
Oblacsinszka	Self-sterile	118.20	93.76a	64.59a	27.00
Pándy272	Auto-incompatible	17.20	12.48c	28.87b	0.36
Csengődi	Partially self-fertile	50.75	23.6b	30.23b	6.60

Auto-incompatible (>1%)*, Partially self-fertile (1-10%)*, Self-fertile (<10%)* fruit set after self-pollination. Values within a row followed by different letter(s) are significantly different ($p<0.05$)

non effective irrigation for trees. Present results were congruent with the results obtained by Bots and Mariani (2005).

Investigating the self-fertility of the cultivars in orchard: The highest fruit set was obtained from free pollination with 58.5% fruit set. Fruit set exceeding 5% can be considered as acceptable degree of self-fertility (Table 4). Nyéki *et al*. (2000) classified the cultivars into three groups: adequate (above 10%), acceptable (5-10%) and weak (below 5%). The fruit set of self pollination was highest in case of cv. *Újfehértói fürtös* and lowest in case of cv. *Pándy272*. The other cultivars were intermediate. Surpasses the desired level of 10% required for safe fruit set. In case of other cultivars, the average degree of self-fertility is between 3 and 6% (Nyéki *et al*., 2008).

However, self-fertile cultivars of sour cherries produced more pollen than auto-incompatible ones. The partially self-fertile varieties displayed less pollen than the latter ones; we cannot state a general rule concerning the correlation between fertility relations and the number of pollen grains per anther. Present observations match the statement of Kozma *et al*. (2003), which the auto-incompatible sour cherry cultivars produce less pollen per anther than the self-fertile cultivars.

CONCLUSION

Testing the viability and germination of pollen grains of sour cherry cultivars are the most important properties in cherry tree fertilization for a fruitful cultivation, especially when the new cultivars are used as pollen donor cultivars purpose, especially in terms of selecting which cultivars should be used by growers. *Érdi bõtermõ*, *Debreceni bõtermõ*, *Kántorjánosi*, *Újfehértói fürtös*, *Éva*, *Petri*, sour cherry cultivars selected in Hungary and similar to the old *Pandymeggy*. The low percentage of viability of *Újfehértói fürtös* and *Debreceni bõtermõ* explain the high variation of self pollination in different years. In this context, this study may provide useful information for facilitating the evaluation of sour cherry cultivars based on their pollen performance. All the examined cultivars no produce enough pollen except of *Kántorjánosi* and *Oblacsinszka* which could be used as a good pollen donor for others.

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