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Pollen-pistil interaction influence on the fruit set of sweet cherry

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ABSTRACT

Pollination success and final fruit set of sweet cherry depends on self- and cross- (in)compatibility relations among cultivars in orchard. The objective of this study was to determine the compatibility relationships and to select pollenizers for the most important Croatian sweet cherry cultivars, 'Gomilička' and 'Stonska'. Phenological observations, pollen germination, pollen tube growth and fruit set were assessed following self- and cross-pollination treatments in two flowering seasons. In general, we found overlap of flowering periods of all cultivars included in the study, but low synchronization was observed for the full-bloom period among traditional cultivars, particularly 'Stonska' (early cultivar) and 'Tugarka' (late cultivar). There was a significant difference in fruit set among the type of pollinations and pollen donors. 'Gomilička' and 'Stonska' are self-incompatible, and cross-pollination is necessary for good fruit set. According to the overlap in flowering time and fertilization level in 'Gomilička', cross-pollination with 'Garnet', 'Isabella' and 'Starking Hardy Giant' was more effective than that of the traditionally grown cultivars 'Stonska' and 'Tugarka', and introduction of these cultivars as pollenizers is a good choice that can provide regular and abundant fruit set. Despite the different S-allele combinations of traditional cultivars, overlap in flowering time with some of the introduced cultivars, and favourable climatic conditions, we found also significant effects of pollen-pistil interactions in the style and ovary on the dynamics of pollen tube growth and final fruit set. Inter-incompatibility reactions between cultivars were not observed.

1. Introduction

Sweet cherry cultivars are mostly self-incompatible, and only a few are cross-incompatible. Incompatible pollen is capable of germinating on the stigmatic surface, penetrating the stigma, and growing into the style, where pollen tube growth can be arrested as a result of the gametophytic incompatibility system with multi-allelic S-loci (Crane and Lawrence, 1929). Identification of self-incompatible genotypes provides a significant basis for the choice of appropriate pollination cultivars during orchard planning (Ercisli et al., 2012).

Pollination success often varies among years, but inclusion of simultaneously flowering compatible pollinizers is recommended to ensure better fruit set. In addition, successful pollen transfer, pollination, and fertilization are influenced by bees (Thompson, 1996) and temperature (Hedhly et al., 2007).

Pollen germination on the stigma and pollen tube growth through the pistil to the ovule are regulated by pollen-pistil interactions (Linskens, 1986). Pollen tube growth through the style of the pistil has been used to study compatibility relationships in many fruit crops, including apricot (Burgos et al., 1993; Egea and Burgos, 1996), plum (Nikolić and Milatović, 2010), almond (Ben-Njima and Socias i Company, 1995; Ortega et al., 2002; Dicenta et al., 2002), sour cherry (Cerović and Ružić, 1992) and olive (Vuletin Selak et al., 2014).

Information about the most successful cross-compatible combinations of sweet cherry cultivars grown in Croatia and compatibility with introduced cultivars is lacking in the literature. 'Gomilička', 'Stonska' and 'Tugarka' are traditionally grown cultivars in the Mediterranean part of Croatia and represent more than 80% of planted sweet cherry trees. Pomological and chemical characteristics (Radunić et al., 2008) and S-allele composition (Ercisli et al., 2012) of local cultivars grown in Croatia have been determined in previous studies. Despite the different S-alleles found in local cultivars, irregular and low yields are often recorded. It could be assumed that the compatibility between them, or overlap in flowering period, are insufficient for successful pollination and fertilization and better fruit set.

To determine the parameters of flower fruit set, we estimated overlap in time of flowering of cultivars in the orchard and estimated the parameters for testing self- and cross-compatibility and for pollen-

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pistil interactions (pollen tube growth, number of pollen tubes in the ovary and fruit set).

2. Materials and methods

2.1. Plant material

Experiments were carried out in a 10-year-old commercial sweet cherry orchard (43°33′36”N; 16°22′11”E) and in a nine-year-old collection/experimental orchard at the Institute for Adriatic Crops and Karst Reclamation (43°33′22”N; 16°20′56”E), both in Kaštela, Croatia during two flowering seasons. Meteorological stations (La Crosse Technology, France) were placed in the orchards, and data on the main meteorological information were collected during the experiment. Agricultural management practices were carried out using standard local technology for cultivation of sweet cherry orchards, without an irrigation system. The cultivar structure of the commercial orchard is based on the old, traditionally grown cultivars ‘Gomilička’, ‘Stonska’ and ‘Tugarka’, while the collection/experimental orchard is based on introduced cultivars (‘Garnet’, ‘Isabella’ and ‘Starking Hardy Giant’) grown on *Prunus mahaleb* L. rootstock. The cultivars (‘Gomilička’ and ‘Stonska’) were selected in the study as female parents to test compatibility relations and fruit set following self- and cross-pollination treatments. Newly introduced cultivars were used only as pollen donors in the experiment, to study compatibility relations and their influence on the fruit set of ‘Gomilička’ and ‘Stonska’.

2.2. Flowering observation

Observation of the flowering periods of all cultivars included in the study and pollen collection for cross-pollination treatments were conducted in both orchards. The flowering period was monitored by visual examination according to method of Štampar (1956), and phases were divided as follows: beginning of blooming (first open flowers), full bloom (70% open flowers), and the end of blooming (95% of petals have fallen). The observations were conducted on five trees of each cultivar. Season phenograms of flowering were made for all cultivars based on the collected data of flowering period.

2.3. In vitro pollen germination

In vitro pollen germination was performed for all cultivars. Branches with flowers at the balloon stage were collected for each cultivar and left to dry for 24 h under room conditions (at 20 ± 2 °C). The next day, pollen was collected and sowed in the germination medium (1% agar and 15% sucrose concentration) in Petri dishes and then incubated at 20 ± 2 °C for 24 h in dark conditions (Stösser and Anvari, 1981). Two dishes per cultivar were used, and ten fields per dish were observed under the light microscope (Carl Zeiss Axioskop 2 plus). A pollen grain was considered germinated if its pollen tube was longer than the length of the pollen grain.

For cross-pollination in the field, collected pollen was preserved at + 4 °C in small vials.

2.4. Pollination experiment

In the pollination experiment, we tested the success of open-pollination, self-pollination and cross-pollination of two female donor genotypes. Five uniformly sized trees of the cultivars chosen as the female donor (‘Gomilička’ and ‘Stonska’) were selected for field pollination experiments. On each selected tree, four branches of similar size in four main directions were chosen for each combination of pollination. At the time of isolation, flowers on the branches were in the balloon stage. Branches were isolated with hand made cotton muslin bags (width 300 mm, length 600 mm) (Neal and Anderson, 2004) to prevent uncontrolled pollination except for the open-pollination cases, in which no bags were used.

Pollen donors for cross-pollination were selected according to the partial or complete overlap of the flowering period. Flowers of the cultivar ‘Gomilička’ were pollinated with pollen of ‘Garnet’, ‘Isabella’, ‘Starking Hardy Giant’, ‘Stonska’, and ‘Tugarka’. Flowers of the cultivar ‘Stonska’ were pollinated with pollen of ‘Garnet’, ‘Gomilička’, and ‘Isabella’.

Pollinations were carried out by hand at anthesis when the stigma was receptive. Small, previously opened and immature flowers were removed to minimize experimental error. For self-pollination, flowers were pollinated with pollen of the same cultivar. Pollinated flowers were counted at the time of pollination and isolated again. Isolation bags were removed after the end of blooming, approximately 12–15 days after pollination. The fruit set for all types of pollination was recorded 15 days after the end of blooming (at initial fruit set) and at harvest time (at final fruit set) and was expressed as a percentage.

2.5. Pollen performance in vivo

In vivo pollen performance was measured on the flowers from the branches chosen as described in the pollination experiment. Cross-pollinated flowers of female parents (15 flowers per pollen donor per sampling date) were collected 1, 2, 3, 4, and 5 days after pollination (DAP). The pistils were removed and fixed in FAA (formalin:acetic acid:70% ethanol at a ratio 1:1:18) for at least 24 h, after which they were washed with running water and softened with 0.8 M NaOH for 12–24 h (Martin, 1959). After softening, the stigma and the style of each pistil were cut from the ovary in the transverse section at the base of the style. The separated pistils were stained with 0.1% aniline blue in phosphate buffer and examined under the microscope (Axioskop 2 plus, Carl Zeiss, Germany) equipped with UV excitation filters (FT 425 nm, LP 450 nm) to observe pollen tube growth. Pollen tube growth was analyzed in the squashed pistil, and the percentage of the pistil with the pollen tube reaching the base of the style and ovary was determined. For all cross-pollination combinations, the number of pollen tubes in the first part of the style (1/3) and in the ovary was counted.

2.6. Statistical analysis

Statistical analysis was conducted using the SAS GLM procedure (SAS Version 9.2, SAS Institute Inc., Cary, NC). The analysis of variance (ANOVA) was conducted separately for each cultivar. After significant F-tests, the means were separated using the Tukey-Kramer post hoc test for multiple comparisons.

3. Results

3.1. Flowering period

The second growing season was slightly cooler than the first one. The average daily temperatures were 12.4 °C (mean), 9.4 °C (min) and 16.2 °C (max) in the first season and 11.4 °C (mean), 6.5 °C (min) and 15.4 °C (max) in the second season (Fig. 1).

The flowering period and overlap of flowering among female genotypes and pollen donors included in the study are shown in Fig. 2. The flowering period was similar in both years and took approximately from 15 to 23 days depending on the cultivar.

In the traditional orchard, the onset of flowering of the cultivar ‘Stonska’ occurred on 11 March in the first season and on 9 March in the second season and was the first flowering cultivar. The flowering period of ‘Gomilička’ started on 13 March in the first season and on 15 March in the second season. ‘Tugarka’ started to flower approximately two weeks later than ‘Stonska’ in both seasons. These important domestic cultivars overlapped in flowering period, but overlap in the full bloom stage was not observed. Chronologically, when flowering intensity of cultivar ‘Stonska’ occurred in the fall, ‘Gomilička’ was in full bloom, and ‘Tugarka’ was at the beginning of the flowering.