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DETERMINATION OF POLLEN VIABILITY IN SWEET CHERRY

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Keywords: Pollen germination *in vitro*, fluorescein diacetate, fluorescent microscopy, pollination, pollen tube growth *in vivo*

Abstract

Implementing the method of determining pollen viability in sweet cherry is of importance both in assessing a pollinator in a varietal composition and in breeding work. Pollen viability in sweet cherries Van, Bigarreau Burlat, Stella and Asenova Rana, flowering simultaneously with the new Yugoslav cv. Čarna, was tested as follows: pollen germination *in vitro* on agar-sucrose medium, pollen staining with fluorescein diacetate and pollen germination, i.e. pollen tube growth *in vivo* in the style of cv. Čarna by fluorescence microscopy. A certain degree of positive correlation was found to exist between *in vitro* and *in vivo* tests. Between the cultivars studied, potential pollinators, quantitative differences in the length and number of pollen tubes were found in the style of cv. Čarna with the *in vivo* test.

1. Introduction

The choice of the proper method for determining pollen viability is of great importance both for assessing varietal composition and in breeding work with self-sterile sweet cherry cultivars. There has been found variation in pollen quality between cultivars within a fruit species (Stösser et al., 1996). There are three forms of pollen analysis: descriptive examinations, viability assays and physiological tests (Galletta, 1983). The standard tests of viability involve *in vitro* and *in vivo* pollen germination, as well as direct estimation of the viability in ungerminated pollen grains using various chemical tests (Stanley and Linskens, 1974).

The implementation of these assays entails the issue of the criteria used for evaluating their validity in estimating pollen quality, i.e. its ability to germinate on the stigma, effect fertilization and form the hybrid seed (Cerović and Mićić, 1996). The objective of this paper was therefore to estimate pollen viability by comparison of three procedures: *in vitro* pollen germination, staining with fluorescein diacetate and *in vivo* analysis of pollen germination in four pollinator cultivars to evaluate properly their viability. This is of importance both for the determination of varietal composition with the new Yugoslav cultivar Čarna and in further breeding work.

2. Material and methods

The pollen of the following sweet cherry cultivars, flowering simultaneously, was used for the experiment: Van, Bigarreau Burlat, Stella and Asenova Rana. Pollen collection

and storage for testing was carried out in the standard way. For estimation of *in vitro* pollen germination, pollen was placed in petri dishes with artificial medium (1% agar-agar + 12% sucrose), which were then kept at room temperature (20° C) for 24 hr. The germinated pollen grains were counted in three different microscopic field.

Determination of pollen viability with fluorescein diacetate was carried out according to the method of Heslop-Harrison and Heslop-Harrison (1970). Viable pollen grains stained by fluorescein diacetate showed light colored reaction. The percentage of pollen grains stained bright yellow was determined.

Testing pollen germination of pollinator cultivars, i.e. the growth of pollen tubes *in vivo*, was done in the style of cv. Čarna, which was separately pollinated at anthesis with the pollen of the cultivars studied. Samples of 30 pollinated flowers were after 72 hr fixed in the FPA (formaldehyde at 40% : propionic acid : alcohol at 70%, 5 : 5 : 90) fixative for 24 hr. The pistils were then prepared, stained and examined by fluorescence microscopy according to the method of Kho and Baër (1968).

The studies reported in this paper were conducted during 1995.

3. Results

The results of studies on pollen viability using the *in vitro* pollen germination test and fluorescein diacetate test are presented in Table 1. The lowest *in vitro* pollen germination was recorded in cv. Asenova Rana (36.67%), and the highest in cv. Van (57.80%) (Figure 1). In the fluorescein diacetate test, the lowest percentage of viable pollen grains was also recorded in cv. Asenova Rana (50.51%), and the highest in cv. Van (78.94%) (Figure 2).

Tests of significance of differences between these two tests showed that they gave different quantitative values relative to pollen viability at the level of the sweet cherry cultivars studied. On average, in all the cultivars studied, a higher percentage of viable pollen grains was recorded with the fluorescein diacetate test, i.e. the differences were highly significant compared to the *in vitro* pollen germination test.

The analysis of pollen germination, i.e. the *in vivo* growth of pollen tubes in all pollination treatments, revealed the penetration of pollen tubes in the central part of the style in cv. Čarna. Diffferences were found to exist between the pollinators, regarding both the longest pollen tube in the style and the average number of pollen tubes in the upper part of the style (Table 2). In the style of cv. Čarna, the longest pollen tube was recorded in the pollination treatment with cv. Van (6531.7 μm). The shortest pollen tube was recorded in the pollination treatment with cv. Bigarreau Burlat (4932.1 μm). As for the average number of pollen tubes that reached the upper third of the style, the greatest number of them was assessed in the pollination treatment with cv. Stella (49.3), and the lowest with cv. Asenova Rana (32.0) (Figure 3). In the *in vivo* test, no phenomena of incompatibility which might hamper the efficacy of the progamic stage, i.e. of a successful fertilization process in cv. Čarna have been observed in any pollination treatment.

As regards the objectivity of evaluation of pollen viability, i.e. of its capability to effect fertilization, a degree of positive correlation of medium strength was found to exist between the pollen germination tests *in vitro* and *in vivo*, which indicates a certain validity of *in vitro* test despite its relativity (Table 3).