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Short communication

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Abstract

The functionality of embryo sacs in relation to their viability and fertilization success was studied over a 2-year period in the sour cherry cultivar 'Čačanski Rubin' during full bloom under field conditions. The occurrence of abnormal embryo sacs resulted in a varying number of functional embryo sacs assessed at the onset of full bloom and had a direct effect on the number of viable embryo sacs. The functionality of embryo sacs, represented by the number of normally developed embryo sacs, which varies from year to year, is approximately equal to the initial number of embryo sacs with embryo, namely, it is the factor which contributes to fertilization success in this cultivar.

Keywords: Sour cherry; Viability of embryo sac; Embryo; Fertilization success

1. Introduction

'Čačanski Rubin' is the first sour cherry cultivar bred in Yugoslavia. It is a mid-late cultivar with large, dark-red aromatic fruits suitable for processing and fresh use. So far, this cultivar has shown a varying degree of fertility, i.e. irregular bearing (Cerović, 1989). A number of factors appear to be related to irregular bearing. Irregularities during the process of microsporogenesis have been reported as a possible explanation for changes in the degree of fertility, resulting in variability of pollen germination in vitro and in vivo (Cerović, 1991, 1992).

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Temperature at flowering time is one of the major ecological factors affecting pollen tube growth rate in fruit crops. Temperature-dependent degree of pollen tube growth was assessed in sour cherry (Cerović and Ružić, 1992), pear (Vasilakakis and Porlingis, 1985), apple (Child, 1966) and almond (Socias i Company et al., 1976; Vasilakakis and Porlingis, 1984). Under field conditions, low temperatures at flowering can have a substantial impact on pollen tube growth rate in the pistil, i.e. the efficiency of the progamic phase, which can result in a considerable reduction in the number of fruits set, e.g. in some plum cultivars (Thompson and Liu, 1973).

On the other hand, the regularity with which macrosporogenesis and macrogametogenesis takes place is closely related to the formation of a normal and functional embryo sac. The occurrence of the early degeneration of megaspores, sterility of the egg apparatus, and disturbances during fertilization have been observed in sour cherry (Potemkina, 1973). In avocado, the occurrence of varying numbers of nuclei in the embryo sac results in the termination of megagametogenesis (Tomer et al., 1976).

Embryo sac viability is a major factor which has a direct influence on the effective pollination period, and thereby the fertility in fruit crops (Williams, 1970). In some apricot cultivars this viability is short and accompanied by early degeneration (Eaton, 1959; Eaton and Jamont, 1965). A too short period of egg apparatus viability restricts the number of fertilized egg cells and the central nucleus in some sour cherry cultivars (Dys, 1984).

The aim of the present study was to consider the functionality of embryo sacs in sour cherry 'Čačanski Rubin' as related to their viability and fertilization success under field conditions at full bloom, as the major parameter, which may affect the degree of fertility.

2. Materials and methods

The sour cherry 'Čačanski Rubin' ('Chase Morello' × 'Köröser') was used for the experiment which was conducted in the field on selected trees using uniform branches with flowers at the late balloon stage. A group of flowers was emasculated and pollinated with 'Šumadinka' ('Köröser' × 'Heimanns Konservenweichsel') at the beginning of full bloom, whereas another group was left unpollinated to test embryo sac viability. Flowers from both groups were isolated by bagging. Twenty flowers per combination were taken every other day from onset of full bloom to 10 days later. The ovaries were fixed in FPA, (formalin : propionic acid : 70% ethyl alcohol, 5 : 5 : 90) and stored at 4°C. The material was dehydrated in an ethyl alcohol series and then embedded in paraffin wax. Paraffin embedded material was sectioned at 10 μm longitudinally and transversally and stained with safranin, crystal violet and green light SF,

according to Gerlach (1969). Daily temperatures were recorded during full bloom. The study was conducted over the 2-year period (1992–1993).

3. Results

As with other stone fruits, the sour cherry contains two ovules. One of the ovules, the primary one, develops into the seed if fertilized, whereas the other, the secondary one, atrophies and eventually degenerates. In this paper we analyzed only the primary ovule. The embryo sac of sour cherry belongs to the monosporial 8-nucleate bipolar *Polygonum* type. Early degeneration of individual cells, or of the entire embryo sac, was observed during the formation of the 8-nucleate embryo sac or immediately after the completion of this process. Such cells lose their normal shape, which is usually accompanied by a strong colour reaction (Fig. 1(a)). Sometimes preserved nucleoli can be seen. In some cases the whole embryo sac shows a strong reaction, i.e. its degeneration is complete (Fig. 1(b)).

3.1. Viability of embryo sac

The viability of embryo sacs following normal development (8-nucleate; 5-nucleate, which means with degenerated antipodals and 4-nucleate with fused polar nuclei) was tested during full bloom, including balloon stage (Fig. 2).

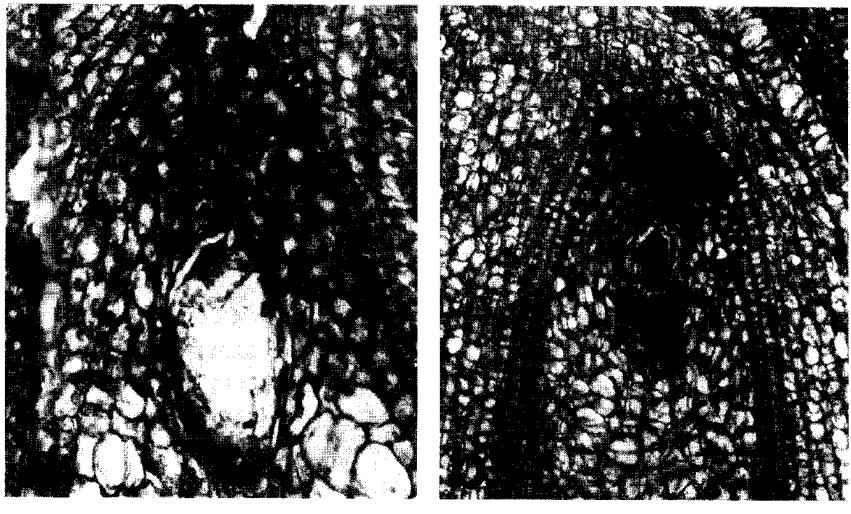

Fig. 1. Embryo sacs of sour cherry 'Čačanski Rubin' in the days following anthesis. A strong colour reaction of the degenerated synergid in the egg apparatus indicates degeneration of this structure (a) (× 500), and of the entire content of the degenerated embryo sac (b) (× 660).

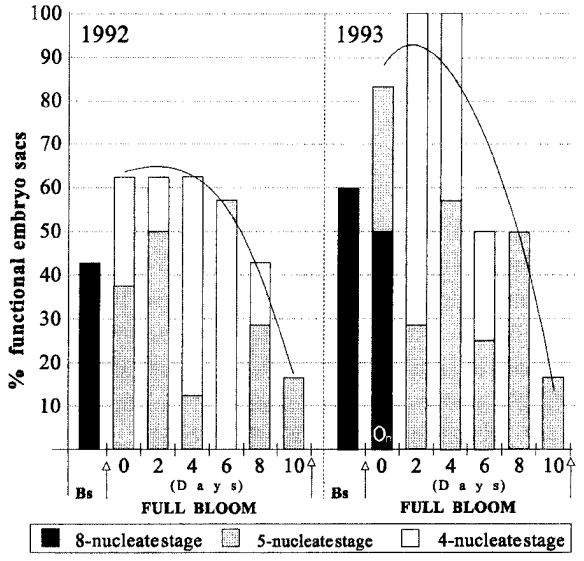

Fig. 2. The ratio of % functional embryo sacs to days at full bloom in unpollinated flowers of sour cherry 'Čačanski Rubin' over 2 years. 1992: $y = 60.46 + 4.20x - 0.84x^2$(R = 0.99); 1993: $y = 89.88 + 3.54x - 1.12x^2$ (R = 0.93). Bs = Balloon stage; On = Onset of full bloom.

Regression lines are best fitted, and their trend in both years shows the maximum between day 2 and day 4 of full bloom. In the first year, the eight-nucleate cytological stage of the embryo sac was observed at balloon stage, and the following year it was also recorded at the onset of full bloom. A difference was noticed between the 2 years in the number of functional embryo sacs. In 1993, a higher percentage of functional embryo sacs was observed compared to the previous year.

In both years, the percentage of functional embryo sacs shows a downward trend after day 4, and at day 10 of full bloom it amounts to only 16%. After day 4 of full bloom, degeneration and irregularity in the spatial distribution of individual cells of the embryo sac were observed, especially of the egg cell (Fig. 3(a)). The phenomenon of complete separation of the egg apparatus cells was sometimes noticed (Fig. 3(b)). In the embryo sac, the central nucleus showed the greatest longevity. Parallel to the above described degenerative and irregular distribution in the embryo sac, the ovule tissue can also show signs of degeneration. In such ovules degeneration is first observed in the integuments, spreading later to the entire ovule.

In both years mean annual daily temperatures at full bloom were approximately the same (14.3°C and 14.8°C in 1992 and 1993, respectively) (Fig. 4), so that they could not be responsible for the differences in embryo sac viability recorded in both years.

Fig. 3. Embryo sacs of sour cherry 'Čačanski Rubin' after day 4 of full bloom. The occurrence of the irregular distribution of the individual elements of the egg apparatus (a) (× 660) and of all cells of the egg apparatus occurs with aging as a sign of degeneration (b) (× 330).

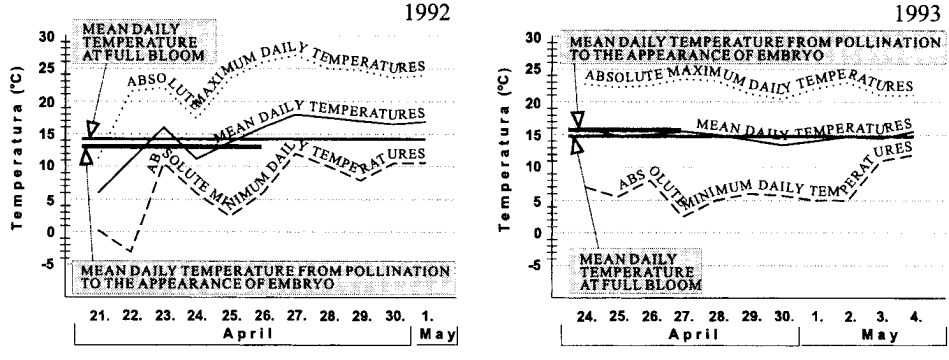

Fig. 4. Dynamics of temperatures at full bloom of sour cherry 'Čačanski Rubin' in 2 years.

3.2. Fertilization success

Fertilization success, expressed as the percentage of embryo sacs in which the embryo is present, was monitored during full bloom (Fig. 5). The globular embryo is the highest developmental stage in the process of embryogenesis, observed during full bloom. The process of endosperm formation started concurrently with the process of early embryogenesis. The dynamics of the percentage of embryo sacs with an embryo follows a linear trend (for 1992) and a parabolic trend (in 1993). The appearance of the embryo in 1992 was observed at

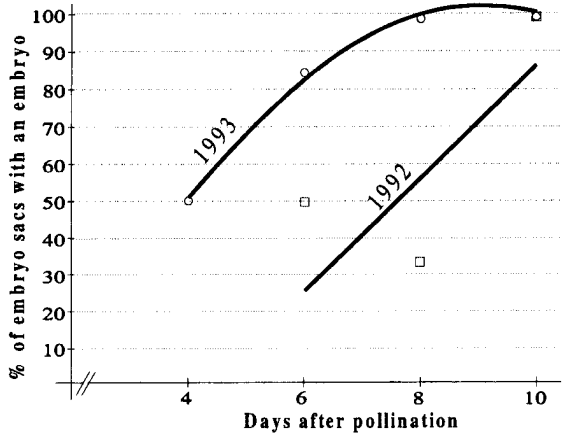

Fig. 5. The ratio of % embryo sacs in which the embryo is present to days after pollination in sour cherry 'Čačanski Rubin' in 2 years. 1992: $y = -38.89 + 4.20x$ (R = 0.98); 1993: $y = -66.66 + 37.50x - 2.08x^2$(R = 0.99).

day 6 after pollination, in contrast to the following year when it was observed 2 days earlier. In 1993 the trend of embryo sacs with an embryo showed a more rapid growth compared to 1992, with the embryo present in all the ovules tested on day 8 after pollination. Mean daily temperature at full bloom was approximately the same in both years. Year-to-year differences were found to exist when observing the interval from the onset of full bloom-pollination, with the appearance of the embryo inclusive. Thus, in 1992 when the appearance of the embryo was observed on day 6 after pollination, mean daily temperature for the interval was 13.1°C. The next year, the embryos were observed on day 4 after pollination, with the mean daily temperature for the interval being 15.3°C (Fig. 4).

4. Discussion

The occurrence of the early degeneration of the embryo sac is a factor that can substantially affect the degree of fertility. In sour cherry 'Montmorency', up to 40% of embryo sacs were found to be incomplete, degenerated, and contained four or fewer nuclei at full bloom (Furukawa and Bukovac, 1989). The degeneration of the egg apparatus, as well as of the entire embryo sacs, was also observed in other sour cherry cultivars (Dys, 1984). Similar phenomena were noticed at the onset of full bloom in our present paper, no matter whether the degeneration of the entire embryo sacs or their individual elements was in question. These phenomena can be directly related to the origin of sour cherry as a plant species. Sour cherry (*Prunus cerasus* L.) is a tetraploid, and its

development involves the genomes of different species of the genus *Prunus* (Olden and Nybom, 1968). Non-homology between sister chromosomes during meiosis results in a certain degree of abnormality in the embryo sac. However, environmental factors also have an influence on this early degeneration of the embryo sac. In our observations, the occurrence of abnormal embryo sacs had a direct effect on the number of functional embryo sacs at the onset of full bloom in sour cherry 'Čačanski Rubin'.

The second parameter concerning the functionality of embryo sac is its longevity at full bloom. The number of functional embryo sacs in sour cherry "Montmorency" was reduced to 8% on day 7 of full bloom (Furukawa and Bukovac, 1989). In some sour cherry cultivars the embryo sac longevity ranges from 3 to 5 days (Anvari and Stösser, 1978; Stösser and Anvari, 1982). The loss of embryo sac viability in some sweet cherry cultivars is much quicker, starting after day 2 of full bloom (Eaton, 1959). In all the mentioned cases, as well as in our present paper, the elements of the embryo sac degenerate as follows: synergids degenerate first, followed by the egg cell and polar nuclei, i.e. the central nucleus. Concurrently with the degeneration of the elements of the embryo sac, the occurrence of irregular spatial distribution of the egg apparatus cells was noticed, i.e. their separation, which is typical of some sour cherry cultivars but only at the beginning of full bloom (Dys, 1984). In our present research, the viability of embryo sacs in 'Čačanski Rubin' in both years was longer compared to the results obtained with other sour cherry cultivars.

The time interval from the moment of pollination or fertilization up to the completely developed embryo varies according to the fruit species. Depending on the year, the appearance of the embryo in our present work was observed on day 4 or day 6 following pollination and can be related to the efficiency of the progamic phase under particular temperature conditions at full bloom (Cerović and Ružić, 1992). On the other hand, if the functionality of embryo sacs is assessed in terms of their viability, the number of viable embryo sacs is approximately equal to the initial number of embryo sacs with an embryo. The progressive development of fertilization success is different in the 2 years, whereas, the viability of embryo sacs in the non-pollinated combination progressively decreases after day 4 of full bloom, and may be related to the effect of the pollination process on activation of the ovary (Linskens, 1973; Deurenberg, 1976). Development and growth of embryo sacs was stimulated by the presence of compatible pollen tubes in the style and final growth elongation of the embryo sac was promoted by cross-pollination in almond (Pimienta and Polito, 1983). In cross-pollinated flowers of both pear and peach, the increased viability period of the embryo sac is accompained by an elongation of the embryo sac itself (Herrero and Gascon, 1987; Herrero et al., 1988). There is also a possibility that prolongation of embryo sac viability, stimulated by pollination exists in sour cherry, and hence chances for fertilization are increased, as in the previously mentioned fruit species

(Herrero et al., 1988). The prolongation of embryo sac viability in 'Čačanski Rubin', as well as the progressive growth trend of fertilization success as reported in this paper, are directly affected by pollination, which was carried out at the onset of full bloom. Pollination at later intervals after anthesis has a drastic effect on the decrease of fertilization efficacy and fruit set in some sweet cherry and sour cherry cultivars (Stösser and Anvari, 1982, 1983).

Our investigations have shown that the functionality of embryo sacs, represented by the number of normally developed embryo sacs, contributes to fertilization success in 'Čačanski Rubin' at full bloom, and has no effect on irregular fertility of this cultivar. Although a direct correlation between fertilization and fruit set in sour cherry (Lech and Tylus, 1983) was observed, the occurrence of abnormality in some sour cherry cultivars at a later stage of embryo and endosperm development has sometimes been observed (Stösser and Anvari, 1978). Furukawa and Bukovac (1989) indicate that nutritional and environmental stresses during early fruit development are the major factors leading to embryo abortion. Further investigations would elucidate whether these factors may be responsible for decrease of fertilization success in 'Čačanski Rubin', namely for differing fertility of this cultivar.

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