Haploid induction from anther culture of stone fruits (Prunus spp.)*

N. Mićić, Gordana Đurić,
ARI *„Serbia" Fruit and Grape Research Centre Čačak*

Mirsada Đulbić, Gordana Dabić,
Faculty of Agriculture, Sarajevo

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Abstract: Haploid induction from anther culture in sour cherry cvs Šumadinka and Čačanski Rubin, plum cv. Požegača and peach accessions BBGVJ with cold pretreatment at 4 - 5°C lasting 0, 5, 14 and 25 days, followed by culturing on Nitsch-Nitsch, Gamborgs'-B5 and two-stage MS media with a great number of differing combinations of IBA, BAP, GA3, glycine and cysteins was not achieved, although there was observed callus formation from the depth of anthers. The monitoring of the process of microgenesis aimed at the application of cold pretreatment and anther culture in the genotypes observed indicated the existence of certain specific traits which might reflect possible obstacles in the induction of embryogenic pollen haploids in these fruit species.

Key words: Haploid, anther, *Prunus*

Introduction

The development of homozygous lines through self-fertilization is practically impossible in most *Prunus* spp. due to self-incompatibility. Therefore, the induction of haploids remains for the present the only procedure with prospects of paving the way for obtaining homozygous lines.

* Experimental stage of the work was carried out in the Institute for Pomology and Viniculture in Sarajevo.

The work on anther culture in *Prunus* is highly aggravated by the fact that anthers can be collected only once a year over a very short period of 7-10 days, which resulted in few reports related to this problem. A successful regeneration of haploids from anther culture 'in vitro' has not been achieved so far in any *Prunus* species: *P.avium* - induced rhizogenic calli (Jordan, 1974); *P. amygdalus* - induced calli (Michellon et al., 1974); *P. armeniaca* - induced callus (Harn and Kim, 1972, cited after Bajaj, 1983); *P. cerasifera* - induced calli and adventitious buds on calli (Serilis et al., 1979); *P. cerasus* and *P. persica* - induced calli (Michellon et al., 1974; Seirlis et al., 1979; Ognjanov, 1989; Đulbić et al., 1990). The results of research conducted to date have not yielded evidence which could reliably set the trends of further work. The only general rule accepted so far was that haploid induction from anther culture was conditioned by a stress pretreatment (Sunderland, 1974; Sunderland and Dunwell, 1977; Huang and Sunderland, 1981).

Material and Methods

The anthers of five genotypes, viz. *P. cerasifera* Ehrh. and *P. persica* - accessions BBGVJ (Gene Bank of Fruit Crops of Yugoslavia), *P. domestica* cv. Požegača and *P. cerasus* cv. Šumadinka and cv. Čačanski Rubin were used for the induction of androgenesis over 1990-1991. Sampling of the fruit species and cultivars chosen for the „in vitro' culture was preceded by a thorough monitoring of the beginning and course of meiosis. Bud samples were collected at 2-day intervals from the moment of differentiation of archesporium till the release of microspores from tetrads and subjected to analysis using the following techniques:

1. Squash technique according to the method of Lespinasse and Salessis (1973);

2. Paraffin technique: fixation after Novashin, embedding and casting in paraffin, sectioning at 7-10 m, staining with Delafield hematoxilin, embedding in Canada balsam.

Twigs with flower buds of the species and cultivars chosen for „in vitro" anther culture were collected from the marked tres at the time when the tetrad stage was prevalent in the sporogenic tissue of anthers. Further procedure with the twigs, ie. the buds can be divided into three basic steps:

1. Cold pretreatment of plant material. Twigs taken from all genotypes were subjected to cold pretreatment in a growth chamber at 4-5^{0}C for 0, 7, 14 and 25 days.

2. Surface sterilization of buds with a part of wood and removed bark and scales was done in 75% of ethanol for 60 sec., in 5% of NaCl for 20min. with three rinses in sterile distilled water.

3. „In vitro" anther culture. The anthers were aseptically excised from flower primordia and placed on culture medium with stomium up. The following culture media were used for the induction of androgenesis:

a) Gamborg's-BP (1968) and Nitsch-Nitsch (1969) with three different combinations and growth regulator concentrations: 1) IBA + BAP + GA3 (0.7 +