Elimination of Plum Pox Virus (PPV) in Plum (*Prunus Domestica* L.) cvs *Kyustendilska Sinya* and *Veljevka* Through In Vitro Techniques

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Abstract

The aim of the present research was to obtain PPV-free plants of *Prunus domestica* cv. 'Kyustendilska Sinya' (KS) and cv. 'Valjevka' (Val) via in vitro techniques.

Mother trees of *'Kyustendilska Sinya'* and *'Valjevka'* were tested through DAS ELISA for PPV as well as other important viruses: Apple chlorotic leaf spot virus (ACLSV), Apple mosaic virus (ApMV), Prune dwarf virus (PDV) and Prunus necrotic ringspot virus (PNRSV). The test results showed that plants have high concentration only of PPV and free of another tested viruses.

Young shoots were collected in May 2000 and surface-sterilized. Explants were cultured on Murashige and Skoog medium, supplemented with 0.56 mg/l BAP, 0.001 mg/l IBA, 30 g/l saccharose and 5.8 g/l agar, pH 5.6. After initiation, small apex buds with 2-3 unfolded leaves were subcultured at 3-weeks intervals on the same medium. DAS ELISA was used to determine the virus content in the plants at each 3th subculture.

To the fourth subculturing 35.3 % of obtained subclones of *KS* and 33.3 % of *Val* were free of PPV. After 8 passages 88 % of established subclones of *KS* and 100 % of Val were PPV-free. After acclimatization the virus-free plants will be retested.

Abbreviations: KS - cv. 'Kyustendilska Sinya'; Val - cv. Valjevka; PPV - Plum Pox Virus; BAP-6-benzylaminopurine; IBA- indolyl-3-butyric acid; MS medium -Murashige and Skoog medium; ApMV - Apple mosaic virus; ACLSV- Apple chlorotic leaf spot virus; PDV- Prune dwarf virus; PNRSV- Prunus necrotic ringspot virus

INTRODUCTION

Plum pox, also known as sharka, is the most devastating viral disease worldwide of stone fruit including peaches, apricots, plums, nectarines. The disease significantly limits stone fruit production in areas where it is established. More than 100 million stone fruit trees in Europe are infected. Sharka is a widespread disease in all parts of Bulgaria, which makes it a limiting factor for growing some susceptible good-quality plum cultivars, such as 'Kyustendilska Sinya' (KS) and 'Valjevka'. In the Plovdiv region, the trees of these cultivars are mass infected by PPV (Milusheva, unpublished data), which makes it difficult to find healthy source plants for producing certified planting material.

Long-distance spread usually occurs as a result of the movement of infected nursery stock or propagative materials. An effective treatment to cure virus infected trees is not available. Control and prevention measures for PPV include field surveys, use of certified nursery materials, use of resistant plants (when available), control of aphids, and elimination of infected trees in nurseries and orchards.

Virus-free stocks are obtained by virus elimination through heat therapy, chemotherapy and/or meristem tissue culture.

The aim of the present research was to obtain PPV-free plants of *Prunus domestica* cv. 'Kyustendilska Sinya' (KS) and cv. 'Valjevka' (Val) through in vitro techniques.

MATERIALS AND METHODS

Plant Materials and Culture Conditions

Young shoot tips were collected in May from the collection orchard of the Fruit-Growing Institute. They were surface-sterilized with 5% Ca hypochlorite for 8 min and three sterile water washes. Tween 20 was added as a surfactant. Explants were cultured on MS medium (Murashige and Skoog, 1962), supplemented with 0.56 mg/l BAP, 0.001 mg/l IBA, 30 g/l saccharose and 5.8 g/l agar, pH 5.6. After culture establishment, small apical and lateral buds with 2-3 unfolded leaves (ca. 3 mm) were transferred at 3-week intervals to the same nutrient medium. Cultures were kept in a growth chamber at 22± 2°C and under a photoperiod of 16 h light (30 µmol m^{-2} s^{-1} PAR, fluorescent lamps OSRAM 40W) and 8 h dark. The proliferated apical buds were separated and transferred to a fresh nutrient medium, and the clones obtained were PPV-tested at every third passage.

Method of Viral Diagnostics

DAS ELISA (Clark and Adams, 1977) was used to detect the virus content both of mother plants and the subclones produced. Diagnostic kits of LOEWE Phytodiagnostica GmbH were used. The adsorption values were measured after 60 min at 405 nm with Titertek Multiskan/MCC (Flow Laboratories). Each test was done in duplicate.

Besides for PPV, the source trees were also tested for other important viruses: Apple chlorotic leaf spot virus (ACLSV), Apple mosaic virus (ApMV), Prune dwarf virus (PDV) and Prunus necrotic ringspot virus (PNRSV) using the aforementioned method. The test results showed that plants had high concentration only of PPV and were free of another tested viruses.

The virus-free subclones obtained from both cultivars were micropropagated and adapted to greenhouse conditions.

RESULTS

The source trees, analyzed by *DAS-ELISA,* showed the presence of high virus concentrations. The absorption value measured for cv. KS was 2.00, and that for cv. 'Valjevka' - 1.40.

After culture establishment, the explants of 'Valjevka' showed a relatively high multiplication rate (Mr), varying in the different subclones within the range of 3 - 5. The PPV testing after the fourth passage showed some variation in the viral content of the analyzed plant tissues. In 16.7% of the subclones produced, the absorption values ranged from 0,300 to 0,400 (high virus content). 44.4% of them had a lower virus content, their absorption values being in the range of 0,100 - 0,200 (low virus content). For the rest 33,3% of the subclones produced, the absorption values were within those of the negative control (Fig.1A).

All subclones of cv. KS were obtained from a single explant left after culture establishment. A very good proliferation was recorded, the second-passage multiplication rate being 3, and that during the third passage increasing from 5 to 8. The results from PPV testing after the 4^{th} passage showed that 52,9% of the isolated subclones had a high virus content, 11,8% - a low one, while 35,3% gave a negative reaction (Fig.1B).

The serological analysis after the 8^{th} passage showed that all subclones produced by cv. 'Valjevka' were free of viruses. For cv. KS, the absorption values in 88% of the plants were almost equal to those of the negative (healthy) control. In 12% of the clones, the absorption values were lower than 0,100, but they were two times higher than those of the negative control. Therefore, these clones could not be assumed to be virus-free.

Plants of selected virus-free subclones were micropropagated, rooted and are currently adapted to ex vitro conditions. These plants are going to be retested.

DISCUSSION

The results from the study showed that the number of subcultures is of significant

importance for tissue virus elimination.

As yet there is no clear concept about the reasons for the virus concentration reduction in newly developed tissues. Most probably, these reasons are of a more complex nature. The presence of cytokinins in the nutrient medium might be one of the essential factors.

It was established (Sanders, 1994) that the presence of cytokinins in plant tissues stimulated the mitotic processes. Furthermore, their inhibiting effect on apical dominance, hence the development of lateral and axillary buds (Helgeson, 1968; Faust, 1989) could suggest that the development rate of in vitro proliferated buds was higher than virus replication, or that in vitro proliferated buds originated from virus-free meristem centres.

The positive results obtained provide a good opportunity to produce PPV-free source trees for establishing mother stock orchards, using the method of clonal micropropagation.

The investigations conducted at the Fruit-Growing Institute with in vitro propagated cv. *Stanley*, grown in orchards (Popov, 1993) showed that the production of own-rooted planting material trees had many advantages over the conventional graft propagation, providing an opportunity for rapid restoration of plum orchards with PPV-free high-tech cultivars.

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Figures

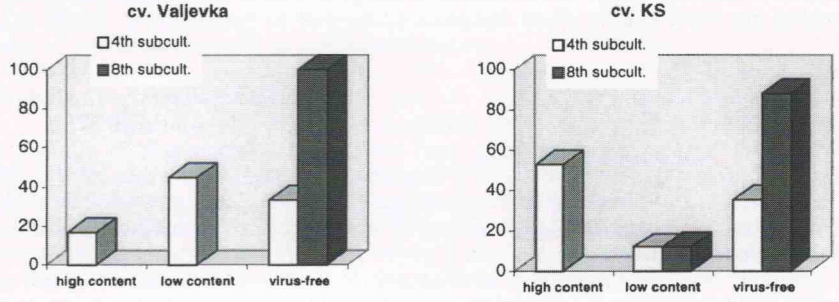

Fig.1. Distribution of the obtained subclones in groups in dependence of PPV content.

A B