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ACHIEVEMENTS IN THE CERTIFICATION OF PLUM PLANTING MATERIAL IN SERBIA

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ABSTRACT

Healthy and true-to-type plant planting material is essential for successful agricultural production. Vegetatively propagated species, such as fruits, are particularly endangered as many pathogens are transmitted through this type of propagation. The certification program for the production of fruit planting material in Serbia started in the mid-2000s. This was the pioneer effort in the country to establish the program and obtain plant material that satisfied all international and newly established national plant material production standards. Since 2005, a total of 26 plum cultivars were introduced into the program. The certification was based on the evaluation of the candidate clones and included the analysis of plants for the presence of prescribed pathogens and their pomological observations. As a result, a nuclear stock of 25 plums, including domestic, autochthonous, and foreign cultivars with a total number of 155 plants was obtained at the Fruit Research Institute, Čačak.

Keywords: plum, cultivars, planting material, certification scheme

INTRODUCTION

A certification scheme or program is a system for the production of vegetatively propagated plants for planting, that can be further propagated or sold, obtained from nuclear stock through several propagation stages (OEPP/EPPO, 2001). During the entire program, plants must be maintained in a protected environment. Two categories of fruit plant material are available on the European market: CAC (*Conformitas Agraria Communitatis*) and certified plant material. Standard material corresponds to the category CAC and must be visually free from harmful organisms. The aim of the certification of fruit planting material is to place on the market high-quality plant material regarding plant health (free from viruses), varietal identity (true-to-type), and homogeneity. A three-stage certification procedure (pre-basic, basic, and certified material) must be used to obtain plant material. There are a number of measures that need to be taken in order to produce certified plum plants. The initial stage is choosing individual plants of each cultivar to be included in the scheme based on their pomological quality. Production of nuclear stock is the second. Each plant from the candidate nuclear stock plants is tested. Only candidate nuclear stock plants that have complied with all standards are promoted to nuclear stock plants, and they are kept in a protected environment (a screenhouse) that prevents pollen and other vectors from spreading infection.

Serbia is a traditionally significant producer of fruit and grapevine planting material. For decades, a large quantity of planting material was produced and exported. The main markets are numerous countries in Europe and the former Soviet Union. Over time, many new private nurseries have been established, and the annual production has increased. For a long time, the only type of material produced was of a standard category. The first mother plantation for the production of virus-free buds of different fruit species and cultivars was

established at the Fruit Research Institute Čačak (FRI) in the late 1970s. Mother plants produced for the plantation were evaluated using biological assays (Ranković, 1981). The implementation of the certification scheme in the production of plum planting material in Serbia started in 2005 (Jevremović and Paunović, 2010).

In this paper, we present the achievements in the certification of plum planting material in Serbia in the past two decades.

MATERIALS AND METHODS

The Fruit Research Institute began its initial effort for the creation of plum nuclear stock in 2005. It included candidate clones of 15 cultivars: 'Boranka', 'Čačanska Lepotica', 'Čačanska Rodna', 'Čačanska Najbolja', 'Čačanski Šećer', 'Čačanska Rana', 'Jelica', 'Krina', 'Mildora', 'Pozna Plava', 'Požegača', 'Valerija', 'Valjevka', 'Timočanka', and 'Stanley'. The second phase was introduced in 2017 with four newly recognized plum cultivars from the FRI breeding program: 'Divna', 'Lana', 'Nada', and 'Petra'. The most recent phase began in 2019 with seven indigenous cultivars, including 'Belošljiva', 'Crnošljiva', 'Crvena Ranka', 'Dragačevka', 'Metlaš', 'Sitnica', and 'Trnovača'. In total, 26 plum cultivars entered the certification scheme with 159 candidate clones, ranging from two to 10 per cultivar. The collections of candidate clones were formed by grafting buds from selected trees of each cultivar onto the virus-free rootstock *Prunus cerasifera*. All plants were planted in pots in the sterile substate and kept in the screenhouse, protected from insects and isolated from the ground.

Evaluation of clones for the certification purpose was carried out according to the following: Law on planting material of fruit trees, vines, and hops; the Regulation on the manner and procedure of production of planting material of fruit trees, vines and hops; the Regulation on the health inspection of crops and facilities for the production of seeds, seedlings, and planting material and the health inspection of seeds, seedlings, and planting material; and the EPPO Scheme for the production of healthy plants for planting, Certification scheme for almond, apricot, peach, and plum (Anonymous, 2006, 2008, 2010; OEPP/EPPO, 2001).

Every candidate clone was repeatedly visually examined for signs of disease and insect damage. Clones were regularly treated with pesticides in order to suppress any possible presence of harmful organisms. Each plant was tested for the presence of the pathogens prescribed in the certification scheme: plum pox virus (PPV), prune dwarf virus (PDV), *Prunus* necrotic ringspot virus (PNRSV), apple chlorotic leaf spot virus (ACLSV), apple mosaic virus (ApMV), Myrobalan latent ringspot virus (MLRSV), and '*Candidatus* Phytoplasma prunorum' (OEPP/EPPO, 2001).

The analysis included biological, serological, and molecular tests. Biological tests were performed on woody indicators (*Prunus tomentosa*, *Prunus persica* GF305, and *P. serrulata* 'Shirofugen') by grafting two buds per tested clone on each indicator. Inoculation of *P. tomentosa* and *P. persica* was performed in the greenhouse (three repetitions per each plant/indicator combination). Inoculation of *P. serrulata* 'Shirofugen' was done in the open field. Indicator plants were visually inspected for the following three months after inoculations.

All candidate clones were tested with serological ELISA tests in three consecutive years (Clark and Adams, 1977). Clones were tested for the presence of ACLSV, ApMV, PDV, PNRSV, and PPV with reagents from BIOREBA AG, Switzerland. The detection of MLRSV was done with the reagents from BIORAD, France. All tests were performed

according to the producers' recommendations. Fresh plum leaf samples were prepared at 1:20 ratio in PBS extraction buffer. Color development was measured at 405 nm on ELISA reader MULTISKAN MCC/340 (Labsystems, Finland) after 60-120 min.

In the first certification phase, each clone was tested for the presence of PPV with molecular RT-PCR test using universal primer pair P1/P2 (Wetzel et al, 1991). In the second and third phase, each clone was also tested with RT-PCR for ACLSV, ApMV, PDV, and PNRSV presence using virus-specific primers as described by Jevremović et al. (2021). For European stone fruit yellows, caused by '*Candidatus* Phytoplasma prunorum' presence, clones were tested with nested-PCR with two sets of primers P1/P7 and R16(X) F1/R16(X) R1 (Schneider et al, 1995; Lee et al, 1995). PCR products were analyzed by 1.5% agarose gel electrophoresis. Gels were stained with ethidium bromide and visualized under UV tray in Gel Doc EZ System (Biorad laboratories, USA). The presence of the fragment of the expected size was considered as a positive result.

RESULTS AND DISCUSSION

Visual inspections

The appearance of symptoms caused by pathogens infecting plums (viruses, viroids, phytoplasmas and virus-like organisms, bacteria, and fungi) and insects was followed by visual inspections. During inspections, no symptoms were observed on candidate clones.

Biological tests

The biological test was conducted in the glasshouse and in the open field on woody indicators *Prunus tomentosa, Prunus persica* GF305, and *P. serrulata* 'Shirofugen'. After the inoculation, the emergence of symptoms was observed for three months. Sharka-like symptoms were evidenced on *Prunus tomentosa* and *Prunus persica* GF305 indicators inoculated with plum 'Jelica' buds taken from all four candidate clones. No other symptoms on indicators were observed in other tested clones.

The application of indicators is compulsory for the certification programs. The indicators used in our program are recommended by EPPO and Serbian bylaws. Indicators *Prunus tomentosa* and *Prunus persica* GF305 are recommended for the detection of a wide range of pathogens. GF305 is suitable for the detection of ACLSV, ApMV, MLRSV, PPV, PDV, and PNRSV. It is also recommended for the detection of '*Candidatus* Phytoplasma prunorum'. The symptoms caused by these pathogens are quite similar, and good expertise is needed to characterize the present pathogen. This indicator is not a suitable for the detection of recombinant PPV strain (PPV-Rec). Recombinant isolates cause no or very mild symptoms in GF305 (Glasa et al., 2005). *Prunus tomentosa* is particularly suitable indicator not only for PPV detection, but also for other viruses infecting stone fruit species (Ranković, 1980; Damsteegt, 1997). *Prunus serrulata* 'Shirofugen' is a recommended indicator for PDV and PNRSV, exhibiting specific localized symptoms in the presence of the virus.

Figure1. Sharka-like symptoms induced by ACLSV on plum leaves

Serological tests

The ELISA method was used for the analysis of leaf samples from candidate clones for the presence of ACLSV, ApMV, MLRSV, PDV, PNRSV, and PPV. The results of the analysis performed in all three phases confirmed that only four plants of plum 'Jelica' were positive for ACLSV presence, and they were excluded from the program. No other viruses were detected in any of the tested clones.

The ELISA test is a very suitable and sensitive method for routine detection of numerous viruses infecting a wide range of crops. Following recommended protocols and using appropriate samples collected at the most convenient time of sampling, this assay ensures reliable detection. Early detection of pathogens in the certification programs with ELISA tests reduces time for virus detection, and infected plants can be promptly removed from the collection.

Molecular tests

Testing for the presence of viruses with RT-PCR revealed that none of the tested clones were infected with the tested viruses (ACLSV, ApMV, MLRSV, PPV, PDV and PNRSV). Also, PCR analysis for '*Candidatus* Phytoplasma prunorum' confirmed that all tested plant were free from phytoplasma.

The PCR method is more sensitive than the ELISA test and has become a major method for virus detection and characterization nowadays. PCR improved the reliability of pathogen detection, and due to its sensitivity, this method is widely used in certification schemes.

Candidate clones were promoted to nuclear stock following the successful completion of all tests, and the creation of the material was reported to the Ministry of Agriculture, Forestry, and Water Management's authorities.

The presented results showed the achievements of the implementation of the certification scheme for the production of plum planting material at the Fruit Research Institute. So far, 25 cultivars have been successfully included in the certification scheme, and pre-basic, basic, and certified material was produced and released.

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