

A Nuclear-Stock Plum Collection Established in Romania

Ioan ZAGRAI^{1)*}, Luminița Antonela ZAGRAI¹⁾

¹⁾Stațiunea de Cercetare-Dezvoltare pentru Pomicultură Bistrița, Drumul Dumitrei Nou, no. 3, 420127, Bistrița, B-N, Romania

^{*)}Corresponding author, e-mail: izagrai@yahoo.com

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ABSTRACT

A program to establish a nuclear-stock plum collection has been started on 2008 at Fruit Research & Development Station Bistrița, Romania. EPPO certification standards and the rules of national regulatory for obtaining nuclear-stock plum material (Prebasic) were successfully implemented. The work was performed in two steps, during 2008-2013. The first step of work was focused on 15 plum cultivars (Iulia, Matilda, Geta, Zamfira, Ivan, Dani, Doina, Romaner, Elena, Jubileu 50, Flora, Renclod Althan, Stanley, Anna Spath and Carpatin) and extended, in the second step, to other six plum cultivars (Agent, Gras ameliorat, Centenar, Minerva, Andreea and Delia). Thus, a total of 21 valuable *Prunus domestica* cultivars were certified as “Prebasic category” and use to establish a nuclear-stock plum collection in appropriate conditions.

Keywords: *EPPO standards, plum, prebasic material, virus free.*

INTRODUCTION

The plum is the dominant fruit specie in Romania (FAOSTAT, 2012), and hence is of great economic importance. On the other hand, some viral pathogens cause serious yield losses in Romanian plum orchards, mainly because the control measures are often poorly implemented. The using of certified virus free planting material is one of the main prophylactic measures to reduce the economic losses caused by the viral pathogens. To secure a virus free status on plum propagation material, it should be produced according to certification scheme and the requirements stated in EPPO (European and Mediterranean Plant Protection Organization) standards [PM 4/30(1)] – (OEPP/EPPO, 2001), [PM 7/32(1)] – (OEPP/EPPO, 2004). Production of nuclear-stock represent the top of storey pyramid of the certification scheme and, therefore, it is the basis for successful of production propagation stock and, subsequently, certified fruit trees. To improve

the current situation in Romania in this field, a certification program for obtaining “Prebasic” plum material and set up a nuclear-stock plum collection was performed at Fruit Research & Development Station Bistrița. A similar work was also recently reported in Serbia (Jevremovic and Paunovic, 2010).

MATERIALS AND METHODS

Plant material. The work for obtaining nuclear-stock plum material was performed in two steps, during 2008-2013. The first step of work was focused on 15 plum cultivars (Iulia, Matilda, Geta, Zamfira, Ivan, Dani, Doina, Romaner, Elena, Jubileu 50, Flora, Renclod Althan, Stanley, Anna Spath and Carpatin) and extended, in the second step, to other six plum cultivars (Agent, Gras ameliorat, Centenar, Minerva, Andreea and Delia). Thus, a total of 21 valuable *Prunus domestica* cultivars were the subject of this work.

Individual trees of each cultivar were visually inspected in experimental orchard for any potential symptoms suggesting possible virus infections, and tested for *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*'. One shoot from each presumed virus free tree was then collected and tested for pathogens listed in EPPO standards [PM 4/30(1)] – (OEPP/EPPO, 2001) to confirm the virus free status. The selected cultivars were grafted onto virus-free Myrobolan 29C into the insect-proof house in

order to produce candidate nuclear-stock. In parallel, biological indexing was performed on *Prunus persica* GF 305. All propagated plants, and also the indicators, were individually tested one year later to all pathogens listed in EPPO standards.

ELISA testing. Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA) – (Clark and Adams, 1977), using commercial polyclonal antibodies, was used as serological method in order to verify the sanitary status of plant material in different stages of certification program. Samples were tested for the presence of PPV, PDV, PNRSV, ACLSV, ApMV

Tab. 1. Summary of the results of serological and molecular tests performed to Candidate nuclear-stock plum.

Cultivar	DAS-ELISA						IC-RT-PCR	Nested-PCR	Status
	PPV	PDV	PNRSV	ACLSV	ApMV	MLRSV	PPV	ESFY	
Iulia	-	-	-	-	-	-	-	-	Vf
Matilda	-	-	-	-	-	-	-	-	Vf
Geta	-	-	-	-	-	-	-	-	Vf
Zamfira	-	-	-	-	-	-	-	-	Vf
Ivan	-	-	-	-	-	-	-	-	Vf
Dani	-	-	-	-	-	-	-	-	Vf
Doina	-	-	-	-	-	-	-	-	Vf
Romaner	-	-	-	-	-	-	-	-	Vf
Elena	-	-	-	-	-	-	-	-	Vf
Jubileu 50	-	-	-	-	-	-	-	-	Vf
Flora	-	-	-	-	-	-	-	-	Vf
Renclod Althan	-	-	-	-	-	-	-	-	Vf
Stanley	-	-	-	-	-	-	-	-	Vf
Anna Spath	-	-	-	-	-	-	-	-	Vf
Carpatin	-	-	-	-	-	-	-	-	Vf
Agent	-	-	-	-	-	-	-	-	Vf
Gras ameliorat	-	-	-	-	-	-	-	-	Vf
Centenar	-	-	-	-	-	-	-	-	Vf
Minerva	-	-	-	-	-	-	-	-	Vf
Andreea	-	-	-	-	-	-	-	-	Vf
Delia	-	-	-	-	-	-	-	-	Vf

and MLRV, according to the manufacturer's instructions (Bioreba, Switzerland for PPV, PDV, PNRSV, ACLSV, ApMV; Sediag, France or Agritest, Italy for MLRV).

PCR testing. To increase the sensitivity of PPV detection, DAS-ELISA was combined with Immunocapture-Reverse Transcription-Polymerase Chain Reaction (IC-RT-PCR) (OEPP/EPPO, 2004). For immunocapture, PPV was trapped with the mentioned polyclonal antibody. Qiagen one-step kit (Qiagen, Germany) and P1/P2 pair of primers (Wetzel et al., 1991) were used for RT-PCR.

To detect 'Candidatus Phytoplasma prunorum', the causal agent of *European Stone Fruit Yellows* (ESFY), Nested-PCR method was performed. Nervures of leaves and/or phloem were grind to a fine powder under liquid nitrogen and, subsequently, total DNA was purified by using DNeasy Plant Mini Kit (Qiagen, Germany). Phytoplasma primers P1/P7 (Deng et al., 1991; Schneider et al., 1995) and R16(X) F1/R16(X) R1 (Lee et al., 1995) were used in the first and second Nested-PCR. GoTaq Flexi DNA Polymerase kit (Promega, USA) was used for DNA amplification.

Growth substrate. The propagated plants have been grown in individual pots filled with commercial soil. To check the potential presence of nematode vectors *Xiphinema* sp. and *Longidorus* sp., the soil in which the plant material has been grown was sampled and analysed by the Central Laboratory for Phytosanitary Quarantine Bucharest (OEPP/EPPO, 2001).

RESULTS AND DISCUSSION

At least one tree from each of the 21 cultivars was selected as initial material after visual monitoring and tests performed in experimental orchards. Serological and molecular diagnosis tests applied to the shoots collected from selected trees were negative for all pathogens listed in EPPO standards [PM 4/30(1)] – (OEPP/EPPO, 2001). That allows the using of these shoots to produce "Candidate nuclear-stock" plants by grafting onto virus-free Myrobalan 29C. One year later, the testing of "Candidate nuclear-stock" derived from 21 selected clones confirmed virus free status in serological and/or molecular assays (table 1).

Biological indexing on *Prunus persica* GF 305 also revealed the absence of pathogens listed in EPPO standards, in all propagated plants.

Soil analysis from pots in which the 'Candidate nuclear-stock' material have been grown was found free of *Xiphinema* sp. and *Longidorus* sp. by the Central Laboratory for Phytosanitary Quarantine Bucharest.

The work done was consistent with EPPO certification standards and the rules of national regulatory. Consequently, plants (three or six) belonging to 21 plum cultivars were certified as "Prebasic category" by Teritorial Inspection for Quality Seeds and Planting Material. This material permitted to establish the first virus free nuclear-stock plum collection at Fruit Research & Development Station Bistrița, Romania. "Prebasic" plants are maintained under strict conditions to avoid potential infection. Currently, "Prebasic" plants are use as precursor for production a larger number of plants of "Basic category".

Because of critical situation in regard to PPV in Romania it is clear that a strategy including propagation of virus free *Prunus* and reinforcing orchards with this kind of planting material is absolutely necessary. The existing a virus free nuclear-stock plum collection represent a good start point for the success of this strategy.

CONCLUSION

A nuclear stock plum collection was established at Fruit Research & Development Station Bistrița. Currently it consists in 'Prebasic' plants belonging to 21 cultivars, most of them originated from Romanian plum breeding.

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