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TECHNOLOGICAL SEQUENCES FOR MULTIPLICATION OF APRICOT PROPAGATION MATERIAL

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ABSTRACT

RSFG Constanța is the owner of the mother stock for biological superior categories of apricot, so in order to align us with the new legislative requirements, starting with 2016 it was introduced in the evaluation process for obtaining Prebase Candidate mother plants for cultivars to which is maintainer: 'Amiral', 'Augustin', 'Auras', 'CMBU', 'De Valu', 'Goldrich', 'Sirena', 'Tudor', 'Umberto', 'Elmar' and 'Olimp'. The evaluation was performed in terms of authenticity based on the test of distinction, uniformity and stability, as well as in terms of phytoviral. The evaluation of the phytoviral status has been done visually, by the biological method and by the DAS-ELISA serological test. The results highlighted the presence of PPV in 'Augustin', 'Sirena' and 'Tudor' cultivars, PDV in 'Auras' and 'Elmar', ApMV viruses in 'Augustin's apricot plants. Healthy plants have been propagated.

INTRODUCTION

In our country a large number of apricot cultivars created in Romania or acclimatized are cultivated; they have with different ripening times, ensuring an assortment of fresh fruits for over three months. The current legislation allows the planting of all existing apricot cultivars in European lists (FRUMANTIS). There is a risk that farmers will set up new plantations with varieties that are not adapt to the climate and soil conditions in our country. As in the case of other fruit species, it is necessary to rehabilitate the apricot through well-thought-out development programs (Trees,fruit-bearing shrubs and strawberries-Technical and Economic Guide, 2014).

Therefore, the quality of the apricot mother plants from high biological categories and of the planting material obtained in the nurseries is very important.

Within SCDP Constanța, the selection of the best clones from the point of view of authenticity, pomological qualities and productivity was done, namely the Prebase candidate mother plants. According to the definition in the legislation in force (Ord. 784/2016, Ord. 119/2020), the mother plant Prebase-Candidate, means a plant that the supplier intends to submit to acceptance as a mother plant in the biological category Prebase. The authentic material follows a protocol that guarantees it is health and is multiplied in a number of desired plants by different methods of propagation.

The choice of biological material was mainly oriented towards the varieties specified in the State Register of Varieties and Rootstocks and in the Official Catalog of Cultivated Plant Cultivars in Romania.

The European Plant Protection Organization (EPPO) has set standards for the certification of Candidate plants, requirements for authenticity, including viral testing methods (PM 4/30 (1), 2001) in order to increase the quality of fruit propagating material, including for apricot.

Regarding the supply with healthy planting material, for apricot, the legislation in the field of production and maintenance of fruit propagation material refers to 8 viral and phytoplasmic pathogens (OM 784/2016 and OM 119/2020): *Apple chlorotic leaf spot virus (ACLSV), Apple mosaic virus (ApMV), Apricot latent virus (ALV), Candidatus phytoplasma prunorum (PHYPPR), Peach latent mosaic viroid (PLMVd), Plum pox virus (PPV), Prune dwarf virus (PDV), Prunus necrotic ringspot virus (PNRSV).*

The research carried out until now on planting material and implicitly on productions has established that in apricot the following have the highest incidence: PPV, PDV, PNRSV and ACLR (Chirilli, et. al, 2016; Milusheva, et. al, 2005).

The serological methods of diagnosis are most preferred for the identification of viruses, because they reduce the time required for testing, allow rapid diagnosis of the virus and immediate elimination of infected plants (Nemeth, 1986).

The aim of the paper is to evaluate the apricot tree cultivars for which RSFG Constanța is maintainer, from the authenticity and phytoviral point of view, in order to obtain Prebase Candidate mother plants with the purpose of supplying the required propagation material used to obtain planting material.

MATERIAL AND METHODS

The biological material was represented by the following cultivars: ‘Amiral’, ‘Augustin’, ‘Auras’, ‘CMBU’, ‘De Valu’, ‘Goldrich’, ‘Sirena’, ‘Tudor’, ‘Umberto’, ‘Elmar’ and ‘Olimp’.

The authenticity analysis was done according to the recommendations of the relevant legislation (OM 1295/2005) regarding the approval of the rules and technical rules on production for marketing, control, quality certification and/or marketing of fruit propagating and planting material.

The data were recorded between 2016 and 2018; 10 trees were evaluated for each cultivar in the maximum fruiting period (year 5 after planting). The analyzed fruit trees are located in the experimental lots from RSFG Constanța.

The paper presents 6 characters considered the most important for the international harmonization of the description of peach cultivars, as follows:

Fruit- size (physiological maturity):- small;- medium;- large. Fruit with an averge size of 21-30 g are considered small, the medium ones are between 31-40 g and the large ones are of 41-50 g.

Fruit- depth of stalk cavity: -shallow; -medium; -deep.

Fruit- ground colour of skin: -white; -cream to yellow; -light orange; -medium orange; -dark orange.

Fruit: colour of flesh: -white; -cream; - light orange; - orange; -dark orange.

Flowering time: - early;- intermediate; - late.

Harvest time: - very early; - early;- intermediate; - late; - very late.

In order to be able to make an accurate assessment of the characteristics of the flesh color, the fruits of each peach cultivar analyzed were harvested at consumption maturity.

The determination of the viral status was accomplished by combining several methods:

1. Field assessment: visual inspections were carried out several times a year.

2. The biological testing was performed according to the recommendations of the International Working Group on Fruit Tree Viruses (Jelkmann, 2001).

The biological testing was performed on 'GF 305' and 'Elberta' seedlings and in accordance with the recommended diagrams, using the structure: number of repetitions / temperature / observation period (w = weeks), as follows:

- 'GF 305' seedlings (5/20^{0}C/8w), to identify: *Apricot latent virus* (ALV), *Candidatus phytoplasma prunorum (PHYPPR), Peach latent mosaic virus viroid (PLMVd);*

- 'Elberta' seedlings (5/20^{0}C/12w), to identify: *Apricot latent virus (ALV), Apricot chlorotic leaf roll phytoplasma (ACLR);*

3. The serological testing was performed to highlight the following viruses: *ACLSV, ApMV, PPV, PDV, PNRSV, SLRSV*, by using the DAS-ELISA method (Clark and Adams, 1977), in accordance with the protocol recommended by the kit manufacturer. The sampling of the test samples was performed at the end of May and beginning of June, in order to avoid high temperatures that would lead to a decrease in viral concentration.

The protocol lasted 2 days and began with the preparation of the biological material, by weighing 1 gram of biological material/sample, grinding in the presence of 5 ml of extraction buffer (Bioreba Kit), thus resulting in the antigen.

The ELISA steps included:

a. Film coating of polystyrene board with antivirus IgG (immunogamaglobulin) solution in coating buffer at pH = 9.6. Antibody fixation occurred by very stable electrostatic bonds in 4 hours at 30 °C.

b. Binding of the viral antigen to the fixed antibody for the formation of the antibody-antigen complex. The obtained antigen from leaves soaked in a buffer solution was introduced into the wells of the plate where the antibody-antigen coupling reaction took place. The placement in the plate was made according to a sketch of the plate that corresponds to the PC reading program of MICROPLATE READER from the endowment of the virology-tissue culture laboratory of RSFG Pitești-Mărăcineni. After distribution of the antigen in the wells (fig. 3), the plate was maintained at 40 °C for 12 hours.

c. Reaction of antigen with immunoenzymatic conjugate (IgG-labeled). In this phase the coupling of the conjugate with the antigen took place: the incubation lasted 5 hours at 30 ^{0}C.

After all the described phases, the plates were washed 3-5 times with a special solution obtained from washing buffer and distilled water.

d. Reaction with the specific substrate. This step was based on the reaction that took place between the substrate and the enzyme in the conjugate to form a colored product. The intensity of the color reaction which measures the amount of specific antigen-bound antibodies present in the serum to be investigated was photometrically determined. The positive evidence is considered to be that which has an extinction value at least 2.5 times higher than the average of negative control.

RESULTS AND DISCUSSIONS

The characteristics of the analyzed apricot cultivars are shown in table 1. The flowering season is an important criterion in choosing apricot cultivars, because the climatic accidents (late frosts or hoar) that occur in the second half of March or early April are becoming more frequent in recent years.

The frost resistance of apricot buds depends of the period of getting out of the dormancy. During the winter the fruit buds can resist up to -20 °C....-22 °C, but the return frosts of -16°C....-18 °C can cause the mass destruction of the fruit buds. The most sensitive phases are full flowering and fruit ripening when temperatures of -2°C....-3 °C and -1 °C....-1.5 °C can totally or partially destroy the current year's harvest (Bălan V. et all., 2008).

In the climatic conditions of the RSFG Constanta during the research years, apricot flowering was recorded between March 14 (in 'Goldrich' and 'Tudor' as cultivars identified for its very early flowering) and March 28 (in 'Olimp' a cultivar with late flowering); the flowering time difference between the earliest and the latest was 14 days.

Harvest time was recorded in mid-June ('Elmar' cv.- very early, fig. 1) until mid-August ('Olimp' cv.- late, fig. 2). The destination of the fruit is mixed as they can be used both for fresh consumption and in the canning industry.

Table 1

Data regarding the characters of the analyzed apricot cultivas, flowering period and harvest time, 2016-2018, Valu lui Traian

Cultivar	Flowering time	Harvest time	Fruit- size (physiological maturity)	Fruit- depth of stalk cavity	Fruit- ground colour of skin	Fruit- colour of flesh
Amiral	16.03	20.06	large	medium	orange	orange
Augustin	17.03	15.08	medium	shallow	dark orange	dark orange
Aurași	15.03	22.06	medium	shallow	light orange	oranj-deschis
Elmar	17.03	13.06	small	medium	dark orange	dark orange
CMBU	22.03	15.07	large	medium	light orange	light orange
De Valu	25.03	11.07	large	deep	light orange	light orange
Goldrich	14.03	07.07	large	deep	light orange	light orange
Olimp	19.03	30.07	large	deep	light orange	light orange
Sirena	28.03	05.08	large	deep	light orange	light orange
Tudor	14.03	10.07	medium	deep	dark orange	dark orange
Umberto	20.03	29.07	large	deep	dark orange	dark orange

The ground color of the skin and of the flesh are important indicators in the evaluation of apricot cultivars and are described in table 1.

The visual field monitoring carried out over the years of observations of the selected plants did not reveal the existence of symptoms that could be associated with diseases caused by viruses, viroids or phytoplasmas.

Biological testing. After the tests accomplished on the apricot cultivars (table 2), no symptoms associated with the targeted diseases were recorded, after indexing on the seedlings GF 305 and Elberta.

There was a good expression of *ACLR* phytoplasma symptoms in the 'Elmar' variety for the selected tree coded T5, on both biological indicators used: GF 305 and Elberta. Also, positive results expressing symptoms produced by the *ACLR*

phytoplasma were identified both on the biological indicator 'GF 305' and on the biological indicator 'Elberta' at the 'Olimp' cultivar in the case of the trees coded T3 and T7. The positive plants were eliminated by burning.

Table 2

Results of the viral evaluation by the biological method

No.	Cultivar	Biological indicator	Symptoms
1.	Amiral	GF 305	-
		Elberta	-
2.	Augustin	GF 305	-
		Elberta	-
3.	Auraș	GF 305	-
		Elberta	-
4.	Elmar	GF 305	T5 -the presence of specific ACLR symptoms
		Elberta	T5 -the presence of specific ACLR symptoms
5.	CMBU	GF 305	-
		Elberta	-
6.	De Valu	GF 305	-
		Elberta	-
7.	Goldrich	GF 305	-
		Elberta	-
8.	Olimp	GF 305	T3; T7– the presence of specific ACLR symptoms
		Elberta	T3; T7– the presence of specific ACLR symptoms
9.	Sirena	GF 305	-
		Elberta	-
10.	Tudor	GF 305	-
		Elberta	-
11.	Umberto	GF 305	-
		Elberta	-

The serological testing by DAS-ELISA to detect viral infections revealed individual infections (Table 3). The *PPV* virus was identified in most samples (trees), namely T3 'Augustin', T2 and T4 'Sirena' and T3 'Tudor'. *PDV* was highlighted on 3 trees, namely T1 'Aura;', T5 'Elmar' 'and T7 'Tudor'. The positive samples were also identified with *ApMV* in T4 'Augustin' and with *PNRSV* in T5 'Sirena'. The trees identified as positive were removed from the selection process and burned.

Table 3

Results of the viral evaluation of the PREBASE-Candidate plants by the DAS-ELISA method in apricot cultivars

No.	Apricot cultivar	Infected trees/virus					
		ACLSV	***ApMV***	***PPV***	***PDV***	***PNRSV***	***SLRSV***
1.	Amiral P1.....P10	-	-	-	-	-	-
2.	Augustin P1.....P10	-	P4	P3	-	-	-

3.	Auraș P1.....P10	-	-	-	P1	-	-
4.	Elmar P1.....P10	-	-	-	P5	-	-
5.	CMBU P1.....P10	-	-	-	-	-	-
6.	De Valu P1.....P10	-	-	-	-	-	-
7.	Goldrich P1.....P10	-	-	-	-	-	-
8.	Olimp P1.....P10	-	-	-	-	-	-
9.	Sirena P1.....P10	-	-	P2; P4	-	P5	-
10.	Tudor P1.....P10	-	-	P3	P7	-	-
11.	Umberto P1.....P10	-	-	-	-	-	-

Figure 1- 'Elmar' cv.

Figure 2- 'Olimp' cv.

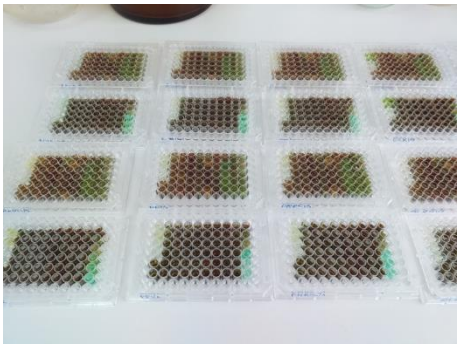

Figure 3. Binding of viral antigen to fixed antibody (Nunc MaxiSorp boards)

CONCLUSIONS

The analyzed apricot cultivars are very diverse in terms of fruit appearance and can be used fresh or in the canning industry from June to mid-August, being a sort that can be recommended to the fruit growers.

Trees of all studied peach varieties correspond in terms of distinctiveness, uniformity and stability.

The increased incidence of peach infections caused by PPV, PDV, ACLR pathogens, identified by testing even in the absence of symptoms in the selected plants is reconfirmed.

Although known with high incidence in apricot, the viruses PPV, PDV, PNRSV and phytoplasma ACLR have not been identified in the varieties 'Amiral',' CMBU', 'De Valu', 'Goldrich', 'Olimp', 'Umberto'.

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Ordin nr. 784/2016 privind cerințele specifice pentru genurile și speciile de plante fructifere menționate în anexa I la Directiva 2008/90/CE a Consiliului din 29 septembrie 2008 privind comercializarea materialului de înmulțire și plantare fructifer destinat producției de fructe, cerințele specifice pe care trebuie să le îndeplinească furnizorii și normele detaliate privind inspecțiile oficiale care intră în domeniul de aplicare al Directivei 2008/90/CE, Monitorul Oficial, Partea I nr. 410 din 31 mai 2016.

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