ELIMINAREA VIRUSULUI PLUM POX PRIN CULTURI *IN VITRO* LA UNELE SOIURI DE PRUN

PLUM POX VIRUS ELIMINATION FROM SOME PLUM CULTIVARS BY *IN VITRO* CULTURE

Plopa Catiţa, Isac Valentina, Butac Mădălina
Research Institute for Fruit Growing Pitesti, Romania

Abstract

For a good management of viral diseases along with the development of sensitive diagnostic techniques substantial attention should be paid to the biotechnological methods for viruses elimination from plants. The experiments conducted at RIFG Pitesti-Romania tried to establish the optimal parameters that would lead to increase efficiency of *in vitro* culture for *Plum pox virus* elimination from 'Carpatin' and 'Pescăruș', plum cultivars. The meristems divided into two groups by size: 0.2-0.4 mm-type A and 0.8-0.9 mm-type B were regenerated on MS and LF basic media with 0.01 to 0.1 mg/l IBA and 0.2 mg/l GA_3 hormonal balance. Multiplication of shoots was developed on the same basal culture media with adapting hormonal balances according to stage. The obtained results showed that the explants size has influenced both the regenerative capacity and degree of virus elimination, irrespective of cultivars and basic media used. Thus in case of 'Carpatin' cultivar with type B explants, regeneration rate increased to 95%, but the *virus free* plants obtained was only 53.57% after six subcultures. With the same cultivar in case when we used type A explants the regeneration capacity obtained was only 33%, but increased the percent of *virus free* plants up to 82.14 %. The leaf samples of the obtained plantlets of each cultivar were tested for *PPV* by DAS –ELISA serological technique.

Cuvinte cheie: Plum pox virus, mediu de cultură, prun
Key words: Plum pox, virus, culture media, plum tree

1. Introduction

Plum pox virus (PPV) also known as Sharka is present in Europe and spread to many countries on other continents. *PPV* is the most devastating viral disease worldwide of stone fruits, especially plum trees.

For obtaining *PPV* free biological material, different methods are used: micrografting (Mosella Chancel et al. 1980); meristem *in vitro* culture (Janečková, 1993; Vertesy 1981), thermotheraphy of *in vitro* cultures (Knapp et al., 1995; Spiegel et al., 1995), chemotherapy (Hauptmanová and Polak, 2011), and or their combinations (Howell et al., 2001).

Obtaining virus free using tissue culture is a difficult process. *In vitro* morphogenesis requires the inducing of some autoregulate phenomena that are autonomous and depend on new heterothrophe conditions that were created. In conformity with literature (Boxus and Druart, 1989) the explants have minim (limit) size to manifest cells totipotentiality under *in vitro* conditions. Another successful factor for *in vitro* culture is represented by media compounds. Sanders (1994) established that the presence of cytochinins in plant tissue stimulated the mitotic process result that the development rate of *in vitro* proliferated buds was higher than virus replication. This is typical for every species or cultivars, the requests are different even depending on the explants used (Isac, 1983). Together with basic culture media, the current methods used for *in vitro* culture in order to induct and sustain of organogenesis have as practical and theoretical base the hormonal balance concept. Dilley (1969) showed that the influence of hormones was manifested as their single action and through the change of ratio between stimulator hormones and inhibitor hormones.

The objectives of this study were to establish some parameters of meristem culture technique to obtain *PPV* virus free plants of *Prunus domestica* for 'Carpatin' and 'Pescăruș', two Romanian plum cultivars.

2. Material and methods

Viral Diagnostics Method DAS-ELISA (Clark and Adams, 1977) was used to detect the virus content in donor plants and for retesting of plants obtained after multiplication phase. Polyclonal antisera from BIOREBA were used for testing. The absorbance values were measured after 120 min at 405 nm with microplate reader PR 1000. The absorbance value recorded was by 1.275 for 'Carpatin' variety and

1.068 for ‘Pescăruș’ variety and cut off value 0.924. Establishing the level of the *PPV* infection after covering periods in multiplication step was evaluated after 3 subcultures, and after six subcultures.

Biological material for micropropagation: was obtained from apically buds of branches of ‘Pescarus’ and ‘Carpatin’ cultivars and was represented by the 0.2-0.4 mm size, type A meristem, and by the 0.8 - 0.9 mm size, type B meristem.

Disinfections of biological material consist of:

- washing with tap water using Tween 20 for 5 minutes;
- immersion in alcohol 96° for 15 minutes;
- immersion in calciu hypoclorite for 10 minutes;
- washing with distilled and sterile water 3 x 10 minutes.

Culture media were represented by Murashihe&Skoog (MS, 1962) and LeeFossard (LF,1977). The organic carbon source was assured using saccharose (30 g/l) and agar like gelifiant agent (6 g /l).

The following hormonal combinations were used:

- for the differentiation phase:

V1= M S + 0.01 mg/l IBA + 0.2 mg/l GA_3

V2 = LF + 0.01 mg/l IBA + 0.2 mg/l GA_3

- for the multiplication phase:

V1 = MS + 1mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l ANA

V2 = LF+ 1mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l ANA

Growth culture conditions consisted of 22-23°C temperature, and 16 hours light (2000-2500 lx) and 8 hours dark photoperiod.

For each treatment 30 explants were observed. Observations consisted in: differentiation capacity (%), multiplication rate (RM), the rate of virus elimination.

3. Results and discussions

Establishment and multiplication of plum cultivars *in vitro*

Many factors influence in vitro regeneration. The experiments conducted showed that in the presence of the same hormonal balance, bazal medium composition influenced significantly the differentiation process of explants in both cultivars tested.

The best results were obtained on LF mineral salts and vitamins (V2). Both explants of ‘Carpatin’ cultivar and ‘Pescăruș’ cultivar showed a high regenerative capacity, situated at 95 % and 85 %, respectively.

The explants differentiation process was lower in conditions of MS minerals salts and vitamins (V1). At the same cultivars percentages of explants differentiation was in these conditions only 33%, fig. 1.

The observations revealed that the explants size significantly influenced the expression of differentiation capacity. On the best basic culture media (V2) in the same culture conditions (temperature and photoperiod), type B explants (0.8-0.9 mm) had a higher capacity of differentiation (95% ‘Carpatin’ and 85% ‘Pescăruș’), fig. 1. Type A explants (0.2-0.4 mm), had a lower capacity of differentiation. The values registered ranged between 30-33 %. After for weeks the differentiation process was finished with the both type of explants.

Results obtained after plantules subculturing procedure on multiplication media revealed that V2 treatment assured a good proliferation. After six subcultures a RM on average of 6 plantlets/explants for ‘Carpatin’ cultivar and 5 plantlets/explants for ‘Pescăruș’ cultivar were obtained.

In culture conditions offered by media from V1 lower results of RM were recorded: 4 plantlets/explants for ‘Carpatin’ cultivar and 2 plantlets/explants for ‘Pescăru’ cultivar.

Viral retesting

Retesting performed with biological material obtained after the third subculture, from type A explants (table 1), revealed a percentage of health material, greater than 50% for both cultivars (‘Carpatin’ 57.14 % and ‘Pescăruș’ 51.71%). When we used type B explants, results showed that percentage of health material was lower: ‘Carpatin’ 42.85% and ‘Pescăruș’ 39.28 %.

Retesting plantlets of ‘Carpatin’ cultivar obtained after six subcultures showed an average of absorbance values ranged between 0.272 and 0.316. That means 82.14 % healthy biological material from type A explants. At ‘Pescăruș’ cultivar with the same explants type the average of absorbance values ranged between 0.254 and 0.307. That represented 85.71%, *PPV* healthy material.

The healthy material percentage obtained was low with type B explants; thus with ‘Carpatin’ cultivar the degree of virus elimination was 53.57 % and with ‘Pescăruș’ cultivar, 64.28 %. Average of absorbance values ranged between 0.380 and 0.413 for ‘Carpatin’ cultivar, and 0.300 and 0.358 for ‘Pescăruș’ cultivar, respectively.

The analysis of absorbance values revealed that with increasing the number of subcultures a decrease in virus concentration resulted.

4. Conclusions

To obtain *PPV* virus free plants from meristem cultures for 'Carpatin' and 'Pescăruș' plum cultivars the best culture media is LF with 0.01 mg/l IBA + 0.2 mg/l GA_3 for differentiation phase and with 1mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l ANA for multiplication phase;

Even small explants had a low regenerative capacity they assured a higher percentage of healthy material;

The number of subcultures seems to be an important factor for virus elimination, result sustained by the higher percentage of healthy material obtained after six subcultures.

5. Acknowledgements

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Tables and Figures

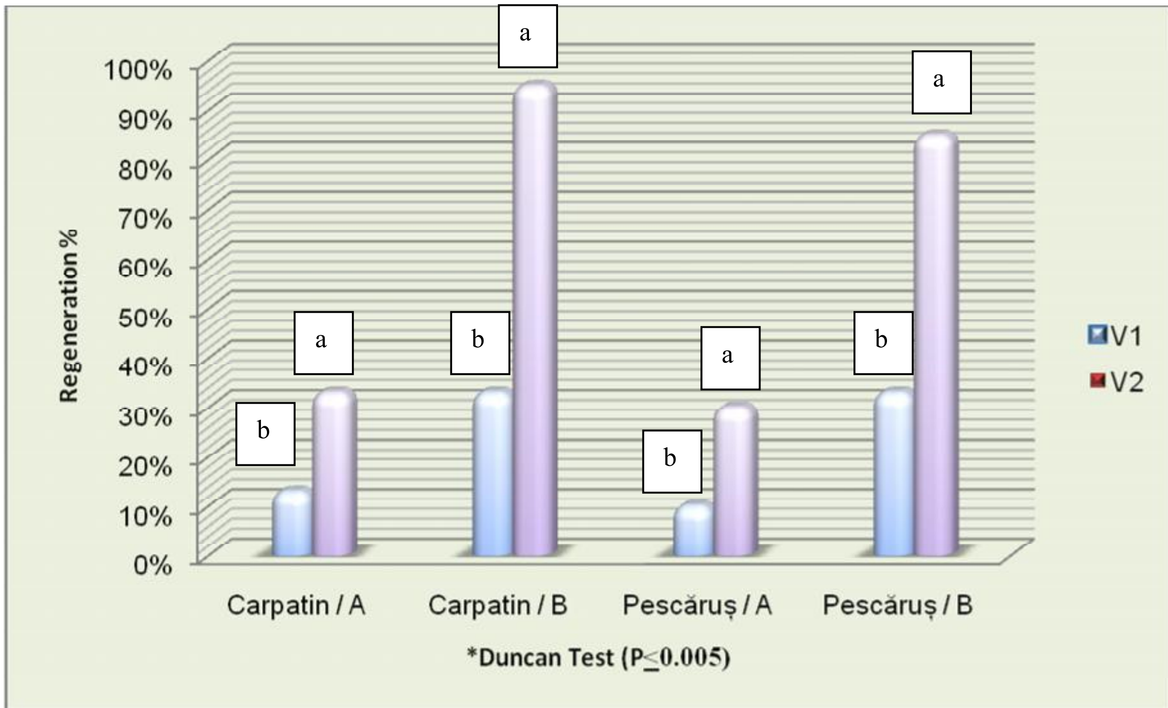

Fig. 1. Cultivars behaviour in differentiation phase

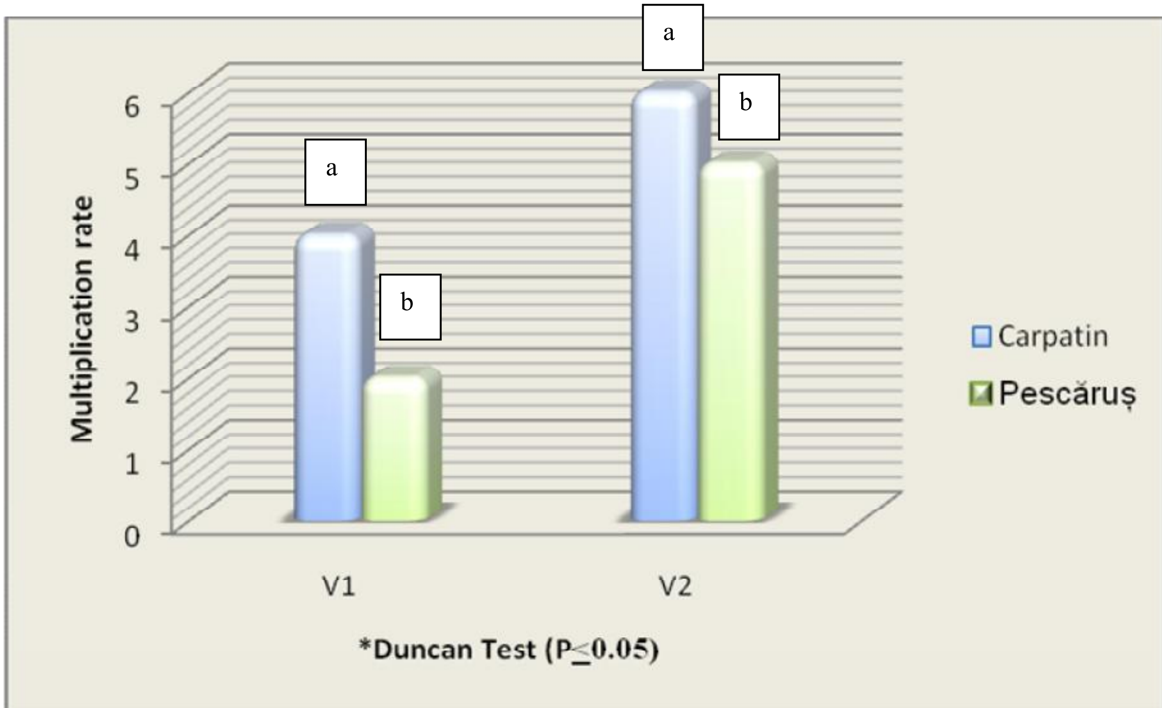

Fig. 2. Results regarding multiplication phas

Table 1. Results of serological test after three subcultures

Cultivar / explant size	Cut off*	% health material	Absorption value range	Positive control means	Negative control means	Test results
Carpatin /type A	0.553	57.14	0.372-0.446	1.086	0.221	negativ
Carpatin/type B	0.553	42.85	0.480-0.513	1.086	0.221	negativ
Pescăruș/type A	0.553	51.78	0.354-0.457	1.086	0.221	negativ
Pescăruș/type B	0.553	39.28	0.500-0.458	1.086	0.221	negativ

Table 2. Results of serological test after six subcultures

Cultivar / explant size	Cut off*	% health material	Absorption value range	Positive control means	Negative control means	Test results
Carpatin /type A	0.591	82.14	0.272-0.316	2.133	0.236	negativ
Carpatin/type B	0.591	53.57	0.380-0.413	2.133	0.236	negativ
Pescăruș/type A	0.591	85.71	0.254-0.307	2.133	0.236	negativ
Pescăruș/type B	0.591	64.28	0.300-0.358	2.133	0.236	negativ

**Cut off value = the average absorbance values of two negative controls x 2.5.*