III. ÎNMULŢIRE, VIROLOGIE ŞI CULTURI DE ŢESUTURI
PROPAGATION, VIROLOGY AND TISSUE CULTURE

OBŢINEREA MATERIALULUI SĂDITOR DE PRUN LIBER DE VIROZE PRIN METODE *IN VITRO*
OBTAINING *VIRUS FREE* PLANTING MATERIAL OF PLUM CULTIVARS BY *IN VITRO* METHOD

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Abstract

In the experiments from R.I.F.G. Pitesti, were established optimal parameters that would lead to the increase effectiveness of *PPV, PDV* and *PNRSV* viruses elimination, using *in vitro* culture. Biological material for meristem culture was represented by plants found infected with virus using TAS-ELISA method from the following varieties 'Pescăruş', 'Silvia', 'Minerva', 'Diana', 'Centenar', 'Tuleu timpuriu', 'Sarmatic', 'Dâmboviţa', 'Tuleu gras', 'Vinete de Italia'. Meristems were grown on MS, WPM, LF and QL with different combinations and concentrations of hormones. The *in vitro* regeneration capacity of meristems was influenced by genotypes and composition of macro and microelements. Another important factor with influence on the regeneration capacity and viruses elimination was the explant size. Explants growth was inversely proportional with number of virus plants obtained: explants with size of 0.2-0.3 mm had a less regenerative capacity than explants with size of 0.6-0.7 mm but assured a great number of *virus free* plants.

Keywords: meristem, culture media, *virus free*, plum
Cuvinte cheie: meristeme, prun, virus free, mediu de cultura

1. Introduction

Plum (*Prunus domestica* L.) is one of the most cultivated species in Romania. From Romanian fruit growing patrimony by 158.000 ha, the plum trees occupy a surface of 75,292 ha. For the quantity and quality of fruit production are responsible some factors. One very important factor is viral health level. *PPV (Plum pox), PDV (Prune dwarf)* and *PNRSV (Prunus necrotic ring spot)* are the most common viruses affecting plum. *PPV* is spread in all country, causing great damage, up to 85% in susceptible varieties (Cociu et al., 1997). Following the same authors, *PDV* can reduce the production with up to 50-82% and *PNRSV* may reduce the production if varieties are susceptible with 15-45%. The propagation through classical methods is one of the propagation ways for these viruses. However the meristems culture technique is the way for obtained healthy plants (Walkey, DGA - 1978).

Optimization technique to obtain *virus free* plants depends on many factors, culture medium, explant type (Ilyefalvi Zsolt Jakab - 2008) and not least a very important factor is the genotype (Isac, 1983).

The present paper exhibits *PPV, PDV* and *PNRSV* elimination level for *in vitro* culture using apical meristems.

These studies were necessary because it is needed to established measures for obtaining *virus free* plants for building new plantation able to produce high quality fruits under the condition of an incresed economic efficiency.

2. Material and methods

The explant source was represented by apically buds from anual branches from **'**Pescăruş', 'Silvia', 'Minerva', 'Diana', 'Centenar', 'Tuleu timpuriu', 'Sarmatic', 'Dâmboviţa', 'Tuleu gras', 'Vinete de Italia' variety. The explants obtained were meristematic dom with 0,6-0,7 mm size and meristematic dom with 0.2-0.3 mm.

Desinfections of biological material consist of:

- washising with water and liquid detergent Tween 80 for 5 min;
- immersion in 6 % (w/v) $Ca(OCl)_2$ for 20 min;
- immersion in 90 % ethanol for 10 min;

- rinsed three times in sterile distilled water.

Culture media were represented of Murashige&Skoog (MS -1962), Lee & Fossard (LF - 1977), Quoirin & Lepoivre (QL-1977) and Woody Plant Medium (Lloyd and McCown -1981). All media contained 40g/l dextrose, 8 g l/l agar and 32 mg/l Na Fe EDTA. Growth regulators in various combinations and concentrations were added to each medium. So resulted variants (Table 1). After dissection and inoculation the cultures were maintained at 22-24^0 C, 16 light / 8 dark hours photoperiod.

The presence of *PPV, PDV* and *PNRSV* in plum material was evaluated several times by TAS-ELISA (Triple-Antybody Sandwich Enzymes Linked Immunosorbent Assay) according to Clark M.F. and Adams M.F., 1977, before placing in the culture and after rooting phase. TAS-ELISA was conducted with SEDIAG reagents applied in recommended dilution. A reaction was considered positive if the absorbance was exceeding double mean value of negative controls.

3. Results and discussion

In vitro explants evolution has been different depending on some factors (Table 2):

Culture media: the best results were obtained on the variant V2 with an average of 83.25 % explants differentiated and on V3 with an average of 70.50 % explants differentiated.

The culture media V1 with 24.75 % and V4 with 3.25 %, have provided inadequate conditions for most varieties.

Explant size had a very important influence in the regenerative capacity. The data presented in table 1 demonstrate that between explants size recorded significant differences regarding to regeneration capacity. The values show that the better results were obtained with explants with 0.6-0.7 mm size: that recorded average 24, 75 % on V1, 83, 25 % on V2, 70.50 % on V3 and 3.25 % on V4, explants differentiation. A substantial diminution of the regeneration capacity was obtained with explants with 0.2-0.3 mm: 6.25 % on V1, 34.25 % on V2, 15 % on V3. We have been situation when the explants with the size of 0.2-0.3 mm have not differentiated.

Variety had a great influence in the regeneration ability of meristems. Thus under the same conditions: variant of culture media (V2), explants size (0.6-0.7 mm) the varieties 'Vinete de Italia' and 'Pescarus' recorded values by 95 % regeneration plants compared with Centenar 80 % regeneration plants and Silvia 62.5 % regeneration percentage.

Results obtained in multiplication phase showed that culture media and variety had a influence upon multiplication rate (figure 1). The best multiplication medium was LF (V2) resulting average in four subculture between 2.5 and 2.9 shoots /explant. The multiplication rate on QL (V2) culture media was between 1.6 and 2.0. shoots / explant and for M&S culture media the multiplication rate was between 1.2-1.7 shoots/explants.

The multiplication ability recorded from 'Vinete de Italia' and 'Pescarus' varieties had a higher media 2,9 (V2), 2 (V3) and 1.7 (V1) (figure 1).

After the fourth subcultures the plants were transferred to rooting media and two variants V1 and V2. The effect growth regulators balance was different. The best rooting medium for all varieties was Lee Fossard with 1.5 mg/l IBA (V1) that recorded a media / assortment by 88,6 % rooted plants with 95 %for 'Sarmatic' variety and 82,8 % rooted plants for 'Tuleu timpuriu'. When culture media was supplemented with ANA (1,5 mg/l) recorded a smaller capacity for rooting between 20 and 64,2 % (Fig.2). After the rooting phase TAS-ELISA (Triple-Antybody Sandwich Enzymes Linked Immunosorbent Assay) test was conducted in order to determine the degree of *virus free* biological material at this time.

Analysis of data from figure 3 and figure 4 showed that differences have appeared depending on size explant. Explants of 0,2-0,3 mm size established better results compared with explants with a 0.6-0.7 mm. So, the level of viral infection to plants obtained from the explants with te size of 0,2-0,3 mm was 53,6 % for *PPV*, 41,3 % for *PDV* and 58,9 % for *PNRSV*. The level of viral infection to plants obtained from the explants with the size of 0,6-0.7 mm was 63,65 % for *PPV,* 48,64 % for *PDV* and 68, 97 % for *PNRSV*. Also, different values were registered depending on variety (fig. 3 and fig. 4), Sarmatic variety responded the best in all situation: explant size and virus type.

4. Conclusions

For obtaining *virus free* process has operated a complex of factors: culture media through components explants size and variety.

The use of the explants with 0.2-0.3 mm size to obtain a differentiation slower compared with the explants with 0.6-0.7 mm but get a better yield to *virus free* plants.

Number of *virus free* plants obtained in similarly conditions was variable and depending by virus. After *in vitro* micropropagation *PDV* was track down to smaller number of plants in both cases (0.2-0.3 mm and 0.6-0.7 mm size) comparative with *PPV* and *PNRSV*.

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Tables and figures

Table 1. The experimental variants

Variant	*Basal medium*	***Regeneration phase***		
		Vitamins	Growth regulators	
			GA_3	IBA
V1	MS	MS	0.3 mg/l	0.01 mg/l
V2	LF	LF	0.3 mg/l	0.01 mg/l
V4	QL	Walkey, 1972	0.3 mg/l	0.01 mg/l
V4	WPM	WPM	0.3 mg/l	0.01 mg/l

Variant	*Basal medium*	***Multiplication phase***			
		Vitamins	Growth regulators		
			GA_3	BAP	IBA
V1	MS	MS	0.5 mg/l	1 mg/l	0,1 mg/l
V2	LF	LF	0.5 mg/l	1 mg/l	0,1 mg/l
V3	QL	Walkey, 1972	0.5 mg/l	1 mg/l	0,1 mg/l

Variant	*Basal medium*	***Rooting phase***			
		Vitamins	Growth regulators		
			GA_3	IBA	ANA
V1	LF	Walkey 1972	0.1 mg/l	1.5 mg/l	-
V2	LF	Walkey, 1972	0.1 mg/l	-	1.5 mg/l

Table 2. **Explants differentiated level (%) after 4 weeks of culture**

No	Variety	Size explant (mm)	Culture media variants			
			V1	V2	V3	V4
1	Pescăruş	0.2-0.3	0	30.00	25.00	0
		0.6-0.7	12.50	95.00	70.00	10.00
2	Silvia	0.2-0.3	0	20.00	10.00	0
		0.6-0.7	5.00	62.50	60.00	0
3	Minerva	0.2-0.3	5.00	25.00	10.00	0
		0.6-0.7	20.00	80.00	70.00	12.50
4	Diana	0.2-0.3	10.00	27.50	10.00	0
		0.6-0.7	25.00	82.50	70.00	0
5	Centenar	0.2-0.3	0	32.50	12.50	0
		0.6-0.7	10.00	70.00	60.00	0
6	Tuleu timpuriu	0.2-0.3	15.00	32.50	12.50	0
		0.6-0.7	25.00	80.00	62.50	0
7	Sarmatic	0.2-0.3	7.50	45.00	17.50	0
		0.6-0.7	40.00	90.00	82.50	0
8	Dâmboviţa	0.2-0.3	5.00	37.50	15.00	0
		0.6-0.7	27.50	87.50	70.00	0
9	Tuleu gras	0.2-0.3	10.00	45.00	17.50	0
		0.6-0.7	37.50	90.00	80.00	5.00
10	Vinete de Italia	0.2-0.3	10.00	47.50	20.00	0
		0.6-0.7	45.00	95.00	80.00	5.00
	Diferentiation media/assortment	0.2-0.3	6.25	34.25	15.00	0
	Diferentiation media/assortment	0.6-0.7	24.75	83.25	70.50	3.25

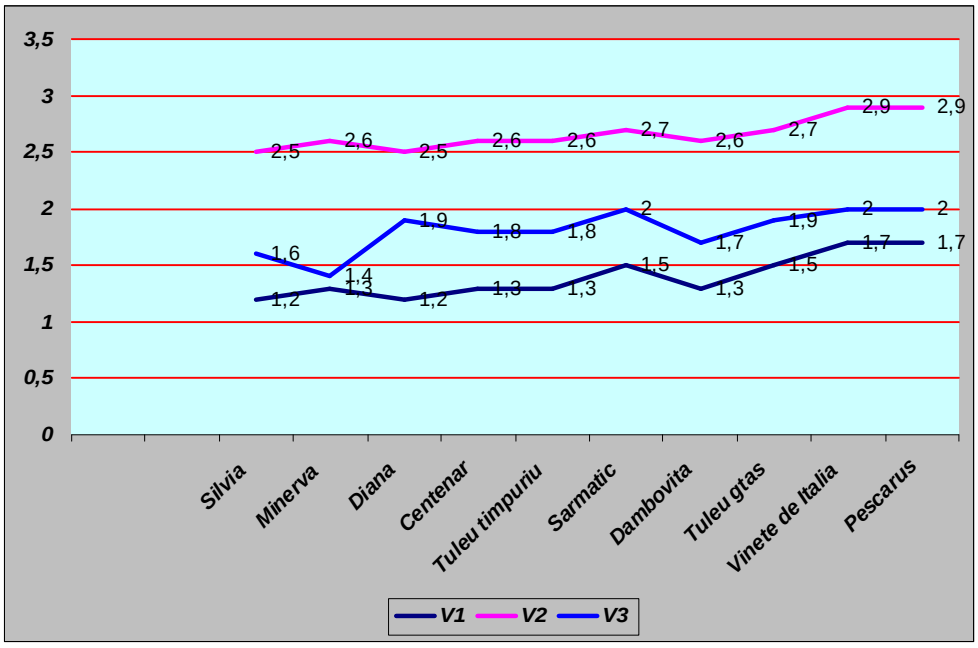

Fig. 1. – Multiplication rate on different culture media

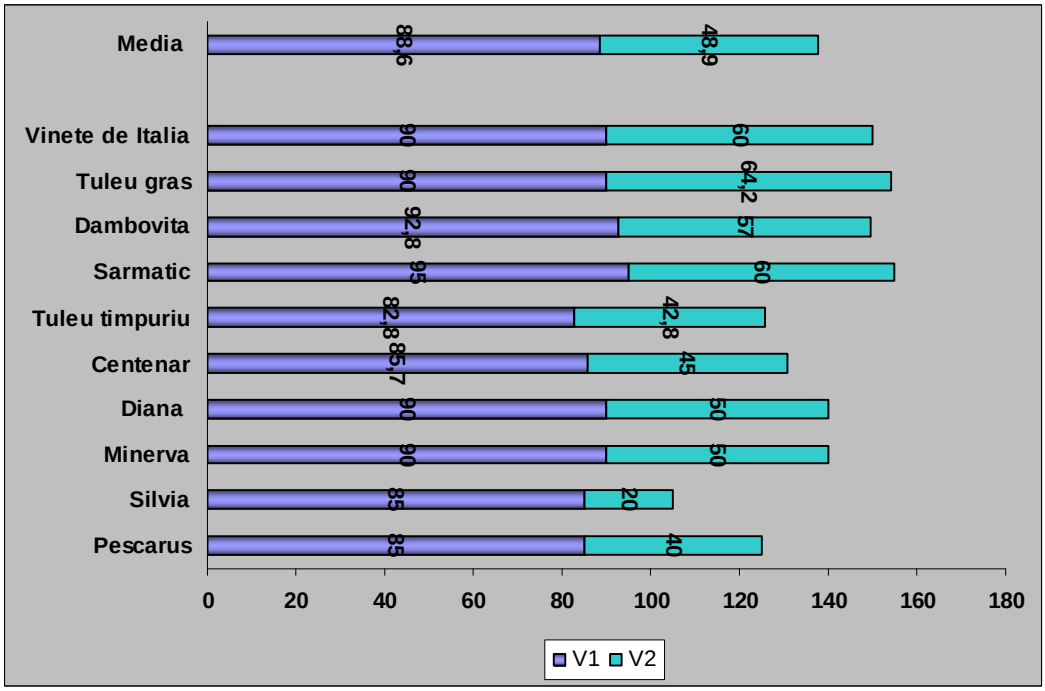

Fig. 2 Rooting percentage (%) to plum trees variety

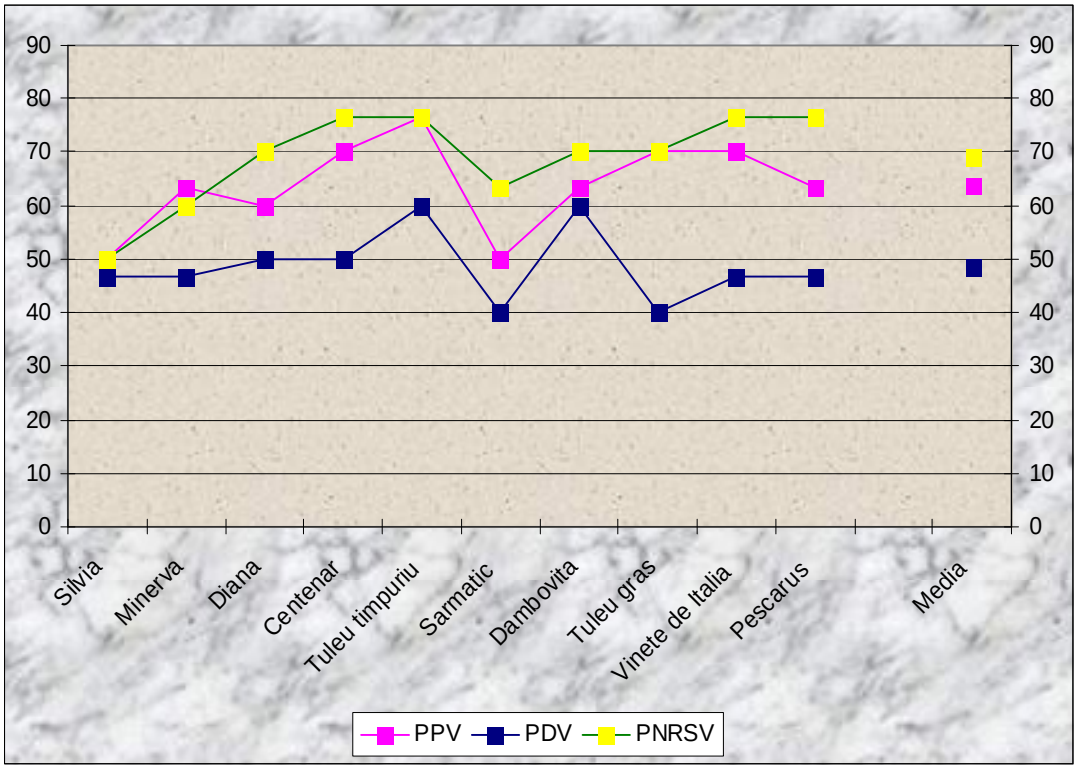

Fig. 3. Average (%) of virus free plants after micropropagation from the explants with the size of 0.6-0.7 mm

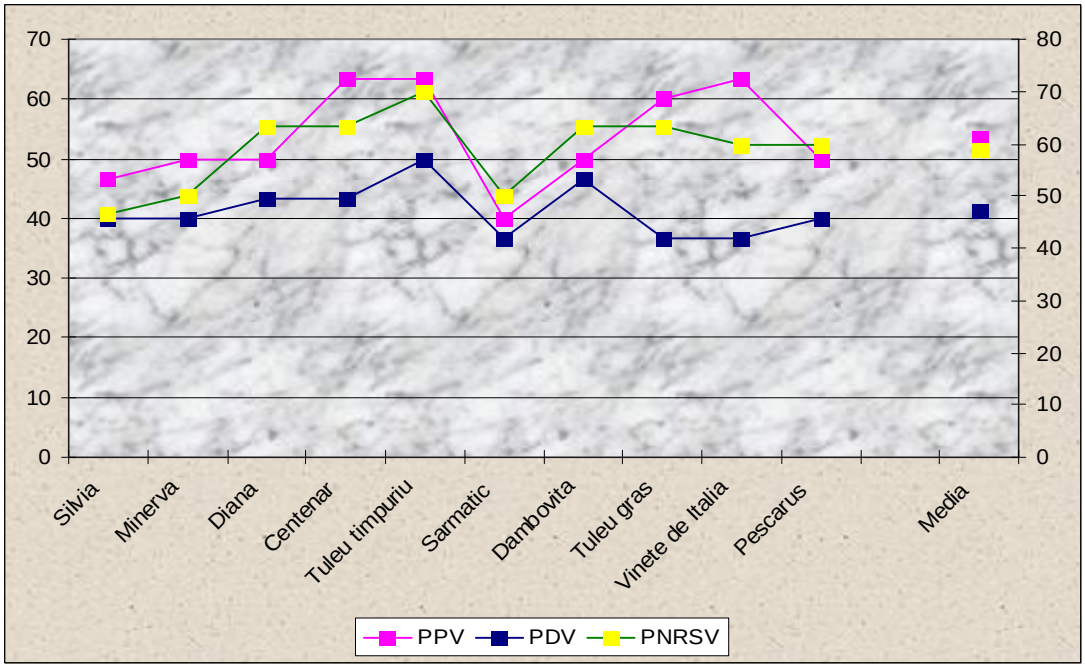

Fig. 4. Average (%) of virus free plants after micropropagation from the explants with the size of 0.2-0.3 mm