RESEARCHES REGARDING THE EPIDEMIOLOGY AND THE DIAGNOSE OF PLUM POX VIRUS AT PLUM

CERCETĂRI PRIVIND EPIDEMIOLOGIA ŞI DIAGNOZA VIRUSULUI PLUM-POX (PPV) LA PRUN

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Abstract. *The researches carried out persue the identification and the diagnose of Plum pox virus (PPV) in the plum orchards. Over 30 plum cultivars and hybrids were studied. The biological material was analysed from the point of view of the plum behaviour to the contamination to PPV in natural conditions and of his reaction to the imuno enzymatic test ELISA and to the imuno cromatografic test AgriStrip. Depending of the plum cultivar symtomps of the PPV appeared distinct on leaves and fruits. He is considerated the most devastating and the most spread virus of plum trees and he has a negative influence in plant production. The serological tests through DAS ELISA and AgriStrip indicated variable viral concentration from 0,333- 0,465 (HNC/1995, Andreea, Vâlcean, Miroval, Corval, Diana, Stanley) to 1,027-2,566 (Silvia, Centenar, Pescăruş, H 57-2-85). The measures against the disease are mostly preventive and consist in using healthy biological material established through control from selected mother plantations witch are periodicaly verified.*

Key words: ELISA, *Prunus domestica*, AgriStrip, *Plum pox.*

Rezumat. *Cercetările întreprinse au urmărit identificarea şi diagnosticarea virusului Plum-pox (PPV) la prun. Au fost luate în studiu peste 28 de soiuri şi hibrizi de prun. Materialul biologic a fost analizat din punct de vedere al comportării lui la infecţiile cu PPV în condiţii naturale şi a reacţiei acestuia la testul imunoenzimatic ELISA şi testul imunocromatografic AgriStrip. În funcţie de genotip, atacul de Plum pox s-a manifestat diferenţiat pe frunze. Este considerat cel mai grav şi răspândit virus la prun, influenţând negativ producţia de fructe. Analizele serologice prin tehnica DAS-ELISA şi AgriStrip, au indicat valori ale absorbanţei variabile de la 0,333-0,465 (HNC/1995, Andreea, Vâlcean, Miroval, Corval, Diana, Stanley) la 1,027-2,566 (Silvia, Centenar, Pescăruş, H 57-2-85). Lupta preventivă a infecţiilor cu virusul PPV este aceea de a utiliza material săditor pomicol stabilit prin control ca fiind sănătos, provenit din plante selecţionate şi verificate periodic din punct de vedere fitosanitar.*

Cuvinte cheie: ELISA, *Prunus domestica*, AgriStrip, *Plum pox.*

INTRODUCTION

The main hosts for Plum pox virus are all cultivated stone fruit species of the genus *Prunus* including apricot (*Prunus armeniaca*), peach (*Prunus persica*), plum (*Prunus domestica*) and the japanese plum (*Prunus salicina*). Susceptible to

the virus infection are also the wild and the ornamental species of the genus *Prunus* like *P. besseyi*, *P. cerasifera*, *P. insititia*, *P. tomentosa* and *P. spinosa*.

The application of chemical treatments in orchards is absolutely necessary for the control of the Plum pox virus specific vectors. Unlike fungal or bacterial plant pathogens that can be controlled chemically, antiviral treatments to control PPV in the field are not available. However, in the countries where the virus is wide spread, between the cultivated varieties exist differences regarding the susceptibility to the virus infection (Hamdorf, 1986). More, the presence of the virus rises problems to the export of biological plant material.

This paper presents the behaviour of the existent plum genotypes in collections in both natural and isolated conditions and the diagnosis of Plum pox virus for the negative fitovirotic selection and also to obtain resistant or tolerant forms to this virus.

MATERIAL AND METHODS

The study and the tests were made in the national collection of plum at the Fruit Growing Research&Extension Vâlcea. The plants were analyzed visual regarding the presence or absence of the PPV symptoms on leaves and fruits in both natural and isolated conditions.

As biological material were used leaves sampled from the inside of the crown from 30 plum varieties, rootstocks and hybrids, samples that didn't manifested any characteristic symptoms of Plum pox virus. The samples collected reprezented the antigen for the imunoenzymatic test DAS-ELISA (Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay). The reagents used for the tests came from the firm Bioreba (figure 1). Also was performed the test for the fast diagnosis of the PPV for 25 from the 30 samples that were studied. The AgriStrip test (figure 2) consists in the detection and confirmation in several minutes of the Plum pox virus for the species of the genus Prunus without the specification of the amount of the viral protein present in the sample analyzed.

Fig. 1. The ELISA test

Fig.2. The AgriStrip test

RESULTS AND DISCUSSIONS

From the evaluations made on the plum biological material studied regarding his behaviour to the PPV infections resulted that only a part of the plants presented symptoms charasteristic to the virus.

The samples analyzed through the two methods and their reactions showed that the virus is present also in latent form.

The spread of the Plum pox virus was rapid and sure from the infected plant to the health plant thanks to the vectors of the virus from the orchards and to the presence of the hosts infected

The datas from the table 1 show that from the 30 sample analyzed through DAS-ELISA using polyclonal antibodies only 9 proved to be infected: the hybrids H 70-25-83, H 74-17-83, H 12-48-85, H 57-2 -85 and the varieties Silvia, Centenar, Tuleu gras, Pescăruş and Tegera. The values of the ELISA absorbance at 404 nm point out variable viral concentration from 1,027/1,091 at the variety Silvia to 2,565/2,566 at the variety Pescăruş comparative with the values of the positive witness (3,000) from the firm producing kits for the diagnose of the PPV Bioreba.

The genotypes HNC/1995, Andreea, Rival, Oltval, Miroval, Carpatin, Stanley, Vânăt românesc, Tita, etc. proved to be healthy, state demonstrated through ELISA values very low from 0,327/0,331 to 0,459/0,581.

The values of the ELISA absorbance regarding the negative witness were 0,423/0,427, values very close to the value of the 21 samples analyzed and proved to be healthy.

A number of 25 samples represented by plum varieties, rootstocks and hybrids were tested for the presence of Plum pox virus through the imunocromatografic method AgriStrip (table 2). Through this method only the variety Tegera proved to be infected. Thus, the positive results obtained through DAS-ELISA weren't confirmed through the AgriStrip method regarding the

hybrids H 70-25-83, H 74-17-83, H 12-48-85, H 57-2-85 and the varieties Centenar, Tuleu gras and Pescăruş.

Probably through AgriStrip the detection of the virus exist only in case the amount of the viral protein is proper to be made evident (values of approximately 2,566).

The tests made through both DAS-ELISA and AgriStrip assured the negative fitovirotic selection regarding PPV. Thus, the plum biological material virus free can be used in the breeding and multiplication programs.

Table 1

The reaction to PPV of the plum biological material through DAS-ELISA

No. crt.	Variety/Hybrid/Rootstock	Place of sampling	ELISA values (at 1 h)	Sample reaction
1	H 70-25-83	G cultureR11/P2	1,435 / 2,137	+
2	HNC/1995	Greenhouse	0,333 / 0,344	-
3	H 74-17-83	G culture R30/P3	1,891 / 2,071	+
4	H 12-48-85	G culture R31/P1	0,993 / 1,015	+
5	Andreea	G culture R1/P2	0,331 / 0,727	-
6	Dobrovika	Greenhouse	0,333 / 0,335	-
7	Andreea	G culture R1/P4	0,327 / 0,338	-
8	H 57- 2 - 85	G culture R35/P1	1,034 / 1,061	+
9	Vâlcean	G culture R14/P3	0,449 / 0,465	-
10	Silvia	G culture R16/P2	1,027 / 1,091	+
11	Centenar	G cultureR18/P2	1,485 / 1,540	+
12	Tuleu gras	G culture R19/P1	2,565 / 2,566	+
13	Pescăruş	G culture R20/P2	2,401 / 2, 554	+
14	Rival	Pl.mother cuttings R1/ P21	0,562 / 0,581	-
15	Oltval	Pl.mother cuttings R2/ P3	0,334	-
16	HNC/1995	Pl.mother cuttings P4	0,338 / 0,343	-
17	Miroval	Pl.mother cuttings P2	0,344 / 0,345	-
18	Pinval	Pl.mother cuttings	0,419 / 0,438	-
19	Oteşani 11	Pl.mother cuttings R1 / P2	0,334 / 0,345	-
20	Oteşani 8	Pl.mother cuttings R 4 / P2	0,372 / 0 ,374	-
21	Corval	Pl.mother cuttings R9 / P2	0,335 / 0,453	-
22	Mirobolan 2 V	Pl.mother cuttings R1 / P18	0,455 / 0,459	-
23	Carpatin / răd.proprii	Bio-depositary 2	0,336 / 0,342	-
24	Tuleu gras /răd. proprii	Bio-depositary 2	0,334 / 0,335	-
25	Stanley / răd. proprii	Bio-depositary 2	0,345 / 0, 354	-
26	Diana	Bio-depositary 2	0,342 / 0,345	-
27	Vânăt românesc / răd. proprii	Bio-depositary 2	0,355 / 0,359	-

28	Tita Cl 1/ răd. proprii	Bio-depositary 2	0,361 / 0,363	-
29	Centenar Cl 15 / mirob.	Bio-depositary 2	0,354 / 0,373	-
30	Tegera	R4/ P7	2,565 / 2,566	+
Negative witness			O,423 / 0,427	-
Positive witness			3,00	+

Note: + = infected sample
- = healthy sample

Table 2

The reaction to PPV of the plum biological material through the imunocromatografic method AgriStrip

No. crt	No. sample	Variety/Hybrid/Rootstock	Sample reaction (After 15 min)
1	1	H 70-25-83	*
2	2	HNC/1995	-
3	3	H 74-17-83	*
4	4	H 12-48-85	*
5	5	Andreea	-
6	6	Dobrovika	-
7	7	Andreea	-
8	8	H 57- 2 - 85	*
9	9	Vâlcean	-
10	10	Dobrovika	-
11	11	Centenar	*
12	12	Tuleu gras	*
13	13	Pescăruş	*
14	14	Rival	-
15	15	Oltval	-
16	16	HNC/1995	-
17	17	Miroval	-
18	18	Pinval	-
19	19	Oteşani 11	-
20	20	Oteşani 8	-
21	21	Corval	-
22	22	Mirobolan 2 V	-
23	23	Carpatin/own roots	-
24	24	Tuleu gras/own roots	-
25	30	Tegera	+

Note: + = infected sample
- = healthy sample
* = doubtful result

CONCLUSIONS

1. The Plum pox virus (PPV) is the most spread and devastading virus that affects plum trees with repercussions on the quality of the fruit-growing biological material and of the fruits. The symptoms induced by the virus on the leaves manifested differently, from low evident to very evident or in certain cases the leaves were without symptoms (latent infection).

2. The imunoenzymatic method DAS-ELISA and the imunocromatografic method AgriStrip showed that the hybrids H 70-25-83, H 74-17-83, H 12-48-85, H 57-2-85 and the varieties Silvia, Centenar, Tuleu gras, Pescăruş şi Tegera were infected with PPV.

3. The negative values showed at the plum analyzed samples HNC/1995, Andreea, Dobrovika, Vâlcean, Rival, Oltval, Miroval, Pinval, Oteşani 11, Oteşani 8, Corval, Mirobolan 2V, Carpatin/own roots, Tuleu gras/own roots, Stanley/own roots, Vânăt românesc/own roots, Tita Cl.1/own roots, Diana, Centenar Cl.15/mirobolan revealed that the analyzed biological material was not infected with the Plum pox virus (PPV).

4. The plum genotypes virus free resulted from the lab tests can be used as healthy biological material in the multiplication process and in the breeding programs.

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