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Short Communication

EVALUATING THE RESISTANCE TO THE PLUM POX VIRUS OF SOME APRICOT TREE CULTIVARS AND HYBRIDS IN SOUTH-EASTERN ROMANIA

Marioara Trandafirescu*, Liana Melania Dumitru*, and Iulian Trandafirescu**

* Research Station for Fruit Growing Constanta, Pepinierei Str. 1, 907300 Valu lui Traian, ROMANIA;
mtrandafirescu@yahoo.com

** Ovidius University of Constanta, Aleea Universitatii, 1, Constanta, ROMANIA

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In Europe, the Plum pox virus causes one of the most serious diseases of stone fruit-growing species (plum, apricot, peach, nectarine, almond and cherry) in all countries where cultivated. Romania is one of the countries in which this virus has been present for several years and where the contamination level is very high. The disease causes considerable damage, as the infected trees have few fruit, which are small, deformed, lacking in flavour, with a low content of sugar and which can neither be consumed nor used in production. In areas where the Plum pox virus is present, the cultivation of highly resistant (tolerant) genotypes is the only possibility to alleviate this problem. The purpose of the research performed in this paper was to identify varieties that are tolerant to the virus and to monitor the manner in which the resistance is transmitted to hybrid descendants. The material employed consisted of apricot tree varieties from the national collection of the Research Station for Fruit-Growing Constanža. The presence of the Plum pox virus was determined by biological method using a GF305 wooden indicator and the serological method through the ELISA test. The varieties identified as having higher resistance to the attack of the Plum pox virus ('Stark Early Orange', 'NJA 17', and 'Sulmona') were used in intraspecific hybridisations for the selection of hybrids to which this characteristic was transmitted. These included: the selections 'VT 48/45', 'VT 51/45', 'VT 47/112' and 'H 9/5'.

Key words: *apricot, sharka, biological method, serological method.*

The Plum pox virus (PPV) causes one of the most damaging diseases of stone fruit species, such as plum, apricot and peach. Almond and cherry trees may become infected with this virus without obvious symptoms (Festic, 1978; Kalashyan *et al*., 1994). The virus was artificially transmitted to cherry tree and sour cherry tree, but the infection remained local without evidence that it might spread (Dosba *et al*., 1987).

The first symptoms of Plum pox virus infection were observed in Bulgaria between 1915 and 1918. Nowadays, the virus is present in most European countries and in Egypt, Syria, Turkey, India, China, Argentina, Chile, USA and Canada.

In Romania, the disease was recorded for the first time in 1922 by Tr. Savulescu and presently, the virus has spread to all the areas where fruit-growing species that have a core are being cultivated, creating massive production losses (Minoiu, 1997; Zagrai *et al*., 2006).

Because of the severity of this disease, within the European Plant Protection Organisation (OEPP) beginning with 1975, the Plum pox virus has been considered to be a phytosanitary quarantine pathogen, given the fact that it represents a serious threat even in the regions where it has not fully spread yet.

The disease is manifested on leaves, flowers, fruit, the stone and sometimes on the trunk and the skeleton branches (Minoiu, 1997). The intensity of the symptoms vary according to *Prunus* species cultivar, PPV strain, season, location, tree age and environmental conditions (Levy *et al*., 2000).

The symptoms are visible during the periods May–June and September–October. During warm summer months, the symptoms are latent and can be observed only on the shady side of trees. Diseased leaves display circular or linear chlorotic spots or light greenish concentric circles and deformations. In general, diseased leaves have the same dimensions as healthy ones.

When fruits are affected, they display circular or elongated spots that are yellow-greenish and with a watery aspect. The diseased fruit are smaller, deformed, with non-uniform colours, less sugar content and a bland taste (Llacer and Cambra, 1986). In addition, they fall from the tree prematurely. Circular spots can be observed on the stone as well, which are yellow at first and become brown as the core becomes dry (Labonne and Dallot, 2006).

There is no available chemical or biological treatment against this virus. The current solution is to eradicate infected trees and to plant material that is free of the virus. The purpose of the study was to identify genitors resistant to the virus and which are capable of transmitting this characteristic to their hybrid descendants, in view of creating new cultivars resistant to Plum pox virus.

The research carried out within this study was divided into two distinct stages. During the first stage, the aim was to identify cultivars resistant to PPV, with the purpose of using them as genitors within the programme for improvement of the apricot tree.

The material included 635 apricot tree cultivars originating from various ecogeographical areas, which form together the national collection of the Fruit-Growing Research and Development Station Constanža. Each cultivar was represented by five trees, planted at a distance of 4.5 × 4.0 m.

The choice of cultivars resistant to PPV was carried out by survey of natural infections in the field. In order to highlight viral infections, visual observations of vegetative organs of the trees (leaves, fruits and stones) were performed. The following scale was used to evaluate the intensity of the symptoms of the PPV: 0 = no symptoms; + = reduced symptoms; + = moderate symptoms; ++ = strong symptoms; +++ = very strong symptoms.

Also, another method was used — indexation based on wooden indicators (GF305). This method consists in passing the virus from an infected individual (the cultivar that is to be tested) to a healthy individual (the indicator) and monitoring the occurrence of specific PPV symptoms.

Subsequent to these tests, the cultivars resistant to PPV were identified and used as genitors for intraspecific hybridisations, which resulted in different hybrid combinations. Then, repeated selections were performed in the hybrid fields F1 and F2, and the hybrids which did not have visual symptoms of PPV were identified. In order to confirm the inheritance of resistance to PPV from their genitors, the hybrids were serologically tested by means of the ELISA test (Clark and Adams, 1977). The advantage of the test is high accuracy for detecting very small quantities of virus (an antigen concentration of 1–10 mg/ml) and a rapid reaction speed (results in 6–24 h).

Summary of the data (Table 1) showed that among the 40 apricot tree cultivars, 15 were free of PPV, shown both by visual observation of vegetative organs (leaves, fruits and stones) and by the wooden biological indicator GF305.

These cultivars were used as genitors resistant to Plum pox virus. In order to obtain hybrid descendants in which the selection of resistance to PPV was effective, the choice of the genitors and correct hybrid combinations are of utter importance. For apricot, the hybridisation formulae must be chosen carefully, especially since some cultivars may manifest a certain level of intersterility, due to which the ability to obtain a F_1 population of adequate dimensions in which there is a genuine chance of identifying elite plants is extremely limited. The 15 cultivars resistant to PPV ('Viceroy', 'NJA 42', 'Mari de Cenad', 'Neptun', 'Stark Early Orange', 'Sam no.1', 'CR 24-12', 'H 9/5', 'Sirena',

Table 1

BEHAVIOUR OF SOME APRICOT CULTIVARS TO INFECTION WITH PPV

Tested cultivar	Symptoms on:			Test on the indicator GF305	
	leaves	fruits	stones	reaction	semnification
Vivagold	0	0	0	-	virus free
Sulmona	0	0	0	0	virus free
H 9/5	0	0	0	-	virus free
Mamaia	(+)	0	0	+	infected
Sirena	0	0	0	0	virus free
Pike	(+)	(+)	++	+	infected
Umberto	++	++	+++	+	infected
NJA 14	++	++	+++	+	infected
Litoral	+	++	(+)	+	infected
Sam no 1	0	0	0	-	virus free
Precoce de Colomer	(+)	(+)	0	+	infected
Earliril	+	++	+	+	infected
Joubert Foulon	(+)	+	+	+	infected
Mari de Cenad	0	0	0	-	virus free
Neptun	0	0	0	-	virus free
C.R. 2-63	+	++	(+)	+	infected
Patriarca Temprano	+++	+++	++	+	infected
NJA 17	0	0	0	-	virus free
R30 P162	0	0	0	-	virus free
R41 P162	0	0	0	-	virus free
Mandula caiszi	+++	+++	++	+	infected
Warley's peach	0	0	0	-	virus free
Rouge de Rousillon	++	+++	+	++	infected
Băneasa 32/29	+++	+++	++	++	infected
CR 24-12	0	0	0	-	virus free
Stark Early Orange	0	0	0	-	virus free
Mărculști 72	++	+++	++	++	infected
Moongold	+++	+++	+++	+++	infected
R9 P53	0	0	0	-	virus free
Farmingdale	++	(+)	(+)	+	infected
Viceroy	0	0	0	-	virus free
NJA 42	0	0	0	-	virus free
Băneasa 3/15	+++	+++	++	+	infected
RR 15-20	+++	+++	++	+	infected
Roșii timpurii	+	+++	+++	+	infected
Băneasa 33/11	(+)	++	(+)	+	infected
Sulina	0	0	0	-	virus free
Baracca	(+)	++	(+)	+	infected
Mărculești	+++	+++	(+)	+	infected
Canino	+++	+++	+++	+++	infected

Table 2

HYBRID COMBINATIONS OF APRICOT TREE USING GENITORS RESISTANT TO PPV

Hybrid combination	Pollinated flowers	Tied fruit		Harvested fruit		Number of planted stones
		no	%	no	%	
Viceroy × NJA 42	949	379	39.9	283	29.8	272
Mari de Cenad × Stark E. Orange	1609	378	23.5	254	15.8	140
Sam no 1 × Neptun	964	401	41.5	302	31.32	294
Sam no 1 × Mari de Cenad	932	397	42.5	298	31.9	260
Mari de Cenad × C.R. 24-12	773	337	43.6	292	37.8	286
H 9/5 × Sirena	819	314	38.3	276	33.6	234
R30P162 × R41P162	951	346	36.3	220	23.1	217
Warley's peach × Sulmona	614	265	43.1	207	33.7	110
NJA 17 × R9P53	571	163	28.5	93	16.28	86
TOTAL	8182	2973	x	2295	x	1899

'R30P162', 'R41P162', 'Warley's peach', 'Sulmona', 'NJA 17' and 'R9P53') , were used to create nine hybrid combinations, presented in Table 2, with a total of 8182 pollinated flowers.

The pollinated flowers resulted in 2973 hybrid fruit, from which we obtained 1899 good stones that were planted in the field. The 15 apricot tree genotypes used in hybridisations included other important features, apart from resistance to PPV, such as: medium and low vigour; large, high quality fruit; good productivity; resistance to extreme cold, drought and pathogen agents and others.

The apricot tree hybrid descendents were serologically tested by means of the immunoenzymatic ELISA test, which measures the infection with PPV, and were categorised into three groups: sensitive, partially resistant and resistant.

From the group of resistant hybrids (Table 3), we would like to note the following selections: 'R8P71-73.10.74', 'R8P79-73.10.74', 'VT 47/112', 'VT 48/53', 'VT 51/45', 'VT 48/45', 'VT 4/73', 'VT 30/103', 'VT 34/72', '91.07.91', '91.35.59' and '92.02.54'. Some of these selections were registered ('Tudor', 'Traian', 'Auras', 'Danubiu', 'Cristal', 'Fortuna', 'Amiral', 'Orizont' and 'Augustin') in the period between 2003 and 2004, while others ('Euxin', 'Histria' and 'Ceres') were approved in 2010.

Subsequent to this research, it is recommended that improvement programmes use genitors that are resistant or tolerant to PPV, in order to obtain new apricot tree cultivars resistant to the infection with this virus. It is also recommended that all testing techniques be used, including molecular methods, in order to highlight with certainty the presence of the virus within certain genotypes, since this is the only way in which we can be absolutely sure that the spreading of the damaging virus is held effectively under control.

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Table 3

PPV DETECTION ON CERTAIN APRICOT TREE SELECTIONS BY MEANS OF THE ELISA TEST

Hybrid combination	Selection	Name of the registrated variety	ELISA test	
			semnification	value
Viceroy × NJA 42	R8 P71 73.10.74	Tudor	negative	0.421
Viceroy × NJA 42	R8 P79 73.10.74	Traian	negative	0.446
Mari de Cenad × S.E. Orange	VT 47/112	Aura	negative	0.402
Sam no 1 × Neptun	VT 48/53	Danubiu	negative	0.452
Sam no 1 × Mari de Cenad	VT 51/45	Cristal	negative	0.456
Sam no 1 × Mari de Cenad	VT 48/45	Fortuna	negative	0.459
Mari de Cenad × CR 24-12	VT 4/73	Amiral	negative	0.465
Mari de Cenad × CR 24-12	VT 30/103	Orizont	negative	0.473
H 9/5 × Sirena	VT 34/72	Augustin	negative	0.474
R30P162 × R41P162	91.07.91	Euxin	negative	0.474
Warleys peach × Sulmona	91.35.59	Histria	negative	0.483
NJA 17 × R9P53	92.02.54	Ceres	negative	0.537
Positive control	-	-	-	2.254
Negative control	-	-	-	0.579

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APRIKOŽU ŠĶIRŅU UN HIBRĪDU REZISTENCES PRET PLŪMJU BAKU VĪRUSU VĒRTĒJUMS RUMĀNIJAS DIENVIDAUSTRUMU DAĻĀ

Šobrīd Eiropā plūmju baku vīruss izraisa vienu no vissmagākajām slimībām, kas skar kauleņkoku sugas (plūmes, aprikozes, persikus, nektarīnus, mandeles un ķiršus) visās to audzēšanas valstīs. Rumānija ir viena no valstīm, kurā šis vīruss ir sastopams jau vairākus gadus un kurā ir ļoti augsts inficēšanās līmenis. Slimība izraisa ievērojamu kaitējumu, jo inficētajiem kokiem ir maz augļu, tie ir mazi un deformēti, bezgaršīgi, ar zemu cukura saturu, nav izmantojami ne svaigā veidā, ne pārstrādei. Tāpēc reģionos, kur plūmju baku vīruss ir izplatīts, vienīgā pieejamā iespēja vīrusa izraisīto bojājumu samazināšanai ir audzēt augsti izturīgus (tolerantus) genotipus. Šī pētījuma mērķis bija noteikt šķirnes, kas ir izturīgas pret vīrusa infekciju, kā arī novērtēt veidu, kādā izturība tiek pārmantota hibrīdajos pēcnācējos. Pētījumā izmantots aprikožu šķirnes no augļkopības pētījumu stacijas *Constanža* nacionālās kolekcijas. Plūmju baku vīrusa noteikšana veikta, izmantojot bioloģisko metodi, kas ietver testus uz GF305 indikatorauga, un seroloģisko metodi ar ELISA testu. Augstākā izturība pret plūmju baku vīrusa infekciju tika konstatēta šķirnēm ‘Stark Early Orange’, ‘NJA 17’, ‘Sulmona’, kuras tika izmantotas kā vecākaugi iekšsugas krustojumos, lai atlasītu hibrīdus, kas mantojuši izturību. No iegūtajiem hibrīdiem kā izturīgi minami ‘VT 48/45’, ‘VT 51/45’, ‘VT 47/112’, ‘H 9/5’.