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## Segregation of Plum Hybrid Families Bred for Resistance to Plum Pox Virus

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**Abstract:** The main target of the contemporary plum breeding programmes is obtaining of cultivars, resistant or tolerant to Plum pox virus (PPV). The present article reports the results from a segregation of plum hybrids depending on their reaction to PPV in field conditions.

The segregation included 160 plum individuals belonging to six plum (*Prunus domestica* L.) hybrid families obtained from the following parental combinations: 'Stenley' x 'Serdika 2', 'Stenley' x 'Pacific', 'Pacific' x 'Serdika 2', 'Pacific' x 'Stenley', 'Pacific' x 'Green gage' and 'Green gage' x 'Pacific'. During 3 vegetative cycles under natural infection background, the hybrids were observed for Sharka symptoms on the leaves and on the fruits. The symptomless plants were tested by enzyme - linked immunosorbent assay (ELISA) for latent PPV infection. On the base of visual inspections and ELISA results 20 hybrids were selected as follows: 5 numbers from cross 'Stenley' x 'Serdika 2'; 10 from 'Stenley' x 'Pacific'; 3 from 'Pacific' x 'Serdika 2' and 2 from 'Green gage' x 'Pacific'. Representing the results in relative values, PPV infection was not detected in 17 % of individuals from the cross 'Stenley' x 'Pacific', in 13.8 % from 'Stenley' x 'Serdika 2', in 13.3 % from 'Green gage' x 'Pacific' and in 11.5 % from 'Pacific' x 'Serdika 2'. All observed individuals from the families 'Pacific' x 'Stenley' and 'Pacific' x 'Green gage' showed medium or severe PPV symptoms and by reason of that they were discarded of survey. Most of individuals without PPV symptoms originated from combination 'Stenley' x 'Pacific' in contrast to the reverse cross 'Pacific' x 'Stenley'. In all observed hybrid populations prevailed the individuals reacting with medium symptoms on the leaves and mild symptoms on the fruits.

Some of chosen hybrids are under course of artificial inoculation by PPV isolates to resolve of their real reaction to PPV.

**Key words:** plum, breeding, segregation, resistance, Sharka

## Introduction

Sharka disease, caused by Plum pox virus (PPV), is the major limiting factor for the development of the plum (*Prunus domestica* L.) production in Bulgaria more than 75 years. Growing of PPV resistant or tolerant cultivars is the economically most beneficial and ecologically sound approach for minimizing the losses caused by the disease. Because of that, the plum breeding programme of the Fruit Growing Institute in Bulgaria is pointed to obtaining of cultivars, resistant or tolerant to Plum pox virus (PPV) and in the same time those possess good fruit quality and economic parameters.

Up to now the following 3 new plum cultivars have been established: 'Plovdivska renkloda' - partially resistant and 'Ulpia' and 'Sineva' - tolerant to PPV (Milusheva *et al.* 2009, Zhivondov and Bozhkova 2010). They were obtained by open pollination of parental cultivars 'Stanley' ('Plovdivska renkloda' and Ulpia) and 'President' (Sineva). In process of acknowledgement is the elite 21-47 (nominated name Ostromila), originating from the crossing 'Pasific' x 'Serdica 2' and which has showed resistance to PPV after field survey and artificial inoculation (Milusheva and Zhivondov 2011).

The current article reports the results of study on six hybrid families in order to segregate plum hybrids depending on their reaction to PPV in field conditions - the first stage of selection of resistant and tolerant genotypes.

## Material and Methods

The segregation included 160 plum individuals belonging to six plum hybrid families obtained from the following parental combinations: 'Stanley' x 'Serdika 2', 'Stanley' x 'Pacific', 'Pacific' x 'Serdika 2', 'Pacific' x 'Stanley', 'Pacific' x 'Green gage' and 'Green gage' x 'Pacific'. The hybrids were observed for Sharka symptoms on the leaves and on the fruits under natural infection background, during 3 vegetative cycles. The symptoms on the leaves and on the fruits were estimated visually by the following five grade scale.

0 - no symptoms

1 - very mild symptoms - up to 3 little spots on the leaf; spots on the fruit skin

2 - medium symptoms - spots cover up to ½ of the leaf and 1 pox on the fruit

3 - severe symptoms - spots cover up to ¾ of the leaf; poxes on the fruits, prematurely fruit dropping

4 - very severe symptoms - spots cover more than ¾ of the leaf; poxes and internal fruit damages, prematurely fruit dropping

The symptomless plants were tested for latent PPV infection by enzyme-linked immunosorbent assay (ELISA), in variant double antibody sandwich (DAS) according to Clark and Adams (1977). DAS ELISA was performed using commercial polyclonal antibodies against PPV (Bioreba AG).

## Results and Discussion

On the base of visual inspections and ELISA results 20 hybrids were selected as follows: 5 numbers from cross 'Stenley' x 'Serdika 2'; 10 from 'Stenley' x 'Pacific' ; 3 from 'Pacific' x 'Serdika 2' and 2 from 'Green gage' x 'Pacific'. The results represented in relative values mean, PPV infection was not detected in 17 % of individuals from the cross 'Stenley' x 'Pacific', in 13.8 % from 'Stenley' x 'Serdika 2', in 13.3 % from 'Green gage' x 'Pacific' and in 11.5 % from 'Pacific' x 'Serdika 2'. All observed individuals from the families 'Pacific' x 'Stenley' and 'Pacific' x 'Green gage' showed PPV symptoms and by reason of that they were discarded of the survey.

Most of individuals without PPV symptoms originated from the hybrid family 'Stenley' x 'Pacific' in contrast to the reverse cross 'Pacific' x 'Stenley'. Both cultivars are tolerant to PPV, but maybe in that cause, there is significance the direction of crossing.

The studied hybrids could be divided in 9 clusters (table 1) on the base of complex evaluation of their reaction to PPV:

1st cluster – 20 hybrids (12.5 %) showed no symptoms on the leaves and on the fruits and ELISA results were negative;

2nd cluster – 15 hybrids (9.4 %) reacted with very mild symptoms on the leaves and symptoms on the fruits were not observed;

3rd cluster – 20 hybrids (12.5 %) showed very mild symptoms on the leaves and on the fruits;

4th cluster – 15 hybrids (9.4 %) manifested medium to severe symptoms on the leaves and no symptoms were found on the fruits;

5th cluster – 40 hybrids (25 %) reacted with medium symptoms on the leaves and very mild symptoms on the fruits;

6th cluster – 18 hybrids (11 %) showed severe symptoms on the leaves and very mild on the fruits;

7th cluster – 14 hybrids (8.7 %) manifested severe to very severe symptoms on the leaves and medium symptoms on the fruits;

8th cluster – 15 hybrids (9.4 %), in which were recorded severe to very severe symptoms on the leaves and severe to very severe symptoms on the fruits;

9th cluster - 4 hybrids (2.5 %) in which were observed no symptoms on the leaves but on the fruits were recorded severe or very severe symptoms.

Tab. 1. Segregating of the hybrids in clusters

Parental combinations	Number hybrids*	Cluster/Number of hybrids of each cluster								
		1	2	3	4	5	6	7	8	9
'Stenley x Serdica 2'	36	5	3	6	2	10	2	2	5	1
'Stenley x Pacific'	58	10	4	7	5	15	7	5	3	2
'Pasific x Serdica 2'	26	3	3	3	3	5	4	3	2	0
'Pacific' x 'Stenley'	17	0	2	1	2	5	2	2	2	1
'Pacific' x 'Green gage'	8	0	2	1	1	2	1	1	1	0
'Green gage' x 'Pacific'	15	2	1	2	2	3	2	1	2	0
Total	160	20	15	20	15	40	18	14	15	4
%		12.5	9.4	12.5	9.4	25	11	8.7	9.4	2.5

\*Number of hybrids from a population

In all observed hybrid populations prevailed individuals (25 %) reacting with medium symptoms on the leaves and mild symptoms on the fruits (table 1).

During the period of the observations the symptomatic picture was dynamic. In the beginning of the study 35 from 160 progenies were symptomless and ELISA negative and after 3 vegetative cycles 20 ones remained PPV free.

Various methods for testing of PPV resistance have been developed. The most common method is the estimation of genotype reactions by natural infection in the field, under high infection background. However, sometimes it is possible susceptible genotypes to remain uninfected for over a long time, under field conditions (Kegler *et al.* 2000) Because of that, some of the segregated hybrids are under course of artificial inoculation by PPV isolates to resolve of their real reaction to PPV.

According to the recent opinions (Hatmann 1998; Kegler *et al.* 1998), it could be distinguished two types of the resistance of plums to Sharka disease. Quantitative resistant is polygenic based and cultivars develop no or very mild symptoms and show clearly reduces virus accumulation in comparison with susceptible standard genotype.

Qualitative resistance is based on the hypersensitive reaction of the genotypes *i. e* these genotypes can be infected but do not become diseased because the virus is localized in the infection site. In our study it was not observed hybrids reacted hypersensitive to PPV.

## Conclusion

The segregation of the plum hybrids showed the progenies, deriving from the same hybrid families manifested rather varied reactions to PPV.

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## SEGREGACIJA HIBRIDA ŠLJIVE SELEKCIONISANIH NA OTPORNOST PREMA VIRUSU ŠARKE ŠLJIVE

-originalni naučni rad-

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### Rezime

Glavni cilj današnjih programa selekcije šljive jeste dobijanje sorti otpornih ili tolerantnih prema virusu šarke šljive. U ovom radu predstavljeni su rezultati segregacije hibrida šljive u zavisnosti od njihove reakcije na virus šarke šljive u poljskim uslovima.

Segregacijom je obuhvaćeno 160 stabala šljive iz šest hibridnih porodica šljive (*Prunus domestica* L.) dobijenih na osnovu sledećih roditeljskih kombinacija: 'Stenli' x 'Serdika 2', 'Stenli' x 'Pacifik', 'Pacifik' x 'Serdika 2', 'Pacifik' x 'Stenli', 'Pacifik' x 'Green Gage' i 'Green Gage' x 'Pacifik'. Tokom 3 vegetaciona ciklusa u uslovima prirodne zaraženosti, posmatrani su simptomi virusa šarke šljive na listu i plodu hibrida. Biljke koje nisu pokazivale simptome analizirane su primenom enzimskog imunoadsorpcionog testa (ELISA) za latentnu zaraženost virusom. Na osnovu vizuelne kontrole i rezultata ELISA testa, selekcionisano je 20 hibrida: 5 hibrida iz ukrštanja 'Stenli' x 'Serdika 2'; 10 iz 'Stenli' x 'Pacifik'; 3 iz 'Pacifik' x 'Serdika 2' i 2 iz 'Green Gage' x 'Pacifik'. Na osnovu relativnih vrednosti rezultata, utvrđeno je da zaraženost virusom šarke šljive nije otkrivena kod 17% stabala iz ukrštanja 'Stenli' x 'Pacifik', kod 13,8% iz ukrštanja 'Stenli' x 'Serdika 2', kod 13,3% iz ukrštanja 'Green Gage' x 'Pacifik' i kod 11,5% stabala iz ukrštanja 'Pacifik' x 'Serdika 2'. Sva ispitivana stabla iz porodica 'Pacifik' x 'Stenli' i 'Pacifik' x 'Green Gage' ispoljila su srednje ili teške simptome zaraženosti šarkom šljive, usled čega su odbačene iz istraživanja. Većina stabala bez simptoma virusa šarke šljive poreklom su iz kombinacija 'Stenli' x 'Pacifik', za razliku od suprotnog ukrštanja 'Pacifik' x 'Stenli'. U svim posmatranim hibridnim populacijama preovlađivala su stabla sa srednjim simptomima na listu i blagim simptomima na plodu.

Trenutno se vrši veštačka inokulacija izolatima virusa šarke šljive kod nekoliko odabranih hibrida u cilju definisanja njihove stvarne reakcije na virus šarke.

**Ključne reči:** šljiva, selekcija, segregacija, otpornost, šarka