

## Oligogenic Inheritance of Resistance to Plum Pox Virus in Apricots

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**Abstract:** In order to determine the inheritance of resistance to PPV in apricot three crosses between resistant and susceptible cultivars and selections were performed. The B<sub>1</sub> seedlings were inoculated with the PPV-M strain by an infected bud. PPV infection was evaluated over 5 consecutive growth periods through visual symptoms, ELISA and in some cases reverse transcriptase PCR assays. Chi-square analysis of each B<sub>1</sub> progeny was performed to determine if the segregation ratio differed from the expected ratio. PPV resistance segregated in three apricot B<sub>1</sub> progenies in a 1:7 (resistant:susceptible) ratio, indicating that resistance was controlled by three independent dominant complementary genes. All three dominant genes are needed for the resistance to be expressed, and the lack of any one of the dominant alleles will result in susceptibility. This knowledge will help us in effective planning of apricot breeding programs with this subjective.

**Keywords:** *Prunus armeniaca* L.; heritability; sharka disease

Apricot (*Prunus armeniaca* L.) ranks as the third most agronomically important species of the stone fruit crops. Plum pox virus (PPV) causes serious damage in apricots grown in the Czech Republic and other countries where it is present. It is evident from previous studies (KARAYIANNIS 1995; LLÁCER & CAMBRA 1998) that breeding and growing of PPV-resistant apricot cultivars are the only way how to solve one of the most significant phytopathological problems.

PPV is difficult to study in *Prunus* plants because of (1) infection procedure is not easy to control, (2) detection is complicated by low virus concentration, (3) infection distribution is not uniform in a plant, (4) lengthy test procedure requiring several growth-dormancy cycles (GUILLET-BELLANGER & AUDERGON 2001).

Studies on inheritance of resistance to PPV in apricot started at the end of 1980s. Currently, there are three different published hypothesis

suggesting that the resistance is controlled by one (DICENTA *et al.* 2000), two (DOSBA *et al.* 1991; MOUSTAFA *et al.* 2001; VILANOVA *et al.* 2003) or three genes (GUILLET-BELLANGER & AUDERGON 2001; POLÁK *et al.* 2002).

A breeding program aimed at introducing resistance to PPV was initiated in the Czech Republic in 1991 (POLÁK *et al.* 1995). Several apricot cultivars and selections have been determined to be resistant to PPV (POLÁK *et al.* 1997). Genetic determinism of PPV resistance in apricot has been studied since 1998.

The aim of this study was to improve knowledge on the inheritance of PPV resistance in apricot.

### MATERIAL AND METHODS

**Plant material.** Apricot cultivar Stark Early Orange (SEO) and selections LE-3241 and LE-3246 (both Vestar × SEO) were used as donors of the

resistance in crosses with PPV susceptible cultivar Vestar and selection LE-3218 (Vestar × SEO). Following cross combinations between above-mentioned cultivars and selections were made at Faculty of Horticulture, Mendel University of Agriculture and Forestry in Lednice in 1999: LE-3218 × SEO (H868), LE-3241 × Vestar (H869) and LE-3246 × Vestar (H870). Crosses were performed by hand pollination in flower buds after removing petals and anthers.

**PPV inoculation and evaluation of PPV infection.** The B<sub>1</sub> seeds were stratified and the subsequent seedlings were grown in an insect-proof greenhouse. When the stems of the seedlings reached about 5 mm thickness they were inoculated with a chip-bud from the plum infected with the PPV isolate from apricot cultivar Vegama (Marcus strain) (PONCAROVÁ & KOMÍNEK 1998). Seedlings were pruned directly after grafting to promote the growth of the inoculated bud. Plants without sharka symptoms on shoots growing from the inoculated bud and with negative ELISA reaction were re-inoculated. PPV infection was evaluated over 5 consecutive growth periods through visual symptoms and ELISA (POLÁK *et al.* 1997). Pruning was performed at the beginning of each growth period to induce vigorous new shoots for symptom scoring. The plants, in which PPV was not detected by ELISA, were tested by RT-PCR using the specific primers P1 and P2 (WETZEL *et al.* 1991). Plants were classified as resistant if they did not show symptoms and positive ELISA or RT-PCR reaction in last 3 growth periods evaluated.

**Statistical analysis.** Chi-square analysis of the segregation of PPV resistance in B<sub>1</sub> apricot plants was performed and a  $\chi^2$  homogeneity test conducted to compare the segregation ratios between families of the same generation (FISHER 1970).

## RESULTS

An inheritance study of resistance to PPV was conducted in the B<sub>1</sub> progenies of crosses LE-3218 × SEO, LE-3241 × Vestar and LE-3246 × Vestar. The segregation for resistance to *Plum pox virus* in the three studied progenies is given in Table 1.

The segregation (resistant:susceptible) obtained after first chilling treatment was 1:1, 1:4 and 1:7 after second respectively third chilling treatment and then it settled down. These results did not vary after more chilling treatments. A slight excess of resistant individuals with respect to the expected ratio 1:7 was observed in all three B<sub>1</sub> progenies.

To eliminate “false resistant individuals” (unsuccessful inoculation) we evaluated symptoms on shoots from the inoculated bud. Plants without sharka symptoms on shoots from the inoculated bud and with negative ELISA reaction were re-inoculated.

A few individuals in each progeny showed symptoms on several leaves and had positive ELISA reaction the next growth period after inoculation. The symptoms only occurred on single leaves in the shoots near the point of inoculation. No symptoms on leaves have been observed and ELISA reaction has been negative since the second growth period after inoculation. This may be due to plant recovery and elimination of the virus (DECROOQ *et al.* 2005).

The intensity of symptoms in susceptible seedlings was high in first two cycles. A decrease in symptom intensity of the seedlings in the later cycles has been observed, probably because of the age of the plants grown in pots in controlled conditions in a greenhouse.

Observed segregation ratios for each individual progeny ( $\chi^2 = 0.11$ ,  $P = 90\text{--}95\%$ ) and over the total

Table 1. Chi-square analysis of the segregation for PPV resistance in B<sub>1</sub> progenies generated from crosses between PPV resistant and susceptible apricot cultivars and selections

Progeny	Phenotype			$\chi^2$ (1:7)	Probability (%)
	R*	S*	total		
LE-3218 × SEO	9	46	55	0.75	25–50
LE-3241 × Vestar	11	64	75	0.32	50–75
LE-3246 × Vestar	13	67	80	1.03	25–50
Observed	33	177	210	1.99	10–25
Expected	26.25	183.75			
Test of homogeneity among B <sub>1</sub> progenies				0.11	90–95

\*Phenotype classes are R (as resistant parent) and S (as susceptible parent)

210 B<sub>1</sub> plants screened ( $\chi^2 = 1.99$ ,  $P = 10\text{--}25\%$ ) were not significantly different from the predicted 1:7 segregation ratio (Table 1). These findings suggest that PPV resistance in apricot is controlled by three independent dominant complementary genes, where the resistance would be a dominant trait and the resistant parents (SEO, LE-3241 and LE-3246) would be heterozygous for all loci.

## DISCUSSION

Different genetic controls of PPV resistance in apricot have been published. DOSBA *et al.* (1991) studied a progeny of 76 individuals from the cross of Screara (susceptible to PPV) with Stark Early Orange (resistant to PPV). They found the ratio  $\frac{3}{4}$  susceptible and  $\frac{1}{4}$  resistant (tolerant), which led them to think that the genetic control of tolerance (of SEO) to PPV might be determined by two independent dominant genes. DICENTA *et al.* (2000) analysed 291 seedlings from 20 different crosses, where the donor of resistance were cultivars SEO, Lito, Avilara and a selection A2408, resulting in a segregation 1:1 susceptible/resistant, which indicate that the resistance to PPV in apricot is controlled by a single dominant gene, the donor of resistance being heterozygous for this trait. MOUSTAFA *et al.* (2001), from crosses between resistant and susceptible cultivars, obtained segregation 3:1 susceptible/resistant to PPV, which adjusted to the hypothesis of two independent dominant loci. GUILLET-BELLANGER and AUDERGON (2001) observed the segregation for resistance to *Plum pox virus* in progenies SEO × SEO, Bergeron × SEO and Bergeron × Bergeron. Preliminary results suggest that the resistance of SEO cultivar is dominant and controlled by at least 3 genes. POLÁK *et al.* (2002) reported a control of the resistance by two dominant independent loci in two crosses SEO (resistant to PPV) × LE-3218 (susceptible to PPV). Moreover, the segregation 7:1 susceptible/resistant obtained in the cross Lejuna (susceptible to PPV) × Harlayne (immune to PPV) indicated that at least three independent dominant genes were involved in the determination of the resistance to PPV. VILANOVA *et al.* (2003) based on results obtained from the self-pollination of Lito, a resistant cultivar from the cross Stark Early Orange × Tyrintos, suggested that the genetic control relies in two dominant genes.

Our results are in agreement with the hypothesis of GUILLET-BELLANGER and AUDERGON (2001) that

resistance SEO is controlled by at least 3 genes. The number of genes observed to segregate in crosses depends on the genotype of the susceptible parent. Distinct hypotheses suggested by DOSBA *et al.* (1991), DICENTA *et al.* (2000), MOUSTAFA *et al.* (2001), and VILANOVA *et al.* (2003) might be explained by differences in genotypes of parents, methodologies (conditions of evaluation, type of inoculation, number of growth periods evaluated, etc.) and size of progenies. It has also to be taken into consideration that susceptibility or resistance is dependent not only on the plant but also on the virus. Our group is the only one using the Marcus strain in research on the inheritance of PPV resistance in apricot. The Marcus strain is more aggressive and therefore more suitable for the resistance examination (DOSBA *et al.* 1992).

We found out that the correct determination of the trait is the key to establish a hypothesis for the inheritance. According to our experience at least three chilling cycles followed by the symptom observations in the new shoots together with ELISA or RT-PCR are necessary. A standard procedure that allows comparing the results between different laboratories has to be elaborated.

The results presented will be utilised in apricot breeding program at Faculty of Horticulture, Mendel University of Agriculture and Forestry in Lednice. Considering the incomplete explanation of the phenotypic variability (different degrees of susceptibility observed) of the trait we are going to perform quantitative trait loci (QTL) mapping in the progeny LE-3246 × Vestar.

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## Abstrakt

SALAVA J., POLÁK J., KRŠKA B. (2005): **Oligogenní dědičnost rezistence vůči viru šarky švestky u meruňky**. Czech J. Genet. Plant Breed., **41**: 167–170.

Pro zjištění způsobu dědičnosti rezistence meruněk vůči viru šarky švestky (PPV) byla provedena tři zpětná křížení mezi rezistentními a náchylnými odrůdami a novošlechtěními. Byl vyvinut nový postup hodnocení rezistence vůči PPV. Semenáčky byly inokulovány kmenem PPV-M očkováním. Rezistence byla hodnocena po pět po sobě jdoucích vegetačních období pomocí sledování symptomů na listech, ELISA testem a v některých případech RT-PCR. Za rezistentní byly považovány rostliny, u kterých nebyly nalezeny symptomy na listech a které měly negativní výsledky ELISA a RT-PCR testu v posledních třech vegetačních obdobích. Shoda získaného a teoretického štěpného poměru byla prokázána pomocí  $\chi^2$  testu. Štěpné poměry 1 : 7 (rezistentní : náchylní) získané ve všech třech potomstvech ukazují, že rezistence je řízena třemi nezávislými dominantními komplementárními geny. Všechny tři dominantní geny je zapotřebí k tomu, aby se rezistence projevila. Jakákoliv chybějící dominantní alela způsobí náchylnost jedince. Z našich pokusů je zřejmé, že pro vytvoření modelu dědičnosti rezistence vůči PPV je klíčové správné hodnocení rezistence. Získané poznatky pomohou při přípravě šlechtitelských programů meruněk zahrnujících tento šlechtitelský cíl.

**Klíčová slova:** *Prunus armeniaca* L.; *Plum pox virus*; rezistence; dědivost

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