Molecular Genetic Mapping in Apricot

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Abstract: A genetic linkage map for apricot (*Prunus armeniaca* L.) has been constructed using amplified fragment length polymorphism (AFLP) markers in 80 BC_1 individuals derived from a cross LE-3246 × Vestar. From 26 different primer combinations, a total of 248 AFLP markers were scored, of which, 40 were assigned to 8 linkage groups covering 315.8 cM of the apricot nuclear genome. The average interval between these markers was 7.7 cM. One gene (*PPVres1*) involved in resistance to PPV (*Plum pox virus*) was mapped. Two AFLP markers (EAA/MCAG8 and EAG/MCAT14) were found to be closely associated with the *PPVres1* locus (4.6 cM resp. 4.7 cM). These markers are being characterized and they will be studied for utilization in apricot breeding with marker-assisted selection (MAS).

Keywords: *Prunus armeniaca* L.; *Plum pox virus*; resistance; AFLP; genetic mapping

Sharka, the disease caused by *Plum pox virus* (PPV), is one of the most important problems to fruit production worldwide. It is evident from the previous studies in Europe (BADENES *et al.* 1996; GLASA *et al.* 2001), that effective and timely control of PPV will rely on the integration of classical breeding approaches with modern technologies for marking and identifying naturally occurring resistance genes.

The Amplified Fragment Length Polymorphism™ (AFLP) (VOS *et al.* 1995; ZABEAU & VOS 1993) combines the specifity of restriction enzyme analysis with the ease and specificity of the polymerase chain reaction (PCR). The technique is rapid and reliable, allowing the researcher to simultaneously evaluate 50 or more potentional polymorphisms on a single polyacrylamide gel. For plant researchers the technique offers quick linkage map construction and rapid identification of markers linked to genes of interest (WANG *et al.* 1997, 2002; LU *et al.* 1998).

In the last a few years, with the advent of DNA-based markers, genetic studies have been greatly facilitated and several genetic maps have been published for *Prunus*. One of them using apricot progenies (LAMBERT *et al.* 2001) was based on RFLPs. HURTADO *et al.* (2001) produced two apricot maps composed of RAPD and AFLP markers from the cross Goldrich × Valenciano. A total of 117 markers were placed into 8 linkage groups on the Goldrich map, defining 570.3 cM of total map length. A total of 66 markers were placed into 9 linkage groups on the Valenciano map, defining 448.9 cM of total map distance.

The LE-3246 × Vestar progeny and available linkage map will be very useful for tagging, mapping and cloning genes controlling resistance to PPV in apricot. Molecular markers linked to PPV resistance could be used in MAS in apricot and other related species of *Prunus*.

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MATERIALS AND METHODS

Plant material. An apricot selection LE-3246 (Stark Early Orange × Vestar) was crossed as a female parent to Vestar at Faculty of Horticulture of Mendel University of Agriculture and Forestry in Lednice na Moravě in 1999. Stark Early Orange and LE-3246 are resistant to PPV, Vestar is susceptible to PPV. The BC_1 seeds were stratified at 5°C for 3 months and subsequent seedlings were grown in a greenhouse. Eighty BC_1 individuals were used to construct the genetic map.

Resistance evaluation. Each BC_1 seedling was inoculated with PPV-Vegama isolate (PPV-M strain) (PONCAROVÁ & KOMÍNEK 1998) by chip-budding two times. The first inoculation was performed at the end of the first growth period, the second one during the second growth period. Symptoms of sharka infection were visually evaluated on apricot leaves. At the same time enzyme-linked immunosorbent assay (ELISA) was applied to the leaves. The observations were continued over three consecutive growth periods.

DNA extraction. Genomic DNA was isolated from fresh apricot leaves using the CTAB protocol described by ELDREDGE *et al.* (1992). DNA concentrations were measured with a minifluorimeter (TKO100, Hoefer Scientific, San Francisco, USA).

AFLP analysis. AFLP analysis followed VOS *et al.* (1995). Twenty-six (*Eco*RI/*Mse*I) primer combinations were used for the AFLP analysis. Designation of each individual AFLP marker was based on the primers used.

Linkage analysis. A genetic linkage map was constructed with MAPMAKER software (V2.0) for Macintosh (LINCOLN *et al.* 1992). The mapping analysis was conducted using a 4.0 minimum LOD score and 0.25 maximum recombination frequency (θ). The Kosambi mapping function (KOSAMBI 1944) was used for calculation of map distances.

RESULTS AND DISCUSSION

In total, 248 AFLP polymorphic markers from 26 primer combinations were scored. Of the 124 markers segregating as in a backcross (1:1), 60 were heterozygous in the female parent (LE-3246) and 64 in the male parent (Vestar). A hundred and twenty-four markers were presented in both parents and segregated 3:1. Sixty loci segregating 1:1, heterozygous in LE-3246, were used to construct the map.

Sixty-three BC_1 plants were PPV susceptible and 17 were PPV resistant. This segregation ratio fits the expected 3:1 ratio for two dominant complementary genes ($\chi^2 = 0.60$, $P = 25–50\%$).

Linkage analysis of the BC_1 population was performed using the MAPMAKER software (LINCOLN *et al.* 1992). Map distances (centiMorgan, cM) were then calculated using the Kosambi mapping function (KOSAMBI 1944). The linkage groups were obtained by choosing 0.25 as the maximum recombinant fraction (frequency) (θ) and 4.0 as the minimum LOD score value.

The genetic linkage map consisted of eight linkage groups covering more than 315.8 cM of the apricot genome (Fig. 1). It is too early to say if the eight linkage groups found correspond to the eight chromosomes of apricot. This map included the *PPVres1* gene conferring the resistance to PPV and 40 AFLP markers. The average distance between adjacent markers is 7.7 cM. Two large gaps, longer than 20 cM, are located in A4 and one in A6 and A8. Nineteen markers remained unassigned to any linkage group.

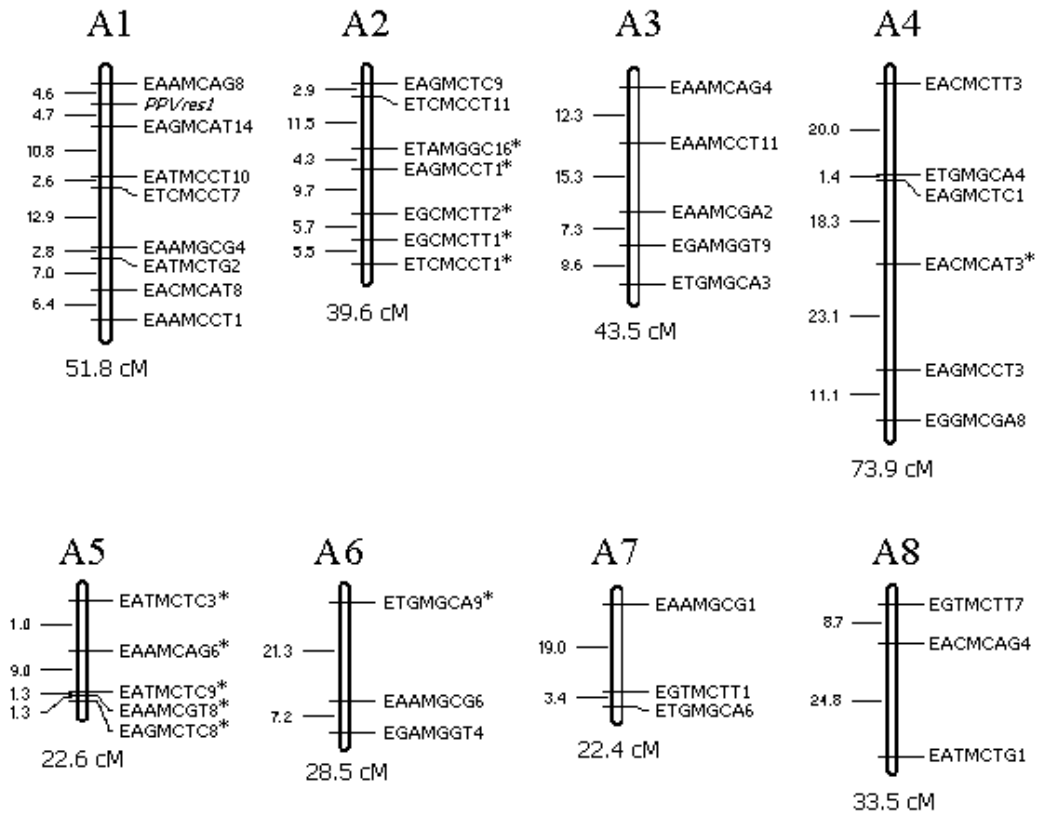
All AFLP markers were evaluated by the χ^2-test for goodness of fit against a 1:1 ratio for the BC_1 type markers and a 3:1 ratio for the F_2 type markers ($P \leq 0.05$). Fifteen loci (22%) had skewed segregation ratio ($P < 0.05$) and 3 of them were unlinked. Clusters of loci with distorted segregations were found in A2 and A5. Skewed segregation was also observed in a locus in A4 and A6. In all the skewed segregations in A2, A4, A5 and A6, the selection was against the homozygotes, the recurrent parent alleles being selected against, the only marker which the recurrent parent alleles being preferred at was EAGMCAT5.

PPV resistance *(PPVres1*) was mapped in A1, 4.6cM away from the cosegregating marker EAAMCAG8 and 4.7cM away from marker EAGMCAT14.

One locus controlling PPV resistance (*PPVres1*) was identified. PPV resistance in apricot is according to MOUSTAFA *et al.* (2001) controlled by two independent dominant loci. GUILLET-BELLANGER and AUDERGON (2001) and POLÁK *et al.* (2001) found, that at least three dominant genes are involved in PPV resistance in apricot. We identified another potentional locus for PPV resistance about 20 cM away from the locus EGGMCGA8 at the bottom of the linkage group A4. It was too far from the AFLP marker and there was not a marker on the other side of the locus so we did not put this locus on the map. It will be necessary to increase the number of molecular markers to saturate this linkage map and map another locus(i) governing PPV resistance.

It was confirmed that markers EATMCCT10 and ETCMCCT7 identified by bulked segregant analysis (BSA) (SALAVA *et al.* 2002) were linked to the PPV resistance trait. The other markers identified by BSA (EAAMCGT and EGCMCTT) were not scorable in progeny LE-3246 × Vestar, did not segregate in the progeny respectively. Four markers closely linked to the *PPVres1* locus were detected in all the resistant individuals and some susceptible individuals too. It shows that the resistance to PPV in apricot is controlled by more than one locus.

Future work will be to construct more saturated genetic linkage map for apricot, to characterize the AFLP markers linked to PPV resistance and to perform comparative



1. Partial genetic linkage map of apricot derived from the population (Stark Early Orange × Vestar) × Vestar. The phenotypic trait (PPV resistance) and the AFLP markers are indicated on the right and genetic distance in centimorgans is indicated on the left. Nineteen markers (EAAMCAG6, EAAMCCT2, EACMCCC2, EACMCCC7, EACMCTT2, EAGMCAT4, EAGMCAT5, EAGMCAT6, EAGMCAT7, EAGMCAT11, EAGMCCT8, EAGMCTC7, EATMCCT8, EATMCTC2, EGAMGGT5, EGGMCGA7, EGGMCGA10, ETAMGGC3, ETGMGCA5) remain independent. Asterisks (*) indicate distorted segregations of markers (χ^2 test, $P < 0.05$). Vertical lines represent linkage groups (A1–A8) with AFLP-defined loci represented by the code for the appropriate AFLP primer combination followed by a number to distinguish different loci recognized by the same primer combination. Total map lengths are represented at the bottom of the linkage groups

analysis among our map and maps of apricot peach, plum, almond and cherry.

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Abstrakt

SALAVA J., WANG Y., KRŠKA B., POLÁK J., KOMÍNEK P., MILLER R.W., DOWLER W.M., REIGHARD G.L., ABBOTT A.G. (2002): **Molekulární mapování genomu meruňky**. Czech J. Genet. Plant Breed., **38**: 65–68.

Genetická vazbová mapa meruňky (*Prunus armeniaca* L.) byla sestavena pomocí AFLP (amplified fragment length polymorphism) markerů a 80 jedinců BC_1 populace po křížení LE3246 × Vestar. Z 26 různých kombinací primerů bylo vyhodnoceno 248 AFLP markerů, ze kterých 40 bylo umístěno do osmi vazbových skupin pokrývajících 315,8 cM jaderného genomu meruňky. Průměrná vzdálenost mezi dvěma sousedními markery je 7,7 cM. Byl zmapován jeden gen (*PPVres1*) ovlivňující rezistenci k viru šarky švestky. Byly nalezeny dva AFLP markery (EAA/MCAG8 a EAG/MCAT14) se silnou vazbou k lokusu *PPVres1* (4,6 cM, resp. 4,7 cM). Tyto markery jsou v současné době charakterizovány a bude zkoumána možnost jejich využití pro MAS (marker-assisted selection) ve šlechtění meruněk.

Klíčová slova: *Prunus armeniaca* L.; virus šarky švestky; rezistence; AFLP; genetické mapování

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