

SSR ANALYSIS CONCERNING THE RESISTANCE TO PPV (*Plum pox virus*) IN SEVERAL ROMANIAN APRICOT PROGENIES

Frăguța CIOMAGA¹, Ligia ION¹, Cristina MOALE²

¹University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59, Marasti Blvd,
011464, Bucharest, Romania

²Research Station for Fruit Growing Constanta, Constanta, Romania

Corresponding author email: ionnagyligia@yahoo.fr

Abstract

Plum pox virus (PPV) is a devastating stone fruit disease of major importance, and better understanding of the genetic control of resistance to this trait would be useful for more efficient development of resistant cultivars. Previous studies have reported a locus major effect from PPV resistance on linkage group 1. The hybrids were grafted simultaneously and subsequently inoculated with the PPV-M and D strains. The symptom scoring on leaves was performed three times over two vegetative cycles. The PPV resistant loci were mapped using composite interval mapping (CIM). This paper presents data from PhD thesis part of the project POSDRU/107/1.5/S/76888, funded by European Social Fund through the Sectorial Operational Programme Human Resources Development 2007-2013.

Key words: disease, markers, *Prunus armeniaca*, PPV, SSR, Sharka.

INTRODUCTION

Plum pox virus (PPV) infection causes the Sharka disease of *Prunus*, has spread from the Balkan countries throughout most of the European subcontinent and around the Mediterranean basin.

Considerable economic loss and significant reduction in productive areas stimulated breeding programs aimed at enhancing resistance to the pathogen in such countries as Greece (Karayiannis et al., 2002), France (Audergon et al., 1994), Italy (Bassi et al., 1995), Spain (Egea et al., 1999) and the Czech Republic (Polak, 1994).

As a result of the intensive search for a source of resistance within available apricot germplasm, some North American cultivars 'Stark Early Orange' (SEO), 'Goldrich', 'Harlayne', 'NJA 45', and others were found to have natural resistance to PPV (Martinez-Gomez et al., 2000). These cultivars were used as donors for a resistance trait in conventional breeding programs based on crosses between resistant and the best local cultivars susceptible to virus.

Recently, resistant selections have been released for regional trials (Badenes and Llacer, 2006; Dosba et al., 1992; Karayiannis et al., 1999; Polak et al., 1997)

Several studies suggest that the resistance is conferred by a limited number (1–3) of genes (Dicenta et al., 2000; Dosba et al., 1992; Krska B et al., 2002; Karayiannis et al., 2007). Development of molecular marker maps for segregating crosses is a significant accomplishment toward understanding the genetics of PPV resistance and developing markers that could potentially be useful in breeding programs. Four molecular genetic maps based on intraspecific crosses introducing PPV resistance from North American cultivars 'Stark Early Orange' and 'Goldrich' have been established to map a PPV resistance in apricot (Lambert et al., 2007; Sicard et al., 2007). On these maps, a major genomic region associated with PPV resistance was located on the *Prunus* G1 at a distance of 20–40 cm. In total, five SSR markers linked to the targeted resistance locus were identified in this region. Three of them have been already successfully tested for marker assisted selection (MAS) in a set of susceptible/resistant cultivars.

MATERIALS AND METHODS

Plant material

We are worked with 5 apricot population:

Pop 3 = Population 3 obtained by crosses between ('Mari de Cenad, x,NJA 21') x 'Kesth Pshor'

Pop 2 = Population 2 obtained by crosses 'Sirena' x 'NJA 42'

Pop 4 = Population 4 obtained by crosses 'Cristal' x ,NJA 21'

Pop 1 = Population 1 obtained by crosses 'Mari de Cenad' x ,SEO'

Pop 5 = population 5 obtained by crosses between ('Viceroy', x,NJA 2') x 'NJA17'

The apricot selection R9 P 53 ('NJA 2' x'Viceroy') was crossed as a female parent to 'NJA 17' a PPV resistant apricot cultivar. The selection R13 VT 8/57 resistant to PPV (issued from 'Mari de Cenad x NJA 21) was crossed as a female parent to 'Kesth Pshor' (susceptible to PPV) in the frame of the Faculty of Horticulture of University of Agronomic Sciences and Veterinary Medicine of Bucharest Romania in 2008. Crosses were performed by hand pollination with isolation of flowers after the petals and anthers removal from the flower buds. The F1 seeds were stratified at 5°C for 3 months and subsequent seedlings were grown in an insect-proof greenhouse.

The young apricot populations sticks were grafted onto inoculated GF305 (used like susceptible rootstock) ready for testing to PPV resistance.

DNA extraction

Genomic DNA was extracted using the modified CTAB procedure [23]. DNA concentrations were measured and working solutions of genomic DNA at 10 ng/μl and at 100 ng/μl in 0.1× TE (0.01M Tris pH 8.0, 0,001 M EDTA) buffer was prepared for the targeted SSR and AFLP analyses, respectively.

SSR analysis

'Stark Early Orange', 'R13 VT 8/57' NJA and R9 P 53 were screened with 3 SSR primer combinations from [2], associated with PPVresistance. PGS 1.21, PGS 1-24 and ppb22-195-F:CTCTTCTCGCCTCCCAATTT and R:GCTTAGCCCTGGGTACAAG and F:ATCTGCTCTTTCCCTCACCT with R:GATTATCCCTCAACCCATCC.

PCR reactions, electrophoresis, and detection of PCR products for the 'Kesth Pshor' × R13 VT 8/57' population were carried out according to conditions specified in (Zhebentyayeva et

all., 2002). SSR primer combinations revealing polymorphism were screened all apricot populations. The mix PCR consist in 10X buffer-2ul, MgCl₂ (25 mM)-1.2 ul, dNTP 10 mM – 0.16 ul, PGS 1.21-F – 0.6ul, PGS 1.21-R – 0.6 ul, PGS 1.24-F – 0.4 ul, PGS1.24-R – 0.4 ul, ppb22-195-F-0.28 ul,ppb22-195-R – 0.28ul and Taq 0.1 ul. A 2-μl aliquot of the PCR reaction was separated by electrophoresis on an agarose gel 2% in order to confirm the amplification of fragments of the expected size and DNA concentration. (Figure 1.).

The PCR-products were diluted (45 ul H₂O and 5 ul DNA) and used to prepare the plate for sequencing.

RESULTS AND DISCUSSIONS

All four SSR primer combinations amplified expected size fragments in germoplasm under study. SSR scores for genotypes are included in supporting documentation, Table 1. For all SSR loci, we recorded genotypes in order of their electrophoretic mobility from fastest to slowest band.

The infection process was different for various plant individuals; in some plants the presence of PPV was detected after the first dormancy period, in some in the three vegetative cycles, while some plants were not infected at all.

Selections 'Kesth Pshor' × 'R13 VT 8/57' and 'R9 P 53' were screened with 3 SSR primer combinations. The products of PCR were separated by electrophoresis on an agarose gel 2% in order to confirm the amplification of fragments of the expected size and DNA concentration. (Figure 1)

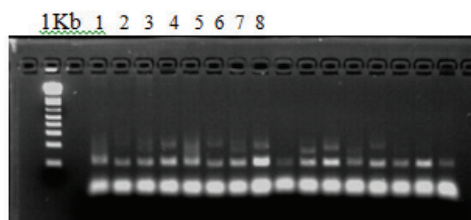


Figure 1. Electrophoresis for PCR with SSR markers in apricot progenies

These SSRs were polymorphic. This presence of polymorphism is slightly higher than that previously reported by Hurtado et al. (2002)

and Vilanova et al. (2003a; 46% and 42%, respectively) in apricot. Additionally, the SSRs, (PGS1_24)-F:CTCTTCTCGCCTCCCAATTT with R:GCTTAGCCCTGGGTACAAG and F:ATCTGCTCTTTCCCTCACCT with R:GATTATCCCTCAACCCATCC were significant for the first screening in a larger population and may be useful for starting a MAS in breeding for PPV resistance. Further evaluation of these loci will be necessary to characterize the genetic control of the PPV resistance trait. Due to the co-dominant nature of SSRs along with their high genetic transportability, the development of SSRs associated with PPV resistance in apricot could facilitate the use of MAS in breeding strategies aimed at breeding for natural resistance.

Table 1. Results concerning the implement of markers PGS 1,24 and Ppb0022-195 in Romanian progenies.

Génotype	Ppb0022-195 Map distance cM	Ppb 0022-195 Map dist.. cM	PGS124 Map dist. cM	PGS124 Map dist. cM
KesthPhor	112.01		129.53	
Viceroy	111.96		100.93	
H2+	107	116.4	98.93	102.9
MariCenad	111.98		100.92	
NJA 17	107.01	112.01	98.9	100.91
Cristal	111.84		100.78	102.73
Sirena	111.81		100.78	127.76
Tabriz	112.01		100.88	
SEO	107.11	111.97	98.9	100.91
Pop3-37	111.9		100.79	
Pop3-38	111.83		129.42	
Pop3-42	111.9		102.73	129.43
Pop3-49	111.93		129.42	
Pop3-64	107.05	111.98	98.89	100.92
Pop3-65	112		129.62	
Pop3-68	107.13		98.88	102.93
Pop3-71				
Pop3-73	107.08		98.94	
Pop3-74	107.12	112.03	98.96	127.93
Pop3-76	107.1	112.04	98.94	129.24
Pop3-77	106.97		98.76	
Pop3-78	111.91		100.81	
Pop3-79	106.94	111.91	98.81	102.76
Pop3-80	106.97	111.92	98.78	100.83
Pop3-81	111.9		100.77	
Pop1-20	107.08	112	98.94	100.96
Pop1-21	111.88		102.75	129.09
Pop1-22	111.89		129.18	
Pop1-55	106.96	111.86	98.8	127.76
Pop1-72	106.98	11.87	9875	137.97

Pop2-106	107.01	111.91	102.81	106.46
Pop2-14	107.03	11.87	98.74	100.78
Pop2-17	11.92		102.85	
Pop2-43	11.93		100.89	102.89
Pop2-47	107.06	111.99	100.87	106.51
Pop2-66	111.91		101.81	102.78
Pop2-63	111.97		100.85	102.83
Pop2-69	107		106.37	
Pop2-70	111.92		100.81	102.74
Pop2-82	111.84		100.75	
Pop4-104	111.92		102.9	129.28
Pop4-19	107.03	111.95	98.88	129.3
Pop4-45	107.07	111.92	98.83	129.22
Pop4-46	107.12	111.98	98.94	129.32
Pop4-48	107.91		98.75	
Pop4-54	106.97	111.91	98.74	129.11
pop4-59	106.94	111.87	98.74	129.13
pop5.51	112.3		100.97	
Pop5-52	112.4		100.95	
pop5-53	107.1	112.01	98.86	100.94

Pop 1 = Population 1 obtained by crosses 'Mari de Cenad x SEO'

Pop 2 = Population 2 obtained by crosses 'Sirena x NJA 42'

Pop 3 = Population 3 obtained by crosses between ('Mari de Cenad, x,NJA 17') x 'Kesth Pshor'

Pop 4 = Population 4 obtained by crosses 'Cristal x NJA 21'

Pop 5 = population 5 obtained by crosses between ('Viceroy', x,NJA 2') x 'NJA17'

For the marker 'Ppb0022-195' the region with resistance is situate between 107 / 112 (Tab. 1)

The success of markers detection of PPV was confirmed to depend on the quality and quantity of isolated DNA during the experiments and (Guilford et al., 1997) applied a similar method of isolation in CTAB buffer.

CONCLUSIONS

Targeted SSR analysis is a very direct way to link the molecular markers to a trait of interest. SSR markers developed for specific genome regions have been used to identify genes of agronomic importance for evidence of selection during domestication in maize (Vigouroux et al., 2002) and to verify wheat-barley introgression (Hernandez et al, 2002). They are the most likely candidates for MAS in crops of economical importance and appear to be suitable for apricot as well (Ribaut and Hoisington, 1998; Badenes and Llacer, 2006).

The identifying of a natural source of resistance to PPV, using this resistant source into new crosses with Romanian commercial cultivars

well adapted in our country, and the implement of marker-assisted selection (MAS), based on markers tightly associated with resistance, as a measure to substantially streamline the breeding process, may be a promising strategy to obtain apricot varieties with natural genetic resistance to PPV.

First couple of markers PGS 1.21 (Reverse and Forward) could be enough for the screening of a larger population of apricot and then start to develop the others SSRs associated with PPV resistance to facilitate the use of MAS in Romanian apricot breeding program

ACKNOWLEDGEMENTS

This paper presents data from PhD thesis part of the project POSDRU/107/1.5/S/76888, funded by European Social Fund through the Sectorial Operational Programme Human Resources Development 2007-2013.

REFERENCES

- Audergon J-M., Dosba F., Karayiannis I., Dicenta F., 1994. Amélioration de l'abricotier pour la résistance à la sharka. EPPO Bul 24, p. 741-748.
- Aranzana M., Pineda A., Cosson P., Dirlwanger E., Ascasibar J., Cipriani G., Ryder C., Testolin R., Abbott A., King G., Iezzoni A., Arús P., 2003. A set of simple-sequence repeat (SSR) markers covering the Prunus genome. Theor Appl Genet 106, p. 819-825.
- Badenes M.L., Llácer G., 2006. Breeding for resistance: breeding for Plum pox virus resistant apricots (*Prunus armeniaca* L.) in Spain. Bulletin OEPP/EPPO Bulletin 36, p. 323-326.
- Bassi D., Bellini E., Guerriero R., Monastra F., Pennone F., 1995. Apricot breeding in Italy. Acta Horticulturae 384, p. 47-54.
- Dicenta F., Martínez-Gómez P., Burgos L., Egea J., 2000. Inheritance of resistance to plum poxvirus (PPV) in apricot, *Prunus armeniaca*. Plant Breeding 119, p. 161-164.
- Dosba F., Orlic S., Dutrannoy F., Maison P., Masson G., Audergon J-M., 1992. Evaluation of resistance to plum pox virus in apricot trees. Acta Horticulturae 309, p. 211-220
- Egea J., Burgos L., Martínez-Gómez P., Dicenta F., 1999. Apricot breeding for sharka resistance at the CEBAS-CSIC, Murcia (Spain). Acta Horticulturae 488, p. 153-157.
- Guilford P., Prakash S., Zhu J.M., Rikkerink E., Gardiner S., Bassett H., Forster R., 1997. Microsatellites in *Malus domestica* (apple): abundance, polymorphism and cultivar identification. Theor. Appl. Genet., 94, p. 249-254.
- Hernández P., Dorado G., Cabrera A., Laurie D.A., Snape J.W., Martín A., 2002. Rapid verification of wheat-Hordeum introgression by direct staining of SCARS, STS, and SSR amplicons. Genome 45, p. 198-203.
- Hurtado M.A., Romero C., Vilanova S., Abbott A.G., Llacer G., Badenes M.L., 2002. Genetic linkage map of two apricot cultivars (*Prunus armeniaca* L.) and mapping of PPV (sharka) resistance. Theor Appl Genet 105, p. 182-192.
- Karayiannis I., Mainou A., Tsaftaris A., 1999. Apricot breeding in Greece for fruit quality and resistance to plum pox virus. Acta Horticulturae 488, p. 111-117.
- Karayiannis I., Thomidis T., Tsaftaris A., 2007. Inheritance of resistance to Plum pox virus in apricot (*Prunus armeniaca* L.). Tree Genetics & Genomes DOI 10.1007/s11295-007-0095-z.
- Krška B., Salava J., Polák J., Komínek P., 2002. Inheritance of resistance to plum pox virus in apricot. Plant Protection Sci 38, p. 180-182.
- Krška B., Vachun Z., Necas T., 2005. The apricot breeding program at the Horticulture faculty in Lednice. Acta Horticulturae 717, p. 145-148.
- Lambert P., Dicenta F., Rubio M., Audergon J.M., 2007. QTL analysis of resistance to sharka disease in the apricot (*Prunus armeniaca* L.) 'Polonais' x 'Stark Early Orange' F1 progeny. Tree Genetics & Genomes 3, p. 299-309.
- Martinez-Gomez P., Dicente F., Audergon J-M., 2000. Behavior of apricot (*Prunus armeniaca* L.) cultivars in the presence of sharka (plum pox potyvirus) : A review. Agronomie-Paris 20, p. 407-422.
- Polák J., 1994. Breeding to resistance to plum pox potyvirus in the Czech Republic. EPPO Bul 24, p. 781-782.
- Polák J., Oukropec I., Komínek P., Krška B., Bittóva M., 1997. Detection and evaluation of resistance of apricots and peaches to plum pox virus. J Plant Dis Protection 104, p. 466-473.
- Ribaut J.M., Hoisington D., 1998. Marker-assisted selection: new tools and strategies. Trends Plant Sci 3, p. 236-239
- Sicard O., Marandel G., Soriano J.M., Lalli D.A., Lambert P., Salava J., Badenes M., Abbott A., Decroocq V., 2007. Flanking the major Plum pox virus resistance locus in apricot with co-dominant markers (SSRs) derived from candidate resistance genes. Tree Genetics & Genomes DOI 10.1007/s11295-007-0114-0.
- Vigouroux Y., McMullen M., Hittinger C.T., Houchins K., Schulz L., Kresovich S., Matsuoka Y., Doebley J., 2002. Identifying genes of agronomic importance in maize by screening microsatellites for 416 Tree Genetics & Genomes (2008) 4, p. 403-417 evidence of selection during domestication. Proc Natl Acad Sci USA 99:9650-965.
- Yang Z-N., Ye X-R., Molina J., Roose M.L., Mirkov T.E., 2003. Sequence analysis of a 282-kilobase region surrounding the Citrus Tristeza Virus resistance gene (Ctv) locus in *Poncirus trifoliata* L. Raf. Plant Physiol 131, p. 482-492.

Zhebentyayeva T.N., Reighard G.L., Krška B., Gorina V.M., Abbott A.G., 2002. Origin of resistance to plum pox virus in apricot: microsatellite (SSR) data analysis. *Plant Protection Sci* 38, p. 117–121.

Vilanova S., Romero C., Abernathy D., Abbott A.G., Burgos L., Llácer G., Badenes M.L., 2003b.

Construction and application of a bacterial artificial chromosome (BAC) library of *Prunus armeniaca* L. for the identification of clones linked to the self incompatibility locus. *Mol Genet Genomics* 269:685–691.

