Origin of Resistance to *Plum Pox Virus* in Apricot: Microsatellite (SSR) Data Analysis

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Abstract

The objective of this study was to establish the genetic relationship among cultivars commonly used as donors for resistance to *Plum pox virus* (PPV) in order to identify the putative sources of resistance to PPV. The plant material tested represented the European, Central Asian and Chinese ecogeographical groups of cultivated apricots as well as the wild Dzhungar-Zailij population of *Prunus armeniaca* L. Forty-eight native accessions as well as the resistant (or tolerant) cultivars Harlayne, Stark Early Orange (SEO), Goldrich, Vestar and two hybrid forms Vestar × SEO (LE 3276) and Velkopavlovická × SEO (LE 2904) were screened by means of SSR analysis. To elucidate genetic relationships among apricot germplasm, a dendrogram was produced using neighbor joining (NJ) analysis of Nei's pair-wise genetic distances over 14 polymorphic SSR markers. On the dendrogram, resistant cultivars were separated into two different clusters suggesting two different sources of resistance to PPV. As was expected from pedigrees, SEO, Vestar, LE 2904 and LE 3276 were grouped together in a cluster adjacent to the European cultivars. Resistant cultivars Harlayne and Goldrich were within another group containing Central Asian apricots and Dzhungar-Zailij form.

Keywords: sharka; *Prunus armeniaca*; microsatellite markers; genetic similarity

INTRODUCTION

Plum pox virus (PPV) infection (or sharka) is the most important disease of *Prunus* species having serious economic impact on apricot production. PPV was first identified by ATANASSOV (1932) in Bulgaria; from there it has spread throughout Europe and around the Mediterranean basin and was recorded in North America and Chile at the end of the last century (ROY & SMITH 1994). In all apricot growing countries, breeding programs, aimed at enhancing resistance to PPV infection in commercial cultivars, were prompted to find sources of disease resistance (KARAYIANNIS *et al*. 1991; AUDERGON *et al*. 1994; BASSI *et al*. 1995; EGEA *et al*. 1999; POLÁK 1994). As a result of intensive research, several North American cultivars of unknown pedigree (Stark Early Orange, Goldrich, Harlayne, Stella) were recognized as immune or tolerant. They were utilized in hybridization with the best of the European commercial cultivars susceptible to virus; and resistant offspring were tested for field performance.

Apricot germplasm distributed worldwide was classified into four main ecogeographical groups with regional subgroups (Central Asian, Irano-Caucasian, European, Dzhungar-Zailij) on the basis of morphological and physiological traits (KOSTINA 1964). North Chinese and East Chinese groups were added recently to the classification (LAYNE *et al*. 1996). Cultivars originating from the United States and Canada represent the North American subgroup within the European group.

Recently, isozymes and AFLP markers were employed to investigate genetic diversity in European and North American apricot cultivars involved in resistance breeding to PPV (BADENES *et al*. 1996; HURTADO *et al*. in press). It was concluded that resistant cultivars are related to European cultivars, but they might share a common ancestral donor of sharka resistance different from that of the European group (HURTADO *et al*. in press).

The objectives of our study were: (1) to investigate the genetic similarity of cultivars involved in breeding for PPV resistance in a set of native cultivars representing all eco-geographical groups of cultivated apricot; and (2) to clarify the genetic relationships of cultivars with respect to PPV resistance.

MATERIALS AND METHODS

Plant material

Forty-eight native apricot accessions representing the European, Chinese and Central Asian ecogeographical groups of cultivated apricot and six wild Dzhungar-Zailij forms belonging to the wild population of *Prunus armeniaca* L. were evaluated (Table 1). The group of cultivars involved in resistance breeding to PPV ("resistant group") was comprised of the North American cultivars Goldrich, Harlayne, Stark Early Orange (SEO), Vestar and two hybrid forms Vestar × SEO (LE 3276) and Velkopavlovická × SEO (LE 2904). Vestar was certified as susceptible; Goldrich, SEO and LE 2904 as tolerant; Harlayne and LE 3276 as immune to PPV infestation (POLÁK *et al*. 1997).

Table 1. Cultivars and forms included in this study

European	**Central Asian/Fergana**	**Cultivars and forms involved in breeding for resistance to PPV**
Ananasnyi Tsuryupinskii	Samyi Rannii	Goldrich (USA)
Velkopavlovická	Kok-Pshar	Harlayne (USA)
Vengerskii Krupnyi	Supkhani	Stark Early Orange (USA)
Krasnoshchekii	Kandak-10	Vestar (Czech)
Nakhichevanskii (Azerbajdzhan)	Kandak-12	LE 2904 (Czech)
Yubilejnyi	Khurmai	LE 3276 (Czech)
Alberge de Tur		
Tilton	Kzyl Khurmai Kannibadam	
Luizet Krupnoplodnyi	Oranzhevo-krasnyi	
Kantsler	Mirsandzhali	
Early Gold	Kolon Boboi	
De Compot	Kzyl Uryuk	
Precoce d'Italia	Tadzhabai	
Real d'Imola		
Vynoslivyi	**Central Asian/Khorezm**	
Bergeron	Nukul Citronnii	
	Kzyl Horezmskii	
Chinese	Paivandi Bukharskii	
Da-chuan-che N1		
Da-chuan-che N2	**Dzhungar-Zailij**	
Yuan-sin	Dzhungarskii 18/55	
Kitaiskii	Dzhungarskii 18/63	
Shantungskii	Dzhungarskii 18/64	
Da-bei	Dzhungarskii 18/68	
Mai-che-sin	Dzhungarskii 18/71	
In-ben-sin	Dzhungarskii 18/75	
Lao-yech-lian		
Pui-sha-sin		
Mi-Bada		

DNA extraction and SSR analysis

Total DNA was extracted according to modified CTAB protocol (SIVOLAP *et al*. 1998). Twelve primer combinations, developed from peach enriched genomic libraries and BAC libraries, were used for PCR amplification. These detected 14 polymorphic SSR loci (pchgms 3; pchcms 5-1; pchgms 10-1; pchgms 10-2; pchgms 11 F1, R1; pchgms 12; pchgms 14; pchgms 17; pchgms 20 F1, R1; pchgms 20 F1, R2; pchgms 26 F2, R2-1; pchgms 26 F2, R2-2; UPD 96-001; UPD 98-406) with apricot DNA. PCR amplification and characteristics of SSR loci in apricot were described previously (ZHEBENTYAYEVA *et al*. 2002). Radio-labeled PCR products were separated on 6% sequencing acrylamide gels with 7.5 M urea and exposed to X-ray films (Biomax MR, Kodak) for 3–5 days.

Data analysis

SSR allelic composition was determined for each accession. Pair-wise genetic distances among accessions were calculated, using the program MICROSAT (MINCH 1997). PHYLIP package version 3.5c (FELSENSTEIN 1989) was employed for cluster analysis with a neighbor joining (NJ) algorithm. A dendrogram was constructed, using the program TREEVIEW (PAGE 1996).

RESULTS

SSR analysis had been previously used for assessment of genetic variability in apricot germplasm, both between and within ecogeographical groups (ZHEBENTYAYEVA *et al*. 2002). The approach was followed to estimate genetic similarity of the cultivars involved in breeding for resistance to PPV. The North American PPV resistant cultivars and their hybrids were added to the European group for a combined analysis with a plant material representing Chinese and Central Asian (Fergana valley) centers of apricot domestication. Twenty six accessions were deleted because mutual enrichment of germplasm originating from two primary centers of origin was revealed in the Irano-Caucasian group, the Zeravshan and Kopet-Dag subgroups.

Cluster analysis of 54 accessions generated a dendrogram with three main groups subdivided into seven clusters (Figure 1). The structure of the dendrogram did not depend on an input order of the samples. Most Fergana cultivars and Dzhungar–Zailij forms were in the first and second groups (1c, 2a, 2b). Chinese cultivars were grouped predominantly in two clusters distributed distantly (1d and 3). European cultivars were scattered across the dendrogram in groups of 1a, 1c, 2b, and the 2b containing the North American cultivar Tilton together with Real d'Imola, Precoce d'Italia and Kantsler. The cultivars involved in breeding for resistance to PPV were separated in two different clusters in the first group. SEO, Vestar, LE 2904 and LE 3276 formed a separate cluster adjacent to European cultivars belonging to the widely distributed genotypes Bergeron – Ananasnyi Tsuryupinskii. North American resistant cultivars Goldrich and Harlayne were within another cluster containing Central Asian (Fergana subgroup) apricots, one of the Dzhungar-Zailij forms and three European cultivars (Vynoslivyi, Early Gold, De Compot), which displayed some traits specific to the Central Asian group (ZHEBENTYAYEVA *et al*. 2002).

DISCUSSION

The history of apricot domestication and dissemination purportedly by Roman soldiers lead FAUST *et al*. (1998) to hypothesize three possible routes of entry into Europe: (1) the northern route from China to the Balkans; (2) the southern route from Armenia through the Near-Eastern region to Greece, Italy and Northern Africa; and (3) a middle route from the Danube valley to Germany (FAUST *et al*. 1998). Distribution of the European cultivars within distant clusters on the SSR-dendrogram is in agreement with this speculation. European cultivars representing the western line, namely, the 'Hungarian apricot' group – Nancy – Royal, Blenheim and Moorpark were thought to be the genetic basis for apricot breeding in the North America. A genetic relatedness among the North American cultivars and European group was confirmed by isozymes and RFLP genotyping (BADENES *et al*. 1996; DE VICENTE *et al*. 1998). Moreover, these authors have found some specific alleles in North American germplasm testifying to introgression of non-European sources for PPV resistance.

As established previously, European cultivars Bergeron, Luizet Krupnoplodnyi, Krasnoshchekii, Yubilejnyi, Velkopavlovická, Vengerskii Krupnyi (= Hungarian Best) shared identical genotypes for isozyme, SSR and AFLP loci and are likely synonymous (ZHEBENTYAYEVA *et al*. 2002). In this study, the position of cultivars involved in breeding for resistance to PPV within the first group supported the importance of 'Hungarian apricots' in forming of the North American contingent. Cultivars involved in resistance breeding to PPV were distributed accord-

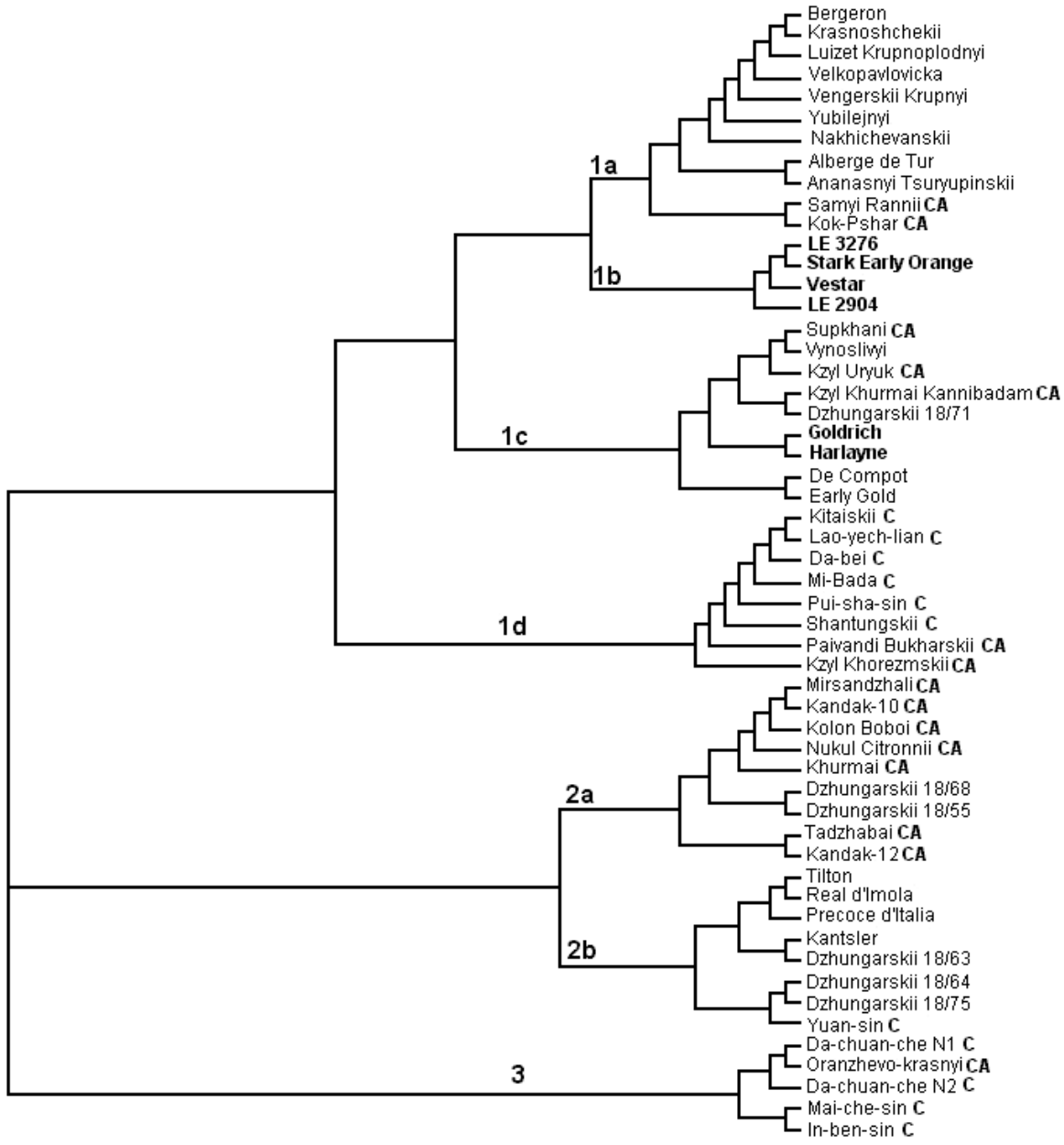

Figure 1. Neighbor joining dendrogram of genetic similarity 54 apricot accessions based on the data of 14 SSR loci. Central Asian (**CA**) and Chinese (**C**) cultivars are indicated

ing to known pedigree. Resistant cultivars Goldrich and Harlayne have a common parental variety Sun Glo (BROOKS & OLMO 1997), and the relatedness of SEO, Vestar, LE 2904 and LE 3276 is documented (POLÁK *et al*. 1997).

Little is known about non-European sources of resistance to PPV in the North American germplasm. Basing on isozyme analysis, BADENES *et al*. (1996) deduced that the Central Asian ecogeographical group is the most likely source of resistant genes in North American donors. On the SSR-dendrogram represented here, positioning of cultivars Goldrich and Harlayne close to the native Fergana forms supported this idea. However clustering of SEO and the remaining accessions from the "resistant group" did not clearly support this hypothesis. Separation of SEO (and LE 3276) from Goldrich and Harlayne on the dendrogram might indicate different ancestral sources of PPV resistance. SEO and some others cultivars of American origin possess most of the rare SSR alleles

and also displayed genetic similarity with the Chinese cultivar Pui Sha Sin (HORMAZA 2002). In our preliminary study, an implication of Chinese apricots in the pedigree of SEO and related cultivars was predicted from isozyme profiling of the accessions under study (data not published). However, the limited number of markers restricts the detecting ability of SSR and isozyme analyses to verify introgressions that have taken place some generations before. Technologies displaying significant more polymorphic loci such as AFLP analysis are currently being pursued to find the specific sources that contribute to PPV resistance in North American cultivars.

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