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NEW APPROACHES FOR MAPPING PPV (*PLUM POX VIRUS*) RESISTANCE IN APRICOT (*PRUNUS ARMENIACA*)[1]

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Abstract

In this study, we have mapped the PPV (*Plum pox virus*) resistance trait as a QTL using a non-parametric mapping method based on the Kruskal-Wallis test. To increase the reliability of this analysis new SSR markers from apricot and peach were incorporated to the F_2 'L×L' linkage map derived from a self-pollination of the resistant cultivar 'Lito'. Several tightly linked AFLP markers, located at the upper region of the linkage group 1, showed high significance levels of the Kruskal-Wallis test statistic, indicating the presence of a putative QTL for the PPV resistance trait.

Key words: PPV, resistance, SSR markers, Kruskal-Wallis test

Introduction

Sharka disease was detected in Spain for the first time in 1984. Since then, the disease spread throughout the country affecting the apricot growing seriously, because all native cultivars are susceptible to the *Plum pox virus*. Consequently, several eradication programs were started, and to date more than one million trees have been removed with a cost higher than 8 million euros. However, the results were not satisfactory and this promoted the development of an apricot-breeding program at the IVIA in 1993, aimed to introduce PPV resistance in native cultivars from North American sources. In addition, two mapping projects were developed

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in order to facilitate the search for molecular markers linked to the PPV resistance in the future (Hurtado et al. 2002, Vilanova et al. 2003). In this work we have introduced new microsatellite markers in the 'Lito' × 'Lito' map (Vilanova et al. 2003) and mapped the PPV resistance as a QTL using a non-parametric approach.

Material and methods

Plant material

An apricot intraspecific F_2 population (N = 76) generated by self-pollination of the cultivar 'Lito', derived from a cross between 'Stark Early Orange', a North American cultivar resistant to PPV, and 'Thyrintos', a Greek susceptible cultivar. This progeny is maintained at the IVIA.

SSR markers

19 SSR markers from apricot (Lopes et al. 2002, Vilanova et al. 2004, Abernathy et al. 2004) and peach (Dirlewanger et al. 2002, Yamamoto et al. 2002) were amplified in the 'Lito' × 'Lito' population. PCRs were performed in a GeneAmp PCR System 9700 thermal cycler (Perkin-Elmer Corp. Calif., USA) following Aranzana et al. (2002). PCR products were separated by electrophoresis in 3% Metaphor-agarose stained with ethidium bromide (0.8 mg/ml) and visualized using UV light.

Evaluation of sharka resistance

Phenotyping of the 'Lito' × 'Lito' progeny had been previously performed according to the Moustafa et al. (2001) biological test, ELISA-DASI and RT-PCR analyses as described by Vilanova et al. (2003).

Linkage analysis

Markers were added to the 'Lito' × 'Lito' ('L×L') map maintaining the framework (fixed order) of the linkage groups established at LOD score 5.0 (Vilanova et al. 2003). The linkage analysis was carried out using Joinmap 3.0 software (Van Ooijen and Voorrips 2001).

Kruskal-Wallis test

The non-parametric mapping method used was based on the rank sum test of Kruskal-Wallis and performed with the MapQTL version 3.0 software (Van Ooijen and Maliepaard 1996).

Results

A total of 19 new SSR markers from different sources showing polymorphism in the 'Lito' × 'Lito' progeny were incorporated to the map (Table 1).

Linkage analysis performed using JoinMap 3.0 software located these markers at the G1, G4, G5, G6, G7 and G8 linkage groups (Table 1). Photo 1 shows the microsatellite marker ssrPaCITA7 segregating in the 'Lito' × 'Lito' population.

The Kruskal-Wallis test was performed for all the markers of the 'L×L' map including those SSRs introduced in this study. Only markers located at the upper part of the linkage group G1 were significant ($P < 0.01$) for the Kruskal-Wallis test statistic (Table 2) suggesting the presence of a putative QTL for the PPV resistance trait in a region between 12.0 and 38.4 cM. Interestingly, all the dominant AFLP markers showing high significance levels come from the resistant parent 'Stark Early Orange' (Table 2).

Phot. 1. Segregation of the microsatellite marker ssrPaCITA7 in the 'Lito' × 'Lito' population. Samples are from left to right: M (100 bp molecular weight marker), individuals from 1 to 18 of the 'Lito' × 'Lito' population, S ('Stark Early Orange'), T ('Thyrintos') and L ('Lito') (photo by J.M. Soriano)

Table 1

Summary of SSRs added to 'L×L' map

SSR marker	Origin	Fragment size (bp)	Linkage group	Reference
SsrPaCITA5	Apricot	220–330	G1	Lopes et al. 2002
SsrPaCITA7	Apricot	195–210	G1	Lopes et al. 2002
SsrPaCITA14	Apricot	170–180	G4	Lopes et al. 2002
SsrPaCITA15	Apricot	195–210	G8	Lopes et al. 2002
SsrPaCITA17	Apricot	175–200	G1	Lopes et al. 2002
SsrPaCITA21	Apricot	200–220	G5	Lopes et al. 2002
MA010a	Peach	125–130	G7	Yamamoto et al. 2002
BPPCT009	Peach	155–190	G6	Dirlewanger et al. 2002
BPPCT012	Peach	155	G8	Dirlewanger et al. 2002
widctlparBPPCT037	Peach	120–125	G5	Dirlewanger et al. 2002
BPPCT038	Peach	160	G5	Dirlewanger et al. 2002
aprigms1	Apricot	220–250	G1	Vilanova et al. 2004
aprigms2	Apricot	165–185	G1	Vilanova et al. 2004
aprigms3	Apricot	260–270	G1	Vilanova et al. 2004
aprigms8	Apricot	180–205	G1	Vilanova et al. 2004
aprigms10	Apricot	280–295	G1	Vilanova et al. 2004
aprigms16	Apricot	220–230	G1	Vilanova et al. 2004
aprigms18	Apricot	200–210	G1	Abernathy et al. 2004

Table 2

Kruskal-Wallis statistic adjusted for ties (K*) for those markers located at the 'L×L' map G1 linkage group coming from the resistant parent 'Stark Early Orange'

Map (cM)	Locus	No.	K*	Df	Sl
0.0	CPPCT-16	72	0.029	2	
12.0	EAT-MCTT (15)	81	7.801	1	***
16.8	aprigms18	78	8.606	2	**
18.4	EAA-MCTA (1)	77	12.039	1	*****
20.1	EAT-MCTC (12)	78	9.625	1	****
21.3	EAA-MCAG (10)	79	6.470	1	**
23.7	EAG-MCTT (1)	76	20.570	1	*******
24.9	EAT-MCTT (10)	81	15.926	1	*******
26.2	EAG-MCAC (7)	80	9.411	1	****
27.2	CITA5	78	13.541	2	****
28.3	CPPCT-27	72	5.513	2	*
29.2	EAC-MCAG (2)	71	7.905	1	****
30.3	EAT-MCTG (3)	73	12.064	1	*****
30.8	aprigms24	73	9.052	2	**
33.0	EAT-MCTC (9)	78	12.708	1	*******
34.7	EAA-MCAC (6)	77	6.080	1	**
35.7	EAT-MCAA (4)	80	6.668	1	***
36.3	CITA17	80	6.585	2	**
36.9	EAA-MCTT (7)	77	6.634	1	**
37.7	96-005 (2)	80	6.585	1	**
38.4	EAC-MCAT (13)	79	8.131	1	****
39.8	EAC-MCAT (7)	78	2.525	1	
40.7	aprigms1	81	1.601	2	
41.7	EAT-MCAG (14)	80	6.585	1	**
43.7	aprigms2	81	2.702	2	
46.2	CPPCT-3	70	2.190	2	
48.1	aprigms3	81	4.800	2	*
51.9	aprigms8	81	7.332	2	**
53.6	EAA-MCAG (2)	78	3.465	1	*
54.8	EAT-MCAC (11)	76	3.899	1	**
55.7	aprigms10	81	3.846	2	
56.8	EAC-MCAT (11)	79	1.831	1	
58.0	CITA7	81	2.395	2	
60.5	EAT-MCAC (8)	76	1.792	1	
63.3	EAA-MCAT (8)	78	3.063	1	*
65.1	CPPCT-26	73	2.775	2	
66.4	EAT-MCTC (13)	78	1.119	1	

Table 2 – cont.

Map (cM)	Locus	No.	K*	Df	Sl
73.3	aprigms16	81	5.125	2	*
78.1	CPPCT-19	70	1.340	2	
88.9	EAC-MCTC (1)	79	0.782	1	
89.3	EAT-MCAC (5)	76	1.452	1	
90.0	EAA-MCTC (1)	77	2.685	1	
93.9	EAG-MCAC (1)	80	2.201	1	
93.9	EAA-MCTA (9)	79	1.623	1	
101.8	EAA-MCTA (16)	78	2.104	1	

No. – number of individuals, Df – degrees of freedom, Sl – significance levels: * – 0.1, ** – 0.05, *** – 0.01, **** – 0.005, ***** – 0.001, ****** – 0.0005, ******* – 0.0001.

Discussion

The inheritance of the PPV resistance in apricot has been a source of controversy for the last years. Since the beginning of the 90's, several hypotheses have been proposed to explain the control of the resistance trait under monogenic, digenic or even polygenic models. Nevertheless, none of them has been generally accepted.

Many disease-susceptible traits are rather qualitative traits, and usually binary response variables, with a polygenic basis. However, in many cases the phenotypes of these traits are not normally distributed, and under this assumption the standard approach to QTL mapping can behave poorly. Several alternative procedures to QTL mapping have been described. The non-parametric mapping method based on the rank sum test of Kruskal-Wallis allows mapping QTLs when a spike in the phenotype distribution occurs and therefore the usual normality assumption cannot be made.

Consistent with this model, the phenotype of the PPV resistance trait is not normally distributed and also shows a binary response, resistance versus susceptibility, pointing out the suitability of the non-parametric method for mapping PPV resistance. This analysis showed that all markers of the 'L×L' map with high significance levels ($P < 0.01$) of the Kruskal-Wallis test statistic are located at the upper part of the G1 linkage group, suggesting that PPV resistance is controlled by at least one major QTL.

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