Identification and mapping of a locus conferring *Plum Pox Virus* resistance in two apricot improved linkage maps

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ABSTRACT

Sharka disease, caused by the *Plum Pox Virus* (PPV), is one of the major limiting factors for stone fruit crops in Europe and America. In particular, apricot is severely affected suffering significant fruit losses. Thus, PPV resistance is a trait of great interest for the apricot breeding programs currently in progress. In this work, two apricot maps, earlier constructed with the F_1 'Goldrich x Currot' ('GxC') and the F_2 'Lito x Lito'-98 ('LxL-98') populations, have been improved including 43 and 37 new simple sequence repeat (SSR) loci, respectively, to facilitate PPV resistance trait mapping. Screening of PPV resistance on the segregating populations classified seedling phenotypes into resistant or susceptible. A non-parametric mapping method, based on the Kruskal-Wallis (KW) rank sum test, was initially used to score marker-trait association and results were confirmed by Interval Mapping. Contrary to the putative digenic model inferred from the phenotypic segregations, all significant markers for the KW statistic

(P< 0.005) mapped in a unique region of ~21.0 and ~20.3cM located on the upper part of the LG1 linkage group in 'GxC' and 'LxL-98' maps, respectively. According to the data, PPV resistance is suggested to be controlled by at least one major dominant locus. The association between three SSRs, distributed within this region, and PPV resistance was tested in two additional populations ('Goldrich x Canino' and 'Lito x Lito'-00) and breeding program parents. The marker ssrPaCITA5 showed the highest KW value (P<0.005) in all cases pointing out its usefulness in marker-assisted selection.

Keywords: Apricot linkage map, Marker-Assisted Selection (MAS), PPV resistance

INTRODUCTION

Sharka disease, caused by the *Plum Pox Virus* (PPV), was detected for the first time in Spain in 1984 (Llácer et al. 1985). Since then, it spread throughout the country seriously affecting apricot cultivation as all native cultivars were susceptible to PPV. Attempts to block the spread of the disease through eradication of the infected trees were unsuccessful. In order to solve this problem in the long term, two breeding programs aimed at introducing PPV resistance from apricot sources, were initiated in Spain (Egea et al. 1999; Badenes et al. 2002), as was done earlier in France (Audergon 1995), Italy (Bassi et al. 1995) and Greece (Karayiannis et al. 1999). A few promising resistant selections have already been obtained by conventional breeding (Badenes et al. 2002), however the PPV resistance screening method used is very time consuming and significantly diminishes the selection efficiency being a 'bottle neck' in the selection process. This screening method is based on a biological test that uses susceptible 'GF-305' peach seedlings, as woody indicators of disease, and involves several cold cycles followed by a Double-Antibody Sandwich Indirect Enzyme-Linked Immunosorbent Assay (ELISA-DASI) (Moustafa el al. 2001).

50 In this context, marker-assisted selection (MAS) for PPV resistance would improve
51 breeding efficiency. Among the different strategies, to search for trait-linked markers
52 genetic maps are one of the most convenient tools. Three *Prunus* linkage maps, based
53 on apricot intraspecific crosses introducing PPV resistance from the North American
54 cultivars 'Goldrich' (Hurtado et al. 2002) and 'Stark Early Orange'('SEO') (Salava et
55 al. 2002; Vilanova et al. 2003), have been used to analyze PPV resistance. Hurtado et al.
56 (2002) and Vilanova et al. (2003) tentatively mapped the PPV resistance trait in the
57 linkage group 1 (LG1) of the F_1 'Goldrich x Currot' ('GxC') and the F_2 'Lito x Lito'-98
58 ('LxL-98') population maps, respectively. A similar location was obtained by Salava et
59 al. (2002) in the BC_1 population map 'LE-3246 x Vestar' where *PPVres1* was flanked
60 by two AFLP-markers (EAA-MCAG8 and EAG-MCAT14) in a region spanning ~9.3
61 cM. Additionally, Decroocq et al. (2005) performed a quantitative trait loci (QTL)
62 analysis for PPV resistance on a F_1 population derived from the interspecific cross
63 *Prunus persica* x *Prunus davidiana*, where resistance was introduced from the *P.*
64 *davidiana* clone P1908. Up to six PPV resistance QTLs were identified on this map and
65 two of them (*PPV-1.1* and *PPV-1.2*) were shown to be located on linkage group 1.
66 In the three apricot populations analyzed, PPV resistance was mapped as a single-
67 locus controlled trait. However, the segregation ratio obtained in these cases deviated
68 significantly from that expected for a single dominant locus and more closely
69 approximated that for two dominant independent loci (Hurtado et al. 2002; Salava et al.
70 2002; Vilanova et al. 2003). Thus, these results supported the hypothesis of a digenic
71 inheritance proposed by Dosba et al. (1991) in contrast with the monogenic control
72 proposed by Dicenta et al. (2000) and Karayannis et al. (2006). In addition, as reported
73 above, QTL analysis for PPV resistance on *P. davidiana*, based on an ordinal
74 phenotypic scale to assess PPV infection, also presented evidence supporting the

75 involvement of several loci in the control of the trait (Decrooq et al. 2005).
76 Nevertheless, the apricot response to PPV infection could only be rated on a binary
77 scale (resistant vs. susceptible) (Salava et al. 2002; Vilanova et al. 2003). The standard
78 QTL mapping approaches assume polygenic inheritance and can behave poorly for
79 binary traits. Several adaptations have been proposed to estimate genetic distance
80 between markers and binary trait locus (BTL) including non-parametric methods
81 (McIntyre et al. 2001).

82 In this paper, we report the detection and location of a genomic region associated
83 with PPV resistance scored as binary trait. This region was located on very similar
84 positions in two improved apricot maps derived from the F_1 'GxC' and the F_2 'LxL-98'
85 populations. Several markers linked to PPV resistance were identified and tested for
86 MAS in a set of susceptible/resistant apricot cultivars and two additional segregating
87 populations.

88 **MATERIALS AND METHODS**

89 **Plant material**

90 Two families were used for mapping PPV resistance (Table 1), an F_1 resulting from
91 the cross 'Goldrich x Currot' ('GxC') and an F_2 derived from the self-fertilization of the
92 PPV resistant cultivar 'Lito' ('LxL-98'), earlier used to construct two apricot linkage
93 maps (Hurtado et al. 2002; Vilanova et al. 2003). 'GxC' was previously described by
94 Hurtado et al. (2002) as 'GxV' progeny and 'LxL-98' as 'LxL' by Vilanova et al.
95 (2003). To analyze SSRs selected for MAS, two additional segregating populations
96 were used, 'Goldrich x Canino' ('GxCa') derived from the cross between the PPV
97 resistant cultivar 'Goldrich' and the PPV susceptible cultivar 'Canino' and an extension
98 of the 'LxL-98' family designated as 'LxL-00'. A total of 7 PPV resistant ('SEO',
99 'Lito', 'Goldrich', 'Harcot', 'Sunglo', 'Veecot' and 'Pandora') and 11 PPV susceptible

100 apricot cultivars ('Tyrinthos', 'Currot', 'Ginesta', 'Canino', 'Mitger', 'Palau',
101 'Bergeron', 'Katy', 'Pepito', 'Moniquí' and 'Colorao') (Martínez-Gómez et al. 2000)
102 were also used to test those SSRs selected for MAS.

103 **DNA isolation**

104 DNA was extracted from 50 mg of young leaves following the method of Doyle and
105 Doyle (1987). DNA quantification was performed by comparison with lambda DNA
106 (Promega, Madison, WI).

107 **Screening for PPV resistance**

108 Evaluation of PPV resistance in the studied families was performed according to the
109 biological test described by Moustafa et al. (2001) using the PPV Dideron strain 3.3 RB
110 (Asensio 1996) and 'GF-305' peach seedlings as woody indicators. The virus presence
111 was analysed by visual scoring of symptoms and confirmed by ELISA-DASI (Lommel
112 et al. 1982) using the 5B-IVIA monoclonal antibody against the PPV coat protein
113 (Cambra et al. 1994). A total of twelve replications of each seedling were evaluated.
114 RT-PCR analyses were performed occasionally to verify uncertain results (Wetzel et al.
115 1991). Phenotypic scoring based on presence/absence of leaf symptoms classified
116 seedlings into susceptible (0)/resistant (1).

117 **SSR markers**

118 A total of 170 and 150 SSRs from peach and apricot were screened in the 'GxC' and
119 the 'LxL-98' populations, respectively (Table 1 Suppl.). SSR amplifications were
120 performed in a GeneAmp®PCR System 9700 thermal cycler (Perkin-Elmer Corp.,
121 Freemont, CA) in a final volume of 10 µl containing 75 mM Tris-HCl pH 8.8, 20 mM
122 $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.8 µM of each primer, 20 ng of
123 genomic DNA and 1 Unit of Taq polymerase (Invitrogen, Carlsbad, CA) using the
124 following temperature profile: 94ºC for 2 min, then 35 cycles of 94ºC for 45 s, 50-60ºC

125 for 1 min and 72ºC for 1 min and 15 s, finishing with 72ºC for 5 min. PCR products
126 were separated by electrophoresis in 3% (w/v) MS-8 agarose (Pronadisa, Madrid,
127 Spain).

128 **Linkage groups**

129 The linkage analysis was carried out using JoinMap 3.0 software (Van Ooijen and
130 Voorrips 2001) with the Kosambi mapping function (Kosambi 1944) used to convert
131 recombination units into genetic distances.

132 In the 'GxC' population two separated genetic linkage maps were constructed for
133 each parent following the "two-way pseudo test-cross" model of analysis (Grattapaglia
134 and Sederoff 1994) and setting a "cross-pollinator" data type. The original maps
135 (Hurtado el al. 2002) were updated by adding 43 new SSR markers. In the 'LxL-98'
136 population the linkage analysis was carried out setting F_2 data type and the original map
137 (Vilanova et al. 2003) was updated by adding 37 new SSR markers. Linkage groups
138 were established using as threshold a minimum logarithm of odds (LOD) of 5.0. In
139 general, linkages considered for mapping were those with recombination frequency
140 lower than 0.4 and LOD score larger than 3.0.

141 **Statistical analysis and BTL identification**

142 Binary trait analysis was performed on the two different updated maps ('GxC' and
143 'LxL-98') using MapQTL version 4.0 (Van Ooijen et al. 2000). Since PPV resistance
144 scorings were not normally distributed, the Kruskal-Wallis (KW) rank-sum test
145 (Lehmann 1975), with a threshold value of $P<0.005$, was first applied individually to
146 each segregating locus to test for associations between markers and PPV resistance.
147 Subsequently, Interval Mapping (IM) analysis (Lander and Botstein 1989; Van Ooijen
148 1992) was performed in order to support the detection of putative BTLs by KW test.
149 The LOD chromosome-wide significance threshold to decide upon the presence or

150 absence of a BTL for IM (Van Ooijen 1999) was determined with a 5% significance
151 level by using permutation tests (Churchill and Doerge 1994) carried out on LG1 and
152 corresponded to a value of 3.0 in both maps. A confidence interval around the position
153 of the largest LOD was indicated by two-LOD support interval (Van Ooijen 1992).

154 Putative double recessive epistatic interactions between ssrPaCITA5 and other co-
155 dominant markers were assessed with the KW test (P<0.05) and multiple comparisons
156 (Dunn 1964) considering differences among the four genotype groups categorized by
157 two SSRs (A-B-/A-Bb/aaB-/aabb).

158 **RESULTS**

159 **'GxC' and 'LxL-98' improved linkage maps**

160 In order to facilitate the identification and location of loci associated with PPV
161 resistance, new SSRs were incorporated into two apricot linkage maps earlier
162 constructed with the intraspecific populations F_1 'GxC' (Hurtado et al. 2002) and F_2
163 'LxL-98' (Vilanova et al. 2003).

164 A total of 170 SSRs from different sources were tested in the 'GxC' progeny (Table
165 1 Suppl.) and 148 out of them (87%) could be amplified. Fifty-five out of amplified
166 SSRs were polymorphic (37%), the rest were monomorphic or produced complex
167 patterns. Fourteen out of these polymorphic SSRs were heterozygous in both parents, 26
168 only in 'Goldrich' and 15 only in 'Currot'.

169 Thirty-seven SSR loci were incorporated into the 'Goldrich' map and distributed
170 throughout the genome, ranging from eight markers in LG1 to one marker in LG3
171 (Table 2 Suppl.). Twelve out of them correspond to co-dominant loci and 25 to
172 dominant loci. Two additional SSRs tested by Hurtado et al. (2002) but unmapped in
173 the previous map, UDP98-409 and UDP98-412 (Cipriani et al. 1999), were also
174 incorporated into the 'Goldrich' map. Four markers (10.5%) deviated significantly from

175 the expected F_1 segregation ration at $P<0.01$. The improved 'Goldrich' map is
176 organized in 8 linkage groups covering a distance of 468cM and comprising 139 loci:
177 63 AFLPs, 48 SSRs, 25 RAPDs and 3 RFLPs (Figure 1). The average distance between
178 adjacent markers was 3.4cM, ranging from 2.4cM in LG6G to 6.3cM in LG8G.
179 Seventeen SSR loci were incorporated into the 8 linkage groups of the 'Currot' map,
180 ranging from one marker in LG2 and LG4 to 5 markers in LG5 (Table 2 Suppl.). Ten
181 resulted in co-dominant loci and 7 in dominant loci. Five markers (29%) showed
182 skewed segregation ratios at $P<0.01$. The 'Currot' map covers a distance of 451cM and
183 comprises 89 loci: 42 AFLPs, 26 SSRs, 21 RAPDs and 1 RFLP (Figure 1). The average
184 distance between adjacent markers was 5.1cM, ranging from 3.0cM in LG8C to 7.0cM
185 in LG4C.
186 Fifteen SSRs heterozygous in both parents provided bridges between the two maps. In
187 addition, part of the mapped SSRs allowed us to establish homologies with other
188 *Prunus* maps: twenty-three were held in common with the *Prunus* reference map
189 derived from the almond-peach cross 'Texas' x 'Earlygold' (Dirlewanger et al. 2004a),
190 37 with the map of Dirlewanger et al. (2004b) obtained from a myrobolan plum x
191 [almond x peach] progeny and 23 with the peach map of Yamamoto et al. (2005).
192 A total of 150 SSRs from peach and apricot were tested in the 'LxL-98' progeny
193 (Table 1 Suppl.) and segregation was demonstrated for 47 (31%) of them. The rest of
194 SSRs could not be amplified, produced complex patterns or were monomorphic. From
195 the 47 polymorphic SSRs, 37 were mapped in the 'LxL-98' map (Table 2 Suppl.), 33
196 were co-dominant loci and 4 dominant. One SSR tested by Vilanova et al. (2003) but
197 unmapped in the previous map, pchgms2(1) (Sosinski et al. 2000), was incorporated
198 with the new markers. In total, 38 SSR markers were added to the map being distributed
199 throughout the genome and ranging from 15 markers in LG1 to 3 markers in LG2 and

200 LG3. No markers were mapped on LG4 (Table 2 Suppl.). Nine markers (23%) deviated
201 significantly from the expected F_2 segregation ratio at P<0.01. The map is organized in
202 8 linkage groups covering a distance of 615cM and comprising 231 loci: 154 AFLPs, 63
203 SSRs and 14 AFLP-RGAs (Soriano et al. 2005) (Figure 2). The average distance
204 between adjacent markers was 2.6cM, ranging from 1.3cM in LG4 to 8.4cM in LG3. the
205 mapped SSR markers established homologies with other *Prunus* maps: twenty-five
206 were held in common with the *Prunus* reference map (Dirlewanger et al. 2004a), 38
207 with the map of Dirlewanger et al. (2004b) and 22 with the map of Yamamoto et al.
208 (2005).

209 **Identification of a BTL for PPV resistance**

210 PPV infection in parents and progenies was evaluated after every dormancy cycle by
211 assessing the presence of symptoms and later confirmed by ELISA-DASI. The
212 inoculation efficiency was very high and over 95% of the 'GF-305' rootstocks
213 developed symptoms. However, distribution of symptoms was highly irregular among
214 seedling shoots hampering the rating of viral symptoms in intermediate grades. Thus,
215 PPV resistance phenotype was scored as resistant or susceptible to avoid mis-
216 classifications. Most susceptible seedlings were detected after the first cycle, but about
217 15-40% (depending on the population) were scored resistant in cycle 1 and resulted
218 susceptible in cycle 2 (Table 1). Evaluation after additional cycles did not detect
219 significant variations (data not shown).

220 The resistant/susceptible ratio does not fit with monogenic models neither in the
221 mapping populations nor in the two additional progenies analyses (Table 1). However,
222 all segregations fit better with a digenic model except for 'GxCa', where three genes are
223 necessary to explain the segregation obtained (data not shown). Co-segregation analysis
224 between PPV resistance and markers from the 'GxC' and 'LxL-98' apricot maps

225 showed that only a few markers located on LG1 showed a recombination frequency (θ)
226 lower than 0.4 and LOD > 3.0 (Table 2). However, the inclusion of the PPV resistance
227 gene in the map modified significantly the original distances among the marker loci, as
228 expected for a trait which segregation fit with a digenic model thus being highly
229 distorted in all populations.

230 The possible involvement of several loci in the control of the PPV resistance trait
231 was initially studied following the KW non-parametric test. In agreement with the co-
232 segregation analysis, only markers located on the upper part of LG1 in both maps were
233 significant (P<0.005) for the KW test (Table 2 and Figure 3). The P<0.005 significance
234 intervals comprised regions of ~21.0cM in the 'Goldrich' map, between UDAp415 and
235 ssrPaCITA17 (38.1-59.1cM map positions), and ~20.3cM in the 'LxL-98' map,
236 between EAA-MCTA1 and EAC-MCAT13 (25.2-45.5cM map positions) (Figure 3).
237 All the dominant markers in the 'LxL-98' map showing KW significant values come
238 from the resistant parent 'SEO', since those coming from 'Tyrinthos' were linked in
239 repulsion with the resistance, being not included in the analysis.

240 BTL identification was also performed with the Interval Mapping (IM) procedure in
241 order to support the results obtained with the KW test, and a general agreement was
242 observed between both methods (Figure 3). In spite of the limitation that the binary
243 phenotype distribution supposes for IM, this method confirmed the detection of one
244 BTL on LG1 in both maps. In 'Goldrich' the two-LOD support interval for this BTL is
245 located within a ~11.4cM genomic region (between markers AA-CTT14 and AA-
246 CCC1), and in 'SEO' within a region of ~9.1cM (between EAG-MCTT1 and EAT-
247 MCTC9) (Figure 3). Interestingly, no other BTLs were found by IM. Table 2 shows the
248 statistics summary for the highly significant markers located between two SSRs
249 flanking these intervals (SC6A6 and ssrPaCITA17).

250 Possible epistatic interactions, that might explain PPV resistance segregations, were
251 tested between the highest KW significant SSR within the BTL associated genomic
252 region identified on LG1 (ssrPaCITA5) and other co-dominant markers residing on a
253 different region of the same chromosome or on different chromosomes. These markers
254 showed relatively high KW values but were non-significant since, as reported above, all
255 the KW significant markers were located in the upper part of LG1. Therefore, as
256 expected from the KW test results, no significant epistatic interactions with a threshold
257 value of $P<0.05$ were detected in any of the two maps (data not shown).

258 **MAS in apricot breeding**

259 SSRs flanking the BTL associated region identified on LG1 (SC6A6 and
260 ssrPaCITA17) and the SSR showing highest KW and IM LOD score values
261 (ssrPaCITA5) were screened in a set of resistant/susceptible apricot genotypes
262 (Martínez-Gómez et al. 2000) and two additional apricot populations ('GxCa' and
263 'LxL-00') to validate their association with PPV resistance.

264 Table 3 shows that ssrPaCITA5 and ssrPaCITA17 alleles linked to PPV resistance
265 were detected in all resistant cultivars but not in susceptible ones. However, SC6A6
266 PPV resistance linked-allele was also detected in two susceptible cultivars ('Mitger' and
267 'Palau'). SSRs located on different linkage groups did not show any association with
268 PPV resistance in the set of apricot genotypes analyzed (data not shown).

269 The association between these markers and PPV resistance in 'GxCa' and 'LxL-00'
270 measured with the KW test (Table 4). Two SSRs, M3b located on the lower region of
271 LG1 and CPPCT-13 located on LG5, were used as negative controls. As previously
272 reported for the 'GxC' population, ssrPaCITA5 showed the highest KW statistic value
273 in the 'GxCa' population. In 'LxL-00' the three markers were highly significant for the
274 KW statistic ($P<0.0001$) although when merging 'LxL-98' and 'LxL-00' data

275 ssrPaCITA5 again shows the highest significant value (data not shown). SSR negative
276 controls showed non-significant values in both populations.

277 To test the potential use of these markers for MAS the percentage of mis-classified
278 seedlings was determined in the four populations studied (Table 5). Seedling
279 classification into resistant or susceptible phenotype based on ssrPaCITA5 is noticeably
280 more accurate than with the other markers (Table 5). Particularly, percentages of
281 seedlings without ssrPaCITA5 classified as PPV resistant (2-10%) are lower than those
282 obtained with SC6A6 or ssrPaCITA17 in all cases (from 6% to 21%).

283 **DISCUSSION**

284 **Improved apricot linkage maps**

285 Saturation of the 'GxC' and 'LxL-98' apricot linkage maps (Hurtado et al. 2002;
286 Vilanova et al. 2003) was moderately high, although clearly insufficient in 'GxC' where
287 not all the groups corresponding to the basic chromosome number of *Prunus* (n=8)
288 could be defined. Moreover, the number of co-dominant markers contained in both
289 maps was fairly small. Thus, to facilitate the use of these maps for MAS or synteny
290 studies up to 43 and 37 new SSR markers, respectively, were incorporated in this work.

291 The new 'Goldrich' map covers 468cM with an average distance between markers of
292 3.4cM, significantly lower than the 3.9cM determined in the original map (Hurtado et
293 al. 2002). As cited above, initially only 5 of the 8 expected linkage groups were
294 identified (Hurtado et al. 2002), but in the improved map 8 groups were obtained
295 noticing that two of the 5 previous groups were formed by joining together 2 different
296 groups (LG1G-LG8G and LG2G-LG5G). The new 'Currot' map covers 451cM with an
297 average distance of 5.1cM in contrast with the 5.8cM of the previous map (Hurtado et
298 al. 2002). Sixteen SSRs established bridges between the 8 linkage groups of both maps
299 maintaining the co-linearity in the majority of them. The apricot genome length

300 estimated using the method of Meagher et al. (1988) was between 800 and 1200 cM,
301 therefore the ‘Goldrich’ map may cover from 39% to 58% of the nuclear genome and
302 the ‘Currot’ map from 37% to 56%.

303 The new ‘LxL-98’ map covers 615cM with an average distance of 2.6cM lower than
304 the 3.3cM of the previous map (Vilanova et al. 2003) and lower than those obtained in
305 most of the *Prunus* maps (Bliss et al. 2002; Dirlewanger et al. 2004b; Yamamoto et al.
306 2005) except Dirlewanger el al. (2004a). According to Meagher et al. (1988) this
307 linkage map may cover from the 52% to 77% of the nuclear genome. In these apricot
308 maps LG1 and LG6 were the largest linkage groups in agreement with results observed
309 in most *Prunus* maps (Dirlewanger et al. 2004a; Dirlewanger et al. 2004b; Yamamoto et
310 al. 2005) and with cytogenetic data suggesting the existence of a chromosome larger
311 than the rest in *Prunus* (Jelenkovic and Harrington 1972).

312 Segregation distortion at $P<0.01$ was observed for 17% of markers in ‘Goldrich’ and
313 22% in ‘Currot’. These markers were distributed throughout the genome but on LG5
314 appears a cluster with 9 skewed markers in ‘Goldrich’ and 5 in ‘Currot’ suggesting that
315 this region may be subjected to selection. In the ‘LxL-98’ map 54% of the distorted loci
316 were located on the LG1 upper part. Vilanova et al. (2003) suggested a selection at pre-
317 or post-zygotic level against lethal or sub-lethal genes located in this region. In addition,
318 27% of the distorted loci were placed on LG6 due to the semi-compatible self-
319 fertilization of ‘Lito’ controlled by the *S*-locus located on LG6 (Vilanova et al. 2003).

320 New SSRs on the ‘GxC’ and ‘LxL-98’ maps established homologies with other
321 *Prunus* maps (Dirlewanger et al. 2004b; Yamamoto et al. 2005) and increased the
322 number of anchor markers with the *Prunus* reference map (Dirlewanger et al. 2004a)
323 from 14 to 23 in ‘GxC’ and from 22 to 25 in ‘LxL-98’. These markers were essentially
324 collinear with other *Prunus* maps supporting the high degree of synteny observed within

325 this genus (Dirlewanger et al. 2004a). Comparative mapping studies developed in the
326 last years will facilitate the future use of MAS in fruit breeding.

327 **Genetic control and mapping of PPV resistance trait**

328 To date genetic control system of PPV resistance remains unknown and this is being
329 an important handicap for the breeding programs. In addition, attempts to locate this
330 trait in the available maps have not been completely successful hindering the MAS
331 development. Several reasons may explain this situation: the strong environmental
332 dependence of PPV resistance scoring (Decroocq et al. 2005), the difficulty into
333 evaluating this trait on large-scale experiments and the differences in the methods of
334 evaluation used by research groups (Llácer et al. 2006).

335 In this work we report the analysis of PPV resistance segregation data in four
336 populations: two F_1, 'GxC' (Hurtado et al. 2002) and 'GxCa', and two F_2, 'LxL-98'
337 (Vilanova et al. 2003) and 'LxL-00'. All observed segregations deviated significantly
338 from the expected ratio for a single dominant locus (1:1 in F_1 and 3:1 in F_2) but fit with
339 a model of two dominant independent loci controlling PPV resistance (1:3 in F_1 and 9:7
340 in F_2) except 'GxCa' consistent with a trigenic model. These results suggest that several
341 loci may be involved in the resistance control. The digenic model would correspond to a
342 double recessive epistasy in which the dominant alleles of both genes are necessary to
343 provide resistance. However, a digenic control for plant pathogen resistance does not
344 seem to be very frequent. In one of the few examples available from the literature,
345 Suwabe et al. (2003) initially found two SSRs linked to two possible independent genes
346 involved in the resistance to clubroot in *Brassica rapa* L., but final results supported an
347 oligogenic control.

348 If monogenic control is not assumed it is not possible to map the PPV resistance trait
349 as a single marker and QTL approaches become necessary. Since attempts to determine

350 intermediate phenotypes with certain guarantees were not completely successful, PPV
351 resistance was scored as a binary trait (resistant vs. susceptible). These kind of traits,
352 non-normally distributed, are not infrequent but the use of standard QTL approaches for
353 such traits may lead to low power or unacceptably high false positive rates (Kruglyak
354 and Lander 1995). The non-parametric mapping method based on the KW rank sum test
355 enables mapping QTL when a spike in the phenotype distribution occurs and therefore
356 the usual normality assumption can not be made (Broman 2002). However, the use of
357 parametric statistical methods is recommended to confirm non-parametric QTL
358 mapping results when there is non-normality (Kruglyak and Lander 1995; Caranta et al.
359 1997).

360 Binary traits analysis for PPV resistance performed with the KW test and confirmed
361 by IM revealed the presence of a putative single BTL in the upper region of LG1 in two
362 different apricot improved maps. The KW test defined BTL confidence intervals,
363 comprising markers significant at $P<0.005$, very similar in size (~21.0 cM) and location
364 in the two maps.The consistency of these results is reinforced if we consider that these
365 two maps are based on two different populations F_1 and F_2 derived from two different
366 resistance donors ('Goldrich' and 'SEO'). Moreover, although not totally coincident,
367 map localizations proposed for PPV resistance trait are consistent with those determined
368 by Salava et al. (2002) in the apricot 'LE-3246 x Vestar' cross and Decroocq et al.
369 (2005) in the *P. persica* x *P. davidiana* cross.

370 The identification of only one genomic region involved in PPV resistance by KW
371 and IM analyses disagrees with the digenic (or even trigenic) model suggested by the
372 segregations observed. Two main hypotheses may explain these contradictory results.
373 First, a possible bias in the PPV resistance evaluation due to the mis-classification of
374 resistant seedlings as susceptible. In fact, the latent resistance of seedlings and

375 selections after showing symptoms of PPV infection has been already documented in
376 apricot (Karayiannis 2006). This possibility would approximate segregations to those
377 expected for one single locus. An alternative explanation might be that KW and IM
378 analyses only detected a major gene located on LG1 but not some modifier genes with
379 minor effects located throughout the genome. A similar model has been proposed
380 previously for the resistance against *Cucumber Mosaic Virus* in pepper (Caranta et al.
381 1997), for the mildew resistance in sunflower (Gentzbittel et al. 1998) and for the
382 resistance to *Plasmodiophora brassicae* in Chinese cabagge (Kuniguki et al. 1997).
383 In conclusion, based on the available evidence, we suggest that PPV resistance in
384 apricot is controlled by at least one major dominant gene located in the upper region of
385 LG1 although the involvement of other minor genes can not be discarded at this time.
386 **MAS in apricot breeding**
387 The evaluation of PPV resistance is one of the main limitations for the apricot
388 breeding programs due to the high costs of time and space that are required. At the IVIA
389 since 1993 near 30 progenies comprising more than 4000 individuals have been
390 generated. Every year, if the conditions are favourable, 500 seedlings can be analyzed
391 requiring total of 13 months for each. Four months are necessary to grow the woody
392 indicators and evaluation itself takes 9 months, including the bud grafting, two cold
393 treatments and two periods of symptoms observation. In this context, the use of
394 molecular markers linked to PPV resistance would significantly increase the breeding
395 program efficiency.
396 Among the selected SSR tested for MAS ssrPaCITA5 was the most effective.
397 Depending on the population type the proportion of susceptible seedlings mis-
398 classfication with this marker varies from 41% to 69%. However, if we use this marker
399 to discard those seedlings without the marker ~50% of the F_1 seedlings and ~25% of

400 the F_2 seedlings would be removed, the majority of them susceptible, while preserving
401 most resistant seedlings (>90% in F_1 and >95% in F_2 populations). These results,
402 although modest in comparison with MAS studies in other species like rice (Hittalmani
403 et al. 1995) or apple (Kellerhals 2000; Tartarini et al. 2000) might still be useful in
404 apricot breeding programs considering the huge limitations reported above particularly
405 relevant in fruit tree crops. Saturation of LG1 should be pursued in the future to
406 facilitate MAS in breeding programs and to tackle map based cloning of the major PPV
407 resistance gene.

408 Moreover, the presence of the ssrPaCITA5 and ssrPaCITA17 PPV resistance linked-
409 alleles in all resistant cultivars studied is specially interesting since four different
410 sources of PPV resistance ('SEO', 'Sunglo', 'Reliable' and *Prunus mandchurica sp.*)
411 are represented in the 7 resistant cultivars analysed (Karayiannis 2006). These results
412 suggest a possible common origin for the PPV resistance however, this remains to be
413 investigated.

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577 **Figure legends and tables**
578 **Table 1.-** PPV resistance segregating populations used in this study

Population	Resistance donor	Type	Cross date	Evaluation date	N[b]	Res./Suscep.[c] 1st cycle	2nd cycle	χ^2 (*P*-value)[d] mono. ratio	χ^2 (*P*-value)[e] di. ratio
'GxC'	'Goldrich'	F_1	1995	1998-99 (2)[a]	81	41/40	21/60	18.78 (<0.0001)	0.04 (0.847)
'LxL-98'	'SEO'	F_2	1998	2000-01 (3)	81	54/27	50/31	7.61 (0.006)	0.99 (0.321)
'LxL-00'	'SEO'	F_2	2000	2002-03 (3)	249	195/54	152/97	25.87 (<0.0001)	2.35 (0.125)
'GxCa'	'Goldrich'	F_1	2001	2004-05 (2)	171	51/120	28/143	77.34 (<0.0001)	6.79 (0.009)

579 [a] Number of vegetative cycles analysed
580 [b] Number of seedlings evaluated
581 [c] Number of Resistant/Susceptible seedlings observed after one or two dormancy cycles
582 [d] χ^2 and *P*-value for the expected Resistant/Susceptible ratio in a monogenic model (1:1 in F_1 and 3:1 in F_2)
583 [e] χ^2 and *P*-value for the expected Resistant/Susceptible ratio in a digenic model (1:3 in F_1 and 9:7 in F_2)
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593 **Table 1 Supplementary.-** Origin and sources of SSR markers tested in the 'GxC' and
594 'LxL-98' populations

Loci acronym	**Source**	**SSR origin**	**'GxC' population**		**'LxL-98' population**	
			Tested	**Mapped**	**Tested**	**Mapped**
UDP	Peach	Cipriani et al. 1999	4	2	---	---
pch/ps	Peach	Sosinski et al. 2000	9	1	---	---
pchgms	Peach	Testolin et al. 2000	---	---	17	1
BBPCT	Peach	Dirlewanger et al. 2002	37	11	37	10
CPPCT	Peach	Aranzana et al. 2002	22	3	---	---
MA/M	Peach	Yamamoto et al. 2002	36	9	36	10
ssrPaCITA	Apricot	Lopes et al. 2002	22	8	22	7
aprigms	Apricot	Vilanova et al. 2006	16	1	16	6
UDAp	Apricot	Messina et al. 2004	20	6	20	1
SC	Apricot	Abernathy et al. 2004	4	2	2	2
Total			170	43	150	37

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605 **Table 2 Supplementary.-** Summary of SSRs mapped in 'Goldrich', 'Curot' and 'LxL-
606 98' maps.

SSR marker	Linkage Group	Goldrich (χ^2)[a]	Currot (χ^2)	LxL (χ^2)	SSR marker	Linkage Group	Goldrich (χ^2)[a]	Currot (χ^2)	LxL (χ^2)
aprigms1	LG1	---	---	11.4*	UDAp404	LG4	0.4[D]	---	---
aprigms2	LG1	1.4[D]	---	9.1	UDAp416	LG4	2.1	---	---
aprigms3	LG1	---	---	3.1	UDAp418	LG4	0.9[D]	---	---
aprigms8	LG1	---	---	2.5	UDAp419	LG4	2.6	---	---
aprigms10	LG1	---	---	9.9*	UDP98-024	LG4	0.9[D]	---	---
aprigms16	LG1	---	---	2.0	BPPCT017	LG5	11.4*	11.4*	0.4
BPPCT011	LG1	---	---	6.3	BPPCT020	LG5	0.1[D]	---	---
CPPCT26	LG1	2.5[D]	---	---	BPPCT026	LG5	---	0.9[D]	---
CPPCT34	LG1	1.8[D]	---	---	BPPCT037	LG5	39.2*	39.2*	1.2
MA030a	LG1	---	---	7.0	BPPCT038	LG5	22.3*	22.3*	31.2*[D]
M3b	LG1	0.5[D]	---	3.3	ssrPaCITA21	LG5	---	2.8[D]	2.2
pchgms10	LG1	---	---	1.8	BPPCT009	LG6	1.4[D]	---	2.7
SC6A6	LG1	3.3	3.3	3.9	BPPCT018	LG6	2.0	2.0	---
SC19A6	LG1	6.3	6.3	11.0*	BPPCT042	LG6	---	---	78.0*
ssrPaCITA5	LG1	0.5[D]	---	8.4	MA027a	LG6	5.8	5.8	4.0
ssrPaCITA7	LG1	2.5[D]	---	4.8	MA040a	LG6	0.3[D]	---	18.8*
ssrPaCITA17	LG1	1.7[D]	---	10.3*	M5a	LG6	3.9[D]	---	---
UDAp415	LG1	1.6[D]	---	---	UDP98-412	LG6	0.0[D]	---	---
BPPCT002	LG2	2.0[D]	---	0.0	CPPCT22	LG7	42.3*	42.3*	---
BPPCT030	LG2	0.0[D]	---	---	MA010a	LG7	0.0[D]	---	1.6
MA007a	LG2	0.1[D]	---	---	MA020a	LG7	---	---	17.1*
ssrPaCITA14	LG2	---	---	1.2	pchgms2(1)	LG7	---	---	9.4*
ssrPaCITA16	LG2	0.0[D]	---	1.7	UDAp407	LG7	---	---	0.1
ssrPaCITA19	LG2	0.5	0.5	---	UDP98-405	LG7	---	32.0*[D]	---
UDAp413	LG2	0.0[D]	---	---	BPPCT012	LG8	---	---	0.3[D]

BPPCT007	LG3	---	---	2.5	MA006b	LG8	---	---	3.0
BPPCT024	LG3	---	---	0.5[D]	MA013a	LG8	0.9[D]	---	0.6
BPPCT039	LG3	---	0.2[D]	---	MA019a	LG8	---	0.2[D]	7.2
MA034a	LG3	---	---	0.6[D]	M6a	LG8	---	0.1[D]	---
ssrPaCITA4	LG3	1.4	1.4	---	Ps1h3	LG8	0.4[D]	---	---
BPPCT040	LG4	0.1[D]	---	---	ssrPaCITA15	LG8	---	---	3.7
ssrPaCITA6	LG4	---	0.2[D]	---	UDP98-409	LG8	1.1	---	---

* Markers showing distorted segregation at *P*<0.01.

[a] Chi-square of the goodness of fit for the segregation at *P*<0.01.

[D] SSR dominant markers

Figure 1.- Genetic linkage maps of 'Goldrich' and 'Currot'. Linkage groups were numbered according to the nomenclature of Dirlewanger et al (2004a). New SSR markers are *black boxed*. *Solid circles* indicate anchor markers with other *Prunus* maps: Dirlewanger et al. (2004a), Dirlewanger et al. (2004b), Yamamoto et al. (2005) an the 'LxL-98' map. *Asterisks* (**) indicate markers with distorted segregations at *P*< 0.01. Distances in cM are shown on the left in 'Goldrich' an on the right in 'Currot'.

Figure 2.- Genetic linkage map of 'LxL-98'. Linkage groups were numbered according to the nomenclature of Dirlewanger et al. (2004a). New SSR markers are *black boxed*. *Solid circles* indicate anchor markers with other *Prunus* maps: Dirlewanger et al. (2004a), Dirlewanger et al. (2004b), Yamamoto et al. (2005) an the 'GxC' map. *Asterisks* (**) indicate markers with distorted segregations at *P*< 0.01. Distances in cM are shown on the left.

Figure 3.- Kruskal-Wallis (KW) statistic values (solid line) and Interval Mapping (IM) LOD score (pointed line) at markers on LG1. Horizontal dashed line corresponds to the *P*<0.005 KW significance level. **a** 'Goldrich' map. **b** 'LxL-98' map. Only SSR and highly significant AFLP markers are shown. Bars at the bottom indicate the KW

627 interval for *P*<0.005 significant markers (solid bar) and the IM two-LOD support

628 interval (pointed bar).

629 **Table 2.-** Results of co-segregation, KW and IM analysis for PPV resistance on 'SEO'

630 and 'Goldrich' maps. SSRs flanking significance intervals (SC6A6 and ssrPaCITA17)

631 and highly significant markers are shown.

			Co-segregation		**KW test**		**Interval Mapping**		
Map	**Marker**	**Pos**[a]	**r**[b]	**LOD**[c]	**KW**[d]	***P*(KW)**[e]	**IM**[f]	$\mathbf{R^2}$**(%)**[g]	**a**[h]
SEO	SC6A6	21.0	0.39	1.26	8.61	<0.05	2.74	14.4	0.263
	EAA-MCTA(1)	25.2	0.31	2.56	12.04	<0.001	2.77	14.6	0.206
	EAG-MCTT(1)	31.5	0.27	5.29	20.57	<0.0001	5.14	25.4	0.356
	ssrPaCITA5	33.4	0.38	2.36	13.54	<0.005	3.59	18.5	0.288
	EAT-MCTC(9)	40.5	0.33	2.95	12.71	<0.0005	3.09	16.1	0.325
	ssrPaCITA17	42.9	0.43	0.83	6.59	<0.05	1.43	7.8	0.198
Goldrich	SC6A6	28.7	0.30	0.16	2.78	---	2.03	14.2	
	UDAp415	38.1	0.29	2.21	9.81	<0.005	5.31	34.8	
	AG-CTT6	43.3	0.26	4.17	17.13	<0.0005	6.05	36.8	
	ssrPaCITA5	49.2	0.32	3.40	13.68	<0.001	3.57	21.6	
	AA-CTT7	52.0	0.29	3.72	15.16	<0.0005	3.71	21.8	
	ssrPaCITA17	59.1	0.39	1.92	7.94	<0.005	2.40	18.0	

632 [a] Position in cM on LG1

633 [b] Recombination frequency between markers and PPV resistance trait.

634 [c] LOD score for co-segregations.

635 [d] Kruskal-Wallis test statistic values.

636 [e] Probability associated with the KW value.

637 [f] LOD score under Interval Mapping.

638 [g] Percentage of the contribution to the total variance.

639 [h] Additive effect.

640

641 **Table 3.-** PCR-amplification of selected SSR markers in 7 Resistant (R) and 11
642 Susceptible cultivars (S). The presence (amplification) of the PPV resistance linked
643 allele is indicated by (+) and the absence by (-).

Cultivar	Pedigree	R/S	ssrPaCITA5	ssrPaCITA17	SC6A6
SEO	Unknown[a]	R	+	+	+
Lito	SEO x Thyrinthos[b]	R	+	+	+
Pandora	SEO x Thyrinthos[b]	R	+	+	+
Sunglo	Unknown[a]	R	+	+	+
Goldrich	Sunglo x Perfection[a]	R	+	+	+
Veecot	Reliable (open pollination)[a]	R	+	+	+
Harcot	[(Geneva x Naramata) x Morden 604] x NJA1 (Phelps x Perfection)[a]	R	+	+	+
Tyrinthos	Unknown[c]	S	-	-	-
Bergeron	Unknown[c]	S	-	-	-
Moniquí	Unknown[c]	S	-	-	-
Colorao	Unknown[c]	S	-	-	-
Canino	Unknown[c]	S	-	-	-
Currot	Unknown[d]	S	-	-	-
Ginesta	Unknown[d]	S	-	-	-
Mitger	Unknown[d]	S	-	-	+
Palau	Unknown[d]	S	-	-	+
Pepito	Unknown[d]	S	-	-	-
Katy	Zaiger´s Genetics (USA)[e]	S	-	-	-

644

645 [a] Brooks and Olmo (1997)

646 [b] Syrgianidis and Mainou (1993)

647 [c] Della Strada et al. (1989)

648 [d] IVIA

649 [e] Russell (1998)

650 **Table 4.-** Association between selected SSRs and PPV resistance in ‘GxCa’ and ‘LxL-

651 00’ progenies based on the Kruskal-Wallis test. KW: Kruskal-Wallis test statistic

652 values; DF: Degrees of freedom; P: probability associated with the KW value.

Marker	**Linkage Group**	**‘GxCa’**			**‘LxL-00’**		
		KW	**DF**	***P*(KW)**	**KW**	**DF**	***P*(KW)**
SC6A6	LG1	3.201	2		60.494	2	<0.0001
ssrPaCITA5	LG1	20.169	3	<0.0001	56.274	2	<0.0001
ssrPaCITA17	LG1	12.475	1	<0.0001	51.562	2	<0.0001
M3b	LG1	2.909	1		2.175	2	
CPPCT-13	LG5	0.166	1		2.885	2	

653

654 **Table 5.-** MAS for PPV resistance. Percentages of mis-classified seedlings in the four

655 populations analysed: R- indicates percentage of seedlings without the marker classified

656 as PPV resistant, S+ indicates percentage of seedlings carrying the marker classified as

657 PPV susceptible and Total indicates percentage of total seedlings mis-classified.

Marker	**‘GxC’**			**‘GxCa’**			**‘LxL-98’**			**‘LxL-00’**		
	R-	**S+**	**Total**	**R-**	**S+**	**Total**	**R-**	**S+**	**Total**	**R-**	**S+**	**Total**
SC6A6	21	62	49	11	76	65	6	69	29	8	47	23
ssrPaCITA5	10	41	32	7	47	41	2	69	27	8	49	24
ssrPaCITA17	15	48	39	14	49	44	8	70	31	7	55	25

658

659 **Figure 1**

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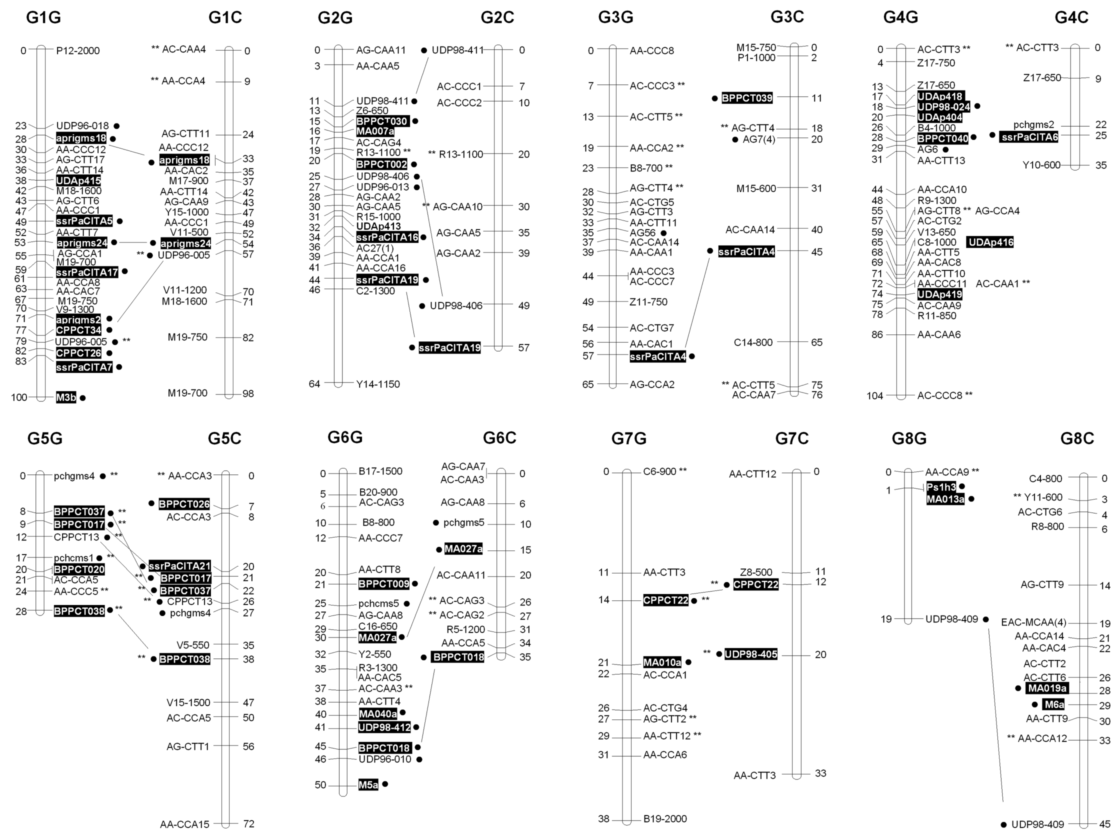

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673 **Figure 2**

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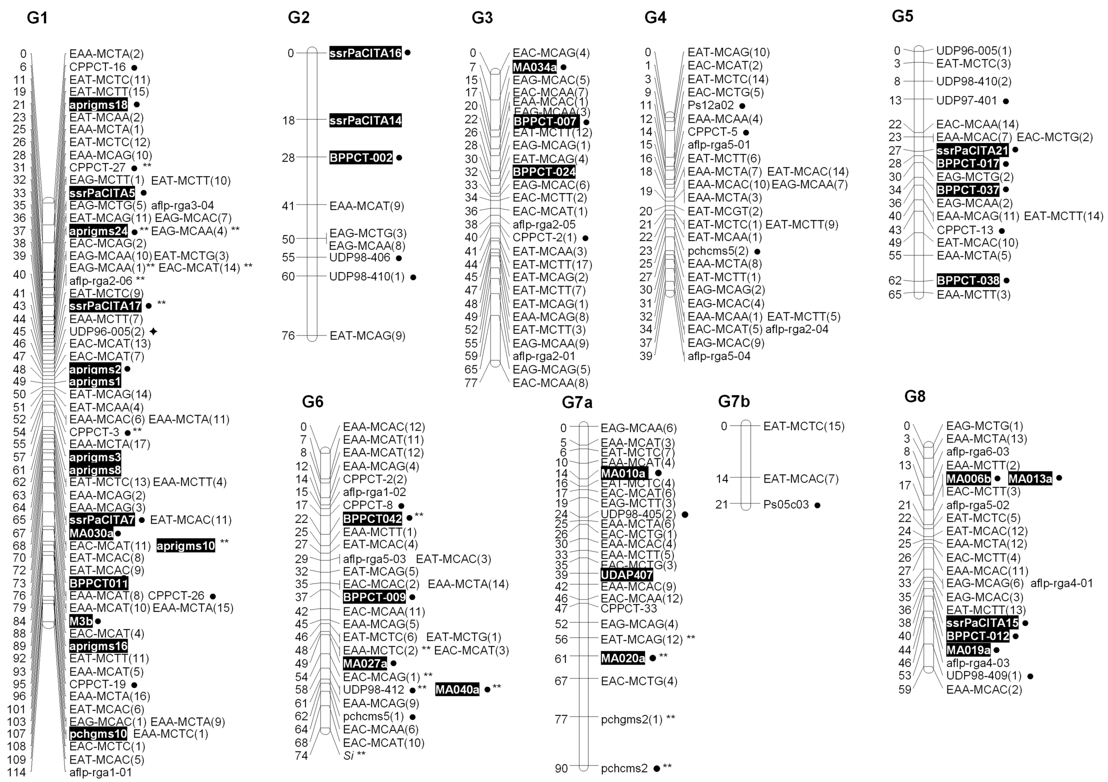

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677 **Figure 3**

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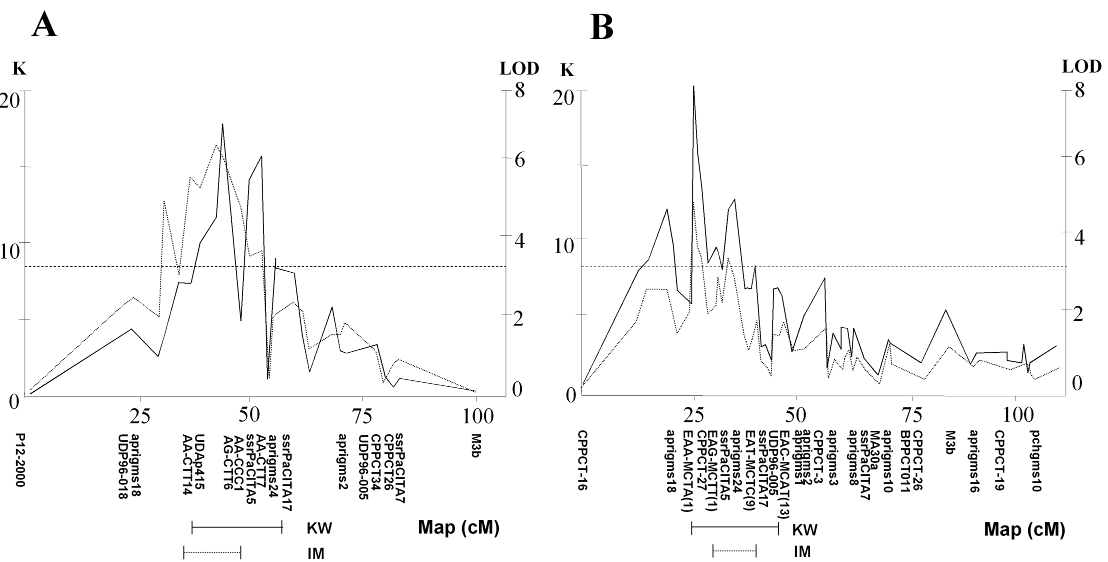

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