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Genetic mapping of Japanese plum

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ABSTRACT - *Genetic linkage maps of two Japanese plum cultivars, Chatard and Santa Rosa, were constructed using the pseudo-testcross mapping strategy and AFLP markers. From the segregating population derived from a cross between these cultivars, 88 plants formed the mapping population, with segregating genes for several traits of agronomical interest, such as the reaction to plum leaf scald. The Chatard map contained 56 markers linked in 11 linkage groups, covering a distance of 905.5 cM with an average distance of 16.2 cM between markers. The map of cultivar Santa Rosa contained 84 markers linked in 14 groups covering a distance of 1349.6 cM and presenting an average distance of 16.1 cM between markers. The maps obtained in the present study can be considered a basic framework and will need to be saturated for a more widespread use in breeding programs for the species.*

Key words: *Prunus*, genetic linkage map, AFLP.

INTRODUCTION

The plum we studied belongs to the genus *Prunus*, Rosaceae family. Most of the plum varieties used for commercial production in the world are classified as European (hexaploid; 2n=48) or Japanese (diploid 2n=16) types (Okie and Weinberger 1995). While the term 'European plum' is applied to cultivars that belong to the species *Prunus domestica*, the term 'Japanese plum' was originally applied to the species *Prunus salicina*. However, most Japanese cultivars launched in the past few years involve a cross between *P. salicina* and several plum species of the same genus. Consequently, the term 'Japanese plum' is currently applied to cultivars of this species and its interspecific hybrids.

The Brazilian commercial production of plums is based on Japanese cultivars since they do not have a high chilling requirement and are able to stand high temperatures in the summer. The cultivars derived from European species are not cultivated commercially in Brazil due to their high chilling requirement (Ducroquet and Mondin 1997).

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The construction of genetic maps is considered one of the most important applications of molecular markers, not only in genetic analysis of species but also in plant breeding. Even though they do not provide information at the molecular level of genes, maps are meaningful because they give a background to comparative and evolutionary studies, as well as the understanding of the biological processes and chromosome organization. In practical terms, genetic linkage maps provide a framework for the identification of QTL-associated markers of agronomic interest and are an auxiliary tool in breeding programs for choosing the parent for crosses as well as in marker-assisted selection (Lande and Thompson 1990).

Among molecular markers, AFLPs have been widely used in genetic mapping because this technique allows the generation of a large amount of polymorphic markers in a relatively short time. In addition, when the mapping population segregates 1:1 (as the pseudo-test cross), although dominant in nature, the AFLPs are as informative as the co-dominant markers (Hemmat et al. 1994, Grattapaglia et al. 1995).

Genetic maps of species belonging to the genus *Prunus* were constructed with the use of molecular markers in almond (Joobeur et al. 2000, Ballester et al. 2001) and peach (Chaparro et al. 1994, Dirlewanger et al. 1998, Lu et al. 1998, Shimada et al. 2000), and also in an interspecific crossing between almond and peach (Foolad et al. 1995). However, no genetic map for plum was found in our literature review.

Among the diseases that attack plum trees, the most important in Brazil is the leaf scald, caused by the bacterium *Xylella fastidiosa*. This bacterium infects the vases of the plants and is transmitted by insects (sharpshooters) causing the death of susceptible plants. It takes three or more years to manifest the disease in contaminated plants. This is a problem for genetic improvement, since breeders have to wait over three years to evaluate and select resistant genotypes. This situation is a perfect opportunity for the development of techniques that allow genotypic selection at the seedling stage, which would save time and costs in comparison to phenotypic selection.

Objective of this study was to construct genetic linkage maps for plum varieties of interest in the state of Santa Catarina (SC), one for the cultivar Santa Rosa (susceptible to leaf scald) and the other for cultivar Chatard (partially leaf scald-resistant).

MATERIAL AND METHODS

Plant material

The segregating population used for the genetic mapping was derived from a crossing involving the cultivars Chatard and Santa Rosa. It was established in 1993 and originally consisted of 223 plants from which 88 were randomly chosen to form the mapping population. The entire population, transplanted in 1995 to the field at the experimental station of EPAGRI in Videira, state of Santa Catarina, is segregating for several genes, among them those for leaf scald-resistance.

DNA Extraction

The genomic DNA was extracted (Doyle and Doyle 1987, with some modifications) from young leaves taken from plants at the beginning of the vegetative cycle, not only from the parents in the cultivars Chatard and Santa Rosa, but also from the 88 plants of the segregating population.

Analysis with AFLP markers

The AFLP analysis was performed with the Invitrogen AFLP analysis system I as recommended by the supplier. In addition, specific primers were synthesized to obtain other markers.

Genomic DNA was digested with the restriction enzymes *Eco*RI (rare cutter) and *Mse*I (frequent cutter), and specific adaptors were connected to the ends of the cleaved segments. Then, the digested and cleaved DNA was pre-amplified using a pair of primers complementary to the adapters *Eco*RI and *Mse*I and containing a selective nucleotide at the 3' end (E + 1 and M + 1). The reaction of selective amplification was set up with additional primers at the end cleaved by *Eco*RI (with two or three selective nucleotides at the 3' end) combined with complementary primers at the end cleaved by *Mse*I (with three selective nucleotides at the 3' end). In the reactions of selective amplification, 30 primer combinations were used (Table 1) in

Table 1. Combinations of primers used in the present study and their respective codes

Primers	M-CTG	M-CTA	M-CAT	M-CAG	M-CAC	M-CAA	M-CTT
E-AGC		1c		1e			1h
E-ACT		2c		2e	2f		2h
E-ACA	3a			3e	3f	3g	
E-AGG	4a	4c	4d		4f	4g	
E-ACG			5d		5f	5g	
E-AAG		6c				6g	
E-ACC							
E-AAC	8a			8e			
E-AC	10a						
E-AA					11f		

E = *Eco* RI and M = *Mse* I

addition to the three primer combinations at the end cleaved by *Eco*RI, with two selective nucleotides at the 3' end.

The fragments amplified in the reaction of selective amplification were electrophoretically separated in denaturing gel of polyacrilamide (6%) at a constant power of 60 W for approximately 1h and 40 min. The silver nitrate staining process of gels was used in accordance with Creste et al. (2001).

Data analysis of marker segregation

According to our initial analysis, the amplified fragments were classifiable in three categories: i) present in both parents; ii) present in cultivar Chatard and absent in cultivar Santa Rosa and segregating in the proportion 1:1 in the progeny and; iii) present in Santa Rosa and absent in Chatard, and segregating in the proportion 1:1 in the progeny. The observed proportions were compared to the expected 1:1 Mendelian proportion by the use of the χ^2 test based on the probability of 1% of significance with software Linkage-1 (Suiter et al. 1983).

Construction of genetic maps

Two genetic maps were constructed, one for the cultivar Chatard and the other for Santa Rosa, using the pseudo-testcross strategy (Hemmat et al. 1994, Grattapaglia et al. 1995). In the first phase of the map construction, the parents were used separately, only for the markers that were present in them showing the Mendelian proportion of 1:1 in the progeny. Afterwards the markers that presented distortion of the 1:1 segregation were added to the map. Mapmaker 3.0 (Lincoln et al. 1992) was the software used to construct the genetic linkage maps. Markers were used in the construction of the linkage groups not only in the repulsion, but also in the association phase. For this purpose, the data matrix was duplicated and the duplicated part inverted. First, a framework of the linkage groups was constructed using a LOD of 3.5 and a maximum distance of 25 cM, the marker distance being defined by the Kosambi function (Kosambi 1944). The first grouping was obtained by the command "order", which was based on the multipoint analysis to establish the best order of the markers within each linkage group. After establishing the framework of the linkage groups, other markers were added using the command "try" under a LOD of 3.0 and a maximum distance of 30 cM. The markers that presented distortions in the 1:1 segregation were added later under the latest stringency parameters.

RESULTS AND DISCUSSION

Analysis with AFLP markers

The mapping population size of 88 used in our study is an in-between value of the 63 and 133 peach plants Dirlewanger et al. (1999) and Shimada et al. (2000), respectively, used to construct genetic linkage maps. In the first one, the authors were able to map QTLs associated to fruit quality traits. In fact, the authors used an existing segregating population established for breeding purposes to develop a genetic linkage map to be used in that breeding program.

The 30 combinations of AFLP primers used in the present study generated 1549 markers, 507 of which were present only in cultivar Chatard, 567 only in cultivar Santa Rosa, and 475 were present in both cultivars. The average number of markers generated by primer combinations was 51.6, which was a value very close to the 46.4 markers found by primer combination in Japanese plum trees by Goulão et al. (2001). However, these values are low in comparison with those brought forth by marker combinations in other species: 74 and 78 in two cultivars *Malus* x *domesticas* (Xu and Korban 2000); 61 and 80 in two cultivars *Prunus persica* (Lu et al. 1998, Cervera et al. 2000); 112 in *Eucalyptus* (Cervera et al. 2000), and 115 in *Pinus* (Cervera et al. 2000).

The discrepancy among the average numbers of AFLP markers generated in the plum tree, which are low in relation to other species may be due to: i) the small size of the plum genome, 0.30 pgc^{-1} (Arumuganathan and Earle 1991); ii) the staining methods: silver nitrate was used in the present study and in that developed by Goulão et al. (2001), and radioactivity in other studies, which has been considered highly efficient.

The average number of markers per primer combinations present in cultivar Santa Rosa was 18.9, while the average number of markers present in cultivar Chatard was 16.9. The t-test performed to compare these averages showed that the differences found between the two were not statistically significant ($P > 0.65$). Not all bands were used for the mapping, either because they were not clear, or because the segregation was a complex one.

The Santa Rosa cultivar also presented a higher number of segregating markers in the proportion 1:1 (88 markers) compared to Chatard (59 markers). The number of markers per primer combination present in one parental and segregating in the proportion 1:1 found in the population were low: an average of 2.0 markers in Chatard and 2.9 in Santa Rosa, i.e. values (Table 2) lower than 7.5 found in *Populus* sp (Cervera et al. 2001).

Genetic maps

Table 2. Number of polymorphic markers detected in the cultivars Chatard and Santa Rosa with the use of 30 combinations of AFLP primers

Primers	Number of markers present			Total of markers	Segregating marker (1:1), P > 0.01	
	Chatard	Santa Rosa	Both		Chatard	Santa Rosa
1b	15	17	9	41	2	2
1c	25	15	12	52	3	1
1e	5	5	23	33		2
1h	3	7	20	30	1	2
2c	18	20	12	50		2
2e	5	7	15	27	2	3
2f	23	22	9	54	2	2
2h	8	15	20	43	2	3
3a	30	30	26	86	4	3
3b	26	33	12	71	1	5
3e	22	21	16	59	3	3
3f	2	9	18	29	2	1
3g	38	30	28	96	3	7
4a	20	20	5	45		5
4b	20	25	12	57	4	5
4c	18	25	12	55	1	3
4d	17	28	11	56	1	8
4f	17	21	8	46	3	3
4g	20	25	13	58	3	4
5b	30	29	15	74	4	6
5d	16	20	10	46		5
5f	17	20	12	49	2	1
5g	23	30	14	67	3	3
6c	7	3	27	37	1	
6g	6	8	17	31	1	3
8a	27	26	11	64	3	1
8e	28	29	17	74	1	2
10a	11	9	27	47	3	2
10b	7	9	19	35	3	
11f	3	9	25	37	1	1
Total	507	567	474	1549	59	88
Mean	16.9	18.9	15.8	51.6	2.0	2.9
Standard deviation	9.5	8.9	6.3	17.1	-	-

Each obtained linkage group received a specific name that consisted of the letters 'F' (Florianópolis) and 'P' (plum) followed by the number of the linkage group. Among the 59 markers generated in cultivar Chatard that segregate in the proportion 1:1, 46 (65%) were mapped into one of the 11 linkage groups along with 10 other markers with distorted segregation (Table 3). Therefore, 13 markers segregating in the proportion 1:1 were not mapped so far. The generated map covered a distance of 905.5 cM, and the proportion of markers linked per group varied from 3.6 to 17.9%. The linkage group sizes varied from 27.9 to 147.6 cM. Among the linkage groups, the average distance between the markers varied from 14 to 22.0 cM (Table 3). Only two intervals between two markers presented distances superior to 30 cM, located in the linkage groups FP2 and FP5. The linkage groups FP1, FP4 and FP5 were the ones that presented the highest number of linked markers: 10, 7 and 7 respectively. The linkage groups FP10 and FP11 consist of two linked markers so far (Table 3 and Figure 1).

Among the 88 polymorphic markers generated in cultivar Santa Rosa that segregated in the proportion 1:1, 67 (76%) were mapped into one of the 14 linkage groups along with 17 other markers with distorted segregation. Thus, 21 markers segregating in the proportion 1:1 were not yet mapped. The generated map covered a distance of 1349.6 cM, and the proportion of markers linked per group varied from 2.4 to 17.9 %. In this map, the sizes of the linkage groups varied from 12.4 cM to 209.8 cM. In the linkage groups, the average distance between the markers varied from 6.2 cM to 19.0 cM. Only four intervals between two markers presented a distance superior to 30 cM, located in the linkage groups FP1 and FP2 and presenting the highest number of markers, i.e. 15 and 13, respectively. The linkage groups FP13 and FP14 consist of two linked markers so far (Table 4 and Figure 2).

Table 3. Number of markers, size of the linkage groups and average distance between markers in cultivar Chatard

Linkage groups	Markers		Length		Average distance cM
	Number	Linked %	Size cM	Percentage	
FP1	10	17.9	147.6	16.3	14.8
FP2	6	10.7	110.3	12.1	18.4
FP3	5	8.9	110.2	12.2	22.0
FP4	7	12.5	107.1	11.8	15.3
FP5	7	12.5	101.1	11.2	14.4
FP6	6	10.7	98.7	10.9	16.5
FP7	4	7.1	77.2	8.5	19.3
FP8	3	5.4	53.3	5.9	17.8
FP9	4	7.1	42.4	4.7	10.6
FP10	2	3.6	29.7	3.3	14.9
FP11	2	3.6	27.9	3.1	14.0
Total	56	100	905.5	100	
Mean					16.2

Table 4. Number of markers, size of the linkage groups and average distance between markers in cultivar Santa Rosa

Linkage groups	Markers		Length		Average distance cM
	Number	Linked %	Size cM	Percentage	
FP1	13	15.5	209.9	15.5	16.1
FP2	15	17.9	203.3	15.1	13.6
FP3	10	11.9	161.7	12.0	16.2
FP4	8	9.5	132.7	9.8	16.6
FP5	7	8.2	128.6	9.5	18.4
FP6	7	8.2	125.3	9.3	17.9
FP7	4	4.8	75.8	5.6	19.0
FP8	4	4.8	73.4	5.4	18.4
FP9	3	3.6	54.8	4.1	18.3
FP10	3	3.6	52.5	3.9	17.5
FP11	3	3.6	49.8	3.7	16.6
FP12	3	3.6	42.8	3.2	14.3
FP13	2	2.4	26.7	2.0	13.4
FP14	2	2.4	12.4	0.9	6.2
Total	84	100	1349.6	100	
Mean					16.1

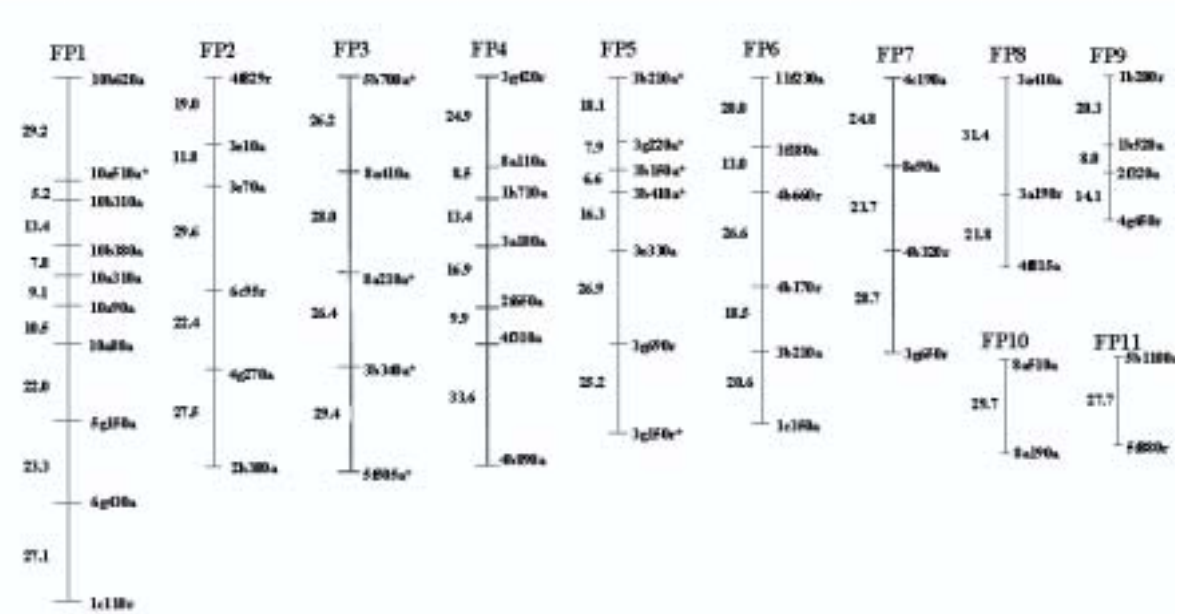

Figure 1. Genetic linkage map of cultivar Chatard; Each linkage group was given a specific name which consists of the letters F (Florianópolis) and P (Plum) followed by the number of the linkage group. Values on the left indicate the distance in relation to the marker of the superior end. The AFLP markers are listed on the right of the linkage groups whose terminology is formed by the combination code of primers to which it belongs (Table 1), being followed by the molecular weight of the marker, the codes (a) association configuration and (r) for repulsion segregation. * Markers with distorted segregation

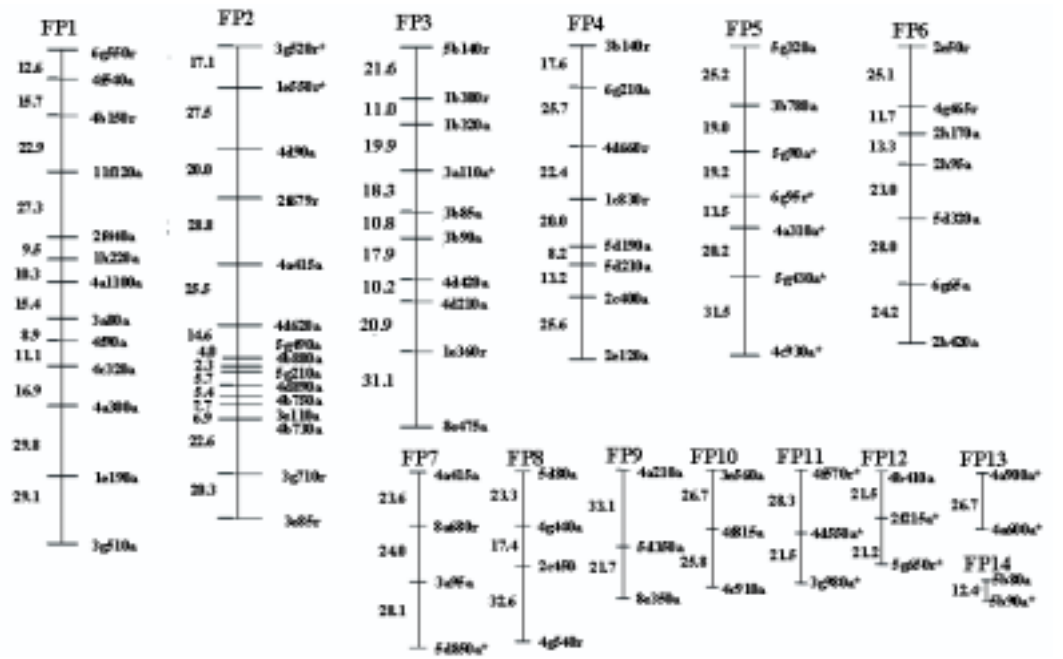

Figure 2. Genetic linkage map of cultivar Santa Rosa; Each linkage group was given a specific name which consists of the letters F (Florianópolis) and P (Plum) followed by the number of the linkage group. Values on the left indicate the distance in relation to the marker of the superior end The AFLP markers are listed on the right of the linkage groups whose terminology is formed by the combination code of primers to which it belongs (Table 1), being followed by the molecular weight of the marker, the codes (a) association configuration and (r) for repulsion configuration. * Markers with distorted segregation

Comparison with other genetic maps constructed for woody species

The number of molecular markers used to construct the maps varied from 62 in *Poncirus trifoliata* (Cristofani et al. 1999) to 566 in *Populus deltoides* (Cervera et al. 2001), demonstrating the small number of markers used for the mapping of the plum tree in the present study, i.e. 56 for cultivar Chatard and 84 for cultivar Santa Rosa (Table 5). The average distance between the linked markers is one of the most important parameters of a linkage genetic map, since it provides the degree of map saturation, which is very important for QTL mapping and the performance of marker-assisted selectio. The average distance of the mapped markers between the compared species (Table 5) varied from 14 cM in *Poncirus trifoliata* (Cristofani et al. 1999) to 2.3 cM in peach (Dirlewanger et al. 1998). The values found for the plum maps were 16.2 cM for Chatard and 16.1 cM for Santa Rosa, demonstrating the opportunity for saturation.

However, concerning the distance covered by the map, the obtained values of 905.5 cM for cultivar Chatard and 1349.6 cM for cultivar Santa Rosa were very similar to those obtained by the mapping of other Prunaceaes that have a haploid genome of the same size as plum, since these values can vary from 1297.0 cM in peach (Lu et al. 1998) to 415.0 cM in the almond tree (Joobeur et al. 2000).

Table 5. Comparison between the genetic maps of the plum and the ones of the woody species taking into consideration the haploid genome size (pgc^{-1}), number of markers (NM), number of linkage groups per haploid number of chromosomes (NGL/NhC), size (S) in cM and average distance between linked markers in cM (AD)

Species	**pgc^{-1}**	**NM**	**NGL/NhC**	**S** cM	**AD** cM	**Reference**
Plum						(Present results)
cv. “Chatard”	0.30	56	11/8	905.5	16.20	
cv. “Santa rosa”	0.30	84	14/8	1349.6	16.10	
Almond						(Joobeur et al. 2000)
cv. “ferragnés”	0.30	127	8/8	415.0	3.20	
cv. “Tuono”	0.30	99	8/8	416.0	4.20	
Citrus						(Cristofani et al. 1999)
Citrus sunki	0.41	63	10/9	732.3	11.60	
Poncirus trifoliata	0.41	62	8/9	866.8	14.00	
Apple						(Hemmat et al. 1994)
cv. “White angel”	0.70	253	24/17	950.0	3.80	
cv. “Rome beuty”	0.70	156	21/17			
Apple						(Conner et al. 1997)
cv. “Wycik McIntosh”	0.70	238	19/17	1206.0	5.10	
cv. “Ny 75441-67”	0.70	110	16/17	692.0	6.30	
cv. “75441-58”	0.70	183	18/17	898.0	4.90	
Peach x Almond	0.30	107	9/8	800.0	7.50	(Foolad et al. 1995)
Peach	0.30	157	15/8	1297.0	8.30	(Lu et al. 1998)
Peach	0.30	249	11/8	712.0	2.30	(Dirlewanger et al. 2000)
Peach	0.30	83	10/8	965.0	11.60	(Shimada et al. 2000)
Picea abies	15.00	413	29/12	2839.0	6.90	(Paglia et al. 1998)
Pinus sp.						(Kubisiak et al. 1995)
Pinus palustri	19	122	18/12	1367.5	11.20	
Pinus elliottii	19	91	13/12	952.9	10.50	
Populos sp.						(Cervera et al. 2001)
Populus deltoides	0.55	566	19/19	2064.0	3.60	
Populus nigra	0.55	339	19/19	1752.0	5.20	
Populus trichocarpa	0.55	369	28/19	1464.0	4.00	
Grape						(Dalbó 2001)
“Horizon”	0.48	157	20/19	1199.0	7.60	
“Illinois 547-1”	0.48	181	20/19	1470.0	8.10	

The coverage of the plum tree map is due to the fact that the markers were spaced and not concentrated. The eleven (cv. Chatard) and 14 (cv. Santa Rosa) obtained linkage groups are above the number of haploid chromosomes of the plum tree (n=8), indicating that two or more linkage groups belong to the same chromosome.

CONCLUSIONS

The genetic maps of plum obtained in the present study can be considered as basic maps of the species, but they need to be saturated in order to increase the possibility of finding associations among molecular markers and loci of agricultural importance. Adding more markers to the map is necessary, not only to obtain the haploid number of chromosomes (n=8), but also to have a complete and uniform coverage of the species genome. Microssatellite markers should be used for this purpose, not only to anchor the linkage groups but also to allow further map fusion for plum as well as for the genus *Prunus*.

Since the current genetic linkage maps are the first ones established for plum tree, they can be used in further studies on chromosome organization, the relative contribution of the different *Prunus* species to commercial plum cultivars, or in comparative map analyses focusing on the location of preserved sinteny regions. Finally, it is expected to that one or more markers shall be found linked to disease-resistance genes, especially to leaf scald.

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Mapeamento genético da ameixeira japonesa

RESUMO - *Mapas genéticos de ligação de duas cultivares de ameixeiras japonesas, Chatard e Santa Rosa foram construídos com marcadores AFLP utilizando-se a estratégia do pseudo-cruzamento teste. Da população segregante oriunda do cruzamento entre estas duas cultivares, 88 plantas formaram a população de mapeamento, onde estão segregando genes para vários caracteres de interesse agronômico, entre eles, a resistência à escaldadura das folhas. O mapa da cv. Chatard apresentou 56 marcadores ligados em 11 grupos de ligação, cobrindo uma distância de 905,5 cM e apresentando uma distância média entre marcadores de 16,2 cM. O mapa da cv. Santa Rosa apresentou 84 marcadores ligados em 14 grupos de ligação, cobrindo uma distância de 1349,6 cM e apresentando uma distância média entre marcadores de 16,1 cM. Os mapas obtidos no estudo podem ser considerados como mapas básicos da espécie e necessitam ser saturados para terem maior aplicabilidade em programas de melhoramento genético.*

Palavras-chave: *Prunus*; mapeamento genético, AFLP.

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