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Coupling linkage maps in F_2 populations of carrot derived from two different crosses

Santos, C. A. F. [1]; Philipp W. Simon[2]

[1]Embrapa-Semi-Arido, C. Postal 23, 56300-970 Petrolina-PE. Brasil. E-mail: casantos@cpatsa.embrapa.br; [2]USDA-ARS- and Department of Horticulture, 1575 Linden Drive, University of Wisconsin, Madison, WI 53706.

ABSTRACT

Linkage grouping and ordering AFLP markers analyses were performed for two different F_2 populations of carrot (*Daucus carota* L.). Included were 277 and 242 dominant AFLP markers and 10 and 8 co-dominant markers assigned to the nine linkage groups of Brasilia x HCM and B493 x QAL F_2 populations, respectively. The overall marker datasets, including repulsion and coupling phases, were consistently assigned to the nine linkage groups at LOD scores ranging from 3.5 to 5.0 in both F_2 populations. Linkage grouping performed with only markers linked in coupling phase showed the nine linkage groups at LOD scores ranging from 3.0 to 7.5, 3.0 to 5.5, 3.5 to 5.0 and 2.5 to 8.5 in the Brasilia-, HCM-, B493- and QAL-coupling F_2 population, respectively. The average marker spacing was 4.78, 4.80, 5.54 and 5.13 cM in the Brasilia-, HCM-, B493 and QAL-coupling phase maps, respectively. Some gaps, greater than 10 cM, were observed more frequently in the coupling linkage maps of B493 and QAL than in the Brasilia- and HCM-coupling maps. Six linkage groups of both populations were merged, based on the conserved AFLP fragments .The excellent genome coverage in this study is suitable to confidently apply single QTL analysis and to perform more powerful QTL analysis considering coupling linkage maps.

Keywords: Daucus carota*, AFLP, genome coverage, molecular markers.*

RESUMO

Mapas de ligação genética em atração em duas populações F_2 de cenoura

Análises de ligações e ordenamento de marcadores AFLP foram realizadas em duas diferentes populações F_2 de cenoura. 277 e 242 dominantes AFLPs e dez e oito marcadores co-dominantes foram distribuídos nos nove grupos de ligações das populações Brasília x HCM e B493 x QAL, respectivamente. Todos os marcadores, incluindo as fases em repulsão e atração, foram consistentemente distribuídos para os nove grupos com valor LOD variando

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de 3,5 a 5,0, em ambas populações. Marcadores em fase de atração foram distribuídos para os noves grupos com valores de LOD variando de 3,0 a 7,5; 3,0 a 5,5; 3,5 a 5,0 e 2,5 a 8,5 nos mapas em atração de Brasília, HCM, B493 e QAL, respectivamente. A distância média dos marcadores foi de 4,78; 4,80; 5,54 e 5,13 cM nos mapas de atração de Brasília, HCM, B493 e QAL, respectivamente. Espaçamento entre os marcadores maior do 10 cM foi mais freqüente nos mapas de B493 e QAL do que nos mapas de Brasília e HCM. Seis dos nove grupos de ligações das duas populações F_2 foram consolidados em grupos únicos. A excelente cobertura do genoma desses mapas é um atrativo para a integração de marcadores co-dominantes de forma a construir mapas que considerem ambas as fases, bem como para estudos de QTL e mapeamento de genes.
***Palavras chaves**:* Daucus carota*, AFLP, cobertura de genoma, marcadores moleculares.*

Unlike many other crops, such as soybean, maize, barley and sugar beet the published genetic maps of carrot have low-density and they are clearly unsaturated and with minimal coverage of the carrot genome. Development of co-dominant markers has been rarely attempted and only a few PCR-based markers have been reported in the carrot literature. Mapping of F_2 populations with exclusively dominant markers has helped little toward the development of reliable marker order within linkage groups. Two-carrot coupling linkage maps, derived from two crosses between wild and white (*Daucus carota* var. *carota*) x cultivated orange (*D. carota* ssp. *sativus*) and cultivated orange x cultivated dark orange and consisting most of AFLPs and a few RAPD, SCARs and microsatellites, are presented. The goals of the present study were to construct two high-density molecular maps, to merge both maps with the largest goal of constructing a cross validated consensus map of carrot.

MATERIAL AND METHODS

The mapping populations were obtained by self-pollinating single F_1 hybrid plants, which originated from a cross between inbred line B493 x QAL (cross 1) and Brasilia x HCM (cross 2). Total genomic DNA was isolated from freeze-dried leaves tissue as described by Boiteux (2000). DNA concentration was determined with a TKO 100 Mini Fluorometer and size, purity, and integrity of total genomic DNA was also determined on 0.8% agarose gels.

The AFLP method was performed essentially as described in Vos et al. (1995), with minor adaptations for carrot DNA introduced by Vivek and Simon (1999). Genomic DNA was separately double digested using the restriction endonucleases enzymes combinations *Eco*R1/*Mse*I and *PstI*/*Mse*I. Each polymorphic AFLP fragment was identified by 1) six letters corresponding to the primer combination, followed by 2) the estimated molecular size of the

DNA fragment in nucleotides, and 3) a letter indicating the parental origin of the fragment: B, H, 4 and Q, respectively for Brasilia, HCM, B493 and QAL.

Specific AFLPs amplicons were excised from the dried polyacrylamide gel to be cloned and to be sequenced in order to develop Sequence Characterized Amplified Regions (SCAR) markers or conserved AFLP in both populations. Ten published microsatellites (Niemann et al., 1997), a RAPD co-dominant marker (Vivek ands Simon, 1999), co-dominant markers reported by Boiteux (2000) and Bradeen and Simon (1998) were also evaluated in both mapping populations.

Linkage analyses were performed with the software MapMaker version 3.0 (Lander et al., 1987) and with the software JoinMap version 2.0 (Stam and van Ooijen, 1995). Linkage groups were assigned primarily with the JoinMap software using LOD score ranging from 3.0 to 6.5 and maximum recombination fraction of 0.5. The recombination fraction frequencies were converted to Kosambi centiMorgans (cM).

RESULTS AND DISCUSSION

A total of 421 and 317 polymorphic AFLP fragments were scored in the Brasilia x HCM and B493 x QAL F_2 populations, respectively. The chi-square p-values were less than 0.02 for 32% and 23% of the AFLP fragments in Brasilia x HCM and B493 x QAL F_2 populations, respectively, and these fragments were not included in the linkage grouping analysis. *Eco*RI/*Mse*I and *PstI*/*Mse*I enzymes pairs revealed 12.0 and 17.8 polymorphic fragments per primer combination, respectively, in the Brasilia x HCM F_2, and 11.3 and 11.9 polymorphic fragment per primer combination, respectively, in the B493 x QAL F_2. In this study, well-defined and easier to screen polymorphic AFLP fragments were usually observed with *Pst*I primers than with *Eco*RI primers. A mapping strategy combining methylation-sensitive and –insensitive enzymes, *Pst*I and EcoRI, with the AFLP technique should provide more complete map coverage (Young et al., 1999).

Two AFLP fragments were “converted” to co-dominant markers when they were amplified by the same primer combination and they presented recombination fraction around 5.0 cM, estimated after individual linkage analysis by primer combination. Among all published microsatellites and PCR-based co-dominant markers, only one microsatellite published by Niemann et al. (1997) yielded co-dominant information in both populations. A microsatellite sequence, identified in routine sequencing of carrot PCR products, provided co-dominant information for the B493 x QAL F_2 population only. Twenty-nine conserved AFLP fragments were used to map the two populations and also to merge the linkage maps.

A total of 287 and 250 molecular markers were used to assign linkage groups in the 160 F_2 plants and 183 F_2 plants of Brasilia x HCM and B493 x QAL, respectively. Those with chi-square values less 0.02 were dropped from the linkage analysis. MapMaker and JoinMap showed exactly the same number of groups and assigned the same markers for a given group using the same LOD score, indicating that algorithms used by these two software packages led to the identical results. Six groups from Brasilia x HCM and B493 x QAL were merged based on two co-dominant markers scored and in 28 conserved dominant AFLP markers (Figure 1).

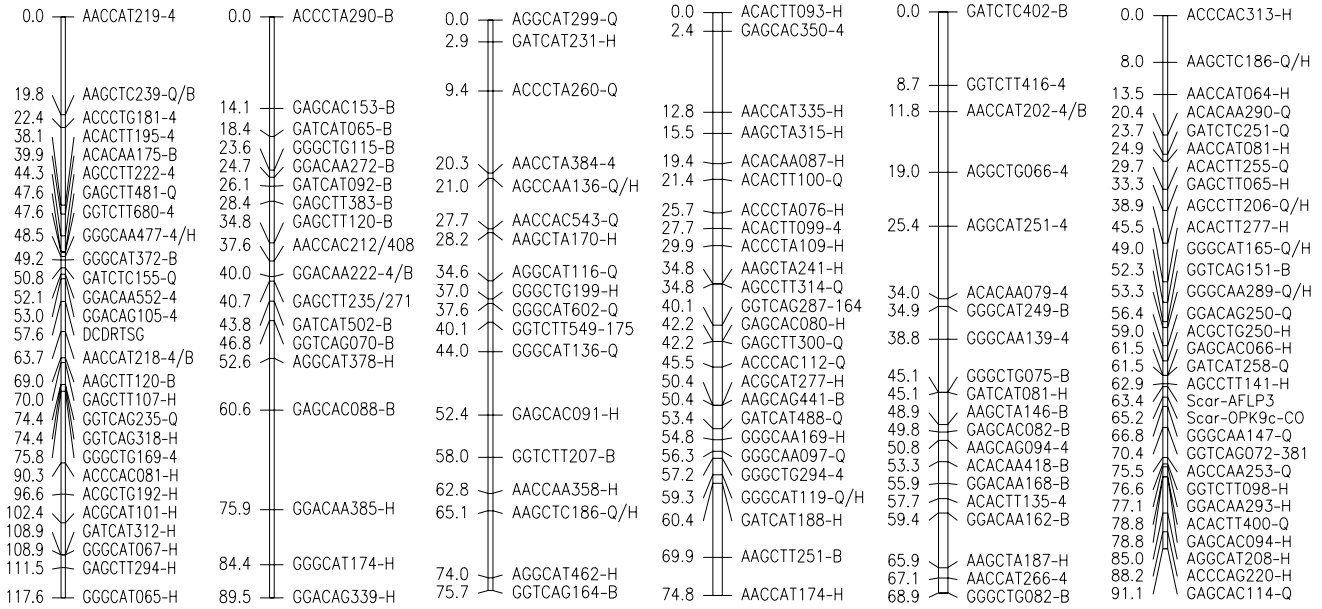

Figure 1. Six merged linkage groups for Brasilia x HCM and B493 x QAL F_2 populations of carrot. AFLP fragments with / and two pair base size represents a conserved AFLP fragment between the two populations and a co-dominant AFLP, respectively

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