

Construction of a RAPD Marker-Based Linkage Map in Ananas Melon

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Introduction: Melon (*Cucumis melo* L.) fruit flesh is a significant source of carbohydrates, ascorbic acid, beta-carotene, folic acid, and potassium. Sucrose is a major factor to determine mature melon fruit sweetness. Ascorbic acid and beta-carotene are major nutrients for human health. Due to consumer preference, these are highly important quality traits of different melon classes. The improvement of these traits is important to breeding programs of most melon types worldwide. Diseases of melon are primary constraints limiting melon production. A yellowing disease, caused by cucurbit yellow stunting disorder virus that is transmitted by *Bemisia tabaci*, is common in South Texas. Molecular tagging and mapping information for these fruit quality traits and disease resistance is expected to be useful to melon breeders because of the possibility to use molecular markers for marker-assisted selection in their breeding programs. Therefore, our initial objective was to construct a RAPD marker-based genetic linkage map in an F₂ population derived from the melon cross of 'Deltex' x TGR1551 for conducting research on the genetics of melon fruit quality and disease resistance.

Materials and Methods: One hundred-eight F₂ plants derived from the cross of 'Deltex' x TGR1551 were planted in a greenhouse at the Texas Agricultural Research and Extension Center-Weslaco in 2003. The 'Deltex' parent is a commercial ananas cultivar with high fruit quality, while the TGR1551 parent is a wild type with low fruit quality. Total genomic DNA was extracted from the leaf tissue of the 108 F₂ plants along with their parents (4). A total of 360 random 10-mer primers (Operon

Technologies, Alameda, Calif.) were used for the RAPD analysis (5). PCR was performed on 96-well plates in an MJ Research thermalcycler (model PTC-0100; MJ Research, Waltham, Mass.). Protocols for PCR and the composition of the final volume of reactants were the same as those described previously (4). A 100-bp DNA ladder (Life Technologies, Grand Island, N.Y.) was used to estimate the length of RAPD markers. The name of each RAPD marker is derived from an "O" prefix for Operon primers, the letters identifying the Operon kit, Operon primer number, and the approximate length (bp) of the marker (3). The 360 primers were used to screen between the parents 'Deltex' and TGR1551. Primers that generated marker polymorphisms between the parents were tested in the F₂ population from the cross between 'Deltex' and TGR1551 to assess genetic linkage of RAPD markers to the traits of interest. To detect segregation distortion of markers, F₂ population marker data were tested for goodness-of-fit to a 3:1 ratio using the chi-square test. The RAPD marker-based linkage map was constructed on the data for the 108 F₂ plants of the 'Deltex' x TGR1551 cross using MAPMAKER version 3.0 (2). On the basis of a LOD score of 3.0 and a linkage threshold of 0.4, linkage groups were displayed using the Group command. To establish a linkage group, a subset of markers was initially selected based on LOD scores and pairwise linkages. The best linkage order within the subset was calculated using the Compare command and then, additional markers were inserted using the Try command. LOD scores of at least 2.0 were considered different between the most and second most likely position for the marker. The Ripple command was finally

used to check the marker order. Map distances (cM) between ordered loci of markers were calculated using recombination fractions and the Kosambi mapping function (1).

Results and Discussion: A total of 208 RAPD markers that segregated in the F₂ population of the 'Deltex' x TGR1551 cross were scored for constructing the genetic linkage map (Table 1). All markers displayed an amplified DNA fragment in the 'Deltex' parent that was absent in the TGR1551 parent. An example of marker OE08.600 obtained from the 'Deltex' parent is shown in Figure 1. Of the 208 markers, 195 (94%) fit the expected 3:1 ratio in the F₂ population on the basis of the chi-square goodness-of-fit test (Table 1). Thirteen markers (6%), however, deviated significantly from the expected 3:1 ratio ($P < 0.05$) in the genetic population. Thus, we excluded the 13 distorted markers in developing the linkage map.

One hundred and ninety-five RAPD markers were used for constructing the genetic map (Table 1). These non-distorted markers were divided into 12 linkage groups, three unlinked pairs (UP), and ten unassigned markers. We developed the molecular marker-based linkage map with 185 RAPD markers (Figure 2). The number of non-distorted markers per linkage group ranged from three on linkage group 12 to 36 on linkage group 6 (Table 1). An average of 14.9 markers were mapped per linkage group. Our linkage map included 157 marker loci spanning a total map distance of 1148 cM. The number of loci per linkage group varied from three on linkage group 12 with a length of 36 cM to 30 on linkage group 6 with a length of 178 cM. An average of 12.6 loci were located per linkage

group. Each linkage group spanned an average length of 91 cM.

This genetic linkage map will be utilized to identify markers linked to QTL controlling mature melon fruit sweetness, quality, size, and shape traits as well as disease resistance, and to determine the genetic relationships among QTL for these important traits in the F₂ population derived from the 'Deltex' x TGR1551 cross.

Literature Cited:

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Table 1. Illustration of the molecular marker-based linkage map on the basis of 208 RAPD markers segregating in 108 F2 plants derived from the 'Deltex' x TGR1551 cross.

Linkage group	No. of markers	No. of loci	Map distance (cM)	Mean distance (cM)	No. of distorted markers ^z
1	23	18	130	7.2	1
2	20	16	106	6.6	1
3	8	7	91	13.0	1
4	14	12	93	7.8	1
5	11	10	82	8.2	1
6	38	30	178	5.9	2
7	11	10	153	15.3	1
8	13	11	40	3.6	0
9	15	11	39	3.5	0
10	17	12	67	5.6	1
11	16	11	80	7.3	1
12	3	3	36	12.0	0
Unlinked pair 1	2	2	27	13.5	0
Unlinked pair 2	2	2	4	2.0	0
Unlinked pair 3	2	2	22	11.0	0
Unassigned markers	13				3
Total	208	157	1148	7.3	13

^zMarkers deviating from the expected 3:1 ratio ($P < 0.05$) were not included in the map.

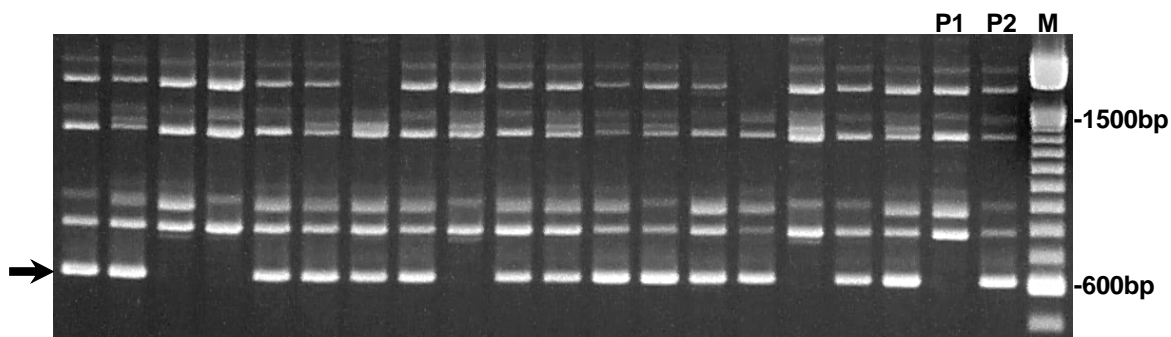


Figure 1. Segregation of RAPD marker OE08.600 amplified from 'Deltex' in an F2 population derived from the melon cross of 'Deltex' x TGR1551. Lines 1-18=F2 plants of the cross, P1=TGR1551, P2='Deltex', and M=a 100-bp DNA marker ladder.

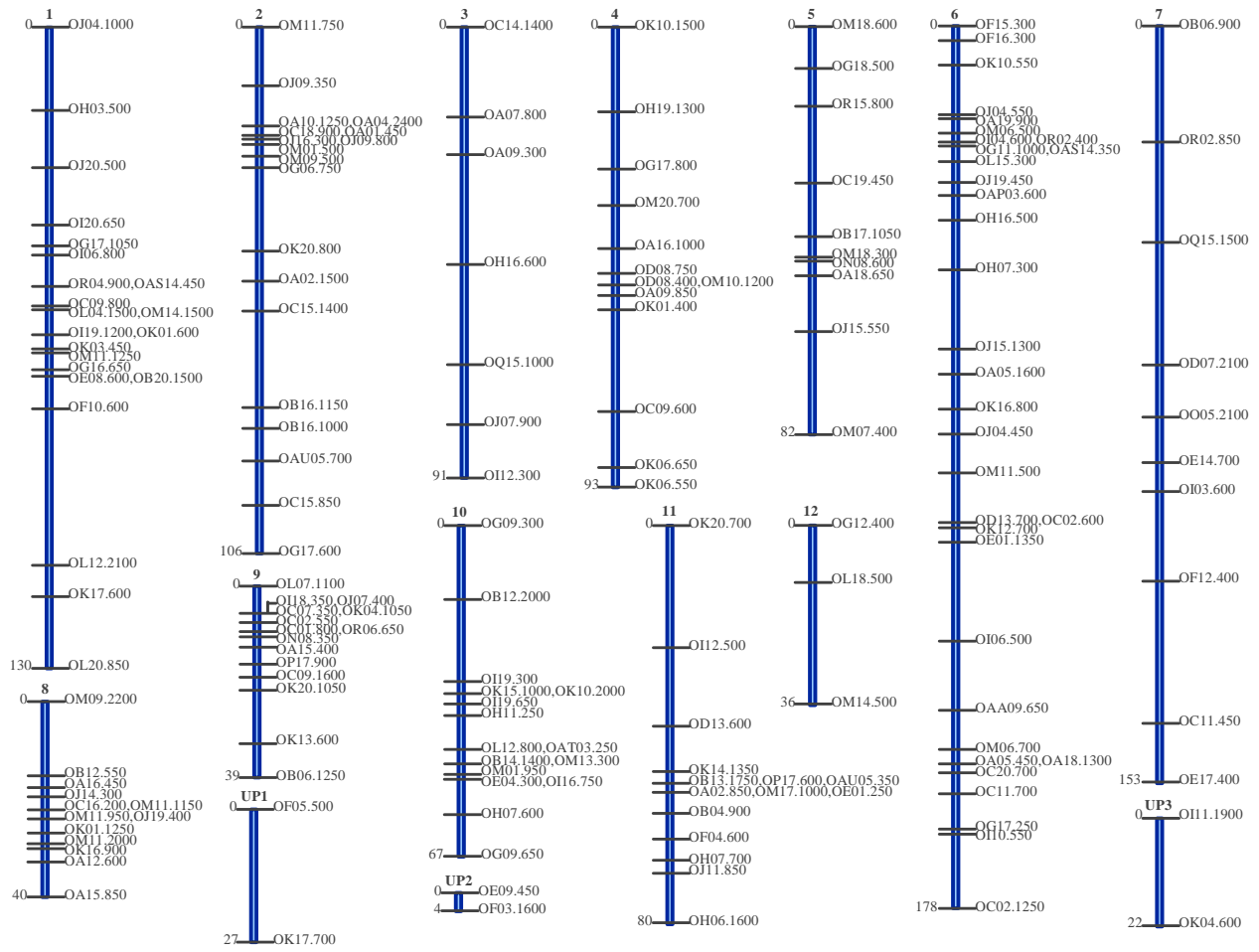


Figure 2. The RAPD marker-based linkage map constructed using an F_2 population of the 'Deltex' x TGR1551 cross. The marker names are given on the right and the length in cM is indicated on the left of each linkage group.