

# A Genetic Linkage Map for Watermelon Based on Randomly Amplified Polymorphic DNA Markers

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**ABSTRACT.** A genetic linkage [randomly amplified polymorphic DNA (RAPD)-based] map was constructed for watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] using a BC<sub>1</sub> population [PI 296341-fusarium wilt resistant x New Hampshire Midget (fusarium susceptible)] x 'New Hampshire Midget'. The map contains 155 RAPD markers, and a 700-base pair sequenced characterized amplified region (SCAR) marker that corresponds to a fragment produced by the RAPD primer GTAGCACTCC. This marker was reported previously as linked (1.6 cM) to race 1 fusarium wilt resistance in watermelon. The markers segregated to 17 linkage groups. Of these, 10 groups included nine to 19 markers, and seven groups included two to four markers. The map covers a genetic linkage distance of 1295 cM. Nine of the 10 large linkage groups contained segments with low (or no) level of recombination (0 to 2.6 cM) among markers, indicating that the watermelon genome may contain large chromosomal regions that are deficient in recombination events. The map should be useful for identification of markers linked closely to genes that control fruit quality and fusarium wilt (races 1 and 2) resistance in watermelon.

Watermelon (*Citrullus lanatus*) is an important horticultural crop that accounts for 2% of the world area devoted to vegetable crops (FAO, 1995). Watermelon production in the United States has increased from 1.2 million tons in 1980 to 3.9 million tons in 1999, with a farm value of \$287 million (USDA, 2000), and there is an ongoing need for genetic improvement of watermelon, particularly with respect to disease and pest resistance.

*Citrullus lanatus* var. *lanatus* (Thunb.) Matsum & Nakai is the progenitor of the cultivated watermelon. *Citrullus lanatus* is one of four known diploid ( $n = 11$ ) species that belong to the xerophytic genus *Citrullus* Schrad. ex Eckl. & Zeyh. It is found in the temperate regions of Africa, central Asia, and the Mediterranean (Jeffrey, 1975; Whitaker and Bemis, 1976; Whitaker and Davis, 1962), and has a haploid genome size of  $4.25 \times 10^8$  base pairs (bp) (Arumuganathan and Earle, 1991).

The cultivated watermelon ( $2n = 2x = 22$ ) (Shimotsuma, 1963) appears to have a narrow genetic base reflected by low levels of isozyme (Navot and Zamir, 1987) and DNA polymorphism among cultivars and accessions of *C. lanatus* var. *lanatus* (Levi et al., 2000). However, higher polymorphism exists among accessions of *C. lanatus* var. *citroides* (Jarret et al. 1997) which is considered the ancestor of *C. lanatus* var. *lanatus* (Navot and

Zamir, 1987). Based on scoring of 636 randomly amplified polymorphic DNA (RAPD) markers, the genetic similarity values among PIs of *C. lanatus* var. *citroides*, PIs of *C. lanatus* var. *lanatus*, and among watermelon cultivars, were 71.0% to 90.5%, 75.0% to 96%, and 92.8% to 98.3%, respectively (Levi et al., 2000, 2001). Despite low DNA polymorphism, vast variation in morphological characteristics, including rind color and thickness, fruit shape and size, flesh texture and color, sugar content, seed shape and color, and days to fruit maturity, exists among watermelon cultivars. Several gene mutants that affect a few of these morphological characteristics have been identified (Rhodes and Dane, 1999).

High-density genetic linkage maps are useful for positioning and tagging genes of interest to facilitate marker-assisted breeding in an increasing number of crop plants. Genetic maps are also useful in gene cloning and in analyzing complex traits (Lee, 1995). Navot and Zamir (1986) reported the first linkage map for watermelon derived from a cross between an accession of the wild species *C. colocynthis* (L.) and the watermelon cultivar Mallali. This map described linkage relationships among 19 protein coding genes in watermelon. Subsequently, they extended the map to 24 loci (including 22 isozyme loci, the locus for fruit bitterness, and the locus for flesh color) that segregate in seven linkage groups, covering 354 cM (Navot et al. 1990). Hashizume et al. (1996) constructed an initial genetic linkage map for watermelon using a BC<sub>1</sub> population derived from a cross between an inbred line (H-7; *C. lanatus*) and a wild accession (SA-1; *C. lanatus*). The map contained 58 RAPD markers, one isozyme, one restriction fragment length polymorphism (RFLP) and two morphological markers segregating in 11 linkage groups, and extending 524 cM. Recently, Hawkins et al. (2001) constructed two linkage maps for watermelon. These maps contained 26 and

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13 RAPD markers segregating in two and in five linkage groups, covering 112.9 and 139 cM, respectively. Still, many markers are required for construction of a saturated map that can be used effectively in watermelon breeding programs, and for locating genes that control important traits like fruit quality and resistance to diseases and pests.

The cultivated watermelon is susceptible to many diseases. Among them, fusarium wilt [incited by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *niveum* (E.F. Sm.) W.C. Snyder & H.N. Hans] and gummy stem blight [incited by *Didymella bryoniae* (Auersw.)] considered most destructive to watermelon (Netzer and Martyn, 1989; Sowell and Pointer, 1962). United States Plant Introduction (PI) 296341 (*C. lanatus* var. *citroides*) showed resistance to races 0, 1, and 2 of *Fusarium oxysporum* f. sp. *niveum* (Martyn and Netzer, 1991). A test with race 2 for progenies of a self-pollinated plant of PI 296341 resulted in a segregation of about three susceptible plants to one resistant plant (Martyn and Netzer, 1991), or 13 susceptible to three resistant plants (Zhang and Rhodes, 1993). These data indicate that race 2 fusarium wilt resistance might be a qualitative trait governed by one or several recessive genes. However, Xu et al. (1999) imply that resistance to race 1 fusarium wilt in PI 296341 may be governed by a dominant gene. Resistant plants of PI 296341 were self-pollinated and selected for resistance to race 2 for three generations. Seeds of the most resistant plants were released by the Texas Agricultural Experiment station as an improved line PI 296341-FR (Martyn and Netzer, 1991). PI 296341-FR also has the following fruit characteristics: a small round fruit with gray-green rind, white flesh, small olive-green to brown seeds, and a period of 80 d from seed germination to fruit maturity. These traits could be useful in genetic studies against the cultivar New Hampshire Midget (NHM), which has an oval shape fruit with red flesh, gray rind, black seeds, and a period of 65 d from seed germination to fruit maturity. Although NHM is a high quality cultivar (developed at the University of New Hampshire in 1951), it is susceptible to all major diseases of watermelon.

This paper describes a genetic linkage map for watermelon using the BC<sub>1</sub> population [PI 296341-fusarium resistant (FR) × NHM] × NHM. This map can be useful in locating genes that control fruit quality, as well as resistance to fusarium wilt in watermelon.

## Materials and Methods

**PLANT MATERIAL.** Parental plants: PI 296341-FR and NHM and their F<sub>1</sub> hybrid, and 78 plants of the backcross progeny [(PI 296341-FR × NHM) × NHM] were grown in the greenhouse (14 h day at 26 °C, and 10 h night at 20 °C). Three weeks after germination young leaves (10 g) were collected from each plant, and stored at -80 °C.

**DNA ISOLATION.** To avoid coisolation of polysaccharides, polyphenols, and other secondary compounds that damage DNA, we used an improved procedure for isolation of DNA from young leaves of watermelon (Levi and Thomas, 1999).

**DNA AMPLIFICATION CONDITIONS AND GEL ELECTROPHORESIS.** Ten decamer oligonucleotides were purchased from the University of British Columbia, Biotechnology Center, Vancouver, British Columbia, Canada, and from Operon Technologies, Inc., Alameda, Calif., and were used for polymerase chain reaction (PCR) amplification as described by Levi et al. (1993) and by Rowland and Levi (1994). RAPD reactions were in 25-μL reaction buffer containing 20 mM NaCl, 50 mM Tris-HCl pH 9, 1%

Triton-X-100, 0.01% gelatin, 1.6 mM MgCl<sub>2</sub>, 200 mM each of dATP, dCTP, dGTP, and dTTP (Sigma, Saint Louis, Mo.), 0.2 mM primer, 7 units Taq DNA Polymerase (Promega, Madison, Wis., supplied in storage buffer A), and 25 ng template DNA. Amplification reactions were carried out for 45 cycles in a thermocycler (PTC-200; MJ Research Watertown, Mass.), programmed for 60 s for DNA to denature at 92 °C, 70 s for DNA annealing at 48 °C, and 120 s for primer extension at 72 °C. DNA amplification conditions for a sequenced characterized amplified region (SCAR) marker used in the present study were the same as for the RAPD primers, except for the DNA annealing temperature which was set at 62 °C. Amplification products were separated by electrophoresis in 1.4% agarose gels in 0.5× Tris borate buffer (Sambrook et al., 1989). The gels were stained with ethidium bromide solution at 0.5 mg·mL<sup>-1</sup> for 30 min and destained for 15 min in distilled water. DNA fragments were visualized under ultraviolet light and photographed using a still video system (Gel Doc 2000; Bio-Rad, Hercules, Calif.). The molecular weights of the amplification products were calculated using 100-bp DNA ladder standards (Gibco BRL, Gaithersburg, Md.).

**MARKER NOMENCLATURE.** The RAPD markers were designated by their serial number and their size. For example, the 500 bp marker produced by primer AW-07 (Operon) was designated as AW07-500, and the 250 bp marker produced by primer No. 101 (University of British Columbia) was designated as 101-250.

**LINKAGE ANALYSIS.** Data were analyzed using Mapmaker version 3.0 (Lander et al., 1987; Lincoln et al., 1992). Markers were first grouped using a minimum log of odds ratio (LOD) score of 4.0 and a maximum recombination value (q) of 0.30. For each linkage group, markers were ordered by using the "Order" command with a minimum LOD score of 3.0 and a maximum recombination value (q) of 0.25. Markers ordered with low confidence were placed again using the "Try" command. The ordered marker sequences were confirmed using the "Ripple" command. Linkage maps were generated with the "Map" command using the "Kosambi" map function. The "Error-Detection" command was used to identify possible marker scoring errors, and putative errors were retested. Chi-square tests were performed to check whether individual markers segregated randomly.

## Results and Discussion

**RAPD MARKER ANALYSIS.** A total of 568 random 10-mer primers with 60% to 90% guanine-cytosine (GC) content were screened in amplification reactions against the parents, PI 296341-FR and NHM, and their F<sub>1</sub> hybrid. Of those, 377 primers yielded between one and 16 DNA amplification products each, ranging in size from 0.1 to 3 kb. Primers with higher GC content produced higher numbers of RAPD bands, as has been reported for other plant species (Fritsch et al., 1993). One hundred and six primers of the 377 primers, produced 179 distinct and reproducible marker bands that could be mapped with high confidence (Table 1). These marker bands were present in the donor parent PI 296341-FR and in the F<sub>1</sub> hybrid, but absent in the recurrent parent NHM (Fig. 1).

PI 296341-FR is a result of three generations of selection (for races 0, 1, and 2, fusarium wilt resistance) and self-pollination (Martyn and Netzer, 1991), and therefore is likely to be highly homozygous. Using the model of Nei and Li (1979), the genetic similarity level  $[2N_{ab}/(N_a + N_b)]$  where  $N_{ab}$  is the number of RAPD fragments shared by two genotypes (a and b) and  $N_a$  and  $N_b$  are the

Table 1. The nucleotide sequences of RAPD primers and the number of markers produced by each primer used in the mapping analysis. Size (bp) of each marker that could not be mapped (unmapped markers), and size of markers skewed toward PI 296341-FR (SP), or toward the cultivar NHM (SC).

Primer <sup>z</sup>	Sequence	No. of markers	Unmapped markers (bp)	SP	SC
002	CCTGGGCTTG	1			
006	CCTGGGCCTA	1			
016	GGTGGCGGGA	1			725
017	CCTGGGCCTC	2			
031	CCGGCCTTCC	1			
034	CCGGCCCCAA	2			425
043	AAAACCGGGC	2			
079	GAGCTCGTGT	1			
081	GAGCTCTAGA	3			1225
084	GGGCGCGAGT	2			
088	CGGGGGATGG	1			
105	CTCGGGTGGG	1			
125	GCGGTTGAGG	2			
147	GTGCGTCCTC	1			
149	AGCAGCGTGG	2		330	
155	CTGGCGGCTG	2			
156	GCCTGGTTGC	2			1500
157	CGTGGGCAGG	3	1200		1425
159	GAGCCCCTAG	3	1100		
173	CAGGCGGCGT	3			
174	AACGGGCAGC	1		500	
184	CAAACGGCAC	1			
186	GTGCGTCGCT	2	2200	2200	
190	AGAATCCGCC	1			
218	CTCAGCCCAG	2			
301	CGGTGGCGAA	1			
308	AGCGGCTAGG	2			
309	ACATCCTGCG	2		650, 750	
312	ACGGCGTCAC	3		850	
329	GCGAACCTCC	1		750	
336	GCCACGGAGA	2			550
338	CTGTGGCGGT	1			
356	GCGGCCCTCT	2			750
359	AGGCAGACCT	4		530	1350, 825
372	CCCCTGACG	1			
383	GAGGCGCTGC	2		825	
384	TGCGCCGCTA	1			
387	CGCTGTCGCC	1			
388	CGGTCGCGTC	2			
389	CGCCCGCAGT	2	850		
402	CCCGCCGTTG	1	650		
411	GAGGCCCGTT	1			
421	ACGGCCCACC	2			
428	GGCTGCGGTA	1			
430	AGTCGGCACC	1			
437	AGTCCGCTGC	4			975, 825,
439	GCCCCTTGAC	1	650		475, 425
456	GCGGAGGTCC	1			
459	GCGTCGAGGG	2			
488	TTCGCTTCTC	1	800		
714	GGGTGGGTGT	1	1300		
731	CCCACACCAC	2			975
758	GGTTGGGTGG	1			1050

<sup>z</sup>Primers are from the University of British Columbia.

Primer <sup>y</sup>	Sequence	No. of markers	Unmapped markers (bp)	SP	SC
C20	ACTTCGCCAC	2	600, 785		
D08	GTGTGCCCCA	1			1375
E14	TGCGGCTGAG	1			
G02	GGCACTGAGG	1			925
G08	TCACGTCCAC	2	750	750	
G10	AGGGCCGTCT	3	575		
G13	CTCTCCGCCA	1			
G17	ACGACCGACA	3			
G18	GGCTCATGTG	2	800		800
G19	GTCAGGGCAA	2			300
H03	AGACGTCCAC	2			
H06	ACGCATCGCA	2	200		
H12	ACGCGCATGT	1	950	950	
H13	GACGCCACAC	1			
I01	ACCTGGACAC	2			275
I07	CAGCGACAAG	1			
I09	TGGAGAGCAG	1		500	
I12	AGAGGGCACA	2			1050
I18	TGCCCAGCCT	1			
L01	GGCATGACCT	2			
L02	TGGGCGTCAA	3			
L05	ACGAGGCAC	1			250
L08	AGCAGGTGGA	2			
L12	GGGCGTACT	1		500	
L17	AGCCTGAGCC	2			
N05	ACTGAACGCC	1			
N09	TGCCGGCTTG	2			
N14	TCGTGCGGGT	1	800		
P01	GTAGCACTCC	1		700	
P02	TCGGCACGCA	4			550
P07	GTCCATGCCA	2			
P08	ACATCGCCCA	1			875
P16	CCAAGCTGCC	2			1400
P06	GTGGGCTGAC	1			
Q06	GAGCGCCTTG	2			1450
R16	CTCTGCGCGT	1	425		
U15	ACGGGCCAGT	3			550
V10	GGACCTGCTG	1			
V15	CAGTGCCGGT	2			400, 650
V19	GGGTGTGCAG	1			225
Y02	CATCGCCGCA	2			
Y13	ACAGCCTGCT	1	600		
Y05	GGCTGCGACA	1			
Z03	CAGCACCGCA	1			
AA11	ACCCGACCTG	1			
AB03	TGGCGCACAC	2	1300		
AB04	GGCACGCGTT	5	575		
AB09	GGGCGACTAC	1			
AB18	TGGTCCAGCC	1	1350		
AC07	GTGGCCGATG	2	500		
AC10	AGCAGCGAGG	1			
AC12	GGCGAGTGTG	2			
AD16	AACGGGCGTC	3	1325, 950,	875	

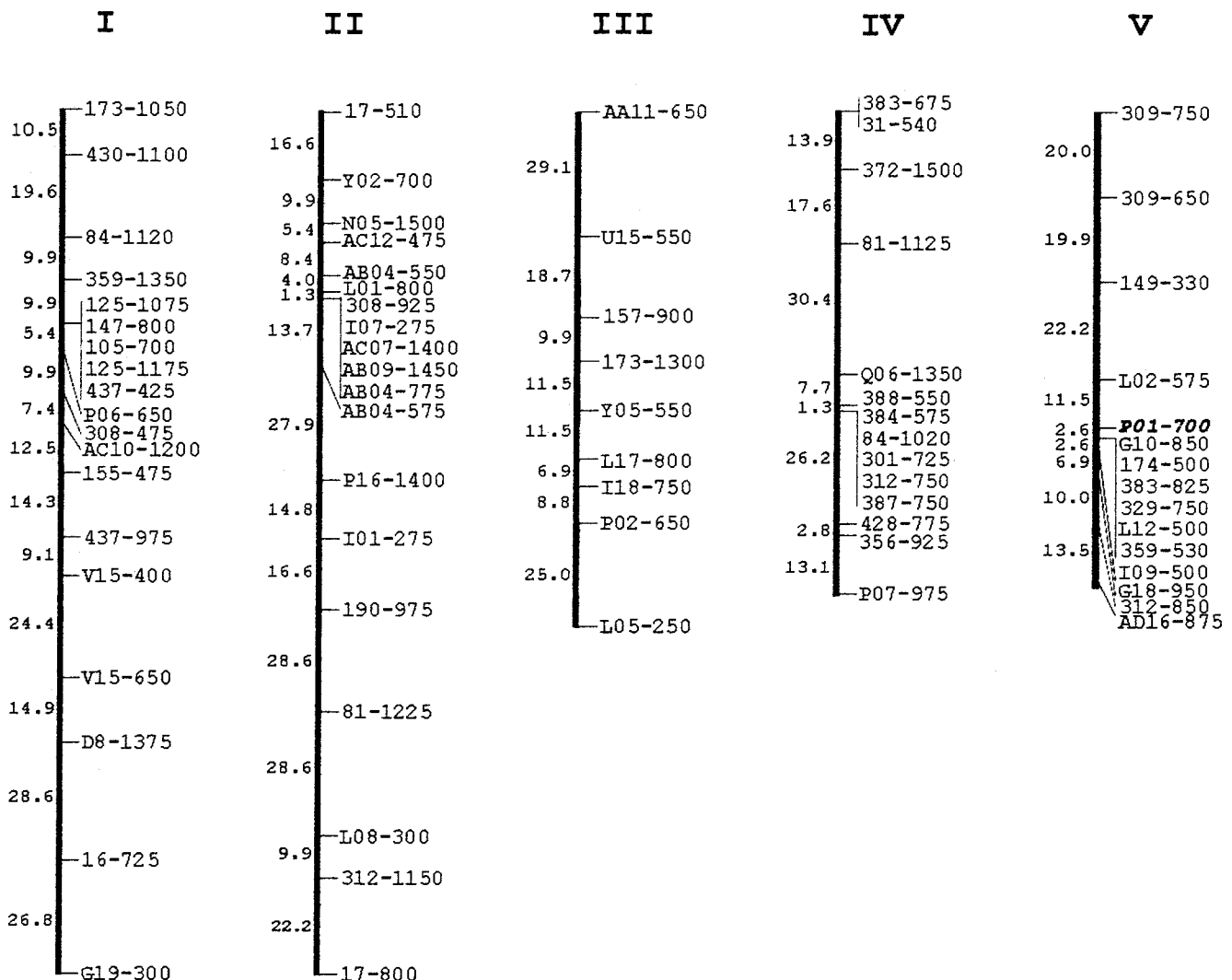
<sup>y</sup>Primers are from Operon Technologies.

total number of RAPD fragments analyzed in each genotype between PI 296341-FR and NHM (based on all fragments produced by 120 primers) is  $2 \times 285 / (563 + 608) = 48.6\%$ . Thus, a high level of polymorphism is expected from the present cross.

**MAP CONSTRUCTION AND MARKER SEGREGATION.** One hundred and fifty-six markers (87.1%) of the 179 markers analyzed could be placed on linkage groups. The map contains 10 major linkage groups, each with nine to 19 markers, and seven minor linkage groups, each with two to four markers. The map covers a distance of 1295 cM with an average distance of 9.3 cM between markers (Tables 1 and 2, Fig. 1).

Of the 179 markers analyzed, 46 (25.7%) showed segregation patterns skewed away from a 1:1 ratio at  $P = 0.05$ . Of these, 31 markers displayed under-presence (skewed towards NHM), while 15 markers displayed over-presence (skewed towards PI 296341-FR) (Tables 1 and 2). Of the 31 markers skewed towards NHM, one marker (G18-800) could not be mapped, eight markers (16-725, 359-1350, 437-425, 437-975, D08-1375, G19-300, V15-400, and V15-650) were assigned to linkage group I, four markers (81-1225, AB04-575, I01-275, and P16-1400) were assigned to linkage group II, two markers (L05-250 and U15-550) were assigned to linkage group III, six markers (34-425, 156-1500, 356-750, 437-475, I12-1050, and P08-875) were assigned to linkage group VI, two markers (V19-225 and 157-1425) were assigned to linkage group VII, one marker (437-825) was as-

signed to linkage group VIII, four markers (731-975, G02-925, P02-550, and Q06-1450) were assigned to linkage group X, and three markers (336-550, 359-825, and 758-1050) were assigned to linkage group XV (Tables 1 and 2, Fig. 1). Most of the skewed markers were clustered in their respective linkage group (Fig. 1). Three of the 15 markers that skewed towards PI 296341-FR (186-2200, G08-750, and H12-950) could not be mapped. The other 12 markers (149-330, 174-500, 309-650, 309-750, 312-850, 329-750, 359-530, 383-825, AD16-875, I09-500, L12-500, and P01-700) were all assigned to linkage group V (Tables 1 and 2, Fig. 1). This linkage group consisted of markers that are highly skewed towards PI 296341-FR, and is likely to represent a chromosome that segregates preferentially in the BC<sub>1</sub> population with the predominant NHM background. Preferential segregation of a chromosome with unique features was first described in a BC<sub>1</sub> population of maize (*Zea mays* L.) (Rhodes, 1942). Preferential segregation may be inferred from a meiotic drive where a chromosome with unique structural or genetic properties renders selective advantage or disadvantage to its respective gametes or zygotes (Buckler et al., 1999; Cameron and Moav, 1957; Sandler and Novitski, 1957; Wendel et al., 1987; Xu et al., 1997). Further studies are needed to determine whether the skewed segregation observed in the current study is a result of a meiotic drive mechanism. Meiotic drive may play a major role in genome evolution, resulting in radical alterations in genome configura-



tion and diversity within short evolutionary periods (Buckler et al., 1999). The skewed segregation of markers in the present study may be a direct result of the wide genetic distance (48.6% genetic similarity) between PI 296341-FR and NHM, giving preference to genotypes that are more compatible with the recurrent parent genotype (NHM). Skewed segregation has been reported mostly in populations derived from interspecific or intergeneric crosses (Bonierbale et al., 1988; Durham et al. 1992; Weeden et al., 1989; Zamir and Tadmor, 1986). However, Wang et al. (1997) reported skewed segregation in a backcross population derived from two melon (*Cucumis melo* L.) cultivars. Hashizume et al. (1996) also reported that 12 (17%) out of 69 RAPD markers mapped had skewed segregation in a BC<sub>1</sub> population of watermelon.

Nine of the 10 major linkage groups possess regions with no, or with low, recombination events (0 to 2.6 cM) between markers (Fig. 1). Some of these regions might be near a centromere. Fewer recombination events occur in the vicinity of a centromere than

in regions distant from it (Dimitrov and Georgieva, 1994; Mather, 1936, 1939). However, variability in crossing-over frequency may also be a result of heterochromatin properties (Mather, 1939). Therefore, further studies are needed to test the properties and locations of these regions deficient in recombination events. In contrast with the large linkage groups, all small linkage groups contained large genetic distances (4.4 to 25.1 cM) between markers (Table 2, Fig. 1), indicating that they might be within chromosomal regions with frequent recombination events.

The present map does not cover all parts of the genome. Twenty-three of the 179 markers analyzed (12.8%) could not be ordered after grouping (Table 1). At least 526 markers (with a maximum distance of 5 cM) are required to saturate 95% of a 1600 cM map (Perin et al., 2000). Recent studies also implied that large population size, and not just the number of markers, would most likely reduce the number of linkage groups identified in a mapping study (Keim et al., 1997; Kesseli et al., 1994).

Although the present map is not saturated, it can be useful for identifying loci around genes of interest, as shown with marker P01-700. This marker was reported as linked (1.6 cM) to fusarium wilt race 1 resistance gene in watermelon (Xu et al. 2000). The same authors sequenced the 700 bp marker produced by the

Fig. 1. RAPD-based genetic linkage map of watermelon derived from a backcross population (PI 294341-FR x NHM) x NHM. Marker names are at the right, while distances between adjacent markers (in cM) are at the left of each linkage group.

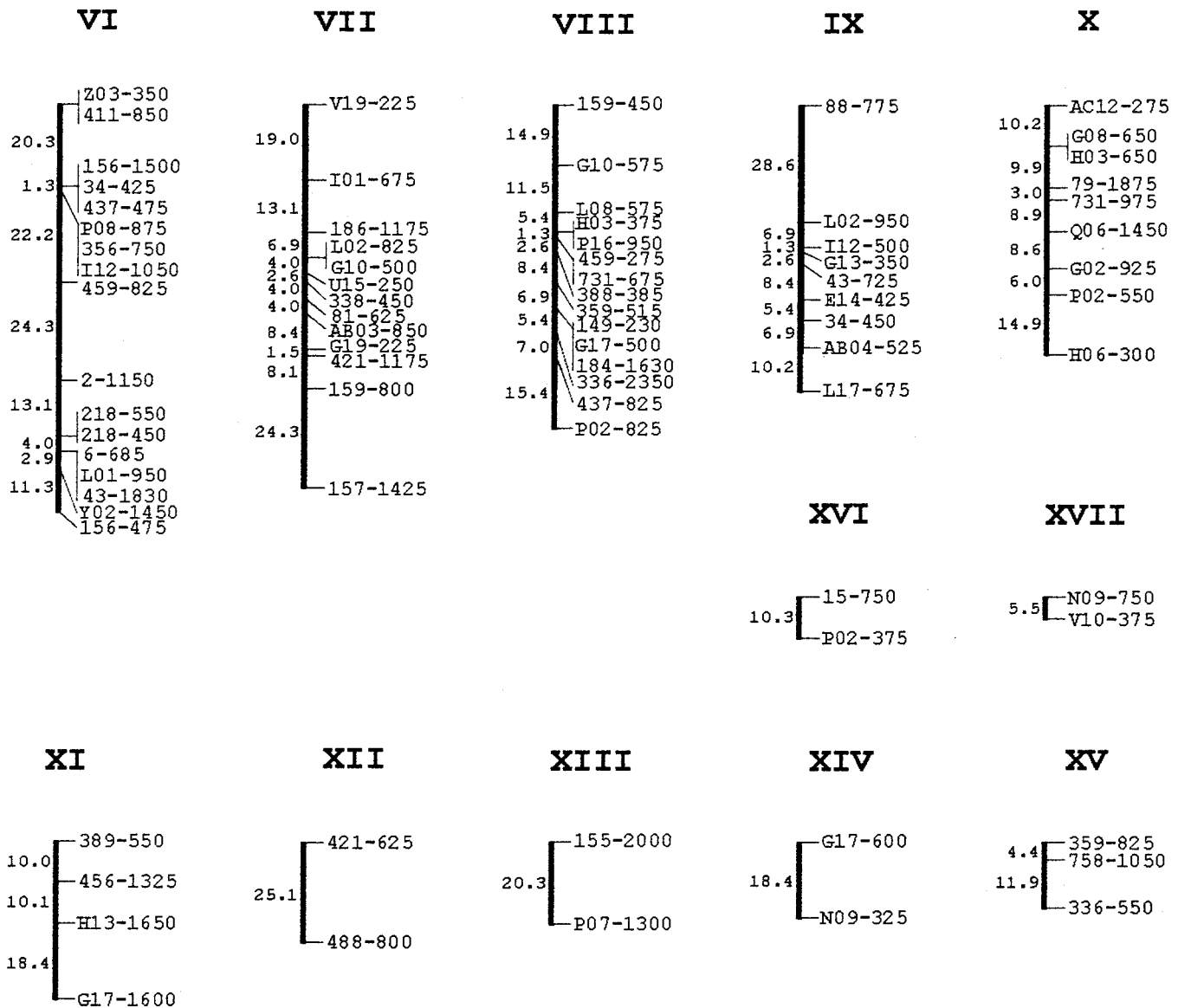


Table 2. Marker distribution among linkage groups.

Linkage group	No. of markers	Length (cM)	Avg distance (cM)	No. of skewed markers
I	19	203.2	11.3	8 <sup>z</sup>
II	19	207.9	11.5	4 <sup>z</sup>
III	9	121.4	15.2	2 <sup>z</sup>
IV	14	113.0	8.7	---
V	15	109.2	7.8	12 <sup>y</sup>
VI	17	99.4	6.2	6 <sup>z</sup>
VII	13	95.9	8.0	2 <sup>z</sup>
VIII	15	78.8	5.6	1 <sup>z</sup>
IX	9	70.3	8.7	---
X	9	61.5	7.7	4 <sup>z</sup>
XI	4	38.5	12.8	---
XII	2	25.1	25.1	---
XIII	2	20.3	20.3	---
XIV	2	18.4	18.4	---
XV	3	16.3	8.2	3 <sup>z</sup>
XVI	2	10.3	10.3	---
XVII	2	5.5	5.5	---
Total	156	1295	9.3 <sup>x</sup>	42

<sup>z</sup>Markers skewed towards NHM.

<sup>y</sup>Markers skewed towards PI 296341.

<sup>x</sup>Average distance between two markers.

RAPD primer, and constructed SCAR primers (5'GTA-GCA-CTCCAACATTTATTCTAATTC, and 5'GTAGCACTCCCAACT-CATACAAAT). In the present analysis, the PCR amplification product of these SCAR primers was entirely consistent with that of the RAPD primer. The marker (P01-700) is assigned to linkage group V (that is skewed towards PI 296341-FR), where it is linked closely to a dense cluster of eight markers (Fig. 1).

Further studies are needed to determine the map distance of these eight markers from the race 1 fusarium wilt resistance gene. Presently F<sub>3</sub> and BC<sub>1</sub>-F<sub>2</sub> families are being constructed, and will be evaluated for resistance to fusarium wilt (races 1 and 2), and for further identification and isolation of molecular markers that are linked closely to fusarium resistance genes. Additionally, the F<sub>3</sub> and BC<sub>1</sub>-F<sub>2</sub> families will be used for evaluation and mapping of genes that control fruit quality. Eventually, the order of assigned markers will be determined when more progenies are used and more markers are developed. RAPD markers in this study will be converted to SCAR primers or to RFLP probes, to facilitate integration with other linkage maps of watermelon, primarily with a linkage map that was recently constructed by Xu and coworkers (personal communication), and which contains 96 loci of RAPD, SSR, isozyme and morphological markers. Additional integration of the present map might be with the linkage groups constructed by Hawkins et al. (2001). Extensive conservation of linkage relationships has been reported in various crops (Gale and Devos, 1998). Therefore, extending the watermelon map and merging it with maps constructed for melon (Baudracco-Arnas and Pitrat, 1996; Brotman et al. 2000; Dogimont et al., 2000; Oliver et al., 2000; Perin et al., 2000; Wang et al., 1997), cucumber (*Cucumis sativus* L.) (Staub and Serquen, 2000), or any other cucurbit species may expedite their genome analysis.

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