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 BC₁ population of ‘296341-fusarium wilt resistant’ x ‘New Hampshire Midget’ (fusarium susceptible). The map contains 155 RAPD markers, and a pair of RAPD primers 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 × 10⁸ 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 Dune, 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 BC₁ 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
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 *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 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 65 d from seed germination to fruit maturity. Although NHM is a high quality cultivar with 60% to 90% guanine-cytosine (GC) content were screened 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 568 random 10-mer primers used in amplification reactions against the parents, PI 296341-FR and NHM, and their F1 hybrid. Of those, 377 primers yielded between 377 primers, produced 179 distinct and reproducible marker bands that could be mapped with high confidence (Table 1).

**Materials and Methods**

**Plant Material.** Parental plants: PI 296341-FR and NHM and their F1 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 MgCl2, 200 μM each of dATP, dCTP, dGTP, and dTTP (Sigma, Saint Louis, Mo.), 0.2 μM 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 amplification 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 0.5x Tris borate buffer (Sambrook et al., 1989). The gels were stained with ethidium bromide solution at 0.5 mg·mL−1 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 F1 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 F1 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 index $\frac{2N_{a}-(N_{c}+N_{n})}{N_{c}+N_{n}}$ where $N_{a}$ is the number of RAPD fragments shared by two genotypes (a and b) and $N_{c}$ and $N_{n}$ 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 | Sequence          | No. of markers | Unmapped markers (bp) | SP  | SC  |
|--------|-------------------|----------------|-----------------------|-----|-----|
| 002    | CCTGGGGCTTG       | 1              |                       |     |     |
| 006    | CCTGGGCCTTA       | 1              |                       |     |     |
| 016    | GTGGGGGCGGGA      | 1              | 725                   |     |     |
| 017    | CCTGGGCGCTC       | 2              |                       |     |     |
| 031    | CCGGCCTTCC        | 1              |                       |     |     |
| 034    | CCGGCCCGCAA       | 2              | 425                   |     |     |
| 043    | AAAACCGGACC       | 2              |                       |     |     |
| 079    | GAGCTCTGTG        | 1              |                       |     |     |
| 081    | GAGCTCCTAGA       | 3              | 1225                  |     |     |
| 084    | CGGCGCGAGTG       | 2              |                       |     |     |
| 088    | CGGGGATGAGG       | 1              |                       |     |     |
| 105    | CTCGGGTGGG        | 1              |                       |     |     |
| 125    | GCAGGTGAGG        | 2              |                       |     |     |
| 147    | GTGCGTCTCTC       | 1              |                       |     |     |
| 149    | AGCACCGTGG        | 2              | 330                   |     |     |
| 155    | CTGGGCGGTG        | 2              |                       |     |     |
| 156    | GCCGCGGTTGC       | 2              | 1500                  |     |     |
| 157    | GTGCGGCGAGG       | 3              | 1200                  | 1425|     |
| 159    | GAGCGCGTGGG       | 3              | 1100                  |     |     |
| 173    | CAGGCCGCGGT       | 3              |                       |     |     |
| 174    | AACCGGCAGC        | 1              | 500                   |     |     |
| 184    | CAACCGGCAC        | 1              |                       |     |     |
| 186    | GTGCGTGCTG        | 2              | 2200                  | 2200|     |
| 190    | AGATCCGGA         | 1              |                       |     |     |
| 218    | CTACAGCCCA        | 2              |                       |     |     |
| 301    | CGTGCGCGA         | 1              |                       |     |     |
| 308    | AGCGGCTTGG        | 2              |                       |     |     |
| 309    | ACATCCTGGG        | 2              | 650, 750              |     |     |
| 312    | ACGCGCTTGG        | 3              | 850                   |     |     |
| 329    | GCCACGCTGC        | 1              | 750                   |     |     |
| 336    | GCCACGGAGG        | 2              | 550                   |     |     |
| 338    | CTTGGGTGCCG       | 1              |                       |     |     |
| 356    | GCGGCCCTCT        | 2              | 750                   |     |     |
| 359    | AGGCGACGCT        | 4              | 530, 1350             | 825 |     |
| 372    | CCCACTGACG        | 1              |                       |     |     |
| 383    | AGGCGCGTGG        | 2              | 825                   |     |     |
| 384    | TCGCCGCGCA        | 1              |                       |     |     |
| 387    | CGCTGTCGCG        | 1              |                       |     |     |
| 388    | CGTGGCGCGT        | 2              |                       |     |     |
| 389    | CGCCCGCGA         | 2              | 850                   |     |     |
| 402    | CCCCGCGTTG        | 1              | 650                   |     |     |
| 411    | GAGGCACGGT        | 1              |                       |     |     |
| 421    | ACGGCCGCCA        | 2              |                       |     |     |
| 428    | GCGTCGGGTA        | 1              |                       |     |     |
| 430    | AGTCGCGACC        | 1              |                       |     |     |
| 437    | AGTCCCGTGG        | 4              | 975, 825              |     |     |
| 439    | GCCCCCTTGAC       | 1              | 650                   | 475 | 425 |
| 456    | GGGAGGTTGG        | 1              |                       |     |     |
| 459    | GGCTGGAGGG        | 2              |                       |     |     |
| 488    | TTTGGCTTCTC       | 1              | 800                   |     |     |
| 714    | GGGTTGGTGG        | 1              | 1300                  |     |     |
| 731    | CCCACCACACC       | 2              | 975                   |     |     |
| 758    | GGTTGGTTGG        | 1              | 1050                  |     |     |

*Primers are from the University of British Columbia.*
| Primer | Sequence       | No. of markers | Unmapped markers (bp) | SP | SC |
|--------|----------------|----------------|-----------------------|----|----|
| C20    | ACTTCGCCAC     | 2              | 600, 785              |    |    |
| D08    | GTGTGCCCA      | 1              |                       | 1375 |    |
| E14    | TGCAGCTGAG     | 1              |                       |    |    |
| G02    | GGCACGTGAG     | 1              |                       | 925  |    |
| G08    | TCACGCTCAG     | 2              | 750                   | 750 |    |
| G10    | AGGCGCGCTCT    | 3              | 575                   |    |    |
| G13    | CTCCTCGCGCA    | 1              |                       |    |    |
| G17    | ACGACGGGACA    | 3              |                       |    |    |
| G18    | GGCTCATGGG     | 2              | 800                   | 800 |    |
| G19    | GTCCGGCGGCA    | 2              |                       | 300 |    |
| H03    | AGACGTTCAG     | 2              | 200                   |    |    |
| H12    | ACGCGAAGATG    | 1              | 950                   | 950 |    |
| H13    | GACGCGACAC     | 1              |                       |    |    |
| I01    | ACCTTGACAC     | 2              |                       | 275 |    |
| I07    | CAGCGGACAG     | 1              |                       |    |    |
| I09    | TGGAGAGCAG     | 1              | 500                   |    |    |
| I12    | AGAGGGCGCA     | 2              |                       | 1050|    |
| I18    | TGCCAGGCTT     | 1              |                       |    |    |
| L01    | GGCTAGGACT     | 2              |                       |    |    |
| L02    | TGCCAGGCTA     | 3              |                       |    |    |
| L05    | ACGCGGACAC     | 1              | 250                   |    |    |
| L08    | AGCAGTTGGA     | 2              |                       |    |    |
| L12    | GGGCGGTATCT    | 1              | 500                   |    |    |
| L17    | AGCGGAGCC      | 2              |                       |    |    |
| N05    | ACTGGAACGC     | 1              |                       |    |    |
| N09    | TGCCGCTTCA     | 2              |                       |    |    |
| N14    | TGCTGGGCGT     | 1              | 800                   |    |    |
| P01    | GTAGCAGCTCC    | 1              | 700                   |    |    |
| P02    | TCGGACGCGG     | 4              |                       | 550 |    |
| P07    | GTTCATGCGCA    | 2              |                       |    |    |
| P08    | ACATCGGCGCA    | 1              | 875                   |    |    |
| P16    | CCAAGCTGCG     | 2              | 1400                  |    |    |
| P06    | GTGGGCCGTCG    | 1              |                       |    |    |
| Q06    | GAGGCGGCTT     | 2              | 1450                  |    |    |
| R16    | CTCTGCGGCT     | 1              | 425                   |    |    |
| U15    | ACGGGCCAGT     | 3              | 550                   |    |    |
| V10    | GACGGTCTGCT    | 1              |                       |    |    |
| V15    | CAGTGGCGGT     | 2              | 400, 650              |    |    |
| V19    | GGTTGCTGAG     | 1              | 225                   |    |    |
| Y02    | CATCGCGGCA     | 2              |                       |    |    |
| Y13    | ACAGGCGTGCT    | 1              | 600                   |    |    |
| Y05    | GGCTGCGACA     | 1              |                       |    |    |
| Z03    | CGACCGGCGA     | 1              |                       |    |    |
| AA11   | ACCCGACCTG     | 1              |                       |    |    |
| AB03   | TGCGGCAACAC    | 2              | 1300                  |    |    |
| AB04   | GGCAGCGCGT     | 5              | 575                   |    |    |
| AB09   | GGCGGACTAC     | 1              |                       |    |    |
| AB18   | TGCTGGCGC     | 1              | 1350                  |    |    |
| AC07   | GTGGCGCGATG    | 2              | 500                   |    |    |
| AC10   | AGCACGGGAGG    | 1              |                       |    |    |
| AC12   | GGCGAGTGCT     | 2              |                       |    |    |
| AD16   | ACGCGCGGCTC    | 3              | 1325, 950, 875        |    |    |

3Primers 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 assigned 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\(_1\) population with the predominant NHM background. Preferential segregation of a chromosome with unique features was first described in a BC\(_1\) 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-

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**Diagram:**

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| I       | II      | III     | IV      | V       |
|---------|---------|---------|---------|---------|
| 10.5    |         | 17-510  |         |         |
| 19.6    | 9.9     | 17-510  |         |         |
| 9.9     | 9.9     | 17-510  |         |         |
| 84-1120 | 9.9     | 17-510  |         |         |
| 9.9     | 9.9     | 17-510  |         |         |
| 359-1350| 9.9     | 17-510  |         |         |
| 125-1075| 9.9     | 17-510  |         |         |
| 147-800 | 9.9     | 17-510  |         |         |
| 105-700 | 9.9     | 17-510  |         |         |
| 125-1175| 9.9     | 17-510  |         |         |
| 437-425 | 9.9     | 17-510  |         |         |
| 7.4     | 9.9     | 17-510  |         |         |
| 9.9     | 9.9     | 17-510  |         |         |
| 12.6    | 9.9     | 17-510  |         |         |
| 155-475 | 9.9     | 17-510  |         |         |
| 14.3    | 9.9     | 17-510  |         |         |
| 9.9     | 9.9     | 17-510  |         |         |
| 437-975 | 9.9     | 17-510  |         |         |
| 15-400  | 9.9     | 17-510  |         |         |
| 190-975 | 9.9     | 17-510  |         |         |
| 28.6    | 9.9     | 17-510  |         |         |
| 16-725  | 9.9     | 17-510  |         |         |
| 26.8    | 9.9     | 17-510  |         |         |

| I       | II      | III     | IV      | V       |
|---------|---------|---------|---------|---------|
| 10.5    |         | 17-510  |         |         |
| 19.6    | 9.9     | 17-510  |         |         |
| 9.9     | 9.9     | 17-510  |         |         |
| 84-1120 | 9.9     | 17-510  |         |         |
| 9.9     | 9.9     | 17-510  |         |         |
| 359-1350| 9.9     | 17-510  |         |         |
| 125-1075| 9.9     | 17-510  |         |         |
| 147-800 | 9.9     | 17-510  |         |         |
| 105-700 | 9.9     | 17-510  |         |         |
| 125-1175| 9.9     | 17-510  |         |         |
| 437-425 | 9.9     | 17-510  |         |         |
| 7.4     | 9.9     | 17-510  |         |         |
| 9.9     | 9.9     | 17-510  |         |         |
| 12.6    | 9.9     | 17-510  |         |         |
| 155-475 | 9.9     | 17-510  |         |         |
| 14.3    | 9.9     | 17-510  |         |         |
| 9.9     | 9.9     | 17-510  |         |         |
| 437-975 | 9.9     | 17-510  |         |         |
| 15-400  | 9.9     | 17-510  |         |         |
| 190-975 | 9.9     | 17-510  |         |         |
| 28.6    | 9.9     | 17-510  |         |         |
| 16-725  | 9.9     | 17-510  |         |         |
| 26.8    | 9.9     | 17-510  |         |         |

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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 melons (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$_1$ 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
RAPD primer, and constructed SCAR primers (5’GTA-GCA-CTCCAACATTTAATCTAATTGC and 5’GTA-GCCTCCCCACTCATACAAAT). 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 F1 and BC1-F2 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 F1 and BC1-F2 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 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 F1 and BC1-F2 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 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 F1 and BC1-F2 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.

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                    |
| II            | 19             | 207.9       | 11.5              | 4                    |
| III           | 9              | 121.4       | 15.2              | 2                    |
| IV            | 14             | 113.0       | 8.7               | --                   |
| V             | 15             | 109.2       | 7.8               | 12                   |
| VI            | 17             | 99.4        | 6.2               | 6                    |
| VII           | 13             | 95.9        | 8.0               | 2                    |
| VIII          | 15             | 78.8        | 5.6               | 1                    |
| IX            | 9              | 70.3        | 8.7               | --                   |
| XI            | 9              | 61.5        | 7.7               | 4                    |
| XII           | 4              | 38.5        | 12.8              | --                   |
| XIII          | 2              | 25.1        | 25.1              | --                   |
| XIV           | 2              | 20.3        | 20.3              | --                   |
| XV            | 2              | 18.4        | 18.4              | --                   |
| XVI           | 2              | 16.3        | 8.2               | 3                    |
| XVII          | 2              | 10.3        | 10.3              | --                   |
| Total         | 156            | 1295        | 9.3               | 42                   |

*Markers skewed towards NHM.
*Markers skewed towards PI 296341.
*Average distance between two markers.

Morphological markers. Theor. Appl. Genet. 93:57–64.
Bonirable, M.W., R.L. Plaisted, and S.D. Tentlesky. 1988. RFLP maps based on common set of clones reveal models of chromosomal evolution in potato and tomato. Genetics 120:1095–1103.
Brotman, Y., L. Silberstein, I. Kovalski, J. Klinger, G. Thompson, N. Katzir, R. and Perel Treves. 2000. Linkage groups of *Cucumis melo*, including resistance gene homologues and known genes. Acta Hort. 510:441–448.
Buckler, E.S. 4th, T.L. Phelps-Durr, C.S. Buckler, R.K. Dawe, J.F. Doebley, and T.P. Holltsford. 1999. Meiotic drive of chromosomal knobs reshaped the maize genome. Genetics 153:415–426.
Cameron, D.R. and R. Moav. 1957. Inheritance in *Nicotiana tabacum* XXVII. Pollen killer, an alien genetic locus inducing abortion of microspores not carrying it. Genetics 42:326–335.
Dimitrov, B. and V. Georgieva. 1994. Comparative analysis of sister-chromatid exchanges in plant and human chromosomes. Mutation Res. 304:187–192.
Dogimont, C., L. Leconte, C. Perin, A. Thabuis, H. Iacoq, and M. Pitrat. 2000. Identification of QTLs contributing to resistance to different strains of cucumber mosaic cucumovirus in melon. Acta Hort. 510:391–398.
Durham, R.E., P.C. Liou, F.G. Gmitter, Jr., and G.A.. Moore. 1992. Linkage of restriction length polymorphisms and isozymes in *Citrus*. Theor. Appl. Genet. 84:39–48.
Food and Agricultural Organization. 1995. Production yearbook for 1994. No. 48. FAO, Rome.
Fritsch, P., M.A. Hanson, C.D. Spore, P.E. Pack, and L.H. Rieseberg. 1993. Constancy of RAPD primer amplification strength among distantly related taxa of flowering plants. Plant Mol. Biol. Rpt. 11:10–20.
Gale, M.D. and K.M. Devos. 1998. Plant comparative genetics after 10 years. Science 282:656–659.
Hashizume, T., I. Skimamoto, Y. Harushima, M. Yui, T. Sato, T. Imai, and M. Hirai. 1996. Construction of a linkage map for watermelon (*Citrus lanatus* (Thunb.) Matsum & Nakai) using random amplified polymorphic DNA (RAPD), Euphytica 90:265–273.
Hawkins, L.K., F. Dane, T.L. Kubisiak, B.R. Rhodes, and R.L. Jarret. 2001. Linkage mapping in a watermelon population segregating for fusarium wilt resistance. J. Amer. Soc. Hort. Sci. 126:344–350.
Jarret, R.L., L.C. Merrick, T. Holmes, J. Evans, and M.K. Aradhya. 1997. Simple sequence repeats in watermelon (*Citrus lanatus* (Thunb.)
Kesseli, R.V., I. Paran, and R.W. Michelmore. 1994. Analysis of detailed
Keim, P., J.M. Schupp, S.E. Travis, K. Klayton, and D.M. Webb. 1997.
Jeffrey, C. 1975. Further notes on Cucurbitaceae: III. Some African taxa.
Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E.
Levi, A. and C.E. Thomas. 1999. An improved procedure for isolation of
Levi, A., C.E. Thomas, A.P. Keinath, T.C. Wehner. 2000. Estimation of
Mather, K. 1936. The determination of position in crossing-over. I.
Martyn, R.D. and D. Netzer. 1991. Resistance to races 0, 1, and 2 of
Navot, N., M. Sarfatti, D. Zamir. 1990. Linkage relationships of genes
Mather, K. 1939. Crossing over and heterochromatin in the X chromo-
Navot, N. and D. Zamir. 1987. Isozyme and seed protein phylogeny of
Netzer, D. and R.D. Martyn. 1989. PI 296341, a source of resistance in

Navot, N., M. Sarfatti, D. Zamir. 1990. Linkage relationships of genes
affecting bitterness and flesh color in watermelon. J. Hered. 81:162–
165.

Navot, N. and D. Zamir. 1986. Linkage relationships of 19 protein-
coding genes in watermelon. Theor. Appl. Genet. 72:274–278.

Navot, N. and D. Zamir. 1987. Isozyme and seed protein phylogeny of
the genus Citrullus (Cucurbitaceae). Plant Systematics and Evolution
156:61–67.

Nei, M., and W. Li. 1979. Mathematical model for studying genetic
variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci.
USA 76:5269–5273.

Netzer, D. and R.D. Martyn. 1989. PI 296341, a source of resistance in
watermelon to race 2 of Fusarium oxysporum f. sp. niveum. Plant Dis.
73:518.

Oliver, M., J. Garcia-Mas, M. Morales, R. Dolcet-Sanjuan, M. Carmen de
Vincente, H. Gomez, H. van Leeuwen, A. Monfort, P. Puigdomenech,
and P. Arus. 2000. The Spanish melon genome project: Construction of
a saturated genetic map. Acta Hort. 510:375–378.

Perin, C., L. Hagen, C, Dogimont, V. de Cunzo, L. Lecomte, and M.
Pitrat. 2000. Construction of a reference map of melon. Acta Hort.
510:367–374.

Rhodes, M.M. 1942. Preferential segregation in maize. Genetics 27:395–
407.

Rhodes, B. and F. Dane. 1999. Gene list for watermelon (Citrullus
lanatus). Cucurbit Genet. Coop. Rpt. 22:61–77.

Rowland, L.J. and A. Levi. 1994. RAPD-based genetic linkage map of
blueberry derived from a cross between diploid species (Vaccinium
araroi and V. elliottii). Theor. Appl. Genet. 87:863–868.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning. A
laboratory manual. 2nd ed. Cold Spring Harbor Lab. Press, Plainview,
New York.

Sandler, L. and E.M. Novitski. 1957. Meiotic drive as an evolutionary
force. Amer. Naturalist 91:105–110.

Shimotsu, M. 1963. Cytogenetic and evolutionary studies in the
genus Citrullus. Seiken Jihou 15:24–34.

Sowell, G., Jr. and G.R. Pointer. 1962. Gummy stem blight resistance of
introduced watermelons. Plant Dis. Rptr. 46:883–885.

Staub, J.E. and F.C. Serquen. 2000. Towards an integrated linkage map
of cucumber: Map merging. Acta Hort. 510:357–366.

U.S. Department of Agriculture. 2000. Agricultural statistics. USDA
Natl. Agr. Stat. Serv., Wash., D.C.

Wang, Y.H., C.E. Thomas, and R.A. Dean. 1997. A genetic map of
melon (Cucumis melo L.) based on amplified fragment length poly-
morphism (AFLP) markers. Theor. Appl. Genet. 95:791–798.

Weeden, N.F. 1989. Application of isozymes in plant breeding. p. 11–54.
In: Janick (ed.). Plant breeding reviews, vol 6. Timber Press, Portland,
Ore.

Wendel, J.F., M.D. Edwards, and C.W. Stuber. 1987. Evidence for
multilocus genetic control of preferential fertilization in maize. Hered-
ity 58:297–301.

Whitaker, T.W. and G.B. Benis. 1976. Cucurbits, p. 64–69. In: N.W.
Simmonds (ed.). Evolution of crop plants. Longman, London.

Xu, Y., X.X. Ouyang, H.Y. Zhang, and Y.J. Wang. 1999. Identification of
molecular markers linked to race 1 fusarium wilt resistance gene in
watermelon wild germplasm PI 296341. Acta Bot. Sinica 41:952

and P. Arus. 2000. The Spanish melon genome project: Construction of
a saturated genetic map. Acta Hort. 510:375–378.

Perin, C., L. Hagen, C, Dogimont, V. de Cunzo, L. Lecomte, and M.
Pitrat. 2000. Construction of a reference map of melon. Acta Hort.
510:367–374.

Rhodes, M.M. 1942. Preferential segregation in maize. Genetics 27:395–
407.

Rhodes, B. and F. Dane. 1999. Gene list for watermelon (Citrullus
lanatus). Cucurbit Genet. Coop. Rpt. 22:61–77.

Rowland, L.J. and A. Levi. 1994. RAPD-based genetic linkage map of
blueberry derived from a cross between diploid species (Vaccinium
araroi and V. elliottii). Theor. Appl. Genet. 87:863–868.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning. A
laboratory manual. 2nd ed. Cold Spring Harbor Lab. Press, Plainview,
New York.

Sandler, L. and E.M. Novitski. 1957. Meiotic drive as an evolutionary
force. Amer. Naturalist 91:105–110.

Shimotsu, M. 1963. Cytogenetic and evolutionary studies in the
genus Citrullus. Seiken Jihou 15:24–34.

Sowell, G., Jr. and G.R. Pointer. 1962. Gummy stem blight resistance of
introduced watermelons. Plant Dis. Rptr. 46:883–885.

Staub, J.E. and F.C. Serquen. 2000. Towards an integrated linkage map
of cucumber: Map merging. Acta Hort. 510:357–366.

U.S. Department of Agriculture. 2000. Agricultural statistics. USDA
Natl. Agr. Stat. Serv., Wash., D.C.

Wang, Y.H., C.E. Thomas, and R.A. Dean. 1997. A genetic map of
melon (Cucumis melo L.) based on amplified fragment length poly-
morphism (AFLP) markers. Theor. Appl. Genet. 95:791–798.

Weeden, N.F. 1989. Application of isozymes in plant breeding. p. 11–54.
In: Janick (ed.). Plant breeding reviews, vol 6. Timber Press, Portland,
Ore.

Wendel, J.F., M.D. Edwards, and C.W. Stuber. 1987. Evidence for
multilocus genetic control of preferential fertilization in maize. Hered-
ity 58:297–301.

Whitaker, T.W. and G.B. Benis. 1976. Cucurbits, p. 64–69. In: N.W.
Simmonds (ed.). Evolution of crop plants. Longman, London.

Xu, Y., X.X. Ouyang, H.Y. Zhang, and Y.J. Wang. 1999. Identification of
molecular markers linked to race 1 fusarium wilt resistance gene in
watermelon wild germplasm PI 296341. Acta Bot. Sinica 41:952–955.

Xu, Y., Y.H. Zhang, G.B. Kang, Y.J. Wang, and H. Chen. 2000. Studies of
molecular marker-assisted selection for resistance to fusarium wilt
in watermelon (Citrullus lanatus) breeding. Acta Genetic Sinica
27:151–157.

Xu, Y., L. Zhu, J. Xiao, N. Huang, and S.R. McCouch. 1997. Chromo-
sonal regions associated with segregation distortion of molecular
markers in F2 backcross, doubled haploid, and recombinant inbred
populations in rice (Oryza sativa L.). Mol. Gen. Genet. 253:535–545.

Zamir, D. and Y. Tadmor. 1986. Unequal segregation of nuclear genes
in plants. Bot. Gaz. 147:355–358.

Zhang, X.P. and B.B. Rhodes. 1993. Inheritance of resistance to races 0,
1 and 2 of Fusarium oxysporum f. sp. niveum in watermelon (Citrullus
sp. PI 296341). Cucurbit Genet. Coop. Rpt. 16:77–78.