An Extended Linkage Map for Watermelon Based on SRAP, AFLP, SSR, ISSR, and RAPD Markers

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ABSTRACT. Seventy-one amplified fragment length polymorphism (AFLP), 93 sequence related amplified polymorphism (SRAP), and 14 simple sequence repeat (SSR) markers were used to extend an initial genetic linkage map for watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. The initial map was based on 151 randomly amplified polymorphic DNA (RAPD) and 30 and inter-simple sequence repeat (ISSR) markers. A testcross population previously used for mapping of RAPD and ISSR markers was used in this study: {plant accession Griffin 14113 [*C. lanatus* var. *citroide* (L.H. Bailey) Mansf.] × the watermelon cultivar New Hampshire Midget (*C. lanatus* var. *lanatus*)} × PI 386015 [*C. colocynthis* (L.) Schrad.]. The linkage map contains 360 DNA markers distributed on 19 linkage groups, and covers a genetic distance of 1976 cM with an average distance of 5.8 cM between two markers. A genomic DNA clone representing 1-amino-cyclopropane-1-carboxylic acid (ACC-) synthase gene, involved in ethylene biosynthesis, was also mapped. As in previous mapping studies for watermelon, a large number of AFLP and SRAP markers were skewed away from the 1:1 segregation ratio, and had to be excluded from the final mapping analysis. The stringent mapping criteria (JoinMap 3.0 mapping program) produced linkage groups with marker order consistent with those reported in previous mapping study for watermelon.

Cultivated watermelon accounts for 2% of the world area devoted to vegetable crops [Food and Agricultural Organization of the United Nations (FAO), 1995]. In the United States, watermelon production has increased from 1.09 million Mg in 1980 to 3.54 million Mg in 2003, with a farm value of \$310 million [U.S. Department of Agriculture (USDA), 2003]. The traditional watermelon cultivars are diploids ($n = 11$; Shimotsuma, 1963). However, in recent years, there has been an increased demand by consumers for seedless (triploid) watermelons, and production has increased considerably. During 2003, over 60% of the watermelons produced in the United States were seedless (USDA, 2003). There is a continuous need to enhance disease and pest resistance in watermelon cultivars, improve fruit quality, and develop new seedless watermelon cultivars suitable to consumer

needs. Molecular markers are useful in identifying and isolating genes controlling disease resistance, or genes controlling fruit quality (Cobos et al., 2005; Liebhard et al., 2003; Mokrani et al., 2002; Soriano et al., 2005). As in other crop plants, including coffee (*Coffea arabica* L.) (Herrera et al., 2002) and banana (*Musa* L.) (Nwakanma et al., 2003), molecular markers can be useful in identifying and in selecting for triploid hybrid plants of watermelon.

High-density genetic maps are being integrated in breeding programs of many crop plants. They are also useful in positional cloning of genes and in elucidating the genetic mode of complex traits that do not display Mendelian segregation (Lee, 1995). *Cucumis sativus* L. (cucumber) and *Cucumis melo* L. (melon) received the greatest attention among cucurbit species with respect to genome mapping (Fazio et al., 2003; Silberstein et al., 2003). Linkage mapping of watermelon genome has been lagging behind the maps constructed for these species. A few linkage maps were constructed for watermelon in recent years. However, these maps are based on isozymes (Navot and Zamir, 1987; Zamir et al., 1984) or RAPD markers (Hashizume et al., 1996, 2003; Hawkins et al., 2001; Levi et al., 2001c, 2002), and

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do not cover all regions of watermelon genome. Many markers (including SRAP, SSR, and AFLP markers) are still required for constructing a dense map that can be used effectively in watermelon breeding programs, and in isolating genes that control fruit quality or confer resistance to diseases and pests. Most of the watermelon cultivars developed in North America during the last two centuries share a narrow genetic base (Levi et al., 2001a, 2001b). Due to the low DNA polymorphism among watermelon cultivars, most of the maps constructed so far for watermelon used genetic mapping populations derived from a wide cross between a watermelon cultivar (*C. lanatus* var. *lanatus*) and a U.S. plant introduction (PI) of the related subspecies *C. lanatus* var. *citroides* (Zhang et al., 2004).

Hawkins et al. (2001) and Levi et al. (2004b) attempted to construct a linkage map for watermelon using F_2 or F_3 populations derived from a wide cross between PI 296341 (*C. lanatus* var. *citroides*) and the watermelon cultivar New Hampshire Midget (NHM). However, most of the markers in these populations were skewed away from the expected segregation ratio (3:1) and could not be mapped with high confidence. In an attempt to reduce the number of skewed markers we used a testcross population [(Griffin 14113 x NHM) x PI 386015] where the testcross parent (PI 386015) is distant from both parents of the F_1 plant (Griffin 14113 and NHM) (Levi et al., 2002). This testcross population was successfully used in our initial mapping of watermelon using RAPD and ISSR markers (Levi et al., 2002). Our objective is to extend the initial map using different types of DNA markers, and in a later stage isolate genes controlling disease and pest resistances and genes controlling watermelon fruit qualities. SRAP, AFLP, and SSR markers have been used in constructing maps for various crops (Li et al., 2003), but not for watermelon. It is vital to experiment with these marker types, determine their polymorphism, segregation, and distribution on watermelon genome, and further explore the linkage regions they cover.

This paper reports the construction of an extended genetic linkage map for watermelon using a testcross population [(Griffin 14113 x NHM) x PI 386015] and examines the efficiency of SRAP, AFLP, and SSR markers vs. ISSR and RAPD markers in constructing the map.

Materials and Methods

PLANT MATERIAL. Parental plants that included Griffin 14113 (provided by R. Jarret, USDA Plant Genetic Resources Conservation Unit, Griffin, Ga.), NHM, their F_1 hybrid plant, and the testcross parent plant (PI 386015) were grown in the greenhouse together with 98 plants of the testcross progeny [(Griffin 14113 x NHM) x PI 386015]. Four weeks after germination young leaves (10 g) were collected from each plant, and stored at -80°C .

ISOLATION OF DNA. To avoid co-isolation 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).

AFLP PROCEDURE. The AFLP procedure developed by Vos et al. (1995) was modified using a commercially available kit (Plant Mapping Kit - Regular Plant Genome; Applied Biosystems, Foster City, Calif.). According to the manufacturer's protocol, a high-quality genomic DNA sampled [500 ng; intact and with a 260/280 optical density (OD) ratio of 1.8] from each plant was used in the AFLP analysis with *EcoRI*-*MseI*, and *PstI*-*MseI* restriction enzymes and primer combinations (Table 1) as described by Levi et al. (2004a). Reactions were analyzed on a 6% acrylamide gel

using a Perkin Elmer ABI-373 Sequencer (Applied Biosystems) as described by Levi et al. (2004a), or on a capillary system using the CEQ8800 DNA sequencer (Beckman Coulter, Fullerton, Calif.). For visualization of DNA fragments on the CEQ8800 the *EcoRI* or *PstI* selective primers were labeled with one of three WellRED dye labels (D2, D3, or D4; Proligo, Boulder, Colo.), as described below for the SRAP analysis.

SRAP ANALYSIS. The SRAP procedure is based on PCR amplification of open reading frames (ORFs), using a forward and reverse primers designed to preferentially amplify exon (rich in C and G nucleotides) and intron regions (rich in A and T nucleotides), respectively. The forward primer is a 14 nucleotide sequence rich in C and G, and three selective bases at the 3' end, while the reverse primer is a 15 nucleotide sequence rich in A and T and three selective bases at the 3' end. The variation in exon, intron or promoter region sequences produces the polymorphism (Li and Quiros, 2001).

Twenty-four SRAP primer combinations were (Table 2) tested with the testcross parents (NHM, Griffin 14113, and PI 386015). For visualization with the CEQ8800 DNA sequencer, the forward primers were labeled with one of three WellRED dyes (D2, D3, or D4) (Proligo). To confirm correct dye balance, the amount of dye-labeled primer added to the PCR reaction was optimized for each primer. Each 10- μL PCR reaction contained 1X PCR buffer (Promega, Madison, Wis.), 2.5 mM MgCl_2 , 200 μM dNTPs (Sigma, St. Louis), 30 ng of reverse primer (IDT, Coraville, Iowa), 0.4 units Taq DNA polymerase (Promega) and 25 ng of DNA. In addition, WellRED-dye-labeled forward primers were added in the following optimized amounts: 15 ng for a D4 primer; 30 ng for a D3 primer; or 75 ng for a D2 primer. Samples were subjected to the following thermal profile for amplification in a PCR thermocycler (MJ): 5 min of DNA denaturing at 94°C , five cycles of three steps: 1 min of denaturing at 94°C , 1 min of annealing at 35°C and 2 min of elongation at 72°C . In the following 30 cycles the annealing temperature was increased to 50°C , with a final elongation step of 5 min at 72°C (Li and Quiros, 2001). The PCR products were analyzed using the CEQ8800 capillary sequencer (Beckman Coulter) by pooling 0.3 μL of the D4 reaction, 0.3 μL of the D3 reaction, 0.5 μL of the D2 reaction, 0.28 μL of D1 size standard, and 30 μL of deionized formamide in a 96-well PCR plate. Samples were run on the CEQ8800 capillary sequencer using the fragment 3 parameters and analyzed using the built-in fragment analysis software provided with the CEQ8800 system. Fragments that ranged in size of 75–400 bp could be scored with high confidence.

SSR ANALYSIS. Microsatellite sequences were isolated from a genomic DNA of the watermelon cultivar Dixielee according to the procedure used by Reddy et al. (2001) for developing cotton SSRs. Following sequencing, primer pairs were designed for these DNA fragments containing SSR motifs (Table 4). The primer pairs were first tested with the testcross parents (Griffin 14113, NHM, and PI386015). PCR was performed in 10- μL reactions containing 1X PCR buffer (Promega), 2.5 mM MgCl_2 , 100 μM dNTPs (Sigma), 0.25 μM of each primer pair (Sigma Genosys, Houston), 0.2 units Taq DNA polymerase (Promega), and 25 ng of DNA. The PCR conditions were identical to that of Saal et al. (2001), and annealing temperatures ranged from 49 to 68°C (Table 1). The PCR products tested first with the parental lines were separated on a 6% native polyacrylamide gel with ethidium-bromide in the running buffer and visualized on an ultraviolet (UV) transilluminator (Saal et al., 2001). The SSR markers that were polymorphic between the parental lines were

Table 1. The AFLP primer pairs, their designation, and the size of markers that were suitable for mapping experiments with the testcross population used for constructing a linkage map for watermelon (E = *EcoRI*, M = *MseI*, P = *PstI*).

Primer pair	Designation	Markers size (bp)
EAAC - MCAG	EACAG	89c, 99c ^z , 101 ^z , 163c, 219c ^z , 248, 312c ^z , 369c, 373c ^z , 425
EACA - MCAC	ECAAC	57c, 91c ^z , 95
EACA - MCTG	ECATG	90, 139c, 174c ^z , 176c, 193 ^z , 195 ^z , 223c
EACC - MCAA	ECCAA	77c, 125, 162, 220c, 238c ^z , 482c ^z
EACC - MCAC	ECCAC	98c, 112c ^z , 161c, 224c ^z , 257c ^z , 454c
EACC - MCAT	ECCAT	90, 92c, 101c, 141c, 145c, 463c
EACC - MCTG	ECCTG	93c ^z , 310c
EAGC - MCAG	EGCAG	94c, 129c, 180, 184c, 215 ^z , 244c ^z , 252c ^z
EAGC - MCTA	EGCTA	79c, 199, 240, 368c
EAGG - MCTT	EGGTT	93, 98c, 188c ^z , 252c, 263c ^z , 382c ^z
EAGG - MCTC	EGGTC	114c ^z , 117 ^z , 120c, 122, 151, 171, 186c, 199c ^z , 223c ^z , 302c, 308c ^z
EAAG - MCTG	EAGTG	118, 213 ^z , 269c, 271, 272c ^z , 408c ^z
EAAG - MCTC	EAGTC	84c, 86, 259, 272c, 492c ^z
EAAG - MCAT	EAGAT	104c ^z , 113c ^z , 121c ^z , 123 ^z , 128c, 135c, 138c ^z , 152c ^z , 165c ^z , 171c ^z , 172 ^z , 197 ^z , 226, 427c ^z , 455 ^z
EAGG - MCAG	EGGAG	93c, 117c, 147c ^z , 202 ^z , 205c, 226c ^z , 237c, 329c, 334c ^z
EAGG - MCAA	EGGAA	91c ^z , 111c, 135c, 157c, 344c
EACC - MCTA	ECCTA	90, 138c, 187c ^z , 257c, 338c
EACA - MCAA	ECAAA	89c, 100
EAAG - MCAG	EAGAG	81c, 91, 100c, 121c ^z , 122 ^z , 125c, 126 ^z , 130c, 160c ^z , 174c ^z , 175 ^z , 181c, 209c, 219c, 226c, 238c, 251c, 259c, 265 ^z , 272, 280c ^z , 288c, 292c ^z , 330c ^z , 332c ^z , 370c, 453c
PACG - MCAT	PCGAT	142 ^z , 347
PACG - MCCT	PCGCT	82c ^z , 89c ^z , 106, 117c ^z , 121c, 178 ^z
PACT - MCAA	PCTAA	93
PACT - MCCG	PCTCG	102c, 151, 153, 175c, 217c, 223c, 301c
PACT - MCGA	PCTGA	120, 176 ^z
PAGA - MCAA	PGAAA	128c, 159 ^z , 183 ^z , 129
PAGA - MCGA	PGAGA	239c, 241 ^z
PAGG - MCCC	PGGCC	82, 95, 104, 131, 135c, 206c, 231
PAGG - MCCT	PGGCT	81c, 89c, 393
PATA - MCCC	PTACC	94c, 137c
PATA - MCGT	PTAGT	138c, 159 ^z , 183 ^z
PAAG - MCAT	PAGAT	181
PACA - MCAA	PCAAA	114
PACT - MCAC	PCTAC	288 ^z
PACT - MCCA	PCTCA	151c
PACT - MCCC	PCTCC	113c, 114c, 130c, 179, 180
PACT - MCCT	PCTCT	100c, 110c, 132, 148c ^z , 152
PATA - MCAA	PTAAA	173c
PATA - MCGA	PTAGA	168c, 296c ^z
PATA - MCGC	PTAGC	114c, 130c, 180
PATA - MCGT	PTAGT	140c, 212, 413c

^zMarkers that could be ordered on the linkage map.

further analyzed for mapping with the testcross population using the CEQ8800 capillary sequencer. For visualization of fragments on the CEQ8800 DNA sequencer the SSR forward primers were labeled with one of three WellRED dye labels (D2, D3, or D4; Proligo), as described above for the SRAP analysis.

RAPD AND ISSR PROCEDURES. The RAPD and ISSR marker analyses were performed as described in previous studies (Levi et al., 1993, 2001c, 2002).

MARKER SCORING. Markers that were unique to one of the parents, Griffin 14113 or NHM, and were present in the F_1 parent, but missing in the testcross parent (PI 386015), could be scored for mapping in the testcross population (Fig. 1). The AFLP markers were scored based on their presence or absence using Genescan/Genotyper software (Applied Biosystems) for AFLP fragments,

or the using the built-in fragment analysis software (provided with the Beckman CEQ8800 system) for SRAP and SSR fragments.

LINKAGE ANALYSIS OF MARKERS IN THE TESTCROSS POPULATION. Prior to linkage analysis segregation rates were scored for each marker. Segregation ratio distortion was evaluated with a chi-square test for goodness of fit based on the segregation expectations of 1:1 for markers in a testcross or BC1 population (Van Ooijen and Voorrips, 2001), and skewed markers were excluded from the analysis (Table 5). Linkage analysis was performed with the JoinMap version 3.0 software (Van Ooijen and Voorrips, 2001) with default options. All markers were analyzed for linkage, and recombination fractions were converted into map distances (centimorgans) with the Kosambi function. LOD scores of 2.0–6.0 were used for grouping of markers, followed by high threshold LOD scores of 7.0–10.0 for final mapping of markers in each linkage group. Loci showing weak or suspect linkages were removed from the analysis (Van Ooijen and Voorrips, 2001).

MARKER NOMENCLATURE. The AFLP markers were designated by the last two nucleotides on the 3' of the *EcoRI* and *MseI* primers, respectively. For example marker EAGAG-122 represents the AFLP marker (with a size of 122 bp) produced by the selective primers *EcoRI*-AAG and *MseI*-CAG. The RAPD or ISSR markers were designated by their serial number and their size. For example, the 500-bp marker produced by primer AW-07 (Operon Biotechnology, Alameda, Calif.), which is unique to Griffin 14113, was designated as AW07-500, and the 250-bp marker produced by primer No. 101 (Univ. of British Columbia, Vancouver), which is unique to NHM, was designated as 101-250c. The SRAP markers were designated by combining the forward and reverse primers (Table 2) with the marker size (Table 3). The SSRs provided by Seminis (Oxnard, Calif) are these beginning with Cgb or CLG, while the SSRs prepared at Alcorn State Univ. (Lorman, Miss.) are beginning with ASUW (Table 4). The RAPD or ISSR markers were designated by their serial number and their size. For example, the 500-bp marker produced by primer AW-07 (Operon Biotechnology), which is unique to Griffin 14113, was designated as AW07-500; and the 250-bp marker produced by primer No. 101 (Univ. of British Columbia), which is unique to NHM, was designated as U101-250c (Fig. 1; Levi et al., 2002).

Results and Discussion

POLYMORPHISM AND MAPPING EFFICIENCY OF MARKERS. The different marker types used in this study (RAPD, ISSR, AFLP, SRAP, and SSR markers) complement each other in the assembly of an extend linkage map. In a previous mapping study (Levi et al., 2002) 168 RAPD and 37 ISSR markers were used for mapping analysis. The AFLP, SRAP, and SSR marker data in this study were combined with these of the RAPD and ISSR markers to produce an extended linkage map (Table 5; Fig. 1). The AFLP, SRAP, or SSR markers were useful in covering linkage regions

Table 2. SRAP forward primers that were labeled with a DNA sequencing dye (FLP) and the reverse unlabeled primers (RUP) used in different combinations to produce the SRAP markers (in Table 3) in watermelon.

FLP	Primer sequence 5' to 3'
me1	TGAGTCCAAACCGGATA
me2	TGAGTCCAAACCGGAGC
me3	TGAGTCCAAACCGGAAT
me4	TGAGTCCAAACCGGACC
me5	TGAGTCCAAACCGGAAG
ab1	AGTGATTCAACCGGAGA
ab2	AGTGATTCAACCGGATA
ab3	AGTGATTCAACCGGAGC
RUP	Primer sequence 5' to 3'
ba1	GTCGAGCTGCCAATTATA
ba2	GTCGAGCTGCCAATTAAT
ba3	GTCGAGCTGCCAATTTGC
ba4	GTCGAGCTGCCAATTTTT
ba5	GTCGAGCTGCCAATTAATA
ba6	GTCGAGCTGCCAATTAAC
ba7	GTCGAGCTGCCAATTAAAG
ba8	GTCGAGCTGCCAATTATT
ba9	GTCGAGCTGCCAATTATC
ba10	GTCGAGCTGCCAATTATG
ba11	GTCGAGCTGCCAATTGGA
ba12	GTCGAGCTGCCAATTTGT
em1	GACTGCGTACGAATTAAT
em2	GACTGCGTACGAATTTGC
em3	GACTGCGTACGAATTGAC
em4	GACTGCGTACGAATTTGA
em5	GACTGCGTACGAATTAAC
em6	GACTGCGTACGAATTGCA

that were not readily covered by the RAPD or ISSR markers, and in merging small (RAPD-based) linkage groups (Levi et al., 2002) into larger linkage groups (as shown with linkage groups I, II, III, V, XI, and XV; Table 5; Fig. 1).

POLYMORPHISM AND SCREENING OF AFLP MARKERS. Although AFLP markers (*MseI-EcoRI*) are highly polymorphic among watermelon cultivars (Levi et al., 2004a), a relatively small number of these markers (142 of 707 markers; 20.1%) were suitable for the mapping experiment with the testcross population. Nineteen AFLP primer pairs (*MseI-EcoRI*) were tested against the parents Griffin 14113 and NHM, their F_1 hybrid, and the testcross parent PI 386015. Each AFLP primer pair produced ≈ 12 to 48 markers (75–450 bp). However, only a few of these markers (2–7) were suitable for the mapping experiment with the testcross population (Table 1). These AFLP markers were uniquely present in one of the donor parents (Griffin 14113 or NHM) and in the F_1 hybrid, but absent in the testcross parent PI 386015. A total of 142 AFLP markers (38 markers unique to Griffin 14113 and 104 markers unique to NHM) were suitable for the mapping analysis using the testcross population (Table 1). However, 76 (54.9%) of these markers were skewed from the expected 1:1 segregation ratio and had to be excluded from the final linkage analysis. Sixty-six AFLP markers were used in the final linkage analysis. Of these, 58 could be mapped with high confidence on the linkage map (Tables 1 and 5; Fig. 1). Of the 58 AFLP markers that could be mapped, 24 markers were clustered on linkage group XII and eight

markers were on linkage group X. The other 26 AFLP markers are mapped on different linkage regions (Table 5; Fig. 1). Clustering of AFLP markers was reported in different mapping studies of crop plants as shown with tomato (*Lycopersicon esculentum* Mill.) (Saliba-Colombani et al., 2000) and melon (*Cucumis melo* L.) genomes (Perin et al., 2002).

As compared with the *MseI-EcoRI* primer pairs, the 21 *PstI-MseI* AFLP primer pairs tested in this study produced a low number (62) of polymorphic markers suitable for the mapping experiment with the testcross population (Table 1). Of these 62 markers, 42 (67.7%) were skewed from the expected 1:1 segregation ratio and had to be excluded from the mapping analysis (Table 1). Of the 20 markers used in the final mapping analysis, 13 could be mapped with high confidence (Tables 1 and 5; Fig. 1). Three of the *PstI-MseI* markers were clustered on linkage group XIX, while the 10 other markers were mapped on different linkage groups (Table 5; Fig. 1).

POLYMORPHISM AND SCREENING OF SRAP MARKERS. The SRAP markers proved to be efficient and reliable in the mapping analysis in this study. Each of the 69 primer pairs tested (Table 2) produced ≈ 12 –28 DNA fragments. About 1–7 of these fragments were suitable for the mapping experiment with the testcross population (total of 223 fragments produced by 69 SRAP primer pairs) (Table 3). However, 120 of the 223 markers were skewed from the expected 1:1 segregation ratio, and had to be excluded from the final mapping analysis (Tables 3 and 5). Of the remaining 103 SRAP markers, 93 could be mapped with high confidence in the final mapping analysis (Table 3 and 5; Fig. 1). In contrast with the AFLP markers, most of the SRAP markers did not cluster and are distributed on most linkage groups (Table 5; Fig. 1).

POLYMORPHISM AND SCREENING OF SSR MARKERS. Forty-three SSR primer pairs were tested for polymorphism between the testcross parents. Of these, 11 primer pairs produced polymorphic markers (total of 20 markers) suitable for the mapping experiment. Two of these markers had distorted segregation, and were excluded from the mapping analysis. Fourteen of the remaining 18 markers, could be ordered on the linkage map (Tables 4 and 5; Fig. 1). Four markers (CLG7996-158, CLG8020-357c, CLG8020-337, and ASUW2-165) are on linkage group V, three (ASUW13-128, ASUW13-105, CLG7992-112) are on linkage group XVII, two (ASUW19-136, and ASUW19-121) are on linkage group VII, and two (CLG8218-281, CLG8218-297) are on linkage group IX. Markers Cgb4765-186, Cgb4767-179, and CLG7996-160 are on linkage groups II, XIII, and XIV, respectively (Table 5; Fig. 1).

MAPPING OF ETHYLENE BIOSYNTHESIS GENE. Trebitsh et al. (1997) have shown that in cucumber the *Female* locus (*F*) is tightly linked to the 1-amino-cyclopropane-1-carboxylic acid (ACC) synthase (*CS-ACS1G*), a key regulatory enzyme in the ethylene biosynthetic pathway. Genomic DNA clones representing four distinct ACC-synthase genes in watermelon were isolated and sequenced (A. Salman and T. Trebitsh, unpublished data). These clones were designated as *Cit-ACS1* (1342 bp), *Cit-ACS2* (892 bp), *Cit-ACS3* (1584 bp), and as *Cit-ACS4* (914 bp). Different primer combinations were designed for each of these clones, and were used in PCR amplification experiments with the mapping parents. However, only clone *Cit-ACS3* (designated in the map as ACS3-345) could be mapped with high confidence on the linkage map (Table 5; Linkage Group XVII; Fig. 1). The forward and reverse primers (5'-GCTAGAGTTTCGAAATGTACTAT and 5'-ACCAATTATTATTAC AATGGTG) produced a 345-bp fragment unique to Griffin 14113, which could be used for map-

Table 3. SRAP primer pairs and the size of their markers used for mapping experiments with a watermelon testcross population.

Primer pair	Marker size (bp)	Primer pair	Marker size (bp)
Me1ba1	88c, 136c, 165c, 185c ^z , 240c ^z , 269c ^z	Ab1ba4	77c, 95c
Me1ba11	76c ^z , 184c ^z , 214, 250 ^z	Ab1ba6	57, 145c, 159c, 211, 342
Me1ba2	116, 316c ^z , 330 ^z	Ab1ba7	71 ^z , 76, 93, 121, 206c ^z
Me1ba4	62c ^z , 79c, 308c, 402c	Ab1ba8	64 ^z
Me1ba5	174 ^z	Ab1ba9	92 ^z
Me1em1	67c ^z , 175, 196c ^z	Ab1em4	155c ^z
Me1em3	103c, 151c, 281c ^z	Ab2ba1	90c, 248c, 421c
Me1em5	90c, 102, 263c	Ab2ba4	68c, 69, 166c, 282 ^z
Me2ba1	352c	Ab2ba6	60, 63c, 175c, 204 ^z , 230 ^z , 356c
Me2ba3	110c ^z , 178c ^z , 376	Ab2ba7	76, 117c
Me2ba4	60c, 172 ^z , 229 ^z , 347	Ab2em1	89c
Me2ba5	88, 172c, 179 ^z , 226c ^z	Ab2em3	358c, 369
Me2em3	67c, 68, 126 ^z	Ab2em4	90c ^z
Me2em4	74c ^z , 88c, 113c ^z , 121c, 136c, 168c ^z	Ab2em5	114, 126c ^z , 219, 222c ^z , 247c ^z , 275, 360c ^z
Me2em5	64, 182c ^z , 203, 267c ^z	Ab2em6	92, 110c ^z , 152, 189c ^z
Me2em6	60, 147c ^z , 230c, 297 ^z	Ab3ba11	159, 213c, 234
Me3ba1	63c ^z , 83c ^z , 129, 173c ^z , 383c	Ab3em3	67, 74c ^z , 208c
Me3ba10	80c, 101c, 320c ^z	Ab3em5	203 ^z , 235 ^z , 422
Me3ba6	181c ^z , 187, 286c	Ab3em6	146c ^z , 148c ^z , 195 ^z
Me3ba8	104	Me1ba7	93c ^z , 116c, 117c
Me3ba9	71, 112c ^z	Me1ba8	192c ^z , 308c ^z , 402c
Me3em1	57, 76c ^z , 129c ^z	Me1em6	154, 401c
Me3em2	91 ^z	Me2ba10	81c ^z , 114c ^z
Me4ba10	57c, 68c, 179 ^z , 234 ^z	Me2ba2	330c
Me4ba2	58c, 64c, 173c ^z , 291c ^z	Me2ba8	123c, 127c ^z , 133c ^z , 147c, 148c ^z , 147c ^z
Me4ba3	79, 99c ^z	Me2ba9	83, 96
Me4ba4	166 ^z , 218c, 222c, 345c ^z	Me3ba11	86c, 170
Me4ba5	57c, 163c ^z , 179 ^z , 234 ^z	Me3ba12	310 ^z , 313 ^z
Me4ba8	58c, 82 ^z , 97c ^z	Me3ba5	57, 74c, 83, 171cz, 239c
Me4em3	64 ^z , 76c, 82c, 260	Me5ba1	167
Me4em5	101, 193, 203, 268 ^z , 359 ^z	Me5ba7	229, 283, 289c
Ab1ba1	68, 93, 141 ^z , 144c ^z , 146c, 256c ^z	Me5em3	204c
Ab1ba10	59c, 65, 71, 77c ^z , 78 ^z , 171c, 179	Me5em5	142c ^z , 204
Ab1ba11	88c, 90c, 242c ^z , 340	Me5em6	77c, 137c, 169 ^z , 259c, 299c ^z , 301c ^z
Ab1ba2	92, 311c, 432c		

^zMarkers that could be ordered on the linkage map.

Table 4. SSR primer pairs that produced markers suitable for the initial mapping analysis of watermelon. Markers designated with “c” at the right of the marker size were derived from the parent ‘New Hampshire Midget.’ Other markers were derived from the parent Griffin 14113.

Forward primer	Primer sequence	Reverse primer	Primer sequence	SSR motif	Fragment size (bp)
Cgb4765-F	TTCTCTTCATTCCTCCCAAAATC	Cgb4765-R	ACGGGTGAGGGAAAACGAG	(CTT)13	186 ^z
CLG8020-F	GGCAAGGATGGAGAGGGTATC	CLG8020-R	GGCCCTATTCCTCCATAAGAC	(GA)7	337 ^z /357c ^z
CLG7996-F	ATACCCTGGGTGAGAGATAGC	CLG7996-R	CCTTCCTTCCTCGATTTC	(GA)6	158 ^z /160 ^z
CLG7992-F	CTAACGCAATTGGAATCACTCAAA	CLG7992-R	GGTAAATGAAATCAATTGTGGAGA	(AAT)11	112 ^z
Cgb4767-F	GAGAGGGGAAAGAAAAGAGGAGAG	Cgb4767-R	AACGGAGGATGATGATTGGTA	(CTT)13	169c/179 ^z
ASUW2-F	GCTTCGTTGTTGCTGCCGTTG	ASUW2-R	GCATAAAATCACACTCAAAAC	(AAG)12	165c ^z /192
ASUW13-F	CTAGAGAAACCCCATC	ASUW13-R	CTCCACTCACATACACAG	(AAG)18	105 ^z / 128 ^z
ASUW19-F	GTGTGTTTTTGCGTGTG	ASUW19-R	GGGCAATCCAATAATCCAG	(AAG)26	121 ^z /136 ^z
Cgb5009-F	CAGTGGCACCCTCATCTAAAG	Cgb5009-R	AGTGGGGGATTCTCTTCCTAAG	(GA)18	216
CLG8288-F	CCCAACTCAATATTTTCGG	CLG8288-R	TTTTTTAATTCGGGCTGG	(GA)20	306
CLG8218-F	GAATTTCAAAATGTAGTTGTGC	CLG8218-R	GTTTTTCCCCTTACATAATTTAT	(AAT)10	281 ^z /297 ^z

^zMarkers that could be ordered on the linkage map.

Table 5. The number of polymorphic markers tested for segregation with the testcross population used for constructing a linkage map for watermelon, the number of skewed markers that were excluded from the mapping analysis, marker distribution on linkage groups, the average distance between markers on each linkage group (DBM), and the total genetic distance (TGD) covered by each linkage group.

Group	RAPDSs	ISSRs	AFLP EcoRI+MseI	AFLP PstI+MseI	SSRs	SRAPs	ACS	Totam	DBM	TGD
Total analyzed	178	38	142	62	20	222	3	665	(cM)	(cM)
Excluded	15	0	76	42	2	120	0	99		
I	19	5	3	3	0	10	0	40	6.8	264.7
II	16	4	0	0	1	11	0	32	6.9	212.1
III	21	7	8	1	0	5	0	42	5.2'	211.2
IV	19	5	2	0	0	8	0	34	4.7	155.2
V	15	0	2	0	4	13	0	34	4.1	133.5
VI	0	0	0	0	0	3	0	3	6.9	13.7
VII	0	0	1	1	2	0	0	4	6.7	20.01
VIII	1	2	0	1	0	0	0	4	12.8	38.4
IX	0	0	0	0	2	2	0	4	18.2	54.6
X	0	0	7	0	0	0	0	7	9.9	59.3
XI	11	0	0	2	0	10	0	23	4.8	105.2
XII	0	0	24	0	0	0	0	24	4.3	98.3
XIII	11	2	1	1	1	4	0	20	5.1	96.8
XIV	14	4	4	0	1	8	0	31	3.1	93.9
XV	14	0	5	1	0	11	0	31	4.2	124.9
XVI	5	1	0	0	0	3	0	9	11.1	88.9
XVII	4	0	0	0	3	3	1	11	7.9	79.5
XVIII	1	0	1	0	0	2	0	4	23.0'	69.1
XIX	0	0	0	3	0	0	0	3	28.5	57.01
Total mapped	151	30	58	13	14	93	1	360	5.8	1976

ping *Cit-ACS3*. Sex expression is greatly affected by ethylene. However, different studies indicate that it may have a complex role in flowering of watermelon (Sugiyama et al., 1998; Trebitsh et al., 1997). Presently, experiments are being conducted to isolate and map genes controlling ethylene biosynthesis and transduction in watermelon (A. Salaman and T. Trebitsh, unpublished data).

MARKER SEGREGATION. In contrast with the RAPD, ISSR, and SSR markers, most of the AFLP (*EcoRI-MseI*; *PstI-MseI*) and SRAP markers tested in this study were skewed away ($P < 0.05$) from the expected 1:1 ratio among testcross progeny (Table 5) and had to be excluded from the mapping analysis. In our recent study with an F_2 mapping population (PI 296341-FR $\times$ NHM) most of the AFLP markers (80%) were skewed from the expected 3:1 ratio (Levi et al., 2004b). Similarly, 47.5% and 48.0% of markers (RAPDs) were skewed in F_2 and F_3 mapping populations derived from the same parental lines (PI 296341-FR and NHM) (Hawkins et al., 2001). In an initial linkage map constructed for watermelon using a BC_1 population [(PI 296341 $\times$ NHM) $\times$ NHM] 25.7% of the RAPD markers were skewed from the expected segregation (Levi et al., 2001c). In a recent mapping study using recombinant inbred lines (RILs; derived from a cross between PI 296341 and the high quality Chinese inbred line 97103) 28% of the mapping markers (RAPDs and ISSRs) were skewed away from the 3:1 segregation ratio ($P < 0.05$) (Zhang et al., 2004). Skewed segregation frequently occurs in populations derived from interspecific crosses and may be a result of differences in genes controlling the reproduction processes (Zamir and Tadmor, 1986), or due to a meiotic drive where a chromosome with unique structural features or genetic properties render selective advantage or disadvantage to its respective gametes or zygotes (Buckler et al., 1999; Cameron and Moav, 1957; Lyttle, 1991; Sandler and

Novitski, 1957), as first shown in maize (*Zea mays* L.) by Rhodes (1942). However, the possibility of amplifying bands of the same size which represent different gene loci (non-single copy loci) may also play a role in the skewed segregation of some of these markers, as has been shown in PCR-RAPD analysis for different plant species (Amarger and Mercier, 1995).

The large number of skewed AFLP and SRAPs as compared with the RAPD, ISSR and SSR markers is likely to be due to distinct properties of the genomic regions covered by these marker types. Higher rate of skewed AFLP or RAPD markers versus RFLP markers were found in mapping population of different crops including tomato (Saliba-Colombani et al., 2000), rice (*Oryza sativa* L.) (Cho et al., 1998), and melon (Perin et al., 2002). In contrast, in barley (*Hordeum vulgare* L.), a larger number of RFLP markers were skewed than AFLP markers (Becker et al., 1995). Highly skewed markers may contribute to overestimation of recombination frequency and to loose linkages between markers. Conversely, they may cause the merging of two linkage groups (Saliba-Colombani et al., 2000). Thus, in this study, the skewed markers had to be excluded from the mapping analysis.

MAP CONSTRUCTION. A total of 653 markers (222 SRAP, 204 AFLP, 168 RAPD, 38 ISSR, and 21 SSR markers) were tested for mapping. Of these, 224 markers were skewed away from the expected 1:1 segregation ratio and had to be excluded from the linkage analysis. Four-hundred and twenty-nine markers were tested for linkage analysis. Of these, 36 could be ordered with high confidence on the linkage map (Table 5; Fig. 1). The map consists of 12 large linkage groups (119.6–373 cM), and 7 small to midsize linkage groups (47.9–91.4 cM) (Table 5; Fig. 1). Marker order is consistent with this on the linkage groups of the first map (Levi et al., 2002). However, in a few linkage groups the order of

Fig. 1. An extended linkage map for watermelon.

Fig. 1. Continued on next page.

Fig. 1. Continued from previous page. An extended linkage map for watermelon.

RAPD or ISSR markers was slightly altered due to the integration of SRAP, AFLP, or SSR markers. A change in the order of a few RAPD markers was observed in linkage group XV (linkage group III in Levi et al., 2002) due to the integration of 6 AFLP and 11 SRAP markers into this group (Fig. 1). The marker order on linkage groups in this map is also consistent with marker order on linkage groups constructed for the F_2 population (PI 296341 x NHM) (as shown by Levi et al., 2004b). A few markers in the

present map (Fig. 1) were also detected in the study with F_2S_7 RIL-based map constructed by Zhang et al. (2004). For example markers 835-325, P01-700, and G10-850 on linkage group V, and markers 824-1475c and 835-450c on linkage group III (Fig. 1) are in comparable genetic distances on linkage group I and II (respectively) on the map constructed by Zhang et al. (2004).

The stringent linkage analysis criteria used with the JoinMap analysis (Van Ooijen and Voorrips, 2001) resulted in a high

number of linkage groups (Fig. 1). These criteria were used to avoid any possible mapping errors, making the map useful in merging experiments with other maps constructed for watermelon (Hashizume et al., 1996, 2003; Hawkins et al., 2001; Levi et al., 2001c; 2002; Zhang et al., 2004).

The total genetic distance of the first map constructed with the present population (141 RAPD and 27 ISSR markers) is 1166.2 with an average genetic distance of 8.1 cM between markers (Levi et al., 2002). In this study, the addition of 93 SRAP, 71 AFLP, 11 RAPD, 3 ISSR, and 14 SSR markers increased the total genetic distance of the map to 1976 cM with an average genetic distance of 5.8 cM between markers.

CONCLUSIONS. Linkage analysis in watermelon is a challenging task because of low DNA polymorphism among watermelon cultivars, and on the other hand, the skewed segregation for large number of markers in mapping populations derived from crosses between PIs of *C. lanatus* var. *citroides* and watermelon cultivars (*C. lanatus* var. *lanatus*). The exclusion of a large number of skewed markers indicates that large regions of watermelon genome are still not mapped. Although AFLPs (*MseI-EcoRI*) produce a high number of polymorphic markers, a relatively small number of these markers was suitable for the mapping analysis. Most of the AFLP markers are ordered on linkage regions different from those discovered by RAPD, ISSR, and SSR markers. Although the large number of skewed SRAP markers, they appeared to be useful in the mapping analysis of watermelon genome. The SSR primers produced a relatively low number of markers useful for mapping. However, like the ISSR markers most of the SSR markers were not skewed from the 1:1 ratio and could be mapped. The large number of skewed AFLP and SRAP markers may reflect the non Mendelian segregation observed for different fruit and seed qualities in F_2 populations derived from crosses between *C. lanatus* var. *citroides* accessions and watermelon cultivars (*C. lanatus* var. *lanatus*). A few markers in this map are also found in maps constructed for watermelon using an F_2 population (Levi et al., 2004b) or a RIL population (Zhang et al., 2004). The order of these markers corresponds with those in the other maps (Levi et al., 2004b). The map presented in this study can be useful in merging the different maps constructed for watermelon (Hashizume et al., 2003; Hawkins et al., 2001; Zhang et al., 2004) and in mapping of markers linked to disease resistance or to fruit qualities.

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