DNA Markers from Different Linkage Regions of Watermelon Genome Useful in Differentiating among Closely Related Watermelon Genotypes

Amnon Levi¹ and Claude E. Thomas

USDA, ARS, U.S. Vegetable Laboratory, 2700 Savannah Highway, Charleston, SC 29414

Additional index words. *Citrullus lanatus*, RAPD, ISSR, AFLP, SRAP, DNA markers, germplasm, vegetables, breeding, triploid

Abstract. A genetic linkage map was previously constructed for watermelon using a wide testcross population [(Plant Accession Griffin 14113; *Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf.) × the watermelon cultivar New Hampshire Midget; NHM [(*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*)] × United States Plant Introduction (PI) 386015 [*Citrullus colocynthis* (L.) Schrad.]]. One-hundred forty-six markers [randomly amplified polymorphic DNA (RAPD), intersimple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), and sequence-related amplified polymorphism (SRAP) markers] unique to NHM and representing different linkage groups on the map were tested for polymorphism among 24 watermelon cultivars limited in genetic diversity. Five (9.4%) of 53 RAPD, six (40.0%) of 15 ISSR, 30 (81.0%) of 37 AFLP, and 33 (80.5%) of 41 SRAP markers tested produced polymorphism among the 24 cultivars. The polymorphic markers used in this study are scattered throughout the watermelon genome. However, a large number (19 of the 30) of AFLP markers clustered on one linkage group on the map. The SRAP markers proved to be most effective in producing polymorphism and in representing different linkage regions of watermelon genome. The polymorphic markers represent all 10 large linkage groups and five of the nine small linkage groups (altogether 15 of 19 linkage groups) of the genetic linkage map constructed so far for watermelon. These polymorphic markers can be useful in DNA fingerprinting of cultivars, in testing seed purity of breeding lines, and in identifying triploid (seedless) hybrid watermelons derived from crosses between closely related tetraploid and diploid lines.

Production of seedless watermelons in the United States has increased significantly in recent years, and there is a need to continue developing new seedless watermelon cultivars suitable to consumer needs. Seedless watermelons are triploids resulting from a cross between a tetraploid (female) plant and a diploid (male) plant (Kihara, 1951). The tetraploid plants are being produced by treating apical meristems of diploid ($2n = 2x = 22$; Shimotsuma, 1963; Shimotsuma and Matsumoto, 1957) watermelon plants with the alkaloid colchicine, which inhibits spindle formation and centromere detachment during cell division (Kihara, 1951). The triploid watermelon seeds are produced in isolation plots where tetraploid plants are pollinated by diploid plants. The seeds produced in isolation plots are mostly triploids. However,

with some tetraploid lines, up to 30% of seeds produced might be tetraploids as a result of self-pollination (Loehrlein and Ray, 1999). Tetraploid seeds are thicker than triploid seeds (Shimotsuma and Matsumoto, 1957). Still, DNA markers can be useful in quality assurance tests to confirm sufficient production of triploid seeds in isolation plots.

DNA markers have been used in genetic studies and in breeding programs of different polyploidy crop plants, including sunflowers (Mokrani et al., 2002), coffee (Herrera et al., 2002), and alfalfa (Barcaccia et al., 2000). Low DNA polymorphism exists among American watermelon cultivars, indicating they are derived from common ancestors (Levi et al., 2001a, 2001b). A set of DNA markers representing different linkage regions of the watermelon genome and producing sufficient polymorphism among genotypes is needed in breeding programs aiming to produce elite triploid (seedless) watermelon lines derived from crosses between closely related diploid and tetraploid breeding lines.

An extended genetic linkage map was constructed for watermelon (Levi et al., 2006). Because of low DNA polymorphism among watermelon cultivars (*C. lanatus* var. *lanatus*) (Levi et al., 2001b), the map con-

structed using a wide cross [a watermelon cultivar (*C. lanatus* var. *lanatus*) and a U.S. Plant Introduction (PI) of the wild *C. lanatus* var. *citroides*]. The map contains 360 DNA markers distributed on 19 linkage groups (10 large and nine small linkage groups) and covers a genetic distance of 1976 cM with an average distance of 5.8 cM between two markers. The map consists mostly of randomly amplified polymorphic DNA (RAPD), intersimple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), and sequence-related amplified polymorphism (SRAP) markers (Levi et al., 2006).

In this study, markers from different linkage groups of the watermelon genetic linkage map (Levi et al., 2006) were examined for polymorphism among watermelon cultivars (diploids $2n = 22$) sharing a narrow genetic background (Levi et al., 2001b). A set of markers representing most of the linkage groups and producing sufficient polymorphism among cultivars was assembled. The polymorphic markers can be useful in DNA fingerprinting of watermelon elite breeding lines, in differentiating among closely related diploid and tetraploid breeding lines, and in confirming the production of true F_1 hybrid triploid (seedless) watermelon lines.

Materials and Methods

Plant material. Twenty-four American heirloom cultivars (diploids; $2n = 22$), with low DNA polymorphism (Levi et al., 2001b), were selected for this study. In addition, BC_5S_1 plants (with a common genetic background) were used for testing the effectiveness of markers for production of polymorphism among closely related genotypes. The BC_5S_1 plants resulted from the following crosses: an F_1 hybrid [U.S. PI 386015 (*C. colocynthis*) × ‘Griffin 14113’ (*C. lanatus* var. *citroides*)] was backcrossed with the watermelon cultivar ‘Allsweet’ (*C. lanatus* var. *lanatus*). Then, four backcrosses were performed using the following watermelon cultivars successively: ‘Sugar Baby’, ‘Charleston Gray’, ‘Charleston Gray’, and ‘Allsweet’ (all are *C. lanatus* var. *lanatus*) to produce BC_5 plants [(*C. colocynthis* × *C. lanatus* var. *citroides*) *C. lanatus* var. *lanatus*]. One BC_5 plant was self-pollinated to produce BC_5S_1 seeds. Seedlings of the watermelon cultivars and the BC_5S_1 were grown in the greenhouse (26 °C/20 °C day/night temperatures). Young leaves were collected from young plants (2–3 weeks old) and stored at –80 °C for DNA isolation.

Isolation of DNA. An improved procedure for isolation of DNA from young leaves of watermelon (Levi and Thomas, 1999) was used in this study.

Randomly amplified polymorphic DNA and intersimple sequence repeat procedures. The RAPD and ISSR markers (Table 1) were analyzed as described in previous studies (Levi et al., 2001b, 2004).

Amplified fragment length polymorphism procedure. The AFLP procedure developed

Received for publication 6 July 2006. Accepted for publication 8 Nov. 2006.

We acknowledge the technical assistance of Laura Pence in developing the molecular markers.

The use of trade names in this publication does not imply endorsement by the USDA of the products named or criticism of similar ones not mentioned.

¹To whom reprint requests should be addressed; e-mail alevi@saa.ars.usda.gov.

Table 1. Sequence of randomly amplified polymorphic DNA and intersimple sequence repeat primers used for producing markers polymorphic among cultivars.

Primer	Sequence (5'-3')
U595 ²	GTCACCGCGC
U610	TTTGCCGCCC
U613	TGCACCCACG
AE04 ³	CCAGCACTTC
G18	GGCTCATGTG
U809 ⁴	GAGGAGAGAGAGAGAGG
U834	AGAGAGAGAGAGAGAGCTT
U835	AGAGAGAGAGAGAGAGCTC
U856	ACACACACACACACCTA
U847	CACACACACACACACAAGC

²Primers starting with "U" are from the University of British Columbia.

³Primers starting with "AE" or "G" are from "Operon."

⁴All intersimple sequence repeat markers are from the University of British Columbia and start with U8.

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* restriction enzymes and primer combinations as described by Levi et al. (2004). AFLP markers (Table 2) were analyzed using a CEQ8800 (capillary system) DNA sequencer (Beckman Coulter, Fullerton, Calif.). For visualization of DNA fragments on the CEQ8800, the *EcoRI* selective primers were labeled with one of three WellRED dye labels (D2, D3, or D4; Prologo, Boulder, Colo.), as described by Levi et al. (2006).

Sequence-related amplified polymorphism analysis. The SRAP markers were tested for polymorphism among the cultivars using the same procedure used for mapping of watermelon genome (Levi et al., 2006). The SRAP markers polymorphic among cultivars (Table 3) were selected for repeated tests.

Selecting and testing linkage markers for polymorphism. The linkage map (Levi et al.,

Table 2. The amplified fragment length polymorphism primer pairs, their designation, and the size of markers that are polymorphic among the watermelon cultivars.

Primer pair	Designation	Marker size (base pair)
EAAC - MCAG	EACAG	99c, 219c, 312c, 373c
EACC - MCAA	ECCAA	482c
EACC - MCAC	ECCAC	112c, 224c, 257c
EACC - MCTG	ECCTG	93c, 310c
EAGG - MCTT	EGGTT	188c, 263c, 382c
EAGG - MCTC	EGGTC	199c, 223c
EAAG - MCTG	EAGTG	272c, 408c
EAAG - MCTC	EAGTC	492c
EAAG - MCAT	EAGAT	104c, 113c, 121c, 138c, 152c, 427c
EAGG - MCAG	EGGAG	147c, 226c, 334c
EACC - MCTA	ECCTA	187c
EAAG - MCAG	EAGAG	121c, 174c

Table 3. The sequence-related amplified polymorphism forward primers labeled with a DNA sequencing dye (FLP) and the reverse unlabeled primers (RUP) used in different combinations to produce the SRAP markers polymorphic among watermelon cultivars (Table 4).

FLP	DYE	Primer sequence (5'-3')
me1	D4	TGAGTCCAAACCGGATA
me2	D3	TGAGTCCAAACCGGAGC
me3	D2	TGAGTCCAAACCGGAAT
me4	D4	TGAGTCCAAACCGGACC
me5	D3	TGAGTCCAAACCGGAAG
ab1	D2	AGTGATTCAACCGGAGA
ab2	D4	AGTGATTCAACCGGATA
ab3	D3	AGTGATTCAACCGGAGC
RUP		Primer sequence (5'-3')
ba1		GTCGAGCTGCCAATTATA
ba2		GTCGAGCTGCCAATTAAT
ba3		GTCGAGCTGCCAATTTGC
ba4		GTCGAGCTGCCAATTTTT
ba5		GTCGAGCTGCCAATTAAA
ba6		GTCGAGCTGCCAATTAAC
ba7		GTCGAGCTGCCAATTAAG
ba8		GTCGAGCTGCCAATTATT
ba9		GTCGAGCTGCCAATTATC
ba10		GTCGAGCTGCCAATTATG
ba11		GTCGAGCTGCCAATTTGA
ba12		GTCGAGCTGCCAATTTGT
em1		GACTGCGTACGAATTAAT
em2		GACTGCGTACGAATTTGC
em3		GACTGCGTACGAATTGAC
em4		GACTGCGTACGAATTTGA
em5		GACTGCGTACGAATTAAC
em6		GACTGCGTACGAATTGCA

2006) contains markers unique to the parent Griffin 14113 (*C. lanatus* var. *citroides*) or to the parent 'New Hampshire Midget' [NHM (*C. lanatus* var. *lanatus*)]. Most of the markers on the linkage map that are unique to the cultivar NHM (Table 1) were tested for their presence or absence in 24 cultivars, and the markers producing polymorphism among cultivars (Table 4) were selected for a repeated test.

Marker nomenclature. The RAPD or ISSR markers (Tables 1 and 4) were designated by their serial number and their size. For example, the 700-base pair (bp) marker produced by primer AE-04 (Operon Biotechnology, Alameda, Calif.), which is unique to NHM, was designated as AE04-700c. The AFLP markers (Tables 2 and 4) were designated by the last two nucleotides on the 3' of the *MseI* and *EcoRI* primers, respectively. For example marker ATGT-157 represents the AFLP marker (with a size of 157 bp) produced by the selective primers *MseI*-ATT and *EcoRI*-AGT. The SRAP markers were designated by combining the forward and reverse primers (Tables 3 and 4) with the marker size (bp). For example, the 316-bp fragment produced in the cultivar NHM by the forward primer Me1 and the reverse primer ba2 (Table 3) is designated as 'Me1ba2-316c' (Table 4).

Results and Discussion

Most of the markers that are unique to the cultivar NHM and mapped on the linkage map constructed for watermelon (Levi et al.,

2006) were tested for polymorphism among the 24 American watermelon cultivars. The markers tested include 53 RAPD, 15 ISSR, 38 AFLP, and 40 SRAP markers. The markers (Table 4) represent all 10 large linkage groups and five of the nine small linkage groups (altogether 15 of the 19 linkage groups) on the genetic map (Levi et al., 2006). Five (9.4%) of 53 RAPD, six (40.0%) of 15 ISSR, 30 (81.0%) of 37 AFLP, and 33 (80.5%) of 41 SRAP markers tested produced polymorphism among the 24 cultivars (Table 4). The markers represent most linkage regions. However, a large number of the AFLP markers (19 of 30 AFLP markers) represent one major linkage region (Table 4). AFLP markers may tend to cluster in certain genomic regions as shown in mapping of tomato (Saliba-Colombani et al., 2000) and melon genomes (Perin et al., 2002). A previous study (Levi et al., 2004) indicated that AFLP and ISSR markers produced higher polymorphisms among watermelon cultivars as compared with RAPDs. In this study, the SRAP markers proved to be as efficient as the AFLPs in producing polymorphism but more effective in representing the different linkage regions of watermelon genome. The SRAP marker may represent gene sequences. Their analysis is based on polymerase chain reaction amplification of open reading frames (ORFs) using 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, whereas 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). Because SRAP markers may represent ORFs, purifying the amplified DNA fragments and sequencing them may connect fingerprint differences to isolated genes. This may form one of the future perspectives of this technique and the base for developing more specialized DNA markers (Li and Quiros, 2001).

The markers in this study represent most of the linkage groups identified so far on the linkage map constructed for watermelon (Table 4). The polymorphic markers can be useful in DNA fingerprinting of watermelon cultivars and breeding lines. Additionally, they can be useful in quality assurance tests to confirm purity of diploid (pollinator) and tetraploid plants used in production of triploid hybrid seeds in isolation plots. Indeed, four RAPD [G18-850c (group III), U610-2100c (group IV), AE04-700c (group V), and U595-725c (group XIV)] and two ISSR markers [U809-450 (group I) and U835-550c (group XIII)] were tested for polymorphism among 30 seedlings with common genetic backgrounds (BC₅S₁) versus 30 seedlings of the watermelon cultivar 'Crimson Sweet'. All four RAPD and two ISSR markers proved effective in producing polymorphism among the BC₅S₁ plants

Table 4. Mapping markers (intersimple sequence repeat, randomly amplified polymorphic DNA, amplified fragment length polymorphism, and sequence-related amplified polymorphism) representing different linkage groups on the genetic map constructed for watermelon polymorphic among American heirloom cultivars (diploids; $2n = 22$): Allsweet (AS), AU-Producer (AP), Black Diamond (BD), Blackstone (BS), Charleston Gray (CY), Coles Early (CE), Congo (CO), Fairfax (FX), Family Fun (FF), Garrisonian (GN), Hawkesbury (HU), Iopride (IP), Ironsides (IS), Jubilee (JB), King & Queen (KQ), Kleckely's Sweet (KS), Klondike (KD), Leesburg (LB), Mickylee (MK), Miles (MS), Minilee (MN), Parker (PK), Picnic (PI), and Stone Mountain (SM).

Linkage groups	AS	AP	BD	BS	CY	CE	CO	FX	FF	GN	HU	IP	IS	JB	KQ	KS	KD	LB	MK	MS	MN	PK	PI	SM
Group I																								
Me1ba1-185c	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	7
Me1ba11-184c	0	1	1	0	0	0	0	1	0	1	0	1	1	1	1	0	1	1	1	0	0	1	1	13
Me3ba1-83c	0	1	1	1	0	1	0	0	0	1	1	1	1	0	0	0	0	1	1	1	1	0	0	12
Ab2em4-90c	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	22
Me4ba8-97c	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	21
ECCTG-93c	1	1	1	1	1	1	0	1	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	20
EAGAT-427c	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	0	1	1	1	20
U809-450c	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	5
Group II																								
Me1em3-281c	1	1	1	1	0	1	0	0	0	1	1	1	1	0	1	1	0	0	1	1	0	0	1	15
Me4ba2-291c	1	1	1	1	0	0	1	0	0	1	1	1	1	0	1	0	1	1	1	1	0	0	1	15
Me3ba6-286c	0	1	1	1	0	0	0	0	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	8
Ab1ba11-242c	0	0	1	0	0	1	1	0	0	0	1	1	1	0	0	1	1	0	1	1	1	1	1	13
Me1ba7-93c	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	5
Me3ba1-173c	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	5
Group III																								
Me1ba2-316c	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	12
Me3ba6-181c	0	1	1	1	0	1	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	1	9
Me2ba8-133c	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	18
ECCTA-187c	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	1	1	1	0	19
EAGAT-121c	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	19
U834-575c	1	0	1	1	0	0	1	0	1	1	0	0	1	0	0	1	0	0	1	0	1	0	0	10
G18-850c	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	21
Group IV																								
Me4ba5-163c	1	1	1	1	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	1	1	1	19
ECCAC-112c	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	21
U610-2100c	1	1	1	0	1	0	1	1	1	0	1	0	1	1	0	1	1	1	1	1	1	0	1	17
Group V																								
Me3em1-76c	1	1	1	1	0	1	0	1	1	0	1	1	0	0	1	1	1	1	1	1	0	1	1	17
Me1ba11-76c	0	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	18
Me2ba8-127c	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	19
Me2ba8-148c	0	0	1	1	0	1	0	1	0	0	0	1	1	1	1	0	0	0	1	1	1	0	1	12
Me4ba2-173c	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	21
Me2em6-147c	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	22
AE04-700c	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	4
Group VIII																								
U856-345c	0	1	0	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	18
Group X																								
EAGAG-174c	0	1	1	1	0	0	1	1	1	1	1	0	0	1	1	0	1	0	0	0	0	0	1	13
EAGAG-121c	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	23
Group XI																								
Me1ba8-308c	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2
Me2em5-267c	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	21
Group XII																								
EACAG-219c	1	1	1	1	1	0	0	1	0	0	1	1	1	0	1	1	1	1	0	0	0	0	1	14
ECCAA-482c	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	4
EAGAT-152c	1	0	0	1	1	0	0	0	0	0	1	1	1	1	0	1	0	0	1	0	1	1	1	12
EGGTC-223c	1	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	20
EGGTT-382c	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	0	1	7
EGGTT-263c	0	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	1	1	0	1	1	1	16
EAGTG-408c	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	1	0	1	1	0	1	1	1	11
EAGAT-138c	1	0	0	1	1	0	0	0	0	0	1	1	1	0	0	1	1	1	1	1	1	1	1	14
EACAG-312c	0	0	1	1	1	0	0	0	0	0	1	1	1	0	0	1	0	1	0	0	0	0	0	8
EACAG-373c	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	4
ECCAC-257c	1	0	1	1	1	1	0	0	0	0	0	1	1	0	0	1	1	0	1	0	1	1	1	14
ECCAC-224c	1	0	1	1	1	0	0	0	0	0	0	1	1	0	0	1	0	0	1	0	1	1	1	12
ECCTG-310c	1	0	1	1	1	1	0	0	0	0	1	1	1	0	1	1	0	1	1	0	1	1	1	16
EAGTC-492c	1	0	1	1	1	1	0	0	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	18
EGGTC-199c	1	0	1	1	1	1	0	0	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	18
EGGTT-188c	1	0	1	1	1	1	0	0	0	0	1	1	0	0	1	1	0	1	1	0	1	1	1	15
EGGAG-226c	0	0	0	1	1	0	0	0	0	0	0	1	1	0	1	1	1	1	1	0	1	1	1	12
EGGAG-334c	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0	1	8
EAGTG-272c	0	0	0	1	1	0	0	0	0	0	1	1	1	0	1	1	1	1	1	0	1	1	1	14
Group XIII																								
Me2ba5-226c	0	0	1	1	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	6
Me3ba1-63c	0	0	1	0	0	1	0	0	0	0	1	0	1	0	1	1	0	1	0	1	0	0	0	8
EAGAT-113c	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	21
U835-550c	1	1	1	1	1	0	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	1	12

(Continued on next page)

Table 4. (continued) Mapping markers (intersimple sequence repeat, randomly amplified polymorphic DNA, amplified fragment length polymorphism, and sequence-related amplified polymorphism) representing different linkage groups on the genetic map constructed for watermelon polymorphic among American heirloom cultivars (diploids; $2n = 22$): Allsweet (AS), AU-Producer (AP), Black Diamond (BD), Blackstone (BS), Charleston Gray (CY), Coles Early (CE), Congo (CO), Fairfax (FX), Family Fun (FF), Garrisonian (GN), Hawkesbury (HU), Iopride (IP), Ironsides (IS), Jubilee (JB), King & Queen (KQ), Kleckely's Sweet (KS), Klondike (KD), Leesburg (LB), Mickylee (MK), Miles (MS), Minilee (MN), Parker (PK), Picnic (PI), and Stone Mountain (SM).

Linkage groups	AS	AP	BD	BS	CY	CE	CO	FX	FF	GN	HU	IP	IS	JB	KQ	KS	KD	LB	MK	MS	MN	PK	PI	SM	
Group XIV																									
Me2ba10-114c	0	1	1	1	1	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	1	0	11
Ab2em6-189c	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	21
EAGAT-104c	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	0	1	1	1	0	20
U834-700c	1	1	1	1	0	0	1	0	1	0	0	0	1	0	0	1	1	0	1	0	1	0	0	0	11
U613-1000c	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	22
U595-725c	1	0	1	0	0	1	1	0	1	1	0	0	0	0	1	1	1	1	1	0	1	1	0	0	13
U847-325c	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	22
Group XV																									
Ab3em6-148c	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	0	0	0	0	1	0	1	1	0	15
Me1ba1-240c	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	5
Me2em5-182c	1	1	1	1	0	1	1	1	0	1	1	0	0	1	1	1	0	1	1	0	0	1	1	1	17
EACAG-99c	0	1	0	1	0	0	1	1	1	1	1	0	1	0	0	1	0	1	0	0	0	0	0	0	10
Group XVI																									
Me4ba4-222c	0	0	1	1	0	1	1	1	1	1	0	1	0	1	1	0	0	0	1	1	0	1	1	1	15
Group XVII																									
Ab2em6-110c	1	0	0	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	19
Group XVIII																									
Me3ba10-320c	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	21
EGGAG-147c	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	0	1	1	1	0	19

Fig. 1. Randomly amplified polymorphic DNA markers U610-2100c (upper figure) and U595-725c (lower figure) are polymorphic among plants of a breeding material (a BC_5S_1 generation, lanes 2–11) and monomorphic among plants of the watermelon cultivar 'Crimson Sweet' (lanes 12–21). Size markers on each side (lanes 1 and 22) are of the 1-kb Plus DNA Ladder (Invitrogen, Carlsbad, Calif.).

and were monomorphic among most of the 'Crimson Sweet' plants (Fig. 1). Although the SRAP markers may produce a number of codominant markers (as indicated by Li and Quiros, 2001), all markers in this study are of dominant nature (present or absent). Codominant markers, including simple sequence repeat (SSR) markers, are considered more effective in breeding programs than dominant markers as shown in melon (Danin-Poleg et al., 2000; Gonzalo et al., 2001). However, it has been a challenging task to develop codominant SSR markers in watermelon as has been indicated in our recent mapping study (Levi et al., 2006). The assembly of markers from different linkage regions of the watermelon genome is vital in differentiating among watermelon genotypes. A saturated linkage map with a large number of polymorphic markers is needed in watermelon breeding programs. The polymorphic markers representing different linkage regions may also be useful in breeding programs enhancing the genetic base of cultivated watermelons through the development of introgression

lines and single-segment substitution lines as shown for rice (*Oryza sativa* L.) (Xi et al., 2006) and tomato (*Solanum lycopersicum*) (Gur and Zamir, 2004).

Literature Cited

- Barcaccia, G., E. Albertini, D. Rosellini, S. Tavoletti, and F. Veronesi. 2000. Inheritance and mapping of $2n$ -egg production in diploid alfalfa. *Genome* 43:528–537.
- Danin-Poleg, Y., N. Reis, S. Baudracco-Arnas, M. Pitrat, J.E. Staub, M. Oliver, P. Arus, C.M. deVicente, and N. Katzir. 2000. Simple sequence repeats in *Cucumis* mapping and map merging. *Genome* 43:963–974.
- Gonzalo, M.J., M. Oliver, J. Garcia-Mas, A. Monfort, R. Dolcet-Sanjuan, N. Katzir, P. Arus, and A.J. Monforte. 2001. Simple-sequence repeat markers used in merging linkage maps of melon (*Cucumis melo* L.). *Theor. Appl. Genet.* 110:802–811.
- Gur, A. and D. Zamir. 2004. Unused natural variation can lift yield barriers in plant breeding. *PLoS Biol.* 2:1610–1615.
- Herrera, J.C., M.C. Combes, H. Cortina, G. Alvarado, and P. Lashermes. 2002. Gene introgression into *Coffea arabica* by way of triploid hybrids

(*C. arabica* × *C. canephora*). *Heredity* 89: 488–494.

- Kihara, H. 1951. Triploid watermelons. *Proc. Amer. Soc. Hort. Sci.* 58:217–230.
- Levi, A. and C.E. Thomas. 1999. An improved procedure for isolation of high quality DNA from watermelon and melon leaves. *Cucurbit Genet. Coop. Rpt.* 22:41–42.
- Levi, A., C.E. Thomas, A.P. Keinath, and T.C. Wehner. 2001a. Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. *Genet. Res. Crop Evol.* 48:559–566.
- Levi, A., C.E. Thomas, T.C. Wehner, and X. Zhang. 2001b. Low genetic diversity indicates the need to broaden the genetic base of cultivated watermelon. *HortScience* 36: 1096–1101.
- Levi, A., C.E. Thomas, M. Newman, O.U.K. Reddy, X. Zhang, and Y. Xu. 2004. ISSR and AFLP markers sufficiently differentiated among American watermelon cultivars with limited genetic diversity. *J. Amer. Soc. Hort. Sci.* 129:553–558.
- Levi, A., C.E. Thomas, T. Trebitsh, A. Salman, J. King, J. Karalius, M. Newman, O.U.K. Reddy, Y. Xu, and X. Zhang. 2006. An extended linkage map for watermelon based on SRAP, AFLP, SSR, ISSR and RAPD markers. *J. Amer. Soc. Hort. Sci.* 131:393–402.
- Li, G. and C.F. Quiros. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in Brassica. *Theor. Appl. Genet.* 103:455–461.
- Loehrlein, M. and D. Ray. 1999. Triploid and tetraploid watermelon (*Citrullus lanatus* [Thunb.] Matsum. and Nakai) seed size and weight. *Cucurbit Genet. Coop. Rpt.* 22:34–37.
- Mokrani, L., L. Gentzbittel, F. Azanza, L. Fitamant, G. Al-Chaarani, and A. Sarrafi. 2002. Mapping and analysis of quantitative trait loci for grain oil content and agronomic traits using AFLP and SSR in sunflower (*Helianthus annuus* L.). *Theor. Appl. Genet.* 106:149–156.
- Perin, C., S. Hagen, V. De Conto, N. Katzir, Y. Danin-Poleg, V. Portnoy, S. Baudracco-Arnas,

- J. Chadoeuf, C. Dogimont, and M. Pitrat. 2002. A reference map of *Cucumis melo* based on two recombinant inbred line populations. *Theor. Appl. Genet.* 104:1017–1034.
- Saliba-Colombani, V., M. Causse, L. Gervais, and J. Philouze. 2000. Efficiency of RFLP, RAPD, and AFLP markers for the construction of an intraspecific map of the tomato genome. *Genome* 43:29–40.
- Shimotsuma, M. 1963. Cytogenetic and evolutionary studies in the genus *Citrullus*. *Seiken Jiho* 15:24–34.
- Shimotsuma, M. and K. Matsumoto. 1957. Comparative studies on the morphology of polyploidy watermelon seeds. *Seiken Jiho* 8:67–74.
- Vos, P., R. Hogers, M. Beeker, M. Reijmans, T. Vandele, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP—a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23:4407–4414.
- Xi, Z.Y., F.H. He, R.Z. Zeng, Z.M. Zhang, X.H. Ding, W.T. Li, and G.Q. Zhang. 2006. Development of wide population of chromosome single-segment substitution lines in the genetic background of an elite cultivar of rice (*Oryza sativa* L.). *Genome* 49:476–484.