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

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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), inter-simple 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 centrometer 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 polyplody crop plants, including sunflowers (Mokrani et al., 2002), coffee (Herrera et al., 2002), and alfalfa (Baraccia 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 constructed 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), inter-simple 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 F1 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, BC3S1 plants (with a common genetic background) were used for testing the effectiveness of markers for production of polymorphism among closely related genotypes. The BC3S1 plants resulted from the following crosses: an F1 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 BC3 plants [(C. colocynthis × C. lanatus var. citroides) C. lanatus var. lanatus]. One BC3 plant was self-pollinated to produce BC5S1 seeds. Seedlings of the watermelon cultivars and the BC5S1 plants 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 inter-simple 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...
Table 1. Sequence of randomly amplified polymorphic DNA and intersimple sequence repeat primers used for producing markers polymorphic among cultivars.

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<th>Primer</th>
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*Primer starting with "U" are from the University of British Columbia.
*Primer starting with "AE" or "G" are from Operon.
*All 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 sample (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 EcoRl-MseJ 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 EcoRl selective primers were labeled with one of three WelRed dye labels (D2, D3, or D4; Proligo, 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., 2006) contains markers unique to the parent {C. lanatus var. citruloides} 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 Msel and EcoRl primers, respectively. For example marker ATGT-157 represents the AFLP marker (with a size of 157 bp) produced by the selective primers Msel-ATT and EcoRl-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 Mel 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 (Pepe 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), A600-700c (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 genomic regions as shown in mapping of tomato (Saliba-Colombani et al., 2000) and melon genomes (Pepe 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).

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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 (diploid; 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), Ironside (IS), Jubilee (JB), King & Queen (KQ), Kleckely’s Sweet (KS), Klondike (KD), Leesburg (LB), Micky Lee (MK), Miles (MS), Minilee (MN), Parker (PK), Picnic (PC), and Stone Mountain (SM).

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(Continued on next page)
through the development of introgression
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 consid-
ered more effective in breeding programs
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 lycopersi-
cum) (Gur and Zamir, 2004).

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grain oil content and agronomic traits
using AFLP and SSR in sunflower (Helianthus
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