RESEARCH ARTICLES
J. Crop Sci. Biotech. 10 (2) : 98 ~ 105

Development of Sequence-Based DNA Markers for Evaluation of Phylogenetic Relationships in Korean Watermelon Varieties

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Abstract

Phylogenetic relationships in Korean watermelons were evaluated by genetic similarity coefficients using 15 SSR, 14 SCAR, and 14 CAPS markers. The SSR markers were selected from previously reported melon and watermelon SSRs through testing polymorphisms within a set of commercial F_1 varieties. The SCAR and CAPS markers were developed from polymorphic AFLP markers between inbred lines 'BN4001' and 'BN4002'. From the AFLP analysis, 105 polymorphic fragments were identified between the inbred lines using 1,440 primer combinations of *Eco*RI+CNNN and *Xba*I+ANNN. Based on the sequencing data of these polymorphic fragments, we synthesized sequence specific primer pairs and detected clear and reliable polymorphisms in 27 primer pairs by indels (insertion/deletion) or RFLP. A total of 43 sequence-based PCR markers were obtained and polymorphic information content (PIC) was analyzed to measure the informativeness of each marker in watermelon varieties. The average PIC value of SCAR markers was 0.41, which was similar to that of SSR markers. Genetic diversity was also estimated by using these markers to assess the phylogenetic relationships among commercial varieties of watermelon. These markers differentiated 26 Korean watermelon varieties into two major phylogenetic groups, but this grouping was not significantly correlated with their morphological and physiological characteristics. The mean genetic similarity was 66% within the complete set of 26 commercial varieties. In addition, these sequence-based PCR markers were reliable and useful to identify cultivars and genotypes of watermelon.

Key words: watermelon, genetic diversity, DNA markers, SSR, AFLP

Introduction

The genus *Citrullus* belongs to the Cucurbitaceae family and contains one commercially important species, *Citrullus lanatus* (Thunb.), and three other wild species, *C. colocynthis* (L.) Schrad, *C. ecirrhosus* Cogn., and *C. rehmii* De Winter. Cultivated *Citrullus lanatus* originated in the dry and sandy areas of southern Africa (Bates and Robinson, 1995). Watermelon is presently cultivated as an important vegetable fruit throughout the world for its sweet flesh. Worldwide gross production of watermelon (*Citrullus lanatus* var. *lanatus*) represented one third of tomato production in 2004 and ranks second after tomato in fruit vegetable cultivation (www.fao.org). The area of watermelon cultivation in Korea is approximately 34,000 ha which is the largest among crops of the Cucurbitaceae family followed by melon, cucumber, and pumpkin. Watermelon is cultivated in approximately 10% of total vegetable cultivation area in Korea and ranks fourth after hot pepper, chinese cabbage, and radish in 2001. Due to the continuously increasing demands for watermelon consumption, it is very important to develop new varieties having enhanced disease resistance, improved adaptability to environmental stress, and high fruit quality. Breeding of new elite watermelon varieties could be accomplished by increasing genetic diversity in the breeding lines through cross hybridization based on the phylogenetic relationships among the germplasm. Genetic diversity among cultivars, genetic purity of F_1 seeds, and identification of specific cultivar are usually determined by phenotypic characteristics of fruits. Although there are wide ranges of phenotypic variations in watermelons, phylogenetic

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studies using isozyme markers indicate that there are narrow genetic diversities in several watermelon cultivars and most of these isozyme markers produced monomorphic band patterns (Biles et al. 1989; Navot and Zamir 1987; Zamir et al. 1984). In contrast, PCR-based DNA markers such as random amplified polymorphic DNA (RAPD) produced a limited number of polymorphisms for analysis of genetic diversity in watermelon (Lee et al. 1996; Levi et al. 2001a, b). Other DNA marker techniques such as amplified fragment length polymorphism (AFLP) (Kang et al. 1997; Levi et al. 2006; Vos et al. 1995) and restriction fragment length polymorphism (RFLP) (Lefebvre et al. 1993; Nam et al. 1997; Prince et al. 1992) have not been primarily applied to the analysis of watermelon because of relatively delicate experimental procedures. Chloroplast and mitochondrial genomes were also analyzed to detect polymorphisms in *Citrullus lanatus* (Dane and Bakhtiyarova 2003; Levi and Thomas 2005a). Dane and Bakhtiyarova (2003) developed two PCR-RFLP markers based on chloroplast DNA sequences to distinguish cultivated watermelon from citron-type watermelon. Levi and Thomas (2005a) detected polymorphisms among five watermelon cultivars and 21 U.S. Plant Introductions (PIs) using 20 chloroplast and 10 mitochondrial RFLP markers. SSR markers detect polymorphisms based on the repeat length of microsatellite sequence (Wang et al. 1994). SSR markers are usually preferred for plant breeding since they are hypervariable, multiallelic, ubiquitous in plant genomes, and easily detectable by simple PCR procedures. SSR or ISSR (inter-simple sequence repeat) markers were developed and characterized for linkage map construction (Hashizume et al. 2003; Levi et al. 2006) or germplasm evaluation in watermelon and Cucurbitaceae family (Guerra-Sanz 2002; Jarret et al. 1997; Joobeur et al. 2006; Katzir et al. 1996; Levi et al. 2005b). However, there are some limitations for molecular genetic research in watermelon because limited numbers of informative and reliable DNA markers are available. In contrast, several relatively dense linkage maps in melon (*Cucumis melo* L.) have been constructed using reliable DNA markers, functionally characterized genes, and agronomically important traits (Brotman et al. 2005; Noguera et al. 2005; Perin et al. 2002; Silberstein et al. 2003). Therefore, application of these informative melon marker systems to watermelon would greatly improve genetic studies in watermelon.

In the present study, we developed sequence-based PCR markers to evaluate phylogenetic relationships in 26 Korean watermelon varieties. Polymorphic and reproducible SSR markers were identified from previously reported SSRs of melon and watermelon, and also informative and reliable marker systems with sequence characterized amplified region (SCAR) and cleaved amplified polymorphic sequence (CAPS) markers were developed from AFLP procedures in watermelon.

Materials and Methods

Plant Materials

Seeds of all commercial F_1 varieties were purchased at a seed market in Korea. Inbred lines 'BN4001' and 'BN4002' were kindly provided by Yoon-Seob Shin, Nongwoo Bio, Nambu Breeding Institute. Table 1 shows all the plant materials used in this study and some phenotypic characteristics of fruit. These plant materials all belong to the species of *Citrullus lanatus* var. *lanatus* and F_1 varieties are commercially cultivated in Korea. All the plants were grown in a greenhouse. Four plants of each cultivar were used for DNA isolation and PCR analysis.

Table 1. Inbred lines and commercial varieties of watermelon used in this study and their phenotypic characteristics.

No	Line or Variety	Source (Company)	Fruit Shape[a]	Fruit Weight[b]	Flesh Color[c]	Rind Color[d]	Seed Size[e]	Seed Color[f]	Seed Size[g]
1	BN4001	Nongwoo Bio	R	L	R	L	MG	M	R
2	BN4002	Nongwoo Bio	O	M	DR	S	G	M	O
3	Speed	Nongwoo Bio	SO	M	DR	L	G	M	SO
4	SpeedP	Nongwoo Bio	SO	M	DR	L	T	M	SO
5	Star	Nongwoo Bio	SO	M	R	L	BR	M	SO
6	GulBD	Nongwoo Bio	SO	M	DR	M	G	E	SO
7	WooRi	Nongwoo Bio	SO	M	R	M	G	L	SO
8	SamBG	Seminis Korea	SO	M	R	M	G	L	SO
9	JoSSB	Seminis Korea	SO	M	R	L	G	M	SO
10	SamDG	Seminis Korea	SO	M	R	M	G	M	SO
11	SeolBG	Seminis Korea	SO	M	R	L	T	M	SO
12	JoEG	Seminis Korea	SO	M	LR	M	G	L	SO
13	KWCH	Seminis Korea	SO	M	R	S	BL	L	SO
14	SemG	Syngenta	SO	M	R	M	T	L	SO
15	InDG	Syngenta	SO	M	R	M	G	L	SO
16	MNDR	Syngenta	SO	M	R	M	BR	L	SO
17	MyGG	Syngenta	SO	M	R	L	G	L	SO
18	MiRG	Takii	SO	M	R	M	G	L	SO
19	JoCG	Takii	SO	M	R	M	G	M	SO
20	DrmG	Dongbu	SO	M	R	L	MB	L	SO
21	SaeRG	Dongbu	SO	M	R	M	G	L	SO
22	SaeCN	Hyundai	SO	M	R	M	BR	M	SO
23	ChaSD	Hyundai	SO	M	R	M	G	M	SO
24	ChoGS	Hyundai	SO	M	R	M	G	M	SO
25	HaeDG	Nonghyeop	SO	M	R	L	BL	L	SO
26	DongBG	Dongwon	SO	M	R	M	BL	L	SO
27	GoNKW	Gonong	SO	M	R	M	G	M	SO
28	BaekMG	Koregon	SO	L	DR	M	T	M	SO

[a] R; round, SO; short oblong, O; oblong
[b] M; middle (8 - 10 kg), L; light (8<kg)
[c] LR; light red, R; red, DR; deep red
[d] GS; green striped, DGS; dark green striped, LGS; light green striped
[e] L; large (0.8> cm), M; middle (0.8 - 0.75 cm), S; small (0.75< cm)
[f] G; gray, T; tan, BL; black, BR; brown, MB; mottled black, MG; mottled gray
[g] E; fruits are matured less than 55 days after flowering, M; 55-60 days, L; more than 60 days

Total DNA isolation

DNA extraction was performed, as previously described (Kang et al. 2001), with a few modifications. Small tissues of young leaves (0.1g) were ground completely in 1.5 ㎖ microtube with 0.5 ㎖ of extraction buffer (0.5 M NaCl, 100 mM Tris-Cl (pH 7.5), 50 mM EDTA (pH 8.0), 0.5% SDS) and tungsten bead using grinding machine TissueLyser (Retsch®, Germany). Each tube was mixed with gentle agitation, and then incubated for 60 min at 65°C. One-third volume of ice-cold 5 M potassium acetate was added to each tube, and mixed thoroughly with gentle agitation. The samples were centrifuged for 15 min at 5,800 g using '4-15C' (Sigma Co, USA). DNA in supernatant solution was precipitated by the addition of an equal volume of ice-cold isopropanol. The precipitated DNA was rinsed twice with 70%

ethanol. Dried DNA was dissolved in sterile water containing RNase H (0.2 mg/㎖) and then used for PCR analysis as template DNA.

Screening of previously developed SSR and SCAR markers

To screen SSR markers, PCR reactions were performed in a final volume of 20 ㎕ with 1 U Prime Taq DNA polymerase (Genet Bio Co, Korea) using 30 ng of DNA as template. The cycling conditions were as follows: an initial cycle at 94°C for 3 min, followed by 35 cycles at 94°C, 30 s, 52-60°C, 30 s and 72°C, 1 min, and a final cycle at 72°C for 5 min. All amplification reactions were performed in a Mastercycler (Eppendorf Co, Germany). Amplification products were visualized with UV illuminator after electrophoresis in 3% high resolution agarose gel (Qbiogene Co, USA) with 1X TBE buffer containing ethidium bromide. Optimum annealing temperature for each primer pair was calculated by the program 'Oligonucleotide Properties Calculator' (www.basic.northwestern.edu) and also tested for reproducible amplification at several temperature conditions. First screening to detect polymorphism in watermelon was conducted between inbred lines 'BN4001' and 'BN4002'. If there was no polymorphism between the inbred lines, we performed a second screening to detect polymorphism within a set of commercial varieties containing Speed, Star, SamBG, and MiRG listed in Table 1. PCR products were digested with restriction enzyme *Nla* III to detect polymorphism among the four varieties.

Nine SCAR markers were also used to detect polymorphisms in watermelon. These markers were developed as selection markers for powdery mildew resistance in the melon breeding program (unpublished data). PCR conditions and procedures to detect polymorphism were same as described in SSR analysis.

Development of SCAR markers derived from AFLP analysis

AFLP techniques developed by Vos et al. (1995) were slightly modified in this experiment. Total DNA (0.5 ㎍) of inbred lines 'BN4001' and 'BN4002' was digested for 2 h at 37°C with 5 U *Eco*RI and 5 U *Xba*I (Roche Co, Germany). After inactivation of the restriction enzyme at 70°C for 15 min, digested DNA was ligated with 10 pmole of *Eco*RI and *Xba*I adapters in 50 ㎕. After ligation, the reaction mixture was diluted to 500 ㎕ with distilled water and 5 ㎕ was used for preselective amplification with *Eco*RI+C and *Xba*I+A primer combination. Preselectively amplified products were diluted to 1:50 with distilled water and 5 ㎕ was used for selective amplification with *Eco*RI/*Xba*I primer combinations having +4 selective nucleotides. PCR reactions were performed in a final volume of 20 ㎕ with 1 U of the Ex Taq DNA polymerase (Takara Co, Japan). PCR conditions for AFLP analysis were same as previously described (Vos et al. 1995). After selective PCR, amplified fragments were fractionated in 2% agarose gel to detect polymorphism. Polymorphic fragments detected between the inbred lines were recovered from agarose gel and cloned into pGEM®-T Easy cloning vector (Promega Co, USA) for sequence analysis. Based on these sequence data, we synthesized sequence specific primer pairs to amplify the internal region of polymorphic fragments. Using these primer pairs, we analyzed polymorphisms between the inbred lines. PCR conditions for SCAR markers were same as described above in SSR marker screening. If there was no polymorphism between the inbred lines, we performed a second screening with the four varieties used in the SSR marker screening. PCR products were also digested with several restriction enzymes to detect polymorphisms among the varieties.

Table 2. Sequence-based markers selected in this study and their primer sequences, annealing temperature, restriction enzymes used to detect polymorphism in watermelon, and calculated PIC value.

Marker Name	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	Tm	Marker Type[a]	PIC Value
CMSSR 30	TCCTTCTTTTCCTCGCTCTG	TGCCCTAAAATCCTCACACT	52℃	SSR-M	0.45
MCPI-03	GCATAAACCACCTGTGAGTGG	ATGGCTTTGCGTTTCATTTC	55℃	SSR-J	0.26
MCPI-04	AGCAAATGCATGGGGAAAAC	TGTTGAATGGAGGCTTTGAG	55℃	SSR-J	0.49
MCPI-07	GGTTATGGCCATCTCTCTGC	GAGAGTGGGCGTAAGGTGAG	55℃	SSR-J	0.50
MCPI-12	GGAGTAGTGGTGGAGACATGG	TCCTTTCTCTTTCGCAAACTTC	55℃	SSR-J	0.43
MCPI-14	TCAAATCCAACCAAATATTGC	GAGAAGGAAACATCACCAACG	55℃	SSR-J	0.47
MCPI-28	AATGTTAAGCAGTAAGCACATGG	ACACCGGAGAAGGTGAATTG	55℃	SSR-J	0.48
MCPI-30	GCTTTGAAGTTTGTTTAATTTTAGTCC	CGCCTCACGCTCTCTCTAAC	55℃	SSR-J	0.45
MCPI-31	TAACCGTCACCAACCCATTC	TCCAAAATTGGTCGGATTTG	55℃	SSR-J	0.26
MCPI-32	AAGGCTGCAGAGACCATGAC	AATGATGAAGAACGGGCAAG	55℃	SSR-J	0.50
MCPI-37	AATCTTCCCCATGCCAAAAC	GACTTCCAAACCCTCCCTTC	55℃	SSR-J	0.47
AB006530.1	TGAGAGGAAAGGAACCATAA	GTCTCTTGCAAAGCTCAAACT	55℃	SSR-G	0.47
EST00675	CTTCCTTTCTCTTCATTCCC	TGAGGGAAAACGAGTTTAGA	55℃	SSR-G	0.26
Cgb4765	TTCTCTTCATTCCCCCAAAATC	ACGGGTGAGGGAAAACGAG	55℃	SSR-L	0.26
ASUW2	GCTTCGTTGTTGCTGCCGTTG	GCATAAAATCACACTCAAAC	55℃	SSR-L	0.47
WMA 1	GGAAAACGCAAAATCAGAGAG	GGACGACATTTTTACCGGTC	55℃	SCAR	0.47
WMA 2	AAAACACCCCCGAAAATAAAACTT	AGGAAGGTGAGAGAGACCTTGCTA	52℃	Tru9 I	0.36
WMA 3	TTCACATAGTGAGGGGAGAGTGTA	TAGAGGAACTGCCCAACGAAGTAG	60℃	Hae III	0.35
WMA 4	AATTCCTTACATATCGGGCATTAT	TCTAGAGGAACTTCATCACCTTCA	52℃	Tru9 I	0.46
WMA 5	ACGAGCAAGTATATCTACATCGTG	TCTAGAGGCTAAGAAGGAGCTGAG	52℃	Tru9 I	0.50
WMA 6	ATTCACATAGTGAGGGGAGAGTG	AAGCGACAACAATACATTCAACC	52℃	Hae III	0.35
WMA 7	GGGCAATTTTTGGCTTTATTATC	ACTCACCACACCAATGAACCTC	52℃	EcoR I	0.31
WM 2	CTAGAAATAAGGTTACCGCCT	AGAAAACTATGGTAGCTGCAG	55℃	SCAR	0.50
WM 11	TCTCATGCAACCTAATTGATTAC	CCTTTCAAAACTTATTCCTTAGGT	55℃	Tru9 I	0.44
WM 13	CTAGAAACAGGGGTAGAGGT	AATTCGTCTGTTGTTGTTGGAG	55℃	Nde I	0.44
WM 15	CTAGAAAGTACTGATCTCGGAG	CAACACCCTTGAACTCTTTCC	55℃	SCAR	0.44
WM 16	TTGGTTCAATATCTACCCGGT	GAATTCTACATGTATGAGAAAG	55℃	EcoR I	0.39
WM 26	CTAGAATTGCAACCATTTTCC	CAAGTGTTGTGTGAACCGAG	55℃	Hinf I	0.48
WM 27	GGATATTTGCTCTTGTGTTCGA	TCGCATGGAAGCAGGAACCA	55℃	SCAR	0.26
WM 33	CTAGAATGGCAAGTCTCAGAC	ATTCTTCCGAGAATCAACAACC	55℃	Taq I	0.45
WM 38	GAACACAAGTACGGAACACC	GAATTCGATGATCGAGTACCTG	55℃	EcoR I	0.70
WM 46	CTAGAAGACGCGGGAATG	ATTCTACCCCAAACCAACATA	55℃	Hae III	0.39
WM 59	AAAACTTTGCAAATTCGAACC	AATTCGGTTGGTTTGTTT	55℃	SCAR	0.14
WM 61	AGATAACTTTCACAAAATCCAAG	AATTCGGTGATGACTTGATAAT	55℃	SCAR	0.46
WM 76	CTAGATAGACATCTAAGAGAG	AATTCGAGCCTCTTTATTTGC	55℃	SCAR	0.39
WM 78	CTAGATTAGAGGTTTGGGTTCA	ATTCACAGTTTCGATATGGCATT	55℃	SCAR	0.39
WM 81	CTAGATTTCTATTACATTTTACACTT	TCAACAGCATTTTGTGACATT	55℃	Hae III	0.43
WM 86	CTAGATTTGGTTTTTCAACTTCC	AATTCAGCCGAGTGGTTATAC	55℃	SCAR	0.26
WM 88	AGATTTGAAATACTTCCAAAGAA	ATTCTACTGGTTCCTTGCTTG	55℃	SCAR	0.72
WM 103	TAGATGCTCTATGAAGGTTACGA	AATTCGACCCCATGAGATAC	55℃	SCAR	0.36
WM 106	AGATCAAGCTTAAAATAGCAAGG	AATTCAAGGTCTGCCACAG	55℃	SCAR	0.50
WM 109	ATACACTTCCATATGTATCATATTTGC	AATTCACAAACATTAAGGAGAAG	55℃	SCAR	0.26
CPM 6	GACAGATGAAGGAAGCCAGAAT	TGGCTTCTGTCTGTACTCTCAA	53℃	SCAR	0.26

[a] SSR-M; SSR markers developed in melon by Lee et al. (2004), SSR-J; SSR markers developed in watermelon by Joobeur et al. (2006), SSR-G; SSR markers developed in watermelon by Guerra-Sanz (2002), SSR-L; SSR markers developed in watermelon by Levi et al. (2006). In case of CAPS marker, restriction enzymes are indicated.

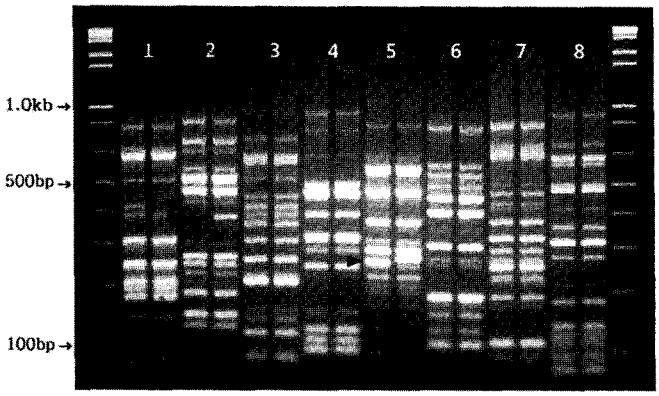

Fig. 1. Identification of polymorphic bands between inbred lines 'BN4001' and 'BN4002' by using AFLP procedures on 2% agarose gel. Arrows indicate polymorphisms between the lines. Numbers above each set of PCR products represent several primer combinations used for AFLP analysis. Selective nucleotide for *Xba*I site is ATTT in all the reaction. Selective nucleotide for *Eco*RI sites are CAAC (1), CATC (2), CATG (3), CACA (4), CAGC (5), CTAG (6), CTTC (7) and CRRG (8).

Estimation of the informativeness of each DNA marker

Forty-three sequence-based DNA markers showing polymorphisms in watermelon were analyzed to estimate the informativeness of each marker in 26 commercial watermelon varieties. The allelic composition of each marker in each genotype was determined. To evaluate the informativeness of each marker, polymorphic information content (PIC) was calculated according to the equation:

$$PIC = 1 - \sum_{i=1}^{n} pi^2$$

where p*i* is the frequency of *i*th allele, and *n* is the total number of different alleles at the marker locus (Guo et al. 1999; Lee et al. 2004).

Analysis of phylogenetic relationships in commercial watermelon varieties

To analyze phylogenetic relationships in 26 commercial watermelon varieties, every polymorphic fragment was treated as a unit character. The presence or absence of a specific fragment was denoted as '1' or '0', respectively, and then transferred into a data matrix over all genotypes and fragments. A pairwise similarity between genotypes was estimated using the Nei-Li similarity index (Nei and Li 1979) according to the formula: Genetic similarity = 2 N_{ab} / (N_a +N_b), where N_{ab} is the number of fragments shared by two genotypes (a, b), and Na and Nb are the total number of fragments analyzed in each genotype. A dendrogram was constructed based on the similarity matrix data by applying UPGMA (unweighted pair-group method with arithmetic average) cluster analysis using computer program Numerical Taxonomic and Multi-Variant Analysis System (NTSYS -version 2) (Rohlf 1993).

Results and Discussion

Screening of SSR and SCAR markers in Korean watermelon varieties

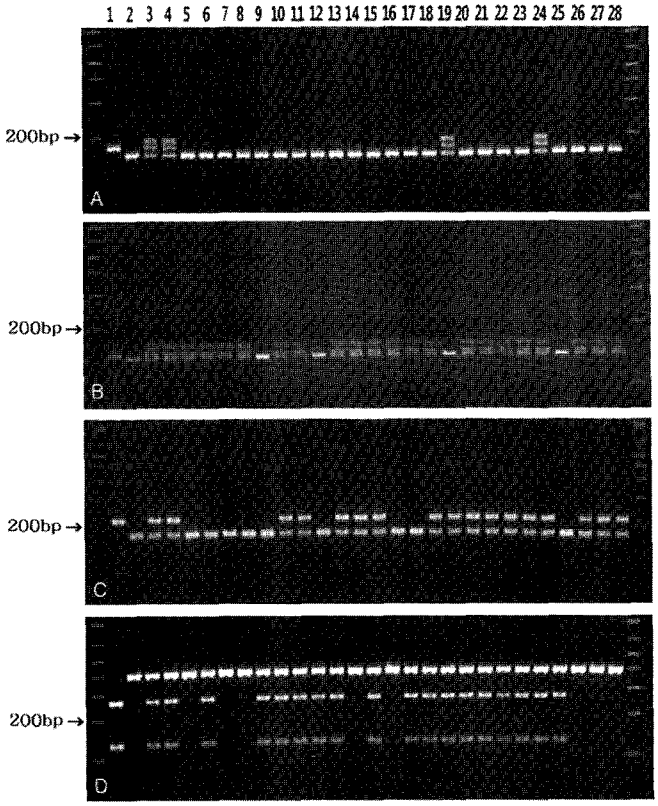

Fig. 2. PCR amplification and restriction pattern on 3% high resolution agarose gel of several sequence-based markers in two inbred lines (lane 1, 2) and 26 commercial varieties (lane 3-28) listed in Table 1. Amplification pattern of SSR marker 'EST00675' derived from watermelon (A), amplification pattern of SCAR marker 'CPM6' derived from melon (B), amplification pattern of SCAR marker 'WM76' derived from AFLPs in watermelon (C), and restriction pattern of CAPS marker 'WM81' with *Hae*III derived from AFLPs in watermelon (D).

Two sets of SSR markers were tested in Korean watermelon varieties. A first set consisted of 94 SSR markers previously developed in melon (Gonzalo et al. 2005; Lee et al. 2004). Among these markers, only 23 markers produced clear and reproducible PCR products in two watermelon inbred lines, and only one marker (CMSSR30) showed polymorphism between the lines (Table 2). Since the levels of polymorphism were low between two inbred lines, another four varieties were analyzed by 23 SSR markers. The PCR products were digested with *Nla* III to find polymorphism among these varieties, but any polymorphism was not detected from the 23 SSR markers. The other 71 markers were not able to produce detectable PCR product on agarose gel or to show reproducible band patterns. Low levels of cross homology in microsatellite sequences were reported between different genera in Cucurvitaceae family. Katzir et al. (1996) tried to assess the possibility of utilizing SSR franking primers from single species to other genera or species of Cucurvitaceae. They used five SSR markers from melon and two SSR markers from cucumber to analyze three watermelon genotypes. Two of the five melon SSR markers and one of the two cucumber SSR markers didn't show any clear signal in watermelon. Low level of cross homology in SSR markers between melon and watermelon may have occurred due to the nature SSR primers requiring a complete matching of DNA sequences.

When we tested the other set of 73 watermelon SSRs (Guerra-Sanz 2002; Jarret et al. 1997; Joobeur et al. 2006; Levi et al. 2006), 68 markers produced clear and reproducible PCR products. Ten (MCPI-03, 04, 07, 12, 28, 30, 31, 32, EST00675, and Cgb4765) of the 68 SSR markers showed polymorphisms between the inbred lines (Table 2), and additional four markers (MCPI-14, 37, AB006530.1, and ASUW2) showed polymorphisms among four watermelon varieties. Although those 73 SSR markers of watermelon were developed and tested for polymorphisms in several morphologically variable genotypes (Guerra-Sanz 2002; Jarret et al. 1997; Joobeur et al. 2006; Levi et al. 2006), low levels of polymorphism (19%) were found in Korean commercial varieties. This result may be due to the different genetic diversity of germplasms used in each experiment. Estimation of the levels of genetic diversity in germplasms is the most important step in breeding programs. Lee et al. (1996) used RAPD markers to detect genetic diversity in 39 watermelon cultivars representing a wide range of origin and important agronomic characteristics. They detected 35 reliable and polymorphic fragments among 162 RAPD fragments (21% polymorphism). Although our markers revealed a slightly low level of polymorphism (19%) in commercial varieties, these sequence-based PCR markers could be used efficiently for the evaluation of genetic diversity, F_1 seed purity test, linkage map construction, and cultivar discrimination in watermelon.

Out of nine SCAR markers linked to powdery mildew resistance in melon, only one marker 'CPM6' showed polymorphism between the two inbred lines. The other eight markers didn't produce clear PCR product in watermelon. DNA sequence analysis revealed that sequences of these eight markers had not originated from the transcribed gene, so these SCAR markers might not produce a clear PCR product due to the low levels of cross homology in non-transcribing sequences between melon and watermelon.

Development of SCAR markers using AFLP analysis

We performed AFLP procedures to develop informative and reliable markers in watermelon. We used 48 *Eco*RI and 30 *Xba*I primer combinations to detect polymorphism between inbred lines 'BN4001' and 'BN4002'. PCR products were separated on 2% agarose gel as illustrated by an example with eight primer combinations (Fig. 1). The number of fragments per primer ranged from three to 17, with an average number of ten. Approximately among more than 14,000 fragments produced in AFLP analysis, 105 fragments showed polymorphisms between the inbred lines. Compared to previously reported data (Levi et al. 2004), the level of polymorphism in our study was low. They detected 118 polymorphic fragments out of 707 among 44 watermelon cultivars. This high level of polymorphism (17%) might be derived mainly from the large number of genotypes (44 cultivars) and the wide range of genetic diversity among the cultivars used for AFLP analysis. All the polymorphic fragments identified in our experiment were cloned and DNA sequence analyzed to convert AFLP markers into specific sequence-based PCR markers. Based on these sequence data, we synthesized specific primer pairs to amplify the internal region of polymorphic fragments. Using these primer pairs, we identified 13 primer pairs (markers designated as SCAR in Table 2, except 'CPM6') showing polymorphisms by size difference of amplified fragments among the two inbred lines and four varieties. To identify more polymorphic markers, we digested the PCR product with several restriction enzymes and identified additional 14 markers (markers indicated

Table 3. Genetic similarity matrix among 26 Korean watermelon varieties using 43 sequence-based markers

Varieties	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
3. Speed	1.00																									
4. SpeedP	0.89	1.00																								
5. Star	0.53	0.48	1.00																							
6. GulBD	0.56	0.54	0.62	1.00																						
7. WooRi	0.57	0.56	0.60	0.85	1.00																					
8. SamBG	0.54	0.46	0.89	0.64	0.62	1.00																				
9. JoSSB	0.57	0.53	0.73	0.67	0.59	0.71	1.00																			
10. SamDG	0.68	0.67	0.59	0.81	0.79	0.54	0.64	1.00																		
11. SeolBG	0.73	0.62	0.54	0.76	0.78	0.56	0.62	0.85	1.00																	
12. JoEG	0.46	0.48	0.65	0.75	0.67	0.60	0.89	0.71	0.67	1.00																
13. KWCH	0.71	0.67	0.65	0.62	0.60	0.67	0.67	0.78	0.70	0.56	1.00															
14. SemG	0.68	0.60	0.75	0.65	0.73	0.82	0.73	0.71	0.70	0.62	0.75	1.00														
15. InDG	0.75	0.73	0.62	0.68	0.67	0.57	0.64	0.84	0.82	0.59	0.78	0.75	1.00													
16. MNDR	0.62	0.57	0.75	0.71	0.67	0.73	0.64	0.65	0.60	0.53	0.67	0.65	0.68	1.00												
17. MyGG	0.54	0.62	0.48	0.76	0.75	0.50	0.56	0.70	0.68	0.67	0.54	0.54	0.60	0.57	1.00											
18. MiRG	0.57	0.53	0.54	0.64	0.62	0.59	0.65	0.67	0.75	0.70	0.60	0.64	0.60	0.57	0.71	1.00										
19. JoCG	0.70	0.62	0.48	0.70	0.59	0.56	0.59	0.67	0.68	0.64	0.57	0.57	0.54	0.48	0.53	0.59	1.00									
20. DrmG	0.75	0.70	0.59	0.68	0.67	0.60	0.70	0.81	0.85	0.62	0.81	0.75	0.93	0.68	0.60	0.64	0.57	1.00								
21. SaeRG	0.75	0.67	0.56	0.78	0.79	0.57	0.67	0.93	0.92	0.71	0.78	0.75	0.78	0.62	0.70	0.82	0.70	0.81	1.00							
22. SaeCN	0.75	0.76	0.65	0.71	0.70	0.60	0.70	0.90	0.76	0.65	0.84	0.78	0.93	0.71	0.64	0.67	0.57	0.90	0.84	1.00						
23. ChaSD	0.75	0.76	0.65	0.71	0.70	0.60	0.67	0.87	0.79	0.62	0.81	0.78	0.96	0.71	0.64	0.64	0.57	0.90	0.81	0.96	1.00					
24. ChoGS	0.71	0.73	0.40	0.46	0.48	0.32	0.39	0.56	0.57	0.31	0.56	0.46	0.65	0.50	0.48	0.35	0.51	0.62	0.53	0.62	0.65	1.00				
25. HaeDG	0.68	0.64	0.65	0.62	0.51	0.67	0.82	0.65	0.64	0.71	0.75	0.68	0.65	0.65	0.60	0.67	0.60	0.68	0.71	0.71	0.68	0.43	1.00			
26. DongBG	0.62	0.54	0.78	0.65	0.60	0.89	0.76	0.65	0.60	0.65	0.78	0.87	0.65	0.68	0.51	0.60	0.60	0.68	0.68	0.71	0.68	0.37	0.78	1.00		
27. GoNKW	0.65	0.57	0.78	0.59	0.60	0.79	0.73	0.68	0.64	0.62	0.75	0.78	0.68	0.75	0.48	0.67	0.57	0.71	0.71	0.75	0.71	0.43	0.71	0.81	1.00	
28. BaekMG	0.51	0.46	0.64	0.67	0.62	0.71	0.62	0.67	0.65	0.67	0.57	0.64	0.51	0.60	0.65	0.81	0.62	0.54	0.73	0.57	0.54	0.29	0.73	0.71	0.73	1.00

with restriction enzymes in Table 2) detecting polymorphism at restriction site. Consequently, we found that 26% of the AFLP clones (27 out of 105) contained at least one restriction site polymorphism or indel (insertion/deletion) among the two inbred lines and four varieties. The frequency in our experiment is low compared to the frequency of EST clones (67%) detecting SNPs (single nucleotide polymorphism) or indels between parental lines for linkage map construction in melons (Morales et al. 2004). Because these parental lines belonged to the most genetically distant groups of cultivated melon, it is not abnormal to have such a high level of polymorphisms between those lines at the expressed sequences. Although these six genotypes (two inbred lines and four varieties) showed significant differences in several morphological characteristics as shown in Table 1, they didn't represent a wide range of genetic diversity in the 105 AFLP clones. This low level of genetic diversity might have been derived partly from the limited number of genotypes used in this study and narrow range of germplasm in Korean watermelon. Another reason for low polymorphisms in these sequence-based markers may be due to the nature of AFLP. Polymorphisms in AFLP analysis are mostly generated from the variation at restriction site or selective nucleotide sequences. using DNA walking method will greatly increase the possibility to detect polymorphism. A total of 27 SCAR or CAPS markers derived from AFLP procedures are listed in Table 2.

Estimation of PIC values of each DNA marker

A total of forty-three sequence-based DNA markers were used to detect polymorphisms among commercial varieties in Korean watermelons. All the markers produced clear and reproducible polymorphic fragments in the 26 varieties. An average of 2.1 alleles per marker was detected. Figure 2 represents PCR amplification patterns of SSR marker 'EST00675' derived from watermelon SSRs (A), SCAR marker 'CPM6' derived from melon (B), SCAR marker 'WM76' derived from AFLPs in watermelon (C), and restriction pattern of CAPS marker 'WM81' derived from AFLPs in watermelon (D) in two inbred lines and 26 commercial varieties. The allelic composition of each sequence-based marker in each genotype was determined to calculate a PIC value of each marker. The average PIC value of all the markers was 0.41 with a maximum of 0.72 for 'WM88' and a minimum of 0.14 for 'WM59' (Table 2). SSR markers showed same level of informativeness as SCAR or CAPS markers on average. Although there was no direct comparison about PIC value between the marker types in watermelon, SSR markers were expected to show a higher level of informativeness than other markers, such as RFLPs, AFLPs, and RAPDs (Prince et al. 1995). Relatively low levels of PIC value among the Korean watermelon varieties might, at least partly, be explained by the fact that Korean watermelons, which originated completely similar part of the world, would be expected to be less heterogeneous than the other wild types or land races. Although the estimate of PIC value was a little low, those sequence-based DNA markers would be used as informative markers in watermelon (Fig. 2). A group of these markers could be used efficiently for DNA fingerprinting purposes, such as identification of specific genotype, analysis of seed contamination in F_1 seed production, and cultivar protection.

Phylogenetic relationships in commercial watermelon varieties

Phylogenetic relationships in 26 commercial varieties of watermelon were established using forty-three sequence-based DNA markers. We generated a dendrogram representing genetic similarity based on the portion of shared fragments between varieties. The dendrogram clustered the 26 varieties into two major groups (Figure 3) at a level of 58% genetic similarity. The first group included 'Speed', 'SpeedP', 'ChoGS', and 'JoCG'. Although these varieties have the same characteristics of fruit maturity, they didn't show significant similarity in other fruit characteristics as shown in Table 1. The second group included all the other varieties and consisted of three subgroups differentiated from each other at a level of 66% genetic similarity, but the subgroups couldn't be significantly differentiated from each other by their fruit characteristics. Commercial varieties clustered in the second subgroup revealed relatively closer genetic

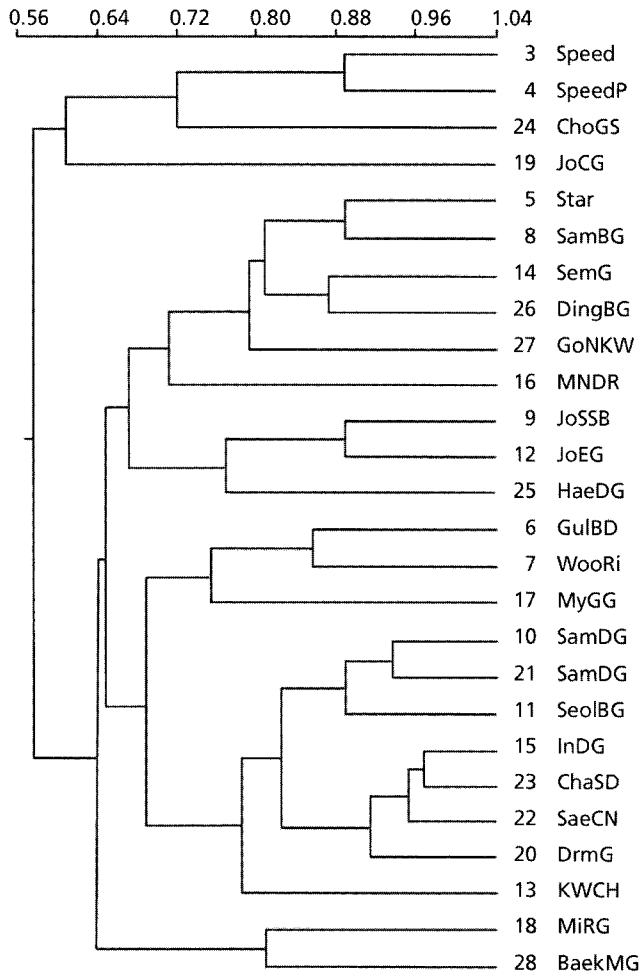

Fig. 3. Dendrogram showing genetic relationships in 26 Korean commercial watermelon varieties. The dendrogram was constructed by UPGMA cluster analysis of the genetic similarity matrix based on the sequence-based polymorphic markers. Numbers on the right are same as the serial number of varieties as shown in Table 1.

relationships than those in the first group and other subgroups. Although differentiation of commercial varieties into several groups or subgroups was detected by DNA polymorphism analysis in watermelon, there would be some differences in genetic diversity among those varieties if analyzed with different types of molecular markers. This would be due to the fact that the DNA markers used in this study were mainly identified by polymorphisms between specific inbred lines 'BN4001' and 'BN4002'. The mean genetic similarity within the complete set of 26 varieties was equal to 66%. Minimum and maximum genetic similarities were found for BaekMG/ChoGS (29%), and ChaSD/InDG and ChaSD/SaeCN (96% each), respectively (Table 3). Evaluation of genetic diversity in commercial watermelon varieties representing a small subset of the genetic pool in Korea could certainly explain the relatively higher level of genetic similarity compared to previously reported data (Lee et al. 1996).

The present study revealed the useful application of 43 sequence-based DNA markers for molecular genetic studies in Korean watermelon. It is also anticipated that these markers could be applied for identifying specific cultivars, assessing genetic diversity among other cultivars and genotypes, and characterizing several morphologically important traits in watermelon.

Acknowledgement

This work was supported by a grant (Code#20050401034824) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.

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