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Genetic Diversity in Watermelon Cultivars and Related Species Based on AFLPs and EST-SSRs

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Abstract

The genetic relationships among 27 watermelon cultigens (*Citrullus lanatus* var. *lanatus*) from different countries of origin and with different horticultural characteristics and 5 related wild-type species and subspecies (*Citrullus colocynthis*, *Citrullus lanatus* var. *citroides*, and *Citrullus rehmii*) were assessed using amplified fragment length polymorphism (AFLP) and expressed sequence tag-simple sequence repeat (EST-SSR) markers. AFLPs were evaluated using 16 *Eco*RI-*Mse*I primer combinations, and 862 alleles (an average of 53.8 alleles per primer combination) were scored. Polymorphisms were found in 806 (93.4%) alleles, whereas 56 monomorphic alleles were identified. Using 16 EST-SSR primer sets, 103 alleles were scored, and all 103 alleles were polymorphic among the 32 genotypes with an average of 6.4 polymorphic alleles per primer pair. However, the high polymorphic ratio among the AFLPs and EST-SSRs was largely due to the wild-type species, while little diversity was observed among the adapted cultivars. Genetic similarity coefficients were calculated based on the 965 polymorphic AFLP and EST-SSR alleles, and a phenetic tree was constructed. The dendrogram contained 2 major clusters. Cluster I included all adapted watermelon cultivars, and the similarity among these cultigens was very high (0.94-0.98), demonstrating cross relationships and a narrow genetic background. Cluster II was composed of 4 wild-type species. The genetic distance between the nodes that comprise these 2 clusters was approximately 0.63, indicating a high level of genetic dissimilarity between the adapted watermelon species and the other related species. The low level of marker polymorphism among the adapted cultivars implies that a severe bottleneck in genetic diversity existed in watermelon during the initial breeding practices.

Key words: *Cucurbitaceae*, DNA fingerprinting, genetic relationship, molecular marker

Introduction

Cucurbitaceae (Cucurbit family) crops are an essential source of food, and include cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), squash (*Cucurbita pepo* L.), and watermelon (*Citrullus lanatus* [Thunb.] Matsum. Et Nakai). Worldwide production of watermelons has increased steadily during the last century, and currently, 6.8% of the world area for vegetable production is devoted to this crop. The production value for the top 20 countries cultivating watermelons was $8.6 billion in 2008, and the amount of fruit produced was 89 million tons. South Korea is the eighth largest watermelon producing country after China, India, and the United States (FAO, 2008), and in 2009, the farm production value for watermelon in South Korea was $930 billion, with a cultivation area of 20,750 ha.

Watermelon belongs to the subfamily *Cucurbitoideae* and is known to originate from Africa (Jeffrey, 1975; 1990). Its genus, *Citrullus*, contains 4 diploid species: an annual, wild, or cultivated species, *Citrullus lanatus* (Thunb.) Matsum and Nakai; 2 perennial, wild species, *C. colocynthis* (L.) Schrad and *C. ecirrhosus* Cogn.; and an annual, wild species *C. rehmii* De Winter. *C. lanatus* is divided into subspecies that include the widely cultivated forms of red sweet watermelon, *C. lanatus* var. *lanatus*, and a preserving melon type of the ancient cultigens, *C. lanatus* var. *citroides*.

A narrow genetic base was detected among cultivated watermelon accessions in *C. lanatus* var. *lanatus*. (Levi *et al.*, 2001a; 2001b). This reduction in genetic diversity in watermelon can be attributed to the process of plant domestication and breeding. The target traits for watermelon breeding have been yield, fruit size and shape, flesh color and texture, sweetness, earliness, dwarf habit, and disease resistance. In South Korea, watermelon-breeding programs in the private seed industry focus on the development and production of F_1 hybrid cultivars. The demands for uniformity in F_1 cultivars and intensive selection based on a few economical traits have resulted in a genetically homogeneous germplasm pool.

Today, diverse characteristics are required for the global seed market. These include tolerance to biotic and abiotic stresses such as new disease pathogens, dry, high temperature, chilling injury, and consumers' demand for health enhancing functions. Therefore, understanding the population structure of adapted and wild-type germplasm and the creation of new traits through diverse cross combinations is a requirement for modern watermelon breeding.

Molecular markers are useful for plant breeding in marker-assisted selection (MAS), genetic diversity assess-

ment, and cultivar identification. The use of molecular markers for germplasm characterization and the construction of genetic maps for tagging important traits have been reported in watermelon (Che *et al.*, 2003; Kwon *et al.*, 2010; Lee *et al.*, 1996; Levi *et al.*, 2001a; 2001b; 2006). Among the different marker types, amplified fragment length polymorphisms (AFLPs) generally reveal a higher number of polymorphisms and do not require DNA sequence information (Vos *et al.*, 1995). These characteristics make AFLPs a good marker choice for genetic diversity studies (Park *et al.*, 2010a) and rapid recovery of chromosomes from the recurrent parent in backcross breeding. In addition, simple sequence repeats (SSRs) are powerful PCR-based DNA markers due to their reproducibility, multi-allelic nature, and co-dominant inheritance. They are highly abundant and distributed throughout the genome with wide genomic coverage. In particular, SSR markers based on transcript sequences, such as expressed sequence Tag-SSRs, or EST-SSRs, have advantages over genomic SSR markers (gSSRs) in that they are locus-specific to the expressed genes and can be used as functional markers. (Park *et al.*, 2010b; Verma and Arya, 2008).

In the present study, the aim was to assess EST-SSRs and AFLPs in a wide range of watermelon germplasm collections. It has been evaluated the DNA fingerprints of 32 watermelon genotypes, including domestic and introduced adapted breeding lines and wild-type germplasm, and their genetic relationships were assessed at the molecular level.

Materials and methods

Plant material

For this genetic relationship study, 27 watermelon cultigens (*Citrullus lanatus* var. *lanatus*), including 19 inbred lines from different countries of origin and 8 domestic lines were used. In addition, 5 different related wild-type species and subspecies (*Citrullus colocynthis*, *Citrullus lanatus* var. *citroides*, and *Citrullus rehmii*) were included as an out-group (Tab. 1). Seed samples for watermelon cultivars and wild-type species (PIs) were obtained from NH seeds, Inc. in South Korea and the US germplasm collection center. Seeds were germinated in a greenhouse, and young cotyledons and true leaves were collected for DNA extraction.

DNA isolation

Leaf tissue was ground in a 1.5 mL micro-centrifuge tube using steel beads and 600 µL of DNA extraction buffer with a TissueLyser (QIAGEN, Venlo, Netherlands). The tube was incubated at 65°C for approximately 45 min, 200 µL of 7.5 M ammonium acetate was added, and then the tube was placed on ice for 15-20 min. The lysate was centrifuged at 14,240 × g for 10 min, and the supernatant was transferred to a new 1.5 mL tube containing 5 µL of glycogen solution (5 mg/mL) and 600 µL of isopropanol. After centrifugation at 14,240 × g for 10 min, the liquid was carefully decanted, and the DNA pellet was washed with 300 µL of 70% Et-OH. The washed pellet was dried and resuspended in 200 µL of 0.1 M Tris. DNA quality and concentration were checked by using a Nanodrop 1000 Spectrophotometer (Thermo Scientific, Pittsburgh, PA, U.S.A.).

AFLP and EST-SSR evaluation

AFLP analysis was conducted following the protocol of Vos *et al.* (1995) with minor modifications. The methods and AFLP primer combinations used in this study have been described in detail by Park *et al.* (2010a).

For EST-SSR analysis, 16 primer pairs were selected from the International Cucurbit Genomics Initiative database (http://www.icugi.org/). These EST-SSR primer pairs showed robust PCR amplification and were polymorphic among the 8 domestic breeding lines used in a previous study by Hwang *et al.* (2011). The primers were synthesized by Bioneer (Deajeon, Korea). The forward primers were labeled with 2 different fluorescence dyes. All PCR amplifications were conducted in a total volume of 20 µL containing 20 ng of genomic DNA, each forward and reverse primer at 0.3 µM, with 1× PCR buffer, 0.2 mM dNTPs, and 0.6 U of Taq polymerase (Solgent, Korea). The touchdown cycling conditions were as follows: 1 cycle of 5 min at 95°C, 10 cycles of 15 s at 95°C, 30 s at 60°C (in decreasing steps of 0.5°C/cycle for cycles 2-10), 30 s at 72°C, and 1 min at 72°C, followed by 35 cycles of 15 s at 95°C, 30 s at 55°C, and 1 min at 72°C. Allele discrimination and detection were conducted by capillary electrophoresis using an ABI3730 DNA Analyzer (Macrogen, Korea).

Polymorphism evaluation and data analysis

The markers were scored based on the presence or absence of the corresponding peaks among the cultigens. Scores of "1" and "0" indicated the presence and absence of the peaks, respectively. The polymorphic information content (PIC) values of EST-SSR markers were determined using the following equation (Botstein *et al.*, 1980):

$$PIC_i = 1 - \sum_{j=1}^{n} ij^2$$

where PIC_i is the PIC of an EST-SSR *I, Pij* is the frequency of the *j*th pattern of EST-SSR *I*, and the summation extends over *n* patterns. Pairwise similarity coefficients among the cultivars were calculated according to the method of Nei-Li (Nei and Li, 1979). The Nei-Li similarity coefficients were calculated as 2a/(n + d), where a is the number of loci in which the peak is absent, and n is the total number of loci. A cluster analysis was performed based on marker data using NTSYS-PC version 2.02 software (Rohlf, 2002) with the unweighted pair group method on arithmetic averages (UPGMA) method.

Tab. 1. List of watermelon cultivar accessions and related species used in the DNA sample panel

No.	Cultivar	Character	Species	Origin	Fruit characteristics					
					Size (kg)	Shape	Color	Rind color	Seed size	Seed color
1	'52192'	Inbred line	*C. lanatus* var. *lanatus*	Korea	7	Round	Red	Black	NS	Black
2	'45NC'	Inbred line	*C. lanatus* var. *lanatus*	Korea	6	Round	Red	'Jubilee' stripe	NS	Black
3	'A216'	Inbred line	*C. lanatus* var. *lanatus*	France	3	Round	Red	'Jubilee' stripe	SS	Dotted brown
4	'Aldf'	Inbred line	*C. lanatus* var. *lanatus*	Thailand	7	Round	Yellow	Green	NS	Dotted brown
5	'BKA'	Inbred line	*C. lanatus* var. *lanatus*	Japan	2	Round	Red	'Jubilee' stripe	NS	Black
6	'BKB'	Inbred line	*C. lanatus* var. *lanatus*	Japan	2	Round	Red	'Jubilee' stripe	NS	Brown
7	'DAH'	Inbred line	*C. lanatus* var. *lanatus*	Japan	2	Round	Red	Green	SS	Brown
8	'DAP'	Inbred line	*C. lanatus* var. *lanatus*	Korea	7	Round	Red	'Jubilee' stripe	NS	Dotted brown
9	'DG4X'	Inbred line	*C. lanatus* var. *lanatus*	USA	8	Round	Red	Dark green	NS	Dotted brown
10	'Drd'	Inbred line	*C. lanatus* var. *lanatus*	Korea	8	Elongated	Red	'Crimson' stripe	TS	Black
11	'Drdfrm'	Inbred line	*C. lanatus* var. *lanatus*	Korea	7	Oblong	Deep red	Dark green	SS	Black
12	'Fdf'	Inbred line	*C. lanatus* var. *lanatus*	Taiwan	6	Round	Red	Green	NS	Brown
13	'JXM'	Inbred line	*C. lanatus* var. *lanatus*	China	6	Round	Red	'Jubilee' stripe	NS	Black
14	'NO1352'	Inbred line	*C. lanatus* var. *lanatus*	Thailand	5	Round	Orange	'Jubilee' stripe	NS	Red
15	'Oto949'	Inbred line	*C. lanatus* var. *lanatus*	Japan	2	Round	Yellow	Green	NS	Black
16	'SBA'	Inbred line	*C. lanatus* var. *lanatus*	Korea	8	Round	Red	'Jubilee' stripe	NS	Brown
17	'SBB'	Inbred line	*C. lanatus* var. *lanatus*	Korea	7	Elongated	Red	'Jubilee' stripe	NS	Dotted brown
18	'Tpdf'	Inbred line	*C. lanatus* var. *lanatus*	Thailand	3	Elongated	Red	Green	SS	Black
19	'Ts34'	Inbred line	*C. lanatus* var. *lanatus*	Korea	7	Elongated	Red	'Jubilee' stripe	TS	Black
20	'Arka Manik'	OP variety	*C. lanatus* var. *lanatus*	India	6	Round	Red	'Crimson' stripe	NS	Dotted brown
21	'All Sweet'	OP variety	*C. lanatus* var. *lanatus*	USA	12	Elongated	Red	'Crimson' stripe	NS	Dotted brown
22	'Bushsugarbaby'	OP variety	*C. lanatus* var. *lanatus*	USA	6	Round	Red	Black	NS	Dotted brown
23	'Charleston Gray'	OP variety	*C. lanatus* var. *lanatus*	USA	10	Round	Red	Green	BS	Dotted brown
24	'Crimson Sweet'	OP variety	*C. lanatus* var. *lanatus*	USA	6	Round	Red	'Crimson' stripe	NS	Dotted brown
25	'Dixilee'	OP variety	*C. lanatus* var. *lanatus*	USA	6	Round	Deep red	'Jubilee' stripe	BS	Dotted brown
26	'Jubilee'	OP variety	*C. lanatus* var. *lanatus*	USA	7	Elongated	Red	'Jubilee' stripe	BS	Dotted brown
27	'Mickylee'	OP variety	*C. lanatus* var. *lanatus*	USA	4	Round	Red	Green	NS	Dotted brown
28	'PI 189225'	Wild type	*C. lanatus* var. *citroides*	Zaire	6	Round	White		GS	Red
29	'PI 254744'	Wild type	*C. lanatus* var. *citroides*	Senegal	4	Round	White		BS	Black
30	'PI 386024'	Wild type	*C. colocynthis*	Iran	0.1	Round			SS	Grey
31	'PI 494817'	Wild type	*C. lanatus* var. *citroides*	Zambia	6	Round	White		GS	Black
32	'PI 632755'	Wild type	*C. rehmii*	France	0.1	Round			MS	Green

Results and discussion

EST-SSR and AFLP evaluation

AFLPs of 32 watermelon genotypes were evaluated with 16 *Eco*RI-*Mse*I primer combinations (Fig. 1, Tab. 2). The results showed a high level of polymorphism among these germplasm. It has been scored 862 alleles with an average of 53.8 alleles per primer combination. Polymorphisms were detected in 806 (93.4%) of the alleles, whereas only 56 monomorphic alleles were identified. For each primer combination, an average of 50.3 polymorphic alleles were detected. The highest number of polymorphic alleles (89) was detected by the *Eco*RI AAC-*Mse*I CAA primer combination, while the *Eco*RI ACT-*Mse*I CAG primer combination detected a relatively low number of alleles (44) and a low polymorphism rate (86.4). When the evaluation was aimed at 27 adapted cultigens by excluding the 5 wild types, 120 of the 862 alleles were polymorphic, with an average of 7.5 per primer combination, indicating that a major portion of the polymorphic alleles were attributable to the genetic unrelatedness between adapted cultigens and wild-type species.

It has been scored 103 alleles using the 16 EST-SSR primer sets. All 103 alleles were polymorphic among the 32 genotypes, with an average of 6.4 polymorphic alleles per primer pair (Fig. 2, Tab. 3). Similar to the observations in the present AFLP evaluation, the high polymorphic ratio (100%) in the EST-SSRs was largely due to the wild-type species, and the polymorphism rate within the 27 adapted cultigens was only 35%. The highest number of polymorphic alleles (10) was detected using the EST-SSR WMU0056 primer pair, while WMU0961 and WMU0580 detected the lowest number of polymorphic alleles (4). WMU0398 and WMU0961 did not reveal any

Tab. 2. Evaluation of amplified fragment length polymorphisms used for DNA fingerprinting of 32 watermelon cultivar accessions and related species

Primer combination	No. of alleles		Polymorphism rate (%)	PIC value
	Total allele	Polymorphic allele		
ACT-FAM				
CAA	82	80	97.6	0.914
CAT	73	67	91.8	0.936
CAG	44	38	86.4	0.473
CAC	25	24	96.0	0.379
CTA	58	56	96.6	0.621
CTT	63	59	93.7	0.678
CTG	37	34	91.9	0.612
CTC	44	41	93.2	0.891
AAC-NED				
CAA	95	89	93.7	0.779
CAT	59	53	89.8	0.844
CAG	37	33	89.2	0.797
CAC	48	46	95.8	0.789
CTA	61	58	95.1	0.764
CTT	53	51	96.2	0.889
CTG	37	32	86.5	0.766
CTC	46	45	97.8	0.674
Total	862	806	93.4	

polymorphisms among the 27adapted cultigens. A functional class was assigned to each EST-unigene based on the degree of similarity (*E*-value) with the closest counterpart sequence found in other plant species. Most of these 16 EST-SSRs exhibited sequence homology to genes with known functions from other organisms, as shown in Tab. 3.

Assessment of genetic similarity and phenetic relationships

In order to estimate the genetic diversity among the watermelon germplasm, genetic similarity coefficients were calculated based on the 965 polymorphic AFLP and EST-SSR alleles, and a phenetic tree was constructed (Fig. 3). The similarity values ranged from 0.2 ('PI 494817' and 'Ts34') to 0.98 ('SBA' and 'JXM'). The dendrogram consisted of 2 major clusters. Cluster I included all adapted watermelon cultivars (*C. lanatus* var. *lanatus*) that originated from countries in Asia, as well as France and the U.S.A. Cluster II was composed of 4 wild-type species. The genetic distance between the nodes in these 2 clusters was approximately 0.63, indicating that a high level of genetic dissimilarity exists between adapted watermelon species and related other species (Levi *et al.*, 2001a).

The similarity values among the cultigens in Cluster I were very high (0.94-0.98), demonstrating cross relationships and a narrow genetic background (Kwon *et al.*, 2010; Jarret *et al.*, 1997; Lee *et al.*, 1996; Levi *et al.*, 2001a; 2008). Cluster I was further divided into 3 subgroups. All Asian cultivars and 4 cultivars from the U.S.A. and France are located in subgroup 1. No significant grouping pattern based on country of origin was observed in this subgroup. This implies that similar ancestral plants might have been used extensively as breeding sources by different countries. This assumption is supported by the close relationship observed in the 'SBA', 'JXM', 'BKA', 'BKB', and '45NC' breeding lines in subgroup 1; although they originated from different Asian countries, they have highly similar morphological characteristics such as fruit shape (round), fruit color (red), rind color ('Jubilee' stripe), seed size (normal), and seed color (black) (Tab. 1). The inbred lines 'Oto949' and 'Tpdf', which originated from Japan and Thailand, respectively, carry very different phenotypic traits except

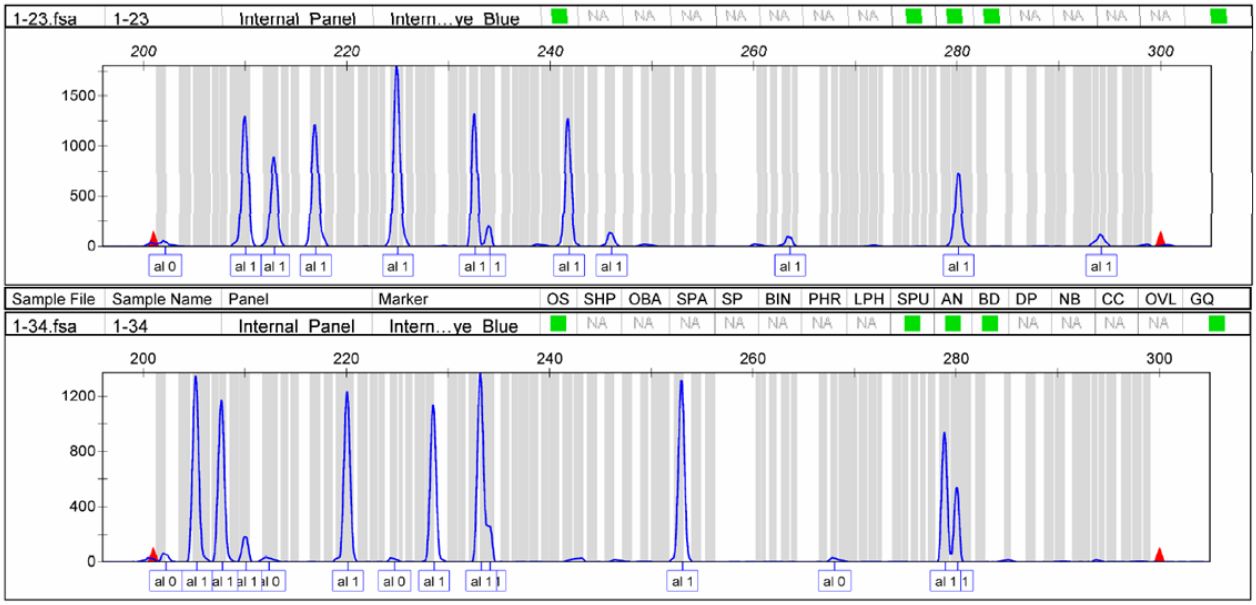

Fig. 1. Phenogram image of amplified fragment length polymorphisms detected by the ACT-FAM/CAA primer combination. Polymorphic peaks are shown between the watermelon cultivar 'All Sweet' (top) and a wild-type subspecies (*C. lanatus* var. *citroides*) 'PI 494817'(down). Only alleles with fluorescence intensities higher than 500 were scored

Tab. 3. Evaluation of expressed sequence tag-simple sequence repeat primer pairs used for DNA fingerprinting of 32 watermelon cultivar accessions and related species

Unigene ID	SSR motif	Product size	Observed size	No. of alleles		PIC value	Putative function	Organism	e value
				Total	Polymorphic				
WMU56	$(TA)_{12}$	256	190-274	10	10	0.785	AAC12676 phloem filament protein	*Cucurbita maxima*	2.00E-06
WMU71	$(CA)_{11}$	167	159-241	6	6	0.568	No hits found	-	-
WMU347	$(TA)_{6}$	187	172-211	6	6	0.488	XP_002302521 predicted protein	*Populus trichocarpa*	3.00E-49
WMU398	$(GA)_{9}$	238	145-280	6	6	0.230	CAN72450 hypothetical protein	*Vitis vinifera*	1.00E-64
WMU400	$(CT)_{19}$-$(CT)_{8}$	212	191-211	8	8	0.649	XP_002281404 PREDICTED: hypothetical protein	*Vitis vinifera*	5.00E-98
WMU503	$(CTT)_{14}$	232	212-241	6	6	0.285	CAO48418 unnamed protein product	*Vitis vinifera*	1.00E-07
WMU580	$(AAG)_{12}$	184	172-196	4	4	0.614	No hits found	-	-
WMU842	$(TA)_{8}(AT)_{6}$	142	122-237	6	6	0.486	XP_002268638 PREDICTED: hypothetical protein	*Vitis vinifera*	3.00E-23
WMU961	$(AG)_{6}$	178	199-351	4	4	0.176	XP_002270335 PREDICTED: hypothetical protein	*Vitis vinifera*	2.00E-78
WMU1085	$(TC)_{13}$	179	174-229	8	8	0.334	No hits found	-	-
WMU1211	$(CT)_{17}$	260	238-280	5	5	0.539	CAN71736 hypothetical protein	*Vitis vinifera*	3.00E-19
WMU2376	$(AG)_{10}$	236	124-238	9	9	0.688	XP_002298707 predicted protein	*Populus trichocarpa*	2.00E-16
WMU3039	$(AT)_{9}$	260	256-283	8	8	0.662	XP_002511257 purine permease, putative	*Ricinus communis*	6.00E-23
WMU4488	$(GCA)_{11}$	141	126-192	5	5	0.594	XP_002517606 lactoylglutathione lyase, putative	*Ricinus communis*	3.00E-68
WMU4452	$(AT)_{16}$	159	142-157	7	7	0.428	AAG25897 silverleaf whitefly-induced protein 3	*Cucurbita pepo*	2.00E-15
WMU4702	$(AG)_{15}$	163	150-174	6	6	0.543	CAN79997 hypothetical protein	*Vitis vinifera*	3.00E-17
Total				104	104				

290

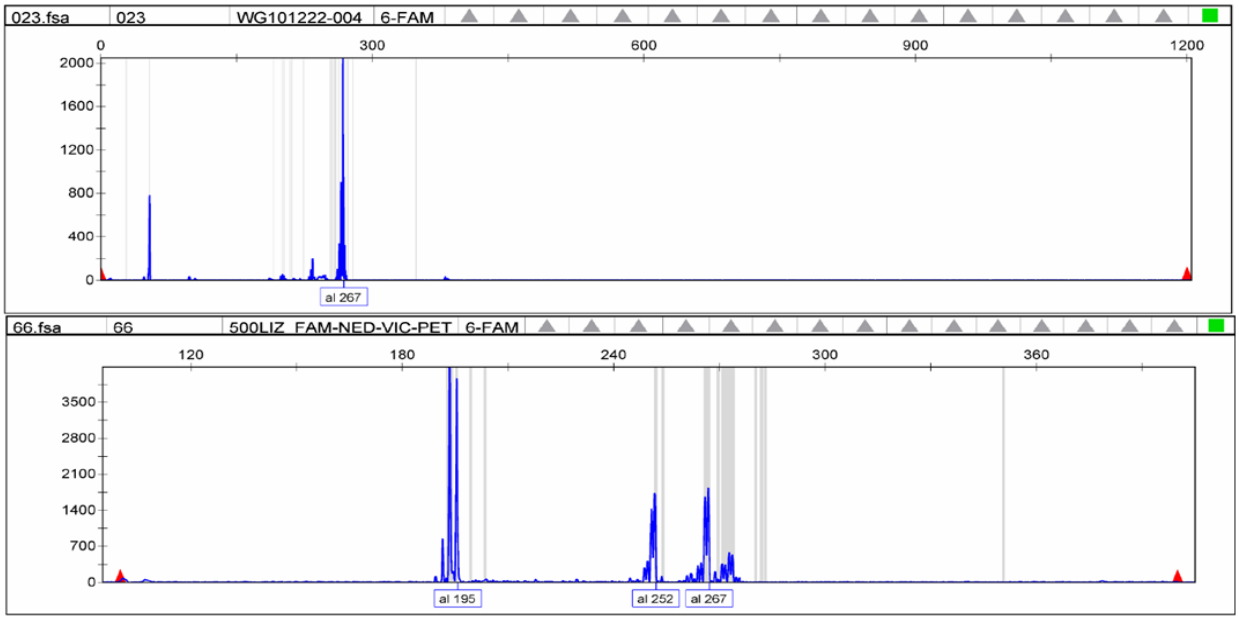

Fig. 2. Phenogram image of expressed sequence tag-simple sequence repeats detected by the WMU056 primer pair. Polymorphic peaks are shown between the watermelon cultivar 'All Sweet' (top) and a wild-type subspecies (*C. lanatus* var. *citroides*) 'PI 494817' (down). Only alleles with fluorescence intensities higher than 1000 were scored

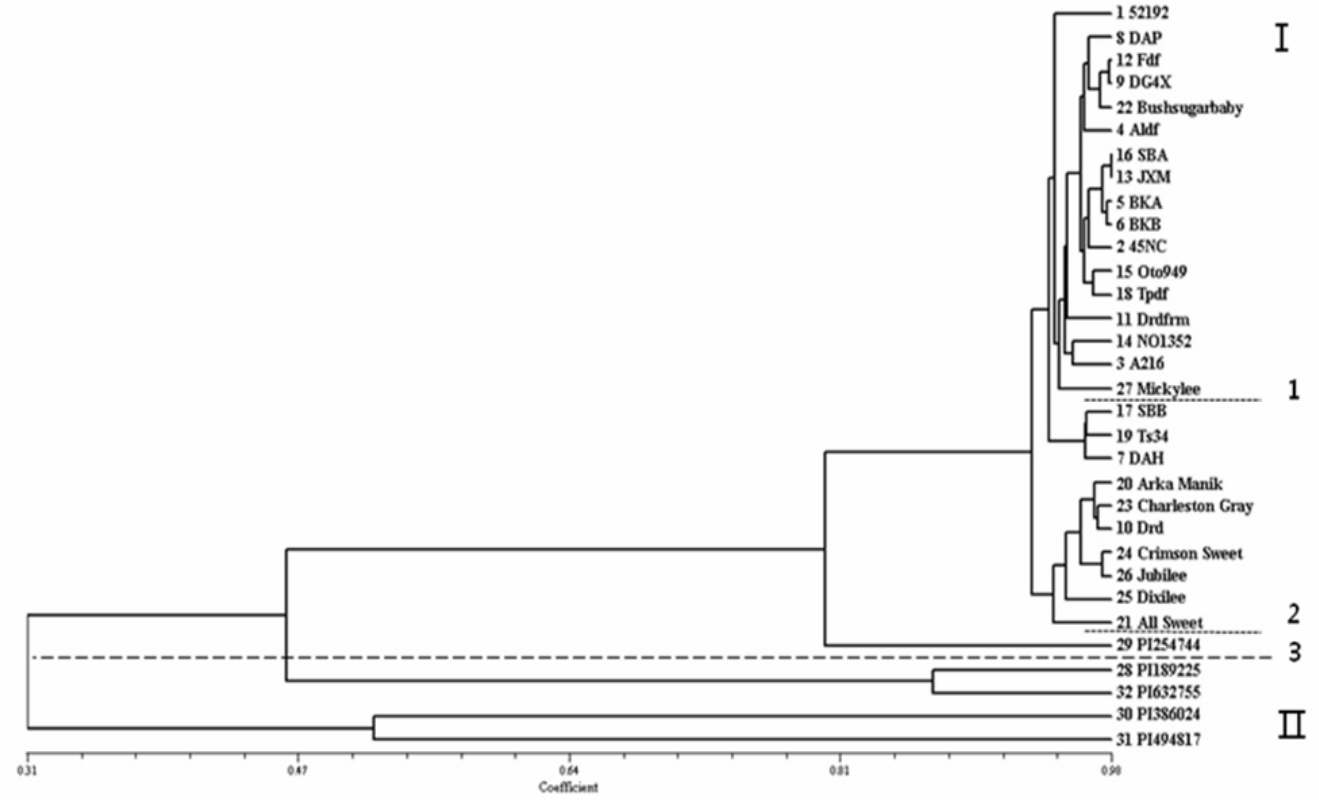

Fig. 3. A dendrogram showing the genetic relationships among 32 watermelon cultivars (*C. lanatus* var. *lanatus*) and 3 related subspecies, *C. lanatus* var. *citroides* ('PI 494817'), *C. colocynthis* ('PI 386024'), and *C. rehmii* ('PI 632755'), as revealed by amplified fragment length polymorphism and expressed sequence tag-simple sequence repeats markers

for green seed color; however, they were closely related in the phenetic tree. The Korean inbred lines were widely dispersed in this subgroup. As determined from parental linage records (personal communication), these cultivars were bred to have diverse combinations of morphological traits as shown in Tab. 1. For example, 'Ts34' and 'Drdfrm' were developed using 'Drd' and 'PI 386024' as parental lines for various seed sizes and fruit shapes.

The plant genotypes comprising subgroup 2 were mostly U.S. open pollinated (OP) varieties with red fresh, crimson stripe, and dotted-brown seed. Diverse seed sizes and fruit shapes were also observed in this group. These OP varieties were reportedly originated from common breeding parentages in order to have highly similar morphological characteristics (Levi *et al.*, 2001b). Although 'Bush Sugarbaby' and 'Mickylee' were also U.S. OP varieties, they grouped more closely to Asian cultivars. As judged from their early-maturing, small-sized fruits, it is possible that these U.S. varieties were developed from breeding materials native to Asian countries. In addition, the fact that they were developed by open pollination implies that pollen contamination with Asian breeding materials cannot be excluded. 'Arka Manik', an OP variety from India also belonged to this subgroup. This cultivar produces round fruits with red fresh and crimson stripe, and carries multidisease resistance.

Cluster II consisted of wild-type germplasm from different species or subspecies. These wild-type PIs possess round and white fruits in common and have diverse rind colors, seed sizes, and seed colors. In general, wild-type species show low plant vigor and small fruit size, much unlike adapted cultivars. Interestingly, although a PI of *C. lanatus* var. *citroides* ('PI 254744') was distantly related to other wild genotypes and allocated independently to the same node of Cluster I, its genetic distance from adapted cultivars was still relatively high (0.31). In field evaluations, 'PI 254744' demonstrated smoother fruit flesh than other PIs of the *citroides* subspecies and plant shape that was similar to that of adapted watermelons. Previous studies by Levi *et al.* (2000 and 2001a) indicated that among *Citrullus* species, the wild *C. colocynthis* species, which has the widest geographic distribution, also has the highest genetic diversity. These studies also demonstrated higher genetic diversity within the wild subspecies *C. lanatus* var. *citroides*, than in *C. lanatus* var. *lanatus*. The PI lines used in this study carry several agronomically important traits that can be utilized for breeding purpose. For example, 'PI 254744' and 'PI 386024' have been reported to be resistant to powdery mildew race 1W and 2W, respectively (Tetteh *et al.*, 2010), and 'PI 494817' is moderately resistant to bacterial fruit blotch (BFB), which is a serious seed-infecting bacterial disease that results in severe economic losses around the world (Hopkins and Thompson, 2002). Interspecific crosses between these PIs and adapted cultigens will enable breeders to develop new breeding sources with diverse characteristics, including resistance to diseases. This process can be facilitated by a backcross that applies molecular markers in both the foreground and background selection. The abundance in polymorphisms shown in this study ensures readiness for molecular marker-based introgression of useful traits from wild-type species.

In conclusion, AFLPs and EST-SSRs were efficient for distinguishing both among and within groups of wide-type PIs and cultivated watermelons. A low level of marker polymorphism among watermelon cultivars implied that a severe bottleneck in genetic diversity existed during the initial breeding practices. The phenetic tree indicated that watermelon accessions cultivated in South Korean were closely related to other cultivars introduced overseas, but were still discernable in terms of grouping. The present DNA fingerprinting data could be useful for choosing parental lines in an effort to broaden genetic pools for watermelon breeding, as well as for cultivar identification and intellectual property protection. In particular, codominant EST-SSRs accessed in this study may contribute to the development of DNA markers that can be used for purity tests in F_1 seed production.

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