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Detection of genetic diversity using RAPD-PCR and sugar analysis in watermelon [*Citrullus lanantus* (Thunb.) Mansf.] germplasm

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Abstract RAPD (random amplified polymorphic DNA) markers generated by 15 arbitrary decamers were used to determine the frequency of DNA polymorphism in 39 watermelon [*Citrullus lanantus* (Thunb.) Mansf.] germplasms. Of the 15 primers tested, all except 1 (primer 275) directed the amplification of polymorphic products. A total of 162 amplification products were generated across all 39 genotypes. Among the 162 fragments, 35 (21%) appeared to be reliable polymorphic markers. The mean value by marker difference in this comparison was 0.24, and the highest, 0.69. Eight RAPD markers could be utilized in the unique variety discrimination 8 watermelon genotypes. From the phenograms constructed by UPGMA based on the comparison of RAPD markers, four clusters were resolved. Each group was also characterized and identified with morphological and genetic characteristics for each genotype. The free sugars of the edible parts of watermelons were analyzed by HPLC (high-performance liquid chromatography). Results from the phylogenetic analysis of band sharing data were consistent with sweetness as measured by HPLC. In conclusion, RAPD assays can be used for providing alternative markers for identifying genotypes and quantitative characteristics in watermelon.

Key words RAPD-PCR · HPLC · Watermelon · *Citrullus lanantus* (Thunb.) Mansf. · Phenogram

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Introduction

Determination of the levels of intraspecific genetic diversity is an important first step in effectively utilizing available germplasm resources. Watermelon [*Citrullus lanantus* (Thunb.) Mansf.] is a poorly described species. Only ten well-defined morphological markers have been characterized and just a few informative isozyme markers are available. Diploid watermelon is a self-compatible but generally outcrossing annual with 11 pairs of chromosomes. Varieties are currently being developed using recurrent selection, except for the seedless triploid varieties, which are being produced by parthenocarpy (Kihara 1951). Many simply inherited traits (Anthracnose resistance, dwarf habit, fruit shape, rind color and earliness) have been characterized and could be easily marked if molecular markers were available for this important crop.

Either RFLP (restriction fragment length polymorphism)- or PCR (polymerase chain reaction)-based approaches can be used to identify molecular markers, but PCR represents the faster and easier alternative (Shin et al. 1990). RAPD (random amplified polymorphic DNA) (Williams et al. 1990; Welsh and McClelland 1990) is the least initially expensive route to molecular marker development and was the route chosen for this project. RAPDs based on the armplification of multiple random segments of the genome using arbitrary primers provide high levels of polymorphisms, some of which are heritable. Recent studies have indicated that RAPDs can provide valuable tools for genotype identification, population and pedigree analysis, phylogenetic studies, the screening of segregating populations for linked markers and genetic mapping (Chaparro et al. 1994; D'Ovidio et al. 1990; Dweikat et al. 1993; Francisco-Ortega et al. 1993; Martin et al. 1991; Rowland and Levi 1994; Szmidt et al. 1993; Welsh and McClelland 1990; Wilkie et al. 1993; Williams et al. 1990; Yu and Nguyen 1994). Although the RAPD procedure is relatively simple and rapid, the first step in

developing genetic maps with those markers has been to examine the level of DNA polymorphism among inbred lines and to identify and select suitable parents showing sufficient DNA polymorphism for efficient mapping.

Due to its economic and agricultural importance, vegetable fruit watermelon is grown as a sub-tropical crop in Korea and many other regions of the world. In Korea, the aims of watermelon breeding of programs are to develop individuals with increased resistance to drought, leaf and stem diseases, rind color and thickness, fruit shape and sweetness. Of these breeding objectives, sweetness is the one most directly related to watermelon's economic value in the market. At present, however, there is no published genetic map of watermelon incorporating both DNA markers and morphological characters. Furthermore, there is very little information available on genetic diversity within the germplasm, and no comprehensive study has been reported. In this paper, we demonstrate that the RAPD assay can be used for accessing genetic diversity, discriminating watermelon germplasm, estimating relatedness and determining sweetness associated with clustered groups. This investigation on DNA polymorphisms provides an essential basis on which to plan future marker-facilitated breeding programs.

Materials and methods

Plant material

Thirty-nine watermelon cultivars were used in this study (Table 1). This germplasm was chosen because it represents a wide range of origin and a range of important agronomic characteristics. All plant materials were grown in a glasshouse and for each, individual leaves of 15-week-old seedlings were removed for DNA extraction. For the sugar analysis, all plants were grown in 1994 at Chonam, Korea. Watermelons were harvested 30 days after artificial selfing.

Total genomic DNA was isolated from fresh leaf material using a modification of the CTAB method (Murray and Thompson 1980; Saghai-Maroof et al. 1984) and microextraction method (Cenis 1992; Dich and Schubert 1993). Leaf material was ground in a 1.5-ml microcentrifuge tube with a pestle in 30 µl extraction buffer [50 m*M* Tris-HCl (pH 8.0), 25 m*M* EDTA (pH 8.0), 0.35 *M* sorbitol, 0.1% *β*-mercaptoethanol]. After grinding, an additional 236 µl of extraction buffer and 36 µl of 5% sarkosyl were added separately to the homogenate, and the tube was placed at room temperature for 5 min. Then, 46 µl 5 *M* NaCl and 37 µl CTAB buffer (8.6% CTAB in 0.7 m*M* NaCl) were added separately, and the homogenate was incubated at 65 °C for 15 min. Two microliters of RNase (1 mg/ml, DNase-free) was added, and the homogenate was incubated at 37 °C for 15 min to remove RNA in the sample.

The samples were extracted with phenol/chloroform (Sambrook et al. 1989). Supernatants were transferred to fresh tubes to which were added a half volume of 7.5 *M* ammonium acetate and 2 volumes of 95% ethyl alcohol. Samples were mixed and left at −20 °C to precipitate the DNA. After centrifugation at 10 000 *g* for 10 min, the supernatant was discarded and the DNA pellet was vacuum-dried. The DNA pellet was resuspended in 20 µl TE buffer [10 m*M* Tris-HCl (pH 8.0), 0.1 m*M* EDTA (pH 8.0)] and stored at 4 °C (Sambrook et al. 1989). Quantification of DNA was accomplished by analyzing samples of the DNA extracts on 0.7% agarose gels alongside diluted uncut and cut lambda DNA standards.

DNA amplification

Arbitrary decamer primers were purchased from the University of British Columbia, Vancouver, Canada. A total of 15 primers were used in this study (Table 2). The optimum reaction mix for a 14-µl PCR reaction comprised approximately 1 ng of DNA template; dATP, dCTP, dGTP and dTTP, each at 200 µ*M* final concentration; 0.27 µ*M* primer; 1.25 × *Taq* polymerase buffer [12.5 m*M* Tris-HCl (pH 8.0), 62.5 m*M* KCl, 0.125% triton X-100], 800 µ*M* $MgCl_2$; 0.5 units of Dynazyme (*Thermus brokianus*, Finnzymes, Finland). Each reaction mix was overlaid with 14 µl of mineral oil to prevent evaporation.

Samples for enzymatic amplification were subjected to 45 cycles of the following thermal profile: 1 min at 94 °C, 2 min at 37 °C and 3 min at 72 °C. Samples were predenatured for 4 min at 94 °C and final extension was for 7 min for 72 °C. Amplification fragments generated by PCR in a water-bath thermal cycler (FINEPCR, Korea) were separated according to size on 1.4% agarose gels, stained with ethidium bromide and visualized by illumination with ultraviolet light.

Nomenclature of markers

Amplification fragment length polymorphism (Amp-FLPs) were observed as the presence versus absence of amplification fragments of the same size. Each amplified band was named by the primer used and its size in base pairs (bp). For example, marker 287-2500 refers to the 2500-bp band amplified by primer 287.

Data analysis

The fraction of bands (F) in common between genotypes was estimated using the formula of Nei and Li (1979); $F = 2M_{XY}/(M_X + M_Y)$, where M_{XY} was the number of total amplified fragments shared between 2 genotypes, and M_X and M_Y were number of total amplified fragments observed in each genotype. From calculated F values, dissimilarity values were derived by subtracting the F value from 1 (i.e. 1–F). The Unweighted Pair Group Method with Arithmetic mean (UPGMA) was used to calculate the pairwise marker difference matrix and to reconstruct the genetic relationships among the genotypes. Marker difference values (MDV) denote the fraction of amplification fragments that were not shared between pairwisely compared cultivars. The computer package program, NTSYS (Rohf 1990), was used for cluster analysis of both marker difference values and the dissimilarity values (1–F) based on the UPGMA method.

Sugars in watermelon cultivars

After the harvesting of the ripe watermelons, a homogenate of the edible part of the middle of each fruit was used for soluble solids determination with a refractometer (Attago, Japan). Monosaccharides and oligosaccharides were isolated from fresh fruit using a modification of the method of Richmond et al. (1981). A 20-g piece of the freshly sliced sarcocarp was homogenized and centrifuged at 10 000 *g* for 10 min. The supernatant was heated at 80 °C for 30 min and filtered first through a C_{18} Sep-pack cartridge and then through a 0.45 µm Millipore filter, and 20 µl of the filtrate was subsequently subjected to high-performance liquid chromatography (HPLC). Sugars were quantitated with a Waters HPLC/510 equipped with RI detector (Waters R-410) and YMC-Pack Polyamine-2 column (250 × 4.6 mm). The solvent was acetonitrile/water (85/25) at a flow rate 1 ml/min.

Results and discussion

RAPD analysis

The DNA samples of 39 individual watermelon cultivars (Table 1) were assayed for RAPD-PCR using 15

Table 1 The 39 accessions of *Citrullus vulgaris* L. genotypes used for the DNA polymorphism investigation

Accession no.	Cultivar name	Important agronomic characteristics[a]				
		Seed size and color[b]	Rind color[c]	Fruit shape[d]	Soluble solids (°Brix)[e]	Rind thickness(mm)
1	Hyosung	M, G	GS	R	11.5	8.3
2	Seolbong (Changheung)	L, G	GS	E	–	–
3	Seounong #8 Sugwa (F_1)	L, G	GS	E	–	–
4	Seoul Seeds #1	S, T	GS	R	9.0	13.5
5	Seoul Seeds #2	M, BL	DGS	R	10.9	7.5
6	Seoul Seeds #3	M, T	GS	R	11.5	13.5
7	Lucky	M, BL	GS	R	10.7	12.5
8	Shindaehwa	M, T	GS	R	11.0	11.0
9	Ukdaewha	M, BR	LGS	–	7.5	11.0
10	Bumin	–	LGS	–	–	–
11	Daewha Creem	–	–	–	–	–
12	Eulier	–	GS	R	13.6	10.5
13	Gabo	M	GS	E	5.4	15.0
14	Sugar Baby	–	DGS	–	–	–
15	Shin Deungtaehwa #1	–	–	–	–	–
16	New Hampshire Midget	L, MB	GS	R	7.0	9.5
17	Jeonseo	–	GS	R	10.6	11.0
18	Giwon	S, T	GS	R	8.0	5.5
19	Buyeon	–	LGS	R	6.2	8.0
20	Clone Dike	–	–	R	10.0	8.5
21	Daewang	–	–	R	8.9	9.5
22	Egypt San	L, BL	DGS	R	9.4	12.8
23	Petite Sweet	M, BL	DGN	R	10.5	5.5
24	Family Fun	–	–	–	–	–
25	Sugar Doll	M, T	DGS	R	9.4	9.0
26	Cheolyong	–	GS	R	–	–
27	New Ascar	M, T	GS	R	11.0	12.0
28	Jeolmil #1	M, T	GS	R	10.3	12.5
29	T4-11-5	–	–	–	–	–
30	T4-11-49	M, T	GS	R	9.9	12.5
31	T4-11-15	–	GS	R	8.9	8.3
32	Trial No. 351	–	DG	R	8.4	13.0
33	Unknown	–	–	–	9.0	–
34	Gold Medal	–	GS	R	8.5	8.0
35	Bok Watermelon	S, BL	GS	B	10.0	4.5
36	Black Star	–	LG	R	–	–
37	Seonong #5	L, BL	LG	E	7.2	10.6
38	Seonong #8	–	–	–	7.8	8.5
39	Whangno	–	GS	R	10.7	10.0

[a] Some genotypes are either of unknown geographic origin or are derivatives of a breeding program. –, not investigated

[b] Seed size: L, Large (>1 cm); M, middle (0.5–1 cm); S, small (<0.5 cm) Seed color: G, Gray; T, tan: BL, black; BR, brown; MB, mottled black

[c] Rind color: GS, Green striped; DGS, dark-green striped; LGS, light-green striped; DGN, dark-green net; DG, dark green

[d] Fruit shape: R, Round; E, elongated; B, block. R (0.4–0.5), E (0.6–0.8); width/length ratio

[e] Soluble solids were determined with a refractometer; 0–25 °Brix scale

primers. Of the 15 primers tested, 14 (with the exception of primer 202) successfully amplified DNA fragments from the watermelon DNA samples (Fig. 1). Each primer produced 3 (primer 202) to 17 (primer 300) fragments from each genotype, and a total of 162 amplification fragments were scored. Among the 162 fragments, 100 (62%) were polymorphic, and the remaining 62 (38%) were monomorphic. However, of the 100 polymorphic fragments, only 35 (22%) turned out to be reproducible and regarded as reliable RAPD markers for further analyses; the remaining 65 fragments were relatively unstable and thus excluded. One of five markers were selected for each primer. The mean number of selected RAPD markers per primer was 2.5 using this criterion. The 35 RAPD markers selected were used to calculate both the dissimilarity value and the marker difference that were used to reconstruct the genetic relationships.

Genetic relationships among watermelon cultivars

From all of the 1-F values obtained using the total bands for 39 watermelon genotypes the mean 1-F value was 0.303. In 36 Sorghum genotypes, this value was 0.117, much lower than our result (Tao et al. 1993). The value was the highest, 0.913, between cvs, 8 ('Shindaehwa') and 30 ('T4-1-49'). The mean MDV was 0.24, and the highest MDV was 0.69 between cvs 7 ('Lucky') in group A and 30 ('T4-11-49') in group C. Cultivar 7 ('Lucky') was also

Table 2 A list of 15 random primers used in this experiment

Primer accession no.	Primer sequences	GC content (%)	Polymorphic bands scored[b]
202	5′-GAGCACTTAC-3′	50	2
203	5′-CACGGCGAGT-3′	70	1
218	5′-CTCAGCCCAG-3′	70	1
222	5′-AAGCCTCCAC-3′	60	1
228	5′-GCTGGGCCGA-3′	80	2
240	5′-ATGTTCCAGG-3′	50	5
250	5′-CGACAGTCCC-3′	70	2
262	5′-CGCCCCCAGT-3′	80	3
266	5′-CCACTCACCG-3′	70	1
275	5′-CCGGGCAAGC-3′	80	0
280	5′-CTGGGAGTGG-3′	70	4
287	5′-CGAACGGCGG-3′	80	4
295	5′-CGCGTTCCTG-3′	70	2
298	5′-CCGTACGGAC-3′	70	3
300	5′-GGCTAGGGCG-3′	80	4

[a] Accession numbers of the University of British Colombia, Vancouver, Canada
[b] Number of scored polymorphic bands produced by each primer

observed to be relatively unique (mean 0.52) when compared to all of the other genotypes (mean 0.24) and appeared to be highly divergent from the others.

Based on the pairwise dissimilarity and MDV data, we carried out cluster analyses using UPGMA (Fig. 2). In both reconstructed phenograms, the four major clusters were resolved, and the overall clustering patterns were similar to each other except for a small number of cultivars (i.e. 4, 23 and 33) that were swapped between groups. For example, cv 4 ('Seoul Seed #1'), which belonged to group D in the phenogram with dissimilarity value (Tree not shown), was switched to group A in the one with MDV (Fig. 2). Cultivar 23 ('Petite Sweet') and 33 (Unknown) were moved from group B (Tree not shown) to group C (Fig. 2) in the phenograms.

In both phenograms, cvs 2 ('Seolbong') and 3 ('Seonong #8'), introduced from China, were clustered together in group B. Commercial cultivars of 'Hyosung', 'Seeds #2', 'Seoul Seeds#3' and 'Lucky', which are related cultivars currently in production, were clustered in group A. Genotypes clustered in groups B, C and D revealed relatively closer genetic relationships than those in group A. Even though the complete comparison of our results with other characteristics could not be done due to a lack of information on the watermelon genotypes, some trends could be identified. For example, 'Seolbong' and 'Seonong #8' in group B have flat and large seeds and 'Petite Sweet' and 'Bok' in group C have a thin rind. Group D consists largely of round-fruited cultivars such as 'Jeonseo', 'Giwon', 'Daewang', 'New Ascar' and 'Whangno'. The elongated fruit shape cultivars of 'Bok' and 'Seonong #5' were clustered together in group C.

Cultivar-specific markers

Nine cultivar-specific RAPD markers were observed, of which 5 were specific for a single cultivar and 4 were shared by 2 genotypes (Table 3). RAPD marker 300–500 is an amplification fragment unique to 'Hyosung' and 300–1400 is unique to 'Lucky'. Similarly, 'Ukdaewha' and 'New Hamphshire Midget' were sufficiently distinguishable from all the others by RAPD markers 240–1200 and 262–1600, respectively. Marker 240–600 was specific to 'Hyosung' and 'Lucky', 300–1300 to 'Hyosung' and 'Shindaewha', 295–800 to 'Seoul Seeds #1' and 'Lucky', 202–3000 to 'Sulbong' and 'Shindaewha' and 202–500 to 'T4-11-49'. Even though a low number of cultivar-specific RAPD markers were found in our study, some cultivars could be distinguished by at least 1-marker difference. RAPD markers have also been successfully applied for identifying celery cultivars (Yang and Quiros 1994). In that study three cultivar-specific markers were screened from 22 celery cultivars analyzed by RAPD-PCR using 28 primers, which distinguished celery cultivars (*Apium graveolens* L. vs '*dulce*'), celeriac (var '*rapaceum*') and annual smallage (var '*secalinum*').

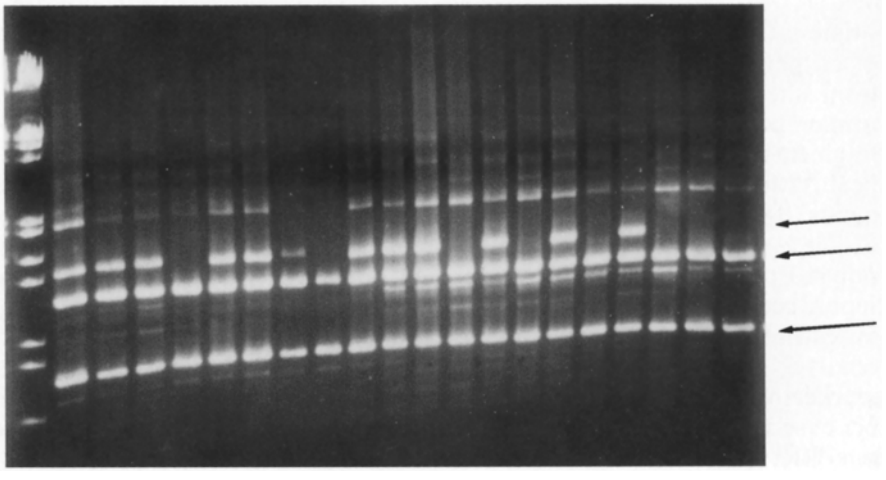

Fig. 1 RAPD profiles generated by primer 298. The marker 298–2000, 298–1400 and 298–1000 (*arrows*) scored as + (present) or − (absent). Molecular weight marker consists of lambda DNA digested with *Eco*RI and *Hin*dIII

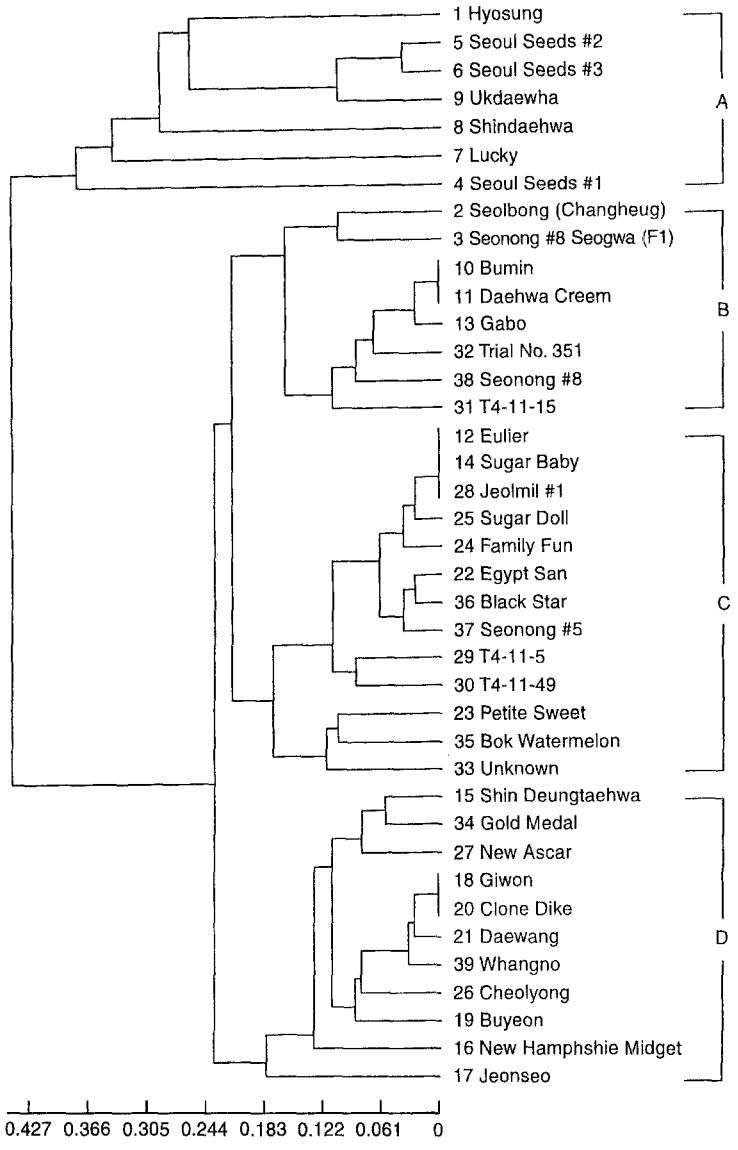

Fig. 2 Phenogram of 39 watermelon cultivars based on marker difference. Numbers are accession numbers of the 39 cultivars shown in Table 1. Values on the *baseline* indicate the average genetic distances between lines

Table 3 List of genotype-specific markers

Genotype	Specific marker
Hyosung	300–500
Lucky	300–1400
Ukdaewha	240–1200
New Hamphshire Midget	262–1600
T4-11-49	202–500
Hyosung & Lucky	240–600
Hyosung & Shindaewha	300–1300
Seoul Seeds #1 & Lucky	295–800
Seolbong & Shindaewha	202–3000

Sugars in watermelon cultivars and phenogram by RAPD analysis

Sugars in 28 of the 39 watermelon accessions were determined by high performance liquid chromatography (HPLC). Free sugars of fructose (reducing), glucose (reducing) and sucrose (nonreducing) were detected. Wide variation was found in the contents of the free sugars among these 28 accessions (Table 4). Though watermelons are known to have a little maltose (Richmond et al. 1981), we did not find any maltose in this study. Since our samples were fully ripe, dissacharides had been drastically broken down into monosaccharides by the ripening processes. Both fructose and glucose content were the highest in cv 37 ('Seonong #5'), and lowest in cv 5 ('Seoul Seeds #2'). Cultivar 12 ('Eulier') showed the highest content of sucrose (6.89%), and the ratio of sucrose to total free sugar [S/(F + G + S)] was 67.4%. No sucrose was detected in the cvs 13 ('Gabo'), 19 ('Buyeon'), 30 ('T4-11-49') and 33 (Unknown). The soluble solids as measured by the refractometer were the highest in cv 12 ('Eulier'), which also showed a high sucrose to total sugar ratio.

Elmstrom et al. (1980) reported that sugar determination with a refractometer provides a reliable value for

Table 4 Content of fructose (F), glucose (G) and sucrose (S) in mature watermelons

Cultivar name	Sugar content(%)				(F+S)/S	Sweetness range[a]
	F	G	S	F+G+S		
Hyosung	2.61(28.7)[b]	2.34(25.8)	4.13(45.5)	9.08	1.09	982
Seoul Seeds #1	3.77(47.1)	2.79(34.9)	1.44(18.0)	8.00	4.56	924
Seoul Seeds #2	1.00(27.5)	0.49(13.5)	2.15(59.1)	3.64	0.69	440
Lucky	3.18(31.5)	2.13(21.1)	4.78(47.4)	10.10	1.11	1122
Shindaewha	4.04(48.6)	2.63(31.6)	1.64(19.7)	8.31	4.07	978
Ukdaewha	3.32(42.0)	1.56(19.7)	3.03(38.3)	7.91	1.61	931
Eulier	2.00(19.6)	1.34(13.1)	6.89(67.4)	10.20	0.48	1094
Gabo	3.73(63.8)	2.12(36.2)	0.00(0.0)	5.85		731
New Hamphshire	3.00(38.9)	1.40(18.1)	3.32(43.0)	7.72	1.33	899
Jeonsoe	2.94(44.5)	2.15(17.4)	2.52(38.1)	7.61	2.02	860
Giwon	3.72(48.9)	2.35(30.9)	1.54(20.2)	7.61	3.94	899
Buyeon	3.26(59.0)	2.27(41.0)	0.00(0.0)	5.53		667
Clone Dike	4.30(52.1)	3.39(41.1)	0.56(6.8)	8.25	13.70	962
Daewang	4.88(60.0)	2.86(35.1)	0.40(4.9)	8.14	19.40	1002
Egypt San	3.40(45.9)	2.53(34.1)	1.48(20.2)	7.41	4.01	854
Petite Sweet	2.38(32.0)	1.21(16.3)	3.85(51.7)	7.44	0.93	842
Sugar Doll	4.42(46.5)	3.42(36.0)	1.66(17.5)	9.50	4.72	1093
New Ascar	2.46(27.5)	1.73(19.4)	4.75(53.1)	8.94	0.88	979
Jeolmil #1	4.01(50.9)	2.16(27.4)	1.71(21.7)	7.88	3.61	948
T4-11-49	4.56(53.0)	4.05(47.0)	0.00(0.0)	8.61		992
T4-11-15	3.79(46.1)	3.51(42.6)	0.93(11.3)	8.23	7.85	927
Trial No. 351	4.33(49.8)	3.20(36.8)	1.16(13.3)	8.69	6.49	1014
Unknown	3.45(48.9)	3.61(51.1)	0.00(0.0)	7.06		787
Gold Medal	3.55(44.5)	3.14(39.3)	1.29(16.2)	7.98	5.19	900
Bok Watermelon	3.74(34.8)	3.70(34.4)	3.31(30.8)	10.80	2.25	1170
Seonong #5	5.26(46.8)	4.44(39.5)	1.55(13.8)	11.30	6.26	1283
Seonong #8	4.21(51.4)	3.64(44.4)	0.34(4.2)	8.19	23.10	943
Whango	3.44(30.6)	2.21(19.6)	5.60(49.8)	11.30	1.01	1251

[a] Average of calculated sweetness range. Values were calculated using the mid-point of relative sweetness range suggested by Eisenberg (1955); S = 100, F = 140–170, G = 60–75

[b] The value between the brockets is the ratio of total sugar

total sugar in mature watermelon fruits. He also suggested that reliable measure of high fructose content was important when screening lines in breeding populations and that the calculated sweetness value provides one alternative criterion for estimating the palatability. However, our results demonstrate that the ratio of sucrose to total free sugars [S/(F + G + S)] shows a highly positive correlation with the refractometer value (Table 5). We therefore recommend that this value be considered as a determinant of the relative sweetness.

The results of the sugar analysis (i.e. total free sugars, sugar ratios, soluble solids and calculated sweetness values) for watermelon accessions clustered in groups A and B by UPGMA (Fig. 2) were averaged and are presented in Table 6. The mean value of sucrose ratio to total free sugar [S/(F+G+S)] in group A (0.373) was much higher than that in group B (0.072). Although the mean values of total free sugar content were similar in both groups (7.84 for group A and 7.74 for group B), group A showed rather higher sweetness than group B on the basis of both refractometer values (10.1 for group A and 7.625 for group B) and the mean value of the sucrose ratio to total free sugar. These observations suggest that the differences in both the sucrose to total free sugar ratio and soluble solids between these two groups might be related to genotypic differences and that the sugar content could be distinguished by specific RAPD markers. Some RAPD markers representing

Table 5 Correlation coefficients estimated among free sugars, their ratio of total sugar and sweetness (*F* fructose, *G* glucose, *S* sucrose, *ST* sweetness was detected by HPLC)

	F	G	S	ST	I	II
G	0.82242**					
S	−0.57461**	−0.53028**				
ST	−0.44369*	−0.27806	0.64035**			
I[a]	0.72097**	0.48233**	−0.90135**	−0.70770**		
II	0.71712**	0.87672**	−0.81160**	−0.47900**	0.73031**	
III	−0.77306**	−0.73009**	0.92091**	0.63819**	−0.93046**	−0.92981**

* 0.05%; ** 0.01%

[a] I; F/(F+G+S); II, G/(F+G+S); III S/(F+G+S)

Table 6 List of sucrose ratio, sweetness, calculated sweetness and total sugar in A and B groups as defined by RAPD analysis (*F* Fructose, *G* glucose, *S* sucrose)

	RAPD analysis[a]	
	A group	B group
S/(F+G+S)	0.373	0.072
Soluble solids[b]	10.1	7.625
Calculated sweetness	896.2	903.8
F+G+S	7.84	7.74

[a] RAPD analysis was calculated by marker difference. A, B group was based on Fig. 2
[b] Soluble solids were determined by refractometer

high free sugar content in watermelon could be identified, although these have to be critically verified in future experiments. RAPD markers 280–800, 280–600, 298–1000, 250–700 and 250–1200 were present in group A, but not in group B. These markers could be thought to be relatively related to determining factors for sweetness.

In conclusion, it is suggested that RAPD markers could be successfully applied for identifying cultivars, studying genetic relationships of cultivars and determining sweetness in watermelon. These results lay the foundation for future research aimed at using RAPD markers to map quantitative trait loci in the watermelon genome.

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