TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2015) 39: 322-331

doi:10.3906/tar-1407-29

Comparative analysis of genetic diversity among Chinese watermelon germplasms using SSR and SRAP markers, and implications for future genetic improvement

Panqiao WANG, Qiong LI, Jianbin HU*, Yan SU
College of Horticulture, Henan Agricultural University, Zhengzhou, P.R. China

Received: 06.07.2014 • **Accepted:** 08.12.2014 • **Published Online:** 06.04.2015 • **Printed:** 30.04.2015

Abstract: The genetic diversity of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] in China, the world's largest producer of watermelon fruits, has not been examined. Two molecular markers, sequence-related amplified polymorphism (SRAP) and simple sequence repeat (SSR), were used to investigate the genetic variation and genetic relationship among 54 Chinese watermelon accessions, as well as 7 accessions from Africa, the United States, and Japan. SRAP assay generated 312 bands, of which 163 were polymorphic, while SSR assay detected 101 alleles with 87 polymorphic alleles and 114 genotypes. A comparative analysis of the diversity index, effective multiplex ratio, and marker index of the 2 markers showed higher efficiency of SRAP markers in watermelon germplasm fingerprinting. The Mantel test resulted in a high correlation between SRAP and SSR data with a value of $\gamma = 0.863$, revealing a good fit between the 2 marker systems. Genetic diversity among the accession set was estimated by a construction of dendrograms using SRAP, SSR, or SRAP-SSR combined data. There was clear consistency between the 3 dendrograms on the positioning of most accessions. The study demonstrated that most Chinese cultivated germplasms (CCGs) were clustered independently of their geographical distribution and horticultural classification, and had a narrow genetic base; most CCGs and foreign cultivars had a high genetic closeness and showed similar lineages; and the 4 wild accessions were the most genetically diverse, followed by the small-fruited accessions from northern China and edible-seeded accessions. We concluded that CCGs lacked genetic variation, and we offer a means of introducing new genetic diversity into the watermelon germplasm pool in China, which is essential to maximizing productivity in watermelon.

Key words: Genetic diversity, genetic improvement, sequence-related amplified polymorphism, simple sequence repeat, watermelon

1. Introduction

The genus *Citrullus* comprises one cultivated species, *Citrullus lanatus* (Thunb.) Matsum. & Nakai, and 3 wild species, *C. colocynthis* (L.) Schrad., *C. ecirrhosus* Cogn., and *C. rehmii* De Winter. The perennial *C. colocynthis*, mainly cultivated for producing the cathartic colocynth, is widely distributed in North Africa, Southwest Asia, and the Mediterranean region, while the perennial *C. ecirrhosus* and annual *C. rehmii* are only endemic to the Namib Desert (Jeffrey, 1975; Levi et al., 2001). The cultivated species *C. lanatus* (Thunb.) Matsum. & Nakai includes cultivated watermelon (*C. lanatus* var. *lanatus*) and C. *lanatus* var. *citroides*, the citron or preserving melon that is cultivated in limited areas around the world (Laghetti et al., 2007). It is generally acknowledged that watermelon originated in the Kalahari Desert of Southwest Africa and was introduced along the Silk Road into China by the tenth century (Walters, 1989; Robinson and Decker-Walters, 1997).

China is the leading watermelon producer in the world. In 2012, 69×10^6 t of fresh fruits were produced in China in an area of 1.9×10^6 ha (http://faostat.fao.org). Watermelon production in China is mainly concentrated in 8 provinces, i.e. Shandong, Henan, Hebei, Anhui, Heilongjiang, Jiangsu, Hainan, and Hubei, which accounts for over 70% of the total cultivation area and approximately 80% of the gross yield (Ma, 2006). Recently, due to the continuously increasing demands for watermelon in both domestic and foreign markets, an increasing number of cultivars have been developed in China, with more than 300 cultivars having been developed during the past 2 decades (Han et al., 2009). It is regrettable, however, that great effort has been expended to make crosses to maintain higher yields while little attention has been given to germplasm study and diverse accession identification. Consequently, the genetic diversity of Chinese watermelon germplasms (CWGs), wild and cultivated, still remains unclear. This results in limited improvement of watermelon in terms of certain agricultural traits, e.g., disease resistance, adaptability to environmental stress, and fruit quality, as a significant portion of the cultivars were developed from closely related parents. Development of new watermelon

* Correspondence: jianbinhu@henau.edu.cn

cultivars with significantly improved traits demands a more diverse range of germplasms. For this purpose, the genetic diversity and relationships among CWGs, particularly for the landraces commonly neglected in modern breeding programs, need to be elucidated.

Molecular markers are powerful tools allowing for the study of genetic variability and relationships in a variety of plant species because they are not influenced by developmental stages or environmental factors. Several nuclear molecular marker systems, e.g., random amplified polymorphic DNA (RAPD) (Lee et al., 1996; Levi et al., 2001; Solmaz et al., 2010), amplification fragment length polymorphism (AFLP) (Che et al., 2003), intersimple sequence repeat (ISSR) (Djè et al., 2010), and simple sequence repeat (SSR) (Jarret et al., 1997; Kwon et al., 2010), have been used to study genetic diversity and phylogenetic relationships in *C. lanatus*. Different estimates for the degrees of genetic variation were obtained from these reports, reflecting the differences in the selected genotypes and marker systems. Several studies showed that it was difficult to detect DNA polymorphism in cultivated watermelon using RAPD (Lee et al., 1996; Levi et al., 2001) and ISSR (Djè et al., 2010), implying a lack of genetic variability in cultivated germplasms. Of the DNA markers used, SSR has been the most informative and has proven to be useful for accession discrimination and diversity assessment in cultivated watermelons. Among the watermelon landraces from Zimbabwe, Mujaju et al. (2010) found that the polymorphic information content (PIC) values of SSRs (0.39–0.97) were markedly higher than those detected by RAPD markers (0.47–0.77). SSRs as DNA markers offer many advantages over other markers, a fact that has been well documented in a number of plant species (Kalia et al., 2011). Sequence-related amplified polymorphism (SRAP) is another effective DNA marker technique based on two-primer amplification that preferentially amplifies open reading frames (Li and Quiros, 2001). This technique was first developed from *Brassica* and proved capable of detecting inter- and intraspecific variations in other crops. Due to the adoption of unique primers, SRAP markers are more reproducible and less complex. Levi et al. (2011) developed a genetic linkage map for watermelon consisting of numerous SRAP markers (the second most prevalent markers after RAPDs), strongly verifying high polymorphism.

Although genetic diversity and relationships in *C. lanatus* germplasms from several countries have been studied, limited information is available on the genetic variability of CWGs. Our previous work revealed low variation among CWGs at chloroplast microsatellite loci (Hu et al., 2011), deepening the understanding of a domestication pattern of CWGs. To obtain more comprehensive information on CWGs, 2 simple and effective nuclear molecular markers, SSR and SRAP, were applied in the present research for a comparative analysis of the genetic diversity and relationships among a set of CWGs from a wide collection. Our study offers information regarding the amount of relevant diversity of CWGs, which will be helpful to identify potentially useful genotypes for genetic improvement, as well as germplasm conservation efforts.

2. Materials and methods

2.1. Plant materials and DNA extraction

Fifty-four morphologically and geographically distant watermelon accessions (Table 1), which were collected from different regions in China, were used in this study. These contained 49 cultivated accessions (accession names start with X), 2 edible-seeded accessions (S1 and S2), and 3 wild accessions (W1, W2, and W3). For the investigation of horticultural characters, all the accessions were grown in the summer of 2008 and 2009 at the farm of Henan Qinfa Seed Company, Zhengzhou, China. Seven foreign accessions, which were introduced from Africa, the United States, and Japan in the last century and maintained by the National Mid-term Genebank for Watermelon and Melon, Zhengzhou, China, were also included in this study to compare the degree of variation in CWGs. Healthy young leaves were sampled from the plants of each accession and subjected to DNA extraction using the cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980).

2.2. SRAP amplification

SRAP primers were selected based on the previous reports of Li and Quiros (2001). All primer combinations were initially screened using a small set of accessions, and those combinations that produced distinct band patterns were selected for subsequent analysis. This screening gave 25 primer combinations that worked well (Table 2). The reaction system and program of PCR amplifications were performed as described by Li and Quiros (2001). PCR products were electrophoresed on 6% nondenaturing polyacrylamide gels (19:1 acrylamide:bis), and the gels were silver-stained according to the method proposed by Creste et al. (2001) and photodocumented. The size of the amplified bands for each marker was estimated by reference to a DNA ladder (pUC19 DNA/*Msp*I marker, Sangon, Shanghai, China).

2.3. SSR amplification

Twenty-three SSR primer pairs were obtained from the published primer sequences (Joobeur et al., 2006). All the primer pairs generated distinct profiles, revealing a high polymorphism among the accession set. PCR amplification was performed in a 15-μL volume containing 1X PCR buffer, 50 ng of sample DNA, 0.5 μM of each

Table 1. List of investigated watermelon accessions in this study and their fruit characters.

Accession	Origin	Fruit character				
		Fruit shape[a]	Fruit weight[b]	Rind color[c]	Flesh color[d]	Flesh firmness[e]
X2	Anhui, China	R	M	GS	R	S
X7	Gansu, China	SO	H	GS	LR	F
X8	Henan, China	SO	H	GS	R	M
X14	Sinkiang, China	R	L	LG	R	M
X19	Guangdong, China	SO	L	GS	O	M
X20	Guangdong, China	R	L	DGS	R	M
X30	Guangdong, China	R	M	DGS	R	M
X24	Hainan, China	R	H	GS	LR	F
X37	Hainan, China	R	L	GS	LR	S
X43	Henan, China	R	M	GS	DR	S
X52	Henan, China	SO	H	GS	R	M
X58	Hubei, China	O	H	GS	O	M
X61	Liaoning, China	R	M	GS	R	M
X66	Gansu, China	SO	L	GS	R	M
X67	Heilongjiang, China	R	L	GS	DR	S
X70	Hebei, China	R	H	LG	DR	F
X80	Zhejiang, China	O	M	LG	LR	M
X82	Taiwan, China	R	H	GS	R	M
X89	Shandong, China	SO	H	GS	LR	S
X103	Gansu, China	GL	L	GS	LR	M
X108	Gansu, China	O	H	GS	LR	M
X114	Sinkiang, China	GL	L	LG	LR	S
X116	Sinkiang, China	GL	L	LG	LR	S
X120	Sinkiang, China	SO	H	GS	LR	F
X134	Jiangsu, China	R	M	LG	O	S
X135	Sichuan, China	R	M	LG	LR	S
X136	Sichuan, China	O	M	LG	LR	S
X145	Zhejiang, China	SO	L	EG	LR	S
X151	Liaoning, China	SO	H	DEG	LR	M
X156	Shaanxi, China	SO	H	LG	LR	M
X159	Jiangsu, China	SO	M	DEG	R	M
X168	Heilongjiang, China	SO	H	DEG	R	S
X176	Hainan, China	R	L	LG	LR	S
X189	Guangdong, China	O	L	DEG	R	S
X192	Shandong, China	O	H	DEG	LR	M
X195	Zhejiang, China	O	L	LG	LR	M
X197	Shandong, China	O	H	LG	DR	F
X202	Hebei, China	O	H	DEG	DR	M
X212	Guangdong, China	SO	L	LG	DR	M
X221	Anhui, China	SO	M	DEG	DR	F
X240	Jiangsu, China	GL	L	GS	R	S
X266	Hubei, China	R	H	DAG	DR	M
X268	Henan, China	R	M	DAG	LR	S
X269	Henan, China	R	H	DAG	LR	M
X314	Hebei, China	R	M	GS	LR	M
X331	Henan, China	O	M	DAG	LR	M
X341	Guangxi, China	R	M	LG	R	M
X435	Shaanxi, China	R	M	GS	O	M
X438	Guangxi, China	R	L	LG	O	F
W1	Sinkiang, China	R	L	GS	LR	F
W2	Sinkiang, China	SO	L	GS	O	F
W3	Heilongjiang, China	R	L	LG	O	F
S1	Guangxi, China	R	L	GS	LR	M
S2	Guangxi, China	R	M	GS	O	M
PI296341	Africa	R	L	LG	O	F
Sugar Baby	US	R	M	DEG	R	M
Crimson Sweet	US	SO	H	GS	R	M
Jubilee	US	O	M	GS	R	M
Zhengyin-2	US	SO	H	LG	R	M
Xindahe-6	Japan	R	M	GS	R	M
Miyako	Japan	R	H	GS	R	M

[a] Fruit shape: R: round, SO: short oblong, O: oblong, GL: gourd ladle; [b] Fruit weight: L: light (<2 kg), M: medium (2–4 kg), H: heavy (>4 kg);
[c] Rind color: GS: green stripes, LG: light green, DEG: deep green, DAG: dark green; [d] Flesh color: LR: light red, R: red, DR: deep red, O: orange.
[e] Flesh firmness: S: soft, M: medium, F: firm.

primer, 200 μM of each dNTP, 1.5 mM $MgCl_2$, and 0.75 unit of Taq DNA polymerase (TaKaRa, Dalian, China). Amplifications were performed in a PTC-200 thermal cycler (MJ Research, Waltham, MA, USA) as follows: 4 min at 94 °C, followed by 30 cycles of 40 s at 94 °C, 40 s at 55 °C, and 1 min at 72 °C, and then 5 min at 72 °C for final extension. The methods of electrophoresis and silver staining were the same as in the SRAP analysis.

2.4. Data collection and analysis

For SRAP or SSR assays, amplified bands were visually scored as present (1) or absent (0) and data were analyzed with NTSYS-pc 2.10e software (Rohlf, 2000). All the distinct monomorphic bands were also included in this investigation. To obtain a measure of the overall utility of the marker systems used in the present study, the diversity index (DI), effective multiplex ratio (EMR), and marker index (MI) were calculated for each SRAP or SSR marker according to Milbourne et al. (1997). SSRs were commonly designed with an EMR of 1 on the assumption that they reveal a single locus (which is not always the case). With the marker data scored, binary matrices (from SRAP, SSR, or SRAP-SSR combinations) were established and used to calculate Jaccard's similarity coefficient (Jaccard, 1908). Cluster analysis was then performed to construct dendrograms based on the similarity matrix data using the unweighted pair group method using arithmetic averages (UPGMA) and the SAHN module of NTSYS-pc 2.10e software. To investigate the congruence among the dendrograms (from SRAP or SSR markers), the Mantel test was conducted using the COPH and MYXCOMP modules in the software (Mantel, 1967). This test gives a cophenetic correlation coefficient (γ) that provides a measure of relatedness between the 2 matrices. Principal coordinate analysis (PCoA) was performed based on the variance–covariance matrix calculated from the marker data using DCENTER and EIGEN modules. The first 3 most informative principal coordinates were used to construct a two-dimensional coordinate plot.

3. Results

3.1. SRAP and SSR analysis

The selected 25 SRAP primer combinations and 23 SSR primer pairs gave distinct band patterns among the 61 watermelon accessions. In SRAP analysis, a total of 312 bands were produced, of which 163 were polymorphic with 52.24% polymorphism (Table 2). The number of

Table 2. Data description of SRAP amplification among 61 watermelon accessions.

Primer combination[a]	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism rate (%)	DI value
me1-em1	9	5	4	44.44	0.594
me1-em2	11	8	3	27.27	0.364
me1-em4	11	6	5	45.45	0.582
me1-em5	15	9	6	40.00	0.757
me1-em6	12	6	6	50.00	0.637
me2-em1	16	6	10	62.50	0.811
me2-em2	12	5	7	58.33	0.575
me2-em3	13	7	6	46.15	0.664
me2-em4	11	6	5	45.45	0.730
me2-em5	13	9	4	30.77	0.590
me2-em6	12	8	4	33.33	0.552
me3-em1	8	4	4	50.00	0.630
me3-em2	10	4	6	60.00	0.663
me3-em3	15	4	11	73.33	0.854
me3-em5	14	5	9	64.29	0.824
me4-em1	10	5	5	50.00	0.640
me4-em2	14	6	8	57.14	0.783
me4-em3	17	7	10	58.82	0.834
me4-em4	12	4	8	66.67	0.790
me4-em5	11	6	5	45.45	0.507
me4-em6	13	3	10	76.92	0.874
me5-em2	18	6	12	66.67	0.887
me5-em3	14	8	6	42.86	0.593
me5-em4	10	6	4	40.00	0.486
me5-em5	11	6	5	45.45	0.587
Mean	12.5	6.0	6.5	52.24	0.672

[a] Primer names are the same as reported by Li and Quiros (2001).

bands per primer combination ranged from 8 (me3-em1) to 18 (me5-em2), with an average of 12.5 bands per primer combination. Each primer combination generated between 3 (me1-em2) and 12 (me5-em2) polymorphic bands, with an average of 6.5 bands per primer combination. The DI for each primer combination varied from 0.364 (me1-em2) to 0.887 (me5-em2), averaging 0.672. As for the SSR assay, 101 alleles were detected and the allelic numbers for primer pairs varied from 2 (MCPI-28 and MCPI-42) to 8 (MCPI-09 and MCPI-13) (mean = 4.4) (Table 3). All the markers generated expected product sizes, implying that SSR polymorphism originated from the variation of SSR length. No evidence of a null gene (defined as no amplification) was observed when using these SSR primers. In total, 87 polymorphic alleles (mean = 3.78) and 114 genotypes (mean = 5.0) were detected among the watermelon accession set. The DI value for each primer pair ranged from 0.132 (MCPI-42) to 0.754 (MCPI-05) and averaged 0.440.

3.2. Comparison of SRAP and SSR marker systems

The amplified results obtained using SRAP and SSR markers are compared and summarized in Table 4. Clearly, the SRAP assay produced more PCR products than the SSR assay, with a mean of 12.48 products per SRAP assay versus a mean of 4.39 products per SSR assay. However, the percentage of polymorphic products of the SSR assay was much higher than that of the SRAP assay (86.14% versus 52.24%), suggesting a high polymorphism of SSR markers. Three genetic parameters, DI, EMR, and MI, were calculated for SRAP and SSR markers (Table 4). In general, the EMR value of SSRs was set as 1.0 per marker. As a significant number of SRAP fragments were detected in one gel lane, SRAP revealed a higher mean number of polymorphic products per assay than SSR and thus had a higher EMR value (4.914). The mean DI value showed similarity to the EMR value. The marker parameter MI considers many possible attributes and offers a measure of overall utility of a given marker system. In our study,

Table 3. Data description of SSR amplification among 61 watermelon accessions.

SSR loci[a]	Product size (bp)	Number of alleles	Number of polymorphic alleles	Polymorphism rate (%)	Number of genotypes	DI value
MCPI-03	217	3	3	100.00	3	0.285
MCPI-04	237	6	5	83.33	7	0.539
MCPI-05	188	7	7	100.00	8	0.754
MCPI-07	249	5	5	100.00	5	0.539
MCPI-09	208	8	5	62.50	5	0.408
MCPI-11	241	5	2	40.00	4	0.266
MCPI-12	246	4	4	100.00	5	0.520
MCPI-13	211	8	6	75.00	7	0.712
MCPI-14	240	5	4	80.00	7	0.685
MCPI-15	241	4	4	100.00	5	0.369
MCPI-16	218	4	3	75.00	4	0.301
MCPI-20	273	3	3	100.00	4	0.364
MCPI-21	193	5	5	100.00	6	0.641
MCPI-23	174	3	3	100.00	4	0.365
MCPI-27	184	3	2	66.67	3	0.182
MCPI-28	285	2	2	100.00	3	0.260
MCPI-30	226	4	4	100.00	6	0.583
MCPI-32	264	3	3	100.00	6	0.449
MCPI-33	271	6	6	100.00	8	0.681
MCPI-37	166	3	3	100.00	4	0.457
MCPI-42	127	2	2	100.00	2	0.132
MCPI-44	158	3	2	66.67	3	0.192
MCPI-47	249	5	4	80.00	5	0.442
Mean		4.4	3.78	86.14	5.0	0.440

[a] Primer names are the same as reported by Joobeur et al. (2006).

Table 4. Comparison of the amplified results of information obtained using SRAP or SSR assays in 61 watermelon accessions.

Marker system	Total number of assays	Total number of products	Number of polymorphic products	Mean number of products per assay	Percentage of polymorphic products	Mean DI	Mean EMR	Marker index
SRAP	25	312	163	12.48	52.24	0.672	4.914	3.302
SSR	23	101	87	4.39	86.14	0.440	1.000	0.440

the MI value of SRAP was nearly 3 times that of SSR, indicating the higher discrimination power of SRAP assay in watermelon.

3.3. Genetic diversity structure determined by SRAP and SSR

Pairwise comparison was performed between the 61 watermelon accessions. Jaccard's similarity coefficients (SCs) were separately calculated using the SRAP and SSR data. The former generated a mean SC of 0.887 and the latter generated a lower value of 0.701, again reflecting polymorphic differences between the 2 marker assays. In the Chinese germplasm pool, SCs calculated from SRAP data ranged from 0.786 (W2 and S1) to 0.960 (X156 and X269) with an average of 0.917, whereas for SSR assay, SCs were between 0.321 (W3 and X61) and 1.00 (X58 and X43), with an average of 0.712.

Dendrograms were constructed based on the SCs from SRAP or SSR data using the UPGMA cluster method, and the 2 markers revealed high similarity in dendrogram topologies (Figures 1a and 1b).

In the dendrogram constructed by SRAP markers (Figure 1a), the 61 watermelon accessions were divided into 5 groups (I, II, III, IV, and V) at a SC of 0.846. Each of the 4 groups I, II, III, and V contained a single accession, i.e. PI296341 (I), W1 (II), W2 (V), and W3 (III). These 4 wild accessions from Africa and China had high genetic diversity and different backgrounds. The remaining 57 accessions, represented by the cultivated accessions from the United States, Japan, and China, were clustered into a broad group (IV). This cluster reflected the close relationships among the cultivated accessions regardless of their geographical heterogeneity and morphological differences. The cultivated accessions from China and foreign origins were mixed together and could not be distinguished further. In this group, 3 subgroups (IV-1, IV-2, and IV-3) could be identified. Subgroup IV-1 consisted of 2 edible-seeded watermelon accessions (S1 and S2), which were characterized by smaller, less sweet fruits with larger seeds. Subgroup IV-2 was heterogeneous; 42 Chinese cultivated germplasms (CCGs) and 6 foreign cultivars (Sugar Baby, Crimson Sweet, Jubilee, Zhengyin-2, Xindahe-6, and Miyako) were closely clustered herein. The accessions in this subgroup had high genetic closeness (SCs varying from 0.875 to 0.960; mean = 0.924), indicating that they had very similar lineages and a narrow genetic base. Interestingly, of the accessions, the diploid accession X43 and its autotetraploid X58 were not genetically identical (SC = 0.925) and belonged to different branches. Subgroup IV-3 consisted of 7 small-fruited watermelon landraces from northeastern China (NE) and northwestern China (NW), each with a higher level of diversity (SCs varying from 0.835 to 0.904; mean = 0.863).

SSR data were used to construct a dendrogram (Figure 1b) that showed a high similarity to the SRAP dendrogram in the positioning of the 61 watermelon accessions. Similarly, the 4 wild accessions (PI296341, W1, W3, and W2) were positioned in 3 groups (I, II, and IV) of the SSR dendrogram. Group III, which corresponded to group IV of the SRAP dendrogram, contained the rest of the 61 accessions. Two subgroups (III-1 and III-2) were identified in this group. The major subgroup, III-1, consisted of the 2 edible-seeded accessions (S1 and S2) and most cultivated germplasms from Chinese and foreign origins (i.e. the accessions from subgroup IV-2 of the SRAP dendrogram), with SCs ranging from 0.637 to 1.00 (mean = 0.822). The 6 cultivars (Sugar Baby, Crimson Sweet, Jubilee, Zhengyin-2, Xindahe-6, and Miyako) introduced from the United States and Japan were scattered among the Chinese accessions, again verifying their close lineages. The 2 accessions, X43 (diploid) and X58 (autotetraploid), that were separated in the SRAP dendrogram were genetically identical in the SSR dendrogram. The subgroup III-2 consisted of 7 diverse small-fruited accessions, which corresponded to subgroup IV-3 of the SRAP dendrogram. The SCs of these accessions varied from 0.423 to 0.651 and averaged 0.566.

3.4. Genetic diversity structure based on combined data of SRAP and SSR

The genetic diversity structure of the watermelon accessions on the basis of SRAP or SSR data separately evidenced a common pattern of molecular markers. First we investigated the goodness-of-fit of the SRAP and SSR systems. The matrices of the 2 markers were compared using the Mantel test (Mantel, 1967). The correlation coefficient of the 2 matrices was high ($\gamma = 0.863$), revealing quite a good fit between the SRAP and SSR systems and the efficiency of combination of the 2 assays in estimating genetic diversity

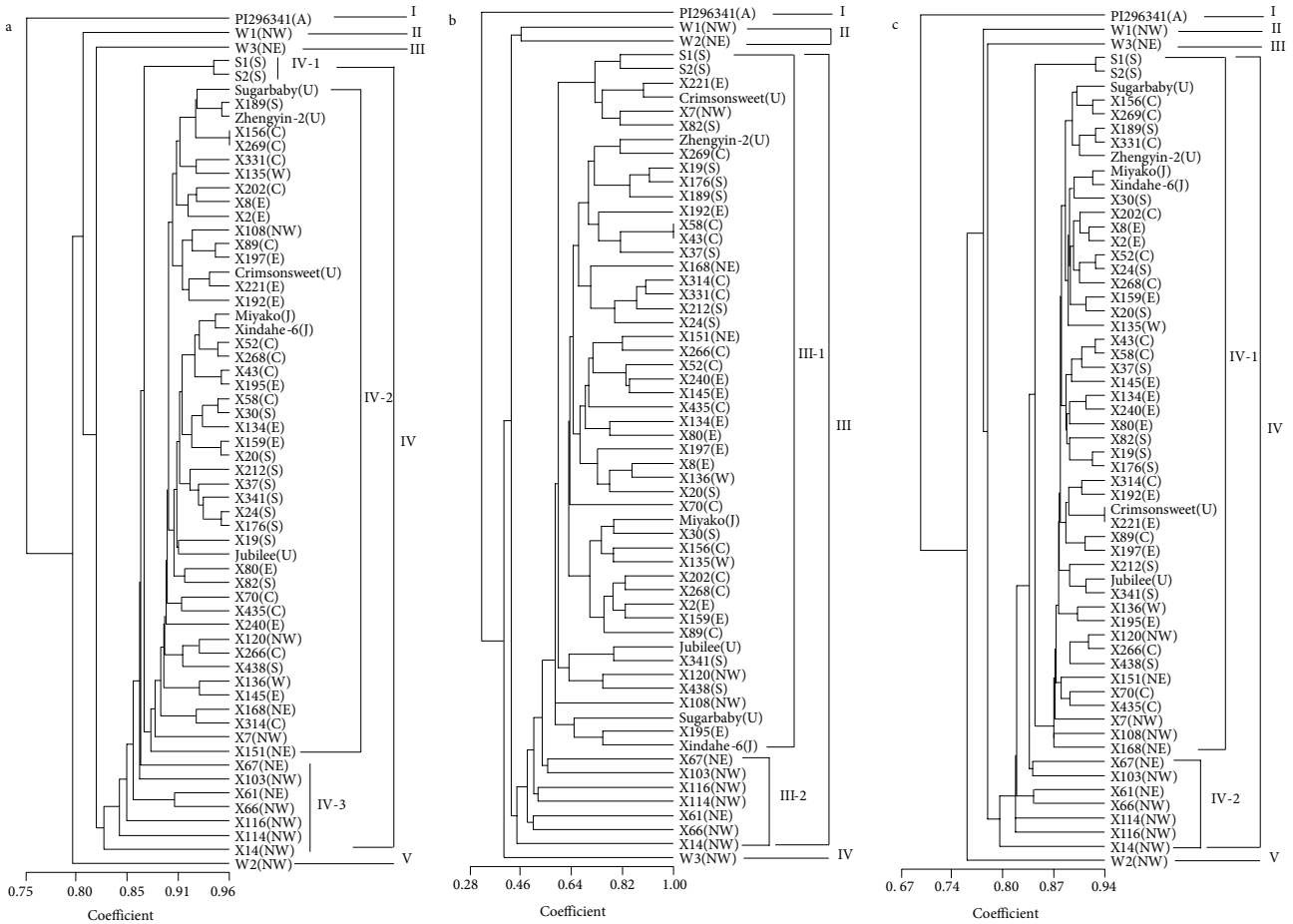

Figure 1. Cluster analysis dendrograms of 61 watermelon accessions using the unweighted pair-group method with arithmetic averages (UPGMA) using **(a)** SRAP data, **(b)** SSR data, and **(c)** SRAP-SSR combined data. Parentheses refer to the region: A: Africa; C: central China; E: eastern China; S: southern China; W: western China; J: Japan; U: United States; NE: northeastern China; NW: northwestern China.

in watermelon. Furthermore, the combined data of the 2 assays were used to reveal the accurate diversity structure of the accession set. The dendrogram constructed from SRAP-SSR combined data (Figure 1c) was very similar to both dendrograms from the SRAP and SSR data alone. As expected, those diverse accessions [the 4 wild accessions (Groups I, II, III, and V), the 2 edible-seeded accessions, and the 7 small-fruited accessions (Subgroup IV-2)] were clearly distinguished from the remaining accessions (78.7% of the total accessions; Subgroup IV-1). The remaining 42 CCGs and 6 foreign cultivars did not further separate based on their geographical origins or horticultural characters. Accession X221 was most similar to the cultivar Crimson Sweet, indicating that X221 might be a domesticated counterpart of Crimson Sweet under specific environmental conditions. To obtain an alternative view of the relationships among the accessions, PCoA was performed based on the SRAP-SSR combined data. The first 3 principal axes accounted for 38.26% of the total variance. In the two-dimensional PCoA diagram (Figure 2), most CCGs and the foreign cultivars were restricted to a small region (Group I), whereas the diverse accessions were dispersed in a wider region (Group II).

4. Discussion

4.1. SRAP and SSR markers

Both SRAP and SSR are effective marker systems and have been widely used to analyze genetic variation in a variety of plant species. SRAP targets open read frames and detects variation from the length of introns, promoters, and spacers in the genome (Li and Quiros, 2001). It is technically simple and requires no prior sequence information. SSR has high stability and can differentiate homozygotes and heterozygotes; however, one drawback is that primer development is time-consuming and expensive. Our results showed that both SRAP and SSR markers revealed a high discriminatory power in that almost all the accessions could be distinguished using

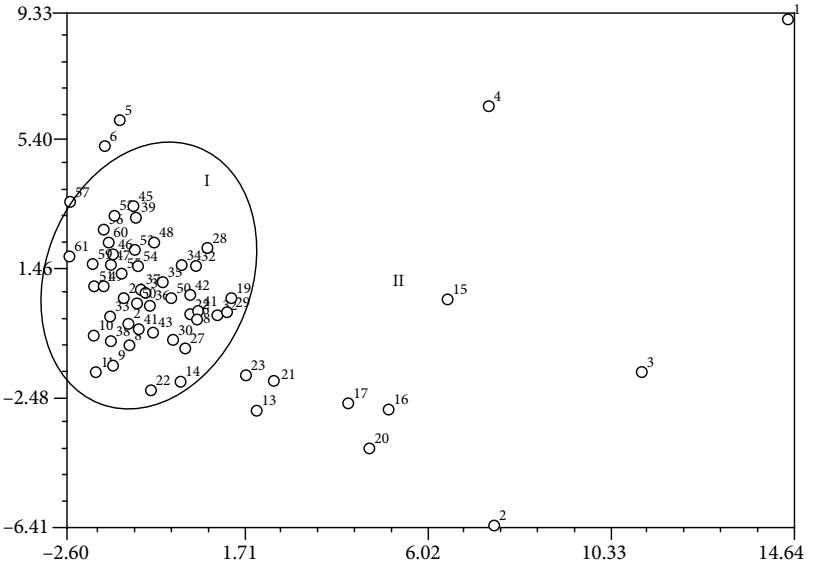

Figure 2. A two-dimensional plot of 61 watermelon accessions based on SRAP-SSR combined data using principal coordinate analysis. The numbers plotted represent individual accessions: 1, PI296341; 2, W1; 3, W2; 4, W3; 5, S1; 6, S2; 7, Sugar Baby; 8, X156; 9, X189; 10, Zhengyin-2; 11, X269; 12, Miyako; 13, X67; 14, X168; 15, X14; 16, X61; 17, X116; 18, X120; 19, X438; 20, X66; 21, X103; 22, X108; 23, X114; 24, X151; 25, X70; 26, X202; 27, X314; 28, Crimson Sweet; 29, X435; 30, X89; 31, X221; 32, X197; 33, X8; 34, X43; 35, X52; 36, X268; 37, Xindahe-6; 38, X331; 39, X58; 40, X266; 41, X2; 42, X192; 43, X135; 44, X136; 45, X134; 46, X159; 47, X240; 48, X80; 49, X145; 50, X195; 51, X19; 52, X20; 53, X30; 54, Jubilee; 55, X212; 56, X341; 57, X7; 58, X82; 59, X24; 60, X37; 61, X176.

the 2 markers, with the exception of X43 and X58, which failed to be distinguished from one another using SSRs. Compared to the locus-specific SSR markers, SRAP generated many more polymorphic bands and thus had higher values of DI, EMR, and MI (Table 4). Such results are in line with several published reports with data showing that SRAP was highly informative due to its high polymorphic percentages or PIC (Amara et al., 2011; Uzun et al., 2011). In detection of genetic diversity among closely related buffalo grass cultivars, Budak et al. (2004) found that SRAP revealed a higher discriminatory power than SSR, ISSR, or RAPD markers. Even if SSRs are preferable to SRAPs because of their reliability and codominant nature, our results indicated that the combination of SRAP and SSR data gave a better estimation of genetic diversity among the accessions. In a diversity study, combinations of different types of molecular markers can enhance the genome coverage and favor the detection of more overall variation in the genome (Mondini et al., 2009).

4.2. Genetic diversity and genetic background of the accession collection

In the present study, although SRAP and SSR markers gave different similarity levels among the accession set, the dendrograms generated from SRAP, SSR, or SRAP-SSR combined data revealed a high similarity for positioning of most accessions. Therefore, each of the molecular data sets seems to be sufficient to explore the genetic diversity of watermelon. All 61 accessions tended to be divided into 5 groups: 4 single accession-containing groups and 1 broad group that nested all the cultivated accessions from China and the other 2 countries (Figure 1). The 4 accessions, PI296341, W1, W2, and W3 (each belonging to a different group), are wild varieties, which are often neglected in modern breeding programs that aim to obtain high-yield cultivars. Certainly, the wild varieties are genetically diverse and have different genetic bases when compared to the cultivated accessions. PI296341 from Africa was the most genetically distant compared to the other accessions. This accession, *C. lanatus* var. *citroides*, is reported to be an important resource of resistance to Fusarium wilt, one of the most severe diseases in watermelon production (Martyn and Netzer, 1991). The 3 wild Chinese accessions (W1, W2, and W3) are found in arid areas or deserts in northern China and have a high tolerance to drought, cold, and infertility. These potentially valuable characters, however, do not seem to have drawn the attention of breeders. All the cultivated watermelon accessions, comprising those with different horticultural characters and geographical origins, were positioned in a broad group in different dendrograms with a high SC (0.902 for SRAP, 0.790 for SSR, and 0.882 for the SRAP-SSR combination). This indicated a narrow genetic base in cultivated watermelon

germplasms, especially in CCGs. This is in agreement with our previous study with chloroplast SSR markers, where 76.1% of CWGs shared a predominant haplotype (Hu et al., 2011). It is speculated that CWGs have experienced a genetic bottleneck in the domestication process, losing some variability. Similar results were also obtained with the watermelon germplasms from different countries by using other molecular tools (Lee et al., 1996; Levi et al., 2001; Che et al., 2003; Mujaju et al., 2010; Solmaz et al., 2010) or enzymes (Zamir et al., 1984). Most CCGs showed genetic closeness to the 6 foreign cultivars (Sugar Baby, Crimson Sweet, Jubilee, Zhengyin-2, Xindahe-6, and Miyako) as they were clustered closely in different dendrograms. Actually, a large number of CCGs have the lineages of foreign cultivars (Han et al., 2009). According to an investigation by Yang (1995), 82.7%, 44.5%, and 6.3% of the watermelon cultivars bred from 1950 to 1994 in China had lineages from the cultivars from Japan, the United States, and the former Soviet Union, respectively. Many cultivars (probably including the 6 foreign cultivars used in this study) introduced to China in the 1950s were frequently used in later breeding programs. This might partially explain the clustering of the cultivated accessions with multiple geographical distributions. Of the CWGs, the 7 small-fruited landraces (from NE and NW) were genetically diverse, as well as the 2 edible-seeded accessions. The 7 small-fruited landraces grow in northern China where the extreme climate conditions (a typical continental climate) may accelerate genomic variation within the accessions, whereas the 2 edible-seeded accessions are grown in southern China in a warm and wet climate and experience a directional selection for seed characters in the breeding process. This may be the reason for their higher diversity levels. These diverse accessions have uncommon morphological characters, e.g., short growth period, small and many-seeded fruits, and a thick rind. Obviously, they show some similar features to wild species, particularly to accession X4, which was assigned a rare haplotype in our previous study (Hu et al., 2011) and also revealed a resistance in field evaluations of disease resistance (data not shown). It is also possible, of course, that they involved rare hybridizations with some wild species.

In general, a diploid and its autotetraploids are genetically identical, but in the SRAP dendrogram (Figure 1a), X43 and its autotetraploid X58 were separated. It is likely that chemical mutagens (e.g., colchicine) caused base variations in the X43 genome in the polyploidization process. DNA sequence variation caused by polyploidization was observed in tobacco (Anssour et al., 2009). Liu et al. (2004) detected the genetic difference between diploid watermelon and its autopolyploids by using AFLP technology.

4.3. Implications for genetic improvement of Chinese watermelons

An understanding of the genetic characterization (both morphological and DNA-based) of crop populations covering representative samples from a wide geographical distribution is essential for the development of efficient breeding strategies seeking to improve agronomic characters. From our diversity analysis, we realized that the major CCGs (large- and medium-sized fruit types and partial small-fruited types) have a low level of genomic variation, contrary to their remarkable phenotypic diversity. This fact reinforces the need for germplasm enrichment (e.g., collection and plant breeding) to broaden diversity. Although we identified some genetically diverse accessions [i.e. the wild accessions, small-fruited accessions (from NE and NW regions), and edible-seeded accessions] that can be a potential resource for Chinese watermelon improvement, their number is relatively limited.

Germplasm collection is still essential for increasing the diversity and future genetic improvement of Chinese watermelons. More attention should be paid to collecting genetically diverse germplasms such as the wild accessions, landraces, and those with specific traits; the germplasms from diversity centers of watermelon should be of major concern as they are probably rich in more desirable genes (Varshney et al., 2005). The introduction of germplasms from the United States, Japan, and Russia is not necessary, as most CCGs already have these lineages in their germplasms. Utilization of genetic materials plays a crucial role in trait improvement, which depends on the introduction of desirable genes. Enhancement of disease resistance and stress tolerance in CCGs will likely be realized by introgression (e.g., backcrossing) of desirable genes from the wild species and the small-fruited landraces. Given the physiological and genetic variability in the accessions from northern China, broad-based population development (e.g., mass selection using the NW/NE accessions and those from other regions in China) might be more effective for trait improvement. As for crossbreeding with the aim of increasing yield, parent materials could be selected from NW and NE regions as much as possible, and the genetic distance of parents should be a first consideration, while geographic distance is not as important. The accessions from central and southern China have limited variability at the DNA level, but with morphoagronomic diversity (e.g., wide variation in the content of sugar, vitamin C, and lycopene) (Zhang et al., 2006), improvement of quality characters could be performed with these accessions by pedigree selection.

Acknowledgment

This work was supported by the National Science & Technology Pillar Program during the 11th Five-Year Plan period (No. 2009BADB8B02).

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