

Identification and validation of a core set of microsatellite markers for genetic diversity analysis in watermelon, *Citrullus lanatus* Thunb. Matsum. & Nakai

Haiying Zhang · Hui Wang · Shaogui Guo ·
Yi Ren · Guoyi Gong · Yiqun Weng · Yong Xu

Received: 2 June 2011 / Accepted: 31 October 2011 / Published online: 6 November 2011
© Springer Science+Business Media B.V. 2011

Abstract Watermelon, *Citrullus lanatus* Thunb. Matsum. & Nakai is an important vegetable crop worldwide. Due to its narrow genetic base, detection and utilization of the genetic variations, cultivar identification and increasing genetic diversity are some important tasks for watermelon breeders. Molecular markers, especially microsatellites or simple sequence repeats (SSRs) are playing increasingly important roles for these purposes. In the present study, a core set of 23 highly informative SSR markers was developed for watermelon genetic diversity analysis. Based on whole genome sequencing of 17 watermelon inbred lines, we identified 3.9 million single nucleotide polymorphisms (SNPs) which were used to construct a SNP-based dendrogram for the 17 lines. Meanwhile, from the sequenced genome, 13,744 SSRs were developed, of which 704 were placed on a high-resolution watermelon linkage map. To develop the core set SSR markers, 78 of the 704 mapped SSRs

were selected as the candidate markers. Using the SNP-based dendrogram as calibration, 23 SSR markers evenly distributed across the genome were identified as the core marker set for watermelon genetic diversity analysis. Each marker was able to detect 2–7 alleles with polymorphism information content values ranging from 0.45 to 0.82. The dendrograms of 17 watermelon lines based on SNPs, the base set of 78 SSRs and the core set of 23 SSRs were highly consistent. The utility of this core set SSRs was demonstrated in 100 commercial watermelon cultivars and elite lines, which could be placed into six clusters that were largely consistent with previous classification based on morphology and parentage data. This core set of SSR markers should be very useful for genotyping and genetic variation analysis in watermelon.

Keywords *Citrullus lanatus* · Fingerprinting · Genetic diversity · Microsatellites · Watermelon

H. Zhang · H. Wang · S. Guo · Y. Ren ·
G. Gong · Y. Xu (✉)
National Engineering Research Center for Vegetables,
Beijing Academy of Agricultural and Forestry Sciences,
Beijing 100097, China
e-mail: xuyong@nercv.org

Y. Weng
USDA-ARS Vegetable Crops Research Unit, Horticulture
Department, University of Wisconsin, Madison,
WI 53719, USA

Introduction

Watermelon, *Citrullus lanatus* Thunb. Matsum. & Nakai is an important vegetable fruit crop. Total yield in 2009 was over 100 million tons accounting for 10.7% of total vegetable production (FAO Statistics 2009, <http://www.fao.org/>). China is currently the top watermelon producer with 68.2 million tons in 2009

which was 67.7% of the world production, Due to its economic and nutraceutical importance, watermelon is gaining more popularity throughout the world (Padmavathi et al. 2010). Each year, a large number of varieties are being released and commercialized (Wehner 2002). However, genetic diversity within watermelon cultivars is low. For example, Levi et al. (2001b) found high genetic similarity values (92–99%) among watermelon cultivars, which make them especially vulnerable to attacks by the large numbers of diseases of insect pests of watermelon. Therefore, in China, there are increasing interests from both growers and watermelon breeders in introducing new germplasm from different geographical regions to enhance watermelon genetic diversity.

Watermelon belongs to the genus *Citrullus* (family Cucurbitaceae) and is the only cultivated species in this genus (Bisognin 2002). It is believed to have originated in Africa (Simmonds 1979) but is now widely spread throughout the world. The primary gene pool for watermelon breeding includes three major forms: *C. lanatus* var. *lanatus* (*C. l.* var. *lanatus* hereinafter), *C. l.* var. *citroides* and *C. colocynthis* (Jeffrey 2001). *Citrullus lanatus* var. *lanatus* includes both wild and cultivated types (Jeffrey 2001). *Citrullus lanatus* var. *citroides*, the cow-melons (Tsamma and citron types) are useful as rootstocks for their disease resistances (Dane and Lang 2004; Thies et al. 2010). *Citrullus colocynthis*, the bitter apple grown for medicinal purposes, is considered a likely ancestor of watermelon (Burkill 1985; Navot and Zamir 1987; Jarret et al. 1997). All of them are valuable genetic resources in watermelon improvement.

Assessment of genetic variation is important for not only watermelon breeding but also efficient management and protection of germplasm resources (Krishna et al. 2004). Meanwhile, cultivar identification is necessary for protecting breeders' rights. Detection and utilization of the genetic variation and cultivar identification are some important tasks for watermelon breeders.

Traditional evaluation of genetic variation and germplasm management is based on a set of morphological descriptors, which, however, are limited and often influenced by environments. In addition, many modern varieties and hybrids are phenotypically less distinct making morphological evaluation more difficult. It is usually difficult to classify more than 200 accessions at one time based solely on their

morphological characteristics. Therefore, the development of reliable methods is necessary to allow for the assessment of genetic diversity and cultivar identification (Naito et al. 2008).

Over the years, a number of molecular marker systems have been used for watermelon genetic diversity and cultivar identification studies including isozymes (Navot and Zamir 1987), random amplification of polymorphic DNA (RAPD) (Williams et al. 1990), amplified fragment length polymorphism (AFLP) (Vos et al. 1995), sequence-related amplified polymorphism (SRAP) (Li and Quiros 2001), and high frequency oligonucleotides targeting active genes (HFO-TAG) (Levi et al. 2010). Analysis using isozymes (Navot and Zamir 1987) and RAPD markers (Levi et al. 2000; Harris et al. 2008) revealed low DNA polymorphism among cultivated watermelons. For example, of 1,309 RAPD markers, only 75 were polymorphic (5.9%) between two watermelon breeding lines (Harris et al. 2008). Other marker systems such as AFLP (Che et al. 2003; Levi et al. 2004), SRAP (Levi et al. 2006; Zhang et al. 2008), EST-PCR (Levi et al. 2009) and HFO-TAG (Levi et al. 2010) produced slightly higher polymorphism among watermelon cultivars than the RAPD markers, but all in general were not breeder friendly. Results from these genetic assays were hardly comparable due to lack of a common core set of reference genotypes and the use of different marker systems. Therefore, it is desirable to develop a more robust, efficient and cost effective marker system that allows integration and amalgamation of data sets gathered at different times and in different laboratories.

Among all marker systems, single nucleotide polymorphism (SNP) (Coryell et al. 1999) is the best marker for marker-based studies. However, at present, the high cost associated with SNP genotyping prevents it from wide use by most watermelon breeding programs. It is especially not practical for small laboratories in developing countries; a gap exists between SNP discovery and reduction to practice (Appleby et al. 2009). At present, a marker of choice is microsatellite or simple sequence repeat (SSR) (Tautz 1989). There are a number of advantages for using SSRs such as their co-dominant and multi-allelic nature, highly reproducibility (Oliveira et al. 2006; Ibrahim et al. 2010), which is very suitable for accession discrimination and assessment of genetic variation (Ebana et al. 2008).

In watermelon, SSRs have recently been used for germplasm characterization (Jarret et al. 1997; Guerra-Sanz 2002; Mujaju et al. 2010), evaluation of genetic diversity (Padmavathi et al. 2010), germplasm management (Zhang et al. 2010), cultivar identification (Levi et al. 2009), and genetic mapping (Levi et al. 2006). For cultivar identification, evaluation of genetic diversity or whole genome genetic mapping studies, full coverage of representative SSRs is needed. In the real world, it is not practical and economic to test hundreds of SSRs (Varshney et al. 2008). Identification of a core set of representative markers with adequate resolving power and genome coverage might be a method of choice for this purpose.

Compared with many other crops (Xie et al. 2010; Huang et al. 2009; Ebana et al. 2008; Federico et al. 2008), watermelon genome-wide SSRs are limited. However, this situation is changing rapidly due to technology development, especially in whole plant genome sequencing. In 2008, The National Engineering Research Center for Vegetables (NERCV) of Beijing, China and its collaborators initiated the International Watermelon Genome Project to sequence the watermelon genome. Using the next-generation Illumina Genome Analyzer (GA) sequencing technology, an elite East Asian ecotype watermelon inbred line 97103 (*C. l.* var. *lanatus*) was sequenced and a draft genome assembly is near completion (Xu et al. manuscript in preparation). To explore genetic diversity for watermelon improvement, we also re-sequenced the genomes of 16 additional watermelon genotypes including three plant introduction (PI) lines of *C. l.* var. *citroides*, two PIs and ten cultivars of *C. l.* var. *lanatus*, and one PI of *C. colocynthis*. This effort has led to genome-wide discovery of SSRs and SNPs. So far, 3,889,080 SNPs and 13,744 SSRs have been detected. Using these markers, a high-density watermelon genetic map was developed. These resources provide us unprecedented opportunities to explore the watermelon genome for breeding purposes.

The objective of this study was to develop a core set of microsatellite markers that can be used for evaluation of genetic diversities of watermelon collections. We first built a dendrogram of the 17 watermelon genotypes with 3.9 million SNPs. Using this dendrogram as a calibration, we identified a core set of 23 SSRs from 704 SSRs that have been mapped in our high-resolution genetic map. The utility of this core set

of SSRs was further demonstrated in 100 commercial cultivars and elite lines.

Materials and methods

Plant materials

Two sets of materials were employed in this study. The first set included 17 *Citrullus* genotypes with diverse genetic backgrounds and horticultural traits, which have been used in whole genome re-sequencing in the senior author's lab. The characteristics of these 17 lines are summarized in Table 1. They were chosen as representatives of the watermelon collection with maximum diversity.

The second set included 100 commercial cultivars and elite lines which were used to assess the potential utility of the core set SSR markers developed in this study. Twenty of these lines were closely related based on their pedigrees. The names and seed sources of these 100 accessions are provided in Table 2.

SNP discovery through whole genome re-sequencing in watermelon

We conducted whole genome sequencing and assembly of an elite East Asian ecotype watermelon inbred line 97103 using Illumina GA (Illumina, Inc., San Diego, USA) at Beijing Genome Institute (BGI)-Shenzhen. The whole genome was sequenced at $107.4 \times$ coverage of the 97103 haploid genome (430 Mbp), and the assembly contained 184 N90 scaffolds (Xu et al. manuscript in preparation). This 97103 watermelon draft genome assembly was used as reference for SNP discovery.

An additional 16 genotypes (Table 1) were re-sequenced at $6.3\text{--}18.8 \times$ depth each using the Illumina GA platform. All high quality (HQ) GA reads of these 16 watermelon lines were aligned against the 97103 draft reference genome using SOAPaligner (Li et al. 2009a) with the following criteria: if an original read could not be aligned to the reference sequence, the first nucleotide at the 5' end and two nucleotides at the 3' end were removed and the modified read was realigned to the reference again. If there was no alignment, two more nucleotides at 3' end were removed from the sequence. This procedure was repeated until alignment was achieved or until the read

Table 1 Characteristics of 17 watermelon lines used for whole genome re-sequencing and genetic diversity analysis

Line #	Line name	Taxonomy status ^a	Ecotype ^b	Seed Source	Fruit shape	Rind features	Fruit weight(kg)	Soluble solid content (Brix)	Flesh color	Taste	Disease resistance*
GS1	97103	<i>Cll</i>	EA	NERCV, China	Round	Medium green with narrow, medium dark green stripes	5.03	11.1	Pink	Crisp and sweet	
GS2	RZ-900	<i>Cll</i>	AM	Rijk Zwaan Co, The Netherlands	Round	Light green with netted	5.30	10.9	Red	Crisp and sweet	
GS3	RZ-901	<i>Cll</i>	EA	Rijk Zwaan Co, The Netherlands	Round	Light green with narrow, medium dark green stripes	4.87	8.3	Red	Crisp and sweet	
GS4	Sugarlee	<i>Cll</i>	AM	NERCV, China	Round	Light green with narrow, medium dark green stripes	4.90	10.4	Pink	Crisp and sweet	AN, race 1; Fw races 0 and 1
GS5	JX-2	<i>Cll</i>	EA	NERCV, China	Round	Medium green with narrow, medium dark green stripes	3.07	10.5	Pink	Crisp and sweet	Fw races 0 and 1
GS6	JLM	<i>Cll</i>	EA	NERCV, China	Round	Medium green with very narrow, medium dark green stripes	2.65	10.3	Yellow	Crisp and sweet	
GS7	JXF	<i>Cll</i>	EA	NERCV, China	Elongated	Medium green with very narrow, medium dark green stripes	1.57	10.8	Red	Crisp and sweet	
GS8	XHBFGM	<i>Cll</i>	EA	NERCV, China	Round	Light green with netted	2.22	10.8	Red	Crisp and sweet	
GS9	Calhoun Gray	<i>Cll</i>	AM	NERCV, China	Elongate	Light green with netted	3.70	8.5	Red	Crisp and sweet	Fw races 0 and 1
GS10	Black Diamond	<i>Cll</i>	AM	NERCV, China	Round	Dark green	3.75	9.4	Red	Crisp and sweet	
GS11	PI296341-FR	<i>Clc</i>		Cape Province, South Africa	Round	Medium green with solid stripes	1.65	0.7	White	Tough and bitter	Fw races 0, 1, and 2
GS12	PI595203	<i>Cll</i>	EG	Georgia, USA	Round	Light green with solid strips	1.90	1.7	White	Tough and not sweet	ZYMV, PRSV, WMV
GS13	PI386019	<i>Cc</i>		Iran	Round	Light green with intermittent stripes	0.09	0.9	White	Tough and bitter	PM, SL
GS14	PI482271	<i>Cll</i>	EG	Zimbabwe	Round	Dark green with hazy, broad, medium dark green stripes.	2.00	7.0	Pink	Soft and little sweet	PM

Table 1 continued

Line #	Line name	Taxonomy status ^a	Ecotype ^b	Seed Source	Fruit shape	Rind features	Fruit weight(kg)	Soluble solid content (Brix)	Flesh color	Taste	Disease resistance*
GS15	PI482303	<i>C/c</i>		Zimbabwe	Oblong	Medium green with solid stripes	3.05	1.4	White and yellow	Tough and bitter	RKN
GS16	PI482276	<i>C/c</i>		Zimbabwe	Round	Dark green with solid strips	0.74	0.7	White	Tough and not sweet	GSB
GS17	Sy-904304	<i>C/l</i>	AM	Syngenta Seeds Inc., USA	Elongate	Light green with wide stripe	8.04	9.0	Red	Crisp and sweet	Fw race 1 and AN

*AN anthracnose; *Fw fusarium* wilt; *GSB* gummy stem blight; *PM* Powdery mildew; *PRSV* papaya ringspot virus; *RKN* root knot nematode; *SW* silverleaf whitefly; *WMV* watermelon mosaic virus; *ZYMV* zucchini yellow mosaic potyvirus

^a *C/l* *C. lanatus* var. *lanatus*, *C/c* *C. lanatus* var. *citroides*, *Cc* *C. citroides*

^b *EA* East Asian, *AM* American, *EG* Egusi

length was shorter than 27 bp. The average sequencing depth and coverage was calculated using the alignment results.

For SNP detection among the 17 watermelon lines, the following three steps were taken. (1) The computer program SOAPSNP (Li et al. 2009b) was used to calculate the likelihood of each individual's genotype based on the alignment results. A Bayesian model was applied to calculate the probability of individual's genotypes with the actual data; (2) All the individual likelihood files were integrated to produce a pseudo-genome for each site in all 17 samples by maximum likelihood estimation (MLE). Sites meeting criteria in copy number, sequencing depth, quality score and minor allele counts were kept for the following rank sum test adjustment. SNPs passing the rank sum test ($P \geq 0.005$) were fixed as members of the HQ SNP set; (3) Base types were allocated back to each individual based on genotypes of HQ SNPs and each individual likelihood file. The genotype with the highest likelihood was chosen as the consensus genotype in each individual. Overall, of the total 64.2 Gb genome DNA sequences generated, 84.4% were aligned to the reference genome. The average sequencing depth was $10.2\times$ and 95.8% of the reference genome was covered by the re-sequenced reads. In total 3,889,080 SNPs were detected across the genomic sequences among the 17 genotypes. The details of resequencing data for the 16 lines are shown in Table 3.

Strategies to develop a core set of SSR markers in watermelon

To identify a core set of SSR markers that can be used for evaluation of genetic diversity in the majority of watermelon collections, we first developed a dendrogram of 17 re-sequenced watermelon lines based on the nearly 3.9 million SNPs, which was then used as a model for validating the efficacy of SSR markers selected. Bioinformatic analyses of the 97103 draft genome identified 13,744 SSRs, 3,759 insertion and deletion (InDel) polymorphisms, and 584 structure variation (SV) markers. These markers were used for the construction of a high-density watermelon linkage map using 103 F₈ recombinant inbred lines (RILs) derived from 97103 \times PI 296341-FR (Ren et al. manuscript in preparation). Line 97103 belongs to *C.l.* var. *lanatus* and PI 296341-FR is an accession of

Table 2 List of watermelon cultivars or breeding lines genotyped by SSR markers

Line #.	Line name
FP1	Mujing
FP2	Jingxin No.1
FP3	98R
FP4	ZhongZi Jingxin
FP5	Jingxin No.2
FP6	WB917
FP7	Jingxin No.3
FP8	WB302
FP9	Jingxin No.4
FP10	DaZi Jingxin
FP11	WB217
FP12	Jingxin No.5
FP13	AU-SWEET-SCARLET
FP14	Jingxin No.6
FP15	LM
FP16	K209
FP17	WB357
FP18	Jingxin No.7-1
FP19	Jingxin No.7-2
FP20	WB303
FP21	WB198
FP22	WB159
FP23	WB102
FP24	Jingxin No.8-1
FP25	Jingxin No.8-2
FP26	MuFENG
FP27	She
FP28	K210
FP29	FuFENG
FP30	Guofeng-1
FP31	Guofeng-2
FP32	Guofeng-3
FP33	Guofeng-4
FP34	Guofeng-5
FP35	T326
FP36	Guobao
FP37	Mu948-1
FP38	Fu948-1
FP39	948
FP40	Mu948-2
FP41	Fu948-2
FP42	948-2
FP43	T2
FP44	Yi

Table 2 continued

Line #.	Line name
FP45	Zaojia
FP46	GreenYi
FP47	Improved Zaojia
FP48	C2
FP49	Mushuang
FP50	Fushuang
FP51	Shuangxing
FP52	Huaxin-1
FP53	Huaxin-2
FP54	Mugui
FP55	Fugui
FP56	Weilong
FP57	New102
FP58	FuHuangJingxin No. 1
FP59	HuangJingxin No.1
FP60	Wbwrite
FP61	Muxiu
FP62	Jingxiu
FP63	MuWang
FP64	Fuwang
FP65	Jingli
FP66	Fulan
FP67	JingLan
FP68	Tianhuang
FP69	T409
FP70	T351
FP71	T409351
FP72	SD
FP73	Jingling-1
FP74	Ming58
FP75	Jingling-2
FP76	Jingling-3
FP77	WB505
FP78	Jingling-4
FP79	Fumo
FP80	Shuangma
FP81	Seedless JingxinNo.1
FP82	Heimi
FP83	SelfHei
FP84	Seedless hei
FP85	MuXin
FP86	FuXin
FP87	Xinyihao
FP88	Improved Fuxin
FP89	Improved Xinyihao

Table 2 continued

Line #.	Line name
FP90	Fengle No.1 ^a
FP91	Zhengkang No.8 ^a
FP92	Heishuai ^a
FP93	Jubao No ^a .1
FP94	Tian Niu ^a
FP95	Fengle No.5 ^a
FP96	Aomi 001 ^b
FP97	Dongmi 001 ^b
FP98	Seedless guangxi ^c No.3
FP99	Seedless Indian
FP100	FuJX

Seed source seeds of all materials were provided by NERCV, China except the following

^a Fengle Seed Co.China

^b Northeast Agriculture University, China

^c Guangxi Academy of Agricultural Sciences, China

C.l. var. citroides. The high-resolution map includes 704 SSRs, 219 InDels and 36 SVs spanning ~800 cM. The 704 SSRs were the starting marker set for developing the core set of SSRs. Our objective

was to eliminate sequentially the SSR markers with low power of discrimination to get a final combination of markers that can be use for genetic diversity analysis.

The 704 SSRs that were polymorphic between 97103 and PI 296341-FR were further tested for polymorphism between two elite cultivated watermelon lines 97103 and Sugarlee (Crall and Alstrom 1983). 97103 is East Asian ecotype whereas Sugarlee is a North American ecotype. The underlying reasoning for this was that watermelon has a narrow genetic base (Levi et al. 2001a, b). Consequently, markers that were polymorphic between the two cultivated elite lines would likely be more useful for genetic diversity studies for other cultivated lines. As a result, 78 SSRs that were polymorphic between 97103 and Sugarlee were identified.

Next, a unweighted pair-group method with arithmetic mean (UPGMA) dendrogram (Sneath and Sokal 1973) was built using the 78 SSRs for the 17 watermelon genotypes (Table 1) to evaluate their ability to differentiate these watermelon lines. Since the 78 SSRs provided the same resolution in resolving these 17 lines, they were used as candidates for selection of the core SSR set. Dendrograms were built by sequentially decreasing the number and varying the combinations

Table 3 Polymorphisms of 16 *Citrullus* genotypes as compared with the watermelon line 97103 based on whole genome re-sequencing

Line name	Sequencing depth (×) ^a	Genome coverage%	# of SNPs ^b	# Indels ^c	Structural variations
JX-2	9.17	98.37	36581	5525	4684
JLM	9.16	98.38	51045	9177	4811
JXF	9.17	98.52	56403	11517	5197
Calhoum Gray	9.15	98.50	68968	13681	5169
PI 482271	7.17	96.73	60978	30983	3853
Black Diamond	9.00	98.39	75784	15792	5053
XHBFGM	9.28	98.52	70517	22401	5760
Sugarlee	9.20	98.52	78897	27380	5536
RZ-900	18.51	99.05	103595	33612	5148
RZ-901	18.79	99.15	131257	31235	5869
Sy-904304	12.99	98.14	126276	39250	5286
PI 595203	9.27	97.74	132517	58064	2973
PI 482303	6.41	89.46	520595	177658	8169
PI 296341-FR	12.5	91.80	1448864	171641	7742
PI 386019	6.30	82.26	1662809	73458	7987
PI 482276	6.75	89.33	1663039	190415	8990

^a Expressed in haploid genome equivalent of the watermelon genome

SNPs single nucleotide polymorphisms, *InDel* insertion/deletion

of SSRs. A final core set was determined based on the following criteria: (1) The SSR-based UPGMA dendrogram resembled the SNP-based dendrogram; (2) Polymorphic information content (PIC) value was over 0.45; (3) all SSRs evenly distributed across the watermelon genome with at least one marker from each linkage group; (4) easy to use in PCR assay (clear PCR product under standard reaction conditions); (5) each SSR detected a single locus. Eventually, 23 SSRs meeting these criteria were selected. Information (marker name, primer sequences, PIC) of all 23 SSR markers is listed in Table 4. Lastly, the utility of this core set of 23 SSRs was validated in 100 commercial cultivars and elite lines.

SSR marker analysis

All tested watermelon lines were grown in the greenhouse at 25/18°C day/night temperatures. For each line, young tender leaves were collected from three 15-day old seedling plants and pooled, and stored at –80°C for DNA isolation following Levi and Thomas (1999).

Each 15 µl PCR reaction mixture contained 20 ng template DNA, 4 µM each of the left and right primers, 2.5 mM MgCl₂, 2 mM each of dNTPs, 0.5 U *Taq* DNA polymerase in 1× PCR buffer (Takara Company, China). The PCR reaction started with 94°C for 5 min followed by 35 cycles of 94°C for 20 s, 55°C for 20 s, and 72°C for 90 s with a final extension at 72°C for 8 min. The PCR products were analyzed using 6% polyacrylamide gel electrophoresis in 1× TBE buffer. The gel was stained with silver staining using the SILVER SEQUENCE DNA Sequencing System (Promega, Madison, WI, USA).

Data analysis

For genetic diversity analysis of SNP data, individual SNPs were used to calculate genetic distances between watermelon lines. The p-distance between two individuals *i* and *j* (Tamura et al. 2004) was defined as follows.

$$D_{ij} = \frac{1}{L} \sum_{i=1}^L d_{ij}^l$$

where *L* is the length of the region in which HQ SNPs were identified. For A/C alleles at position *l*,

$$d_{ij}^l = \begin{cases} 0, & \text{if genotypes of the two individuals are AA and AA} \\ 0.5, & \text{if genotypes of the two individuals are AA and AC} \\ 0.5, & \text{if genotypes of the two individuals are AC and AC} \\ 1, & \text{if genotypes of the two individuals are AA and CC} \end{cases}$$

The neighbor-joining method (Saitou and Nei 1987) was then used to construct the dendrogram on the basis of the distance matrix calculated by the software MEGA5 (Tamura et al. 2011). Bootstrap values were calculated in 1,000 replicates.

Polymorphic SSR markers were scored for the presence or absence of the corresponding bands among the tested accessions. Stutter and background bands were excluded. The scores ‘1’ and ‘0’ indicated the presence and absence of the bands, respectively. The polymorphism information content (PIC) of each marker was calculated according to Smith et al. (1997) as follows: $PIC = 1 - \sum P_i^2$, where *P_i* is the band frequency of the *i*th allele.

Genetic similarities between the 17 re-sequenced genotypes were calculated using Nei and Li’s coefficients index (Nei 1977) with the Freetree software (Pavlicek et al. 1999). The dendrogram was constructed with the same software using UPGMA. The robustness of nodes was inferred by bootstrap analysis of 1,000 replicates.

The dendrogram of all 117 watermelon lines based on 23 SSR markers was constructed using software, Powermarker v3.0 (Liu and Muse 2005; <http://statgen.ncsu.edu/powermarker/>), and visualized with Dendroscope (Huson et al. 2007; <http://www.dendroscope.org>).

Results and discussion

Polymorphisms among 17 *Citrullus* genotypes based on whole genome re-sequencing

The whole genomes of 16 watermelon lines were re-sequenced with the Illumina GA platform with 6.3–18.8× genome coverage. Alignment of the these genome sequences with the 97103 reference draft genome revealed DNA-level sequence polymorphisms which included SNPs, Indels and other structural changes. Details of polymorphisms of each genotype as compared with 97103 are summarized in Table 3. Among these re-sequenced lines, JX-2 and 97103 had the lowest polymorphism (46,790); whereas PI 482276 and 97103 had the highest (186,244, Table 3). In total 3,889,080 SNPs were

Table 4 Information of 23 core set SSR markers for watermelon genetic diversity analysis

SSR marker	Forward primer sequence	Reverse primer sequence	LG*	Scaffold	PIC value
BVW/S00948	TCAAACCGACTGCCATATCA	AGCTTGCTTCCTGGCCTTT	LG1	Scaffold162	0.63
BVW/S00155	TGGATCATTTGACAGATTTAGCGA	CATCACAGTTAAGCATCACAAAGGC	LG1	Scaffold180	0.63
BVW/S00297	ACAACTTTGATTGATTCACGATG	AAGTGAAGACCCCTTTCCCAAAC	LG2	Scaffold1630	0.69
BVW/S00314	GAGGAGAATCGGTTCTTGGACATA	TTGAGCATCCTTGGGACTATCAAT	LG2	Scaffold194	0.60
BVW/S00048	TCAAAGGTTTGGCCTAAATGAAA	TGCTGATCCTCCATCTTAAACCTC	LG3	Scaffold1660	0.55
BVW/S00208	GCAAAGATTGTCTATGAAGCAGCA	GCTCATTTGGCTTCTTGAATCTGTT	LG4	Scaffold170	0.64
BVW/S01734	AAATACATCTTAAATGGGCC	GGAAACATTGACTTCAATCAGCA	LG4	Scaffold125	0.74
BVW/S00441	TGGTTGAAATCAATAAAAAGTGAA	TGGATGTTTTTGGCATTGGA	LG5	Scaffold193	0.69
BVW/S00658	TTAGCCTAAGCAAAGGGTTTTT	AAGTACACATTTTAAACAATCAATCCA	LG5	Scaffold1368	0.60
BVW/S00106	TGGCCTAGAAGATTATTGAGCTGC	CATTATCACATGGCAGATAATGGAAA	LG5	Scaffold108	0.69
BVW/S00233	AAACCATGATTTTACAGGGGATCA	TTTCTGCTTCTTTTGGACCAATGC	LG6	Scaffold260	0.73
BVW/S01897	TTCTTGAAACTCAACCCCTCAA	AAAGCGTGTGAGTGTGAGA	LG6	Scaffold44	0.70
BVW/S00433	TCTTTAAGTTTTGAGGGAGAGC	TTCCCAAGCTAGCCTTTTCA	LG7	Scaffold111	0.82
BVW/S00170	AACGCAGATAGTTAGAAGG	TGACTAATTAACACTACACTCAGACT	LG7	Scaffold505	0.45
BVW/S00369	TGAGAAAATGGAAGATGCAAATGA	TTCTTCTCACTCTCTCTAAGATTTTGC	LG8	Scaffold349	0.73
BVW/S00826	ATGGTTCATTTTTCACGTTCCG	AAAAATCAAAGCAAAGAAACAACAT	LG8	Scaffold12	0.56
BVW/S00209	TGCTTCAAAAATCTAATCACAATTTGC	TTCTTGGTTTCGGGTTTCTTTACA	LG9	Scaffold390	0.79
BVW/S00333	TGTTGAGATTTTIGATTTCAACTGT	TGGGTCAAAGTATTTTTGCTTTTT	LG9	Scaffold7	0.73
BVW/S01843	CCCCCGCCAAAATTAATA	CACCCGTGTAAGGTGGTAAA	LG9	Scaffold109	0.63
BVW/S02048	TCTGTGGATGCAAAATGGT	GCTAATCGAGCCCAAGTTACG	LG10	Scaffold300	0.67
BVW/S00236	CTTGAGCAATTTGGCTTCTAGTGT	GTCAAAATGTCTTTGATTTCCCAA	LG10	Scaffold659	0.62
BVW/S00228	GGAAGATGAGGTGATAAATCAATATGT	AATTGGCCCAATAATCCATATGAC	LG11	Scaffold31	0.74
BVW/S00839	TTCCACACCAAGGAGGTAGG	CATGTCAATTCGATAAAGCAGAAA	LG11	Scaffold116	0.53

* LG linkage group

detected across the genomic sequences among the 17 genotypes.

SNP-based genetic diversity among 17 *Citrullus* genotypes

Based on 3,889,080 SNPs across 17 re-sequenced watermelon genotypes, a UPGMA dendrogram was built (Fig. 1a), which clearly separated 17 *Citrullus* genotypes into two branches. The first branch was *C. colocynthis*, and the second one belonged to *C. lanatus*, which further split into two sister clades of var. *citroides* and var. *lanatus*. The var. *lanatus* subclade had three clusters. The two wild accessions (PI 595203, PI 482271) formed the first and the second cluster, respectively. The third cluster had the cultivated types, which had two sub clusters: the East Asian ecotype and the American ecotype. The East Asian ecotype sub-cluster included 97103, JX-2, JLM, JXF, RZ-901 and XHBFGM, while the American ecotype sub cluster was composed of Black Diamond, Calhoun Gray, Sugarlee, RZ-900 and Sy-904304 (Fig. 1a).

This SNP-based dendrogram was consistent with previous classifications of these 17 lines based on morphological and parentage data (Wehner 1999a, b, 2002). For example, Calhoun Gray, Black Diamond and Sugarlee all belong to the American ecotype although they have different fruit characteristics. Calhoun Gray, derived from Calhoun Sweet × Charleston Gray,

produces elongated fruit resembling to that of Charleston Gray. Black Diamond produces globular fruit with dark green skin which is similar to that of Calhoun Sweet. Calhoun Gray and Black Diamond were closely clustered likely due to their common parent Calhoun Sweet in the pedigrees. Calhoun Gray and Sugarlee (derived from Texas W5, Summit, Charleston Gray, Fairfax, Crimson Sweet, and Graybell) also shared a common parentage with Charleston Gray. These three cultivars of the American ecotype showed close genetic similarity based on SNP clustering (Fig. 1a). Similarly, cultivars of East Asian ecotype 97103 and JX-2 appeared to be closely related. Both were derived from same parent with mid-sized globular fruit and pink flesh. These results also showed that the cultivated watermelons are genetically distant from *C. l.* var. *citroides* and *C. colocynthis*. The genetic distance between *C. lanatus* and *C. colocynthis* was greater than that between *C. l.* var. *citroides* and *C. colocynthis* which was consistent with early findings (Levi et al. 2001a).

Evaluation of genetic diversity among 17 *Citrullus* genotypes using 78 SSRs

From our whole genome mapping effort (Ren et al. manuscript in preparation), we identified 704 polymorphism SSRs between two parental lines, 97103 of *C. l.* var. *lanatus* and PI 296341-FR of *C. l.* var.

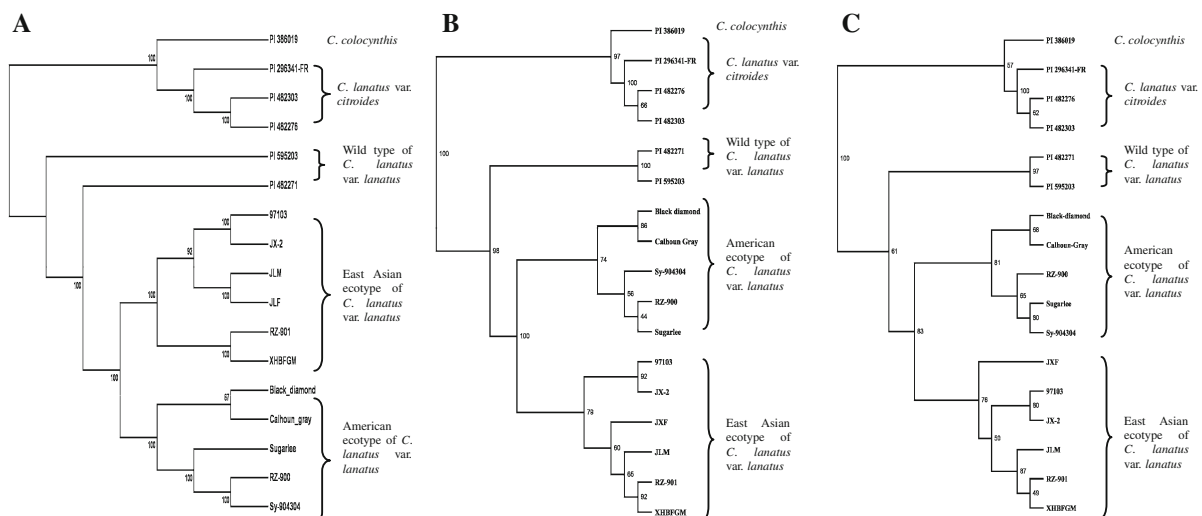


Fig. 1 Dendrograms of 17 watermelon lines based on 3,889,080 SNP loci (a), 78 SSR markers (b), and 23 core set SSR markers (c). Significant bootstrap value after re-sampling data for 1,000 times is shown at each node of the dendrogram

citroides. These 704 SSRs were subjected to polymorphism screening between two elite cultivated watermelon lines 97103 (East Asian ecotype) and Sugarlee (American ecotype), and 78 polymorphic SSRs were identified.

Genetic diversity was evaluated with the 78 SSRs among the 17 *Citrullus* genotypes with whole genome re-sequencing data (Table 1). In total 285 alleles were detected ranging from 2 to 7 alleles and an average of 3.7 alleles per locus. The PIC value ranged between 0.11 and 0.82 with an average of 0.60.

Of the 285 alleles, 57, 22 and 32 were unique to var. *lanatus*, var. *citroides* and *C. colocynthis*, respectively. Forty-eight alleles were shared between var. *lanatus* and var. *citroides*; 22 shared between var. *lanatus* and *C. colocynthis* and 21 between *C. colocynthis* and var. *citroides*. Of the 57 polymorphic alleles in var. *lanatus*, 10 presented in the *Citrullus* wild types and 47 in the cultivated watermelons. Among the 47 cultivar-specific alleles, there were no polymorphic alleles that only existed in East Asian ecotype lines or only in American ecotype lines suggesting a narrow genetic background of cultivated watermelons.

Cluster analysis using UPGMA based on similarity coefficients confirmed the genotypic relationships among the 17 *Citrullus* lines (Fig. 1b). This 78 SSR-based UPGMA dendrogram was the same as the one developed from 3.9 million SNPs suggesting that these SSRs were highly representative of the watermelon genome, had high resolving power and were as effective for genetic analysis as the original 3.9 million SNPs. (Fig. 1b).

Development and validation of a core set watermelon SSRs for genetic diversity analysis

Using the procedure and criteria described early (“Materials and methods” section) we were able to identify 23 SSRs as the core set for genetic diversity analysis in watermelon. Detailed information of the 23 SSR markers is summarized in Table 4. The 23 SSRs detected 97 alleles among the 17 watermelon lines (Table 1) ranging from 2 to 7 alleles per marker and an average of 4.2 alleles per locus. The PIC value ranged from 0.45 to 0.82 with the average of 0.66. These markers were largely evenly distributed across the genome. Each linkage group had at least two markers

except Linkage Group 3 with only one marker (Table 4).

The 23 SSRs in the core set seem to be highly representative and informative which was evidenced from the following two observations. First, the correlation coefficient between genetic similarity matrices of the core set (23 SSRs) and the base set (78 SSRs) was highly significant ($r = 0.96$, $P < 0.005$). Second, the dendrogram based on the core set 23 SSRs among the 17 watermelon lines (Table 1; Fig. 1c) was identical to that created with 78 SSR markers (Fig. 1b), which in turn was largely consistent with the dendrogram based on 3.9 million SNPs. In all three dendrograms (Fig. 1), the majority of the branches were supported by high bootstrapping values.

We attempted to further reduce the number of SSRs in the core set by selecting only one marker from each linkage group (11 in total) with high PIC value (on average 0.66) to construct a UPGMA dendrogram. Although the resulting dendrogram of the 17 genotypes (not shown) was largely consistent with their classification based on morphological and parentage data, Calhoun Gray, the typical American ecotype was placed in a clad with watermelon lines of East Asian ecotype, which was not correct. Bootstrapping values of some nodes were very low. This suggested that the core set of 23 SSR markers was suitable and sufficient to discriminate the 17 *Citrullus* genotypes at the species level.

The high consistency of clusters based on the three sets of marker data (3.9 million SNPs, 78 SSR base set and 23 SSR core set) clearly support our idea of establishing a smaller set of SSRs that capture the majority of genetic diversity in *Citrullus*. Of the 11 linkage groups, each LG had at least two SSRs except LG3 which had only one marker (BVWS00048, Table 4). On our high-resolution genetic map, 74 SSR loci were mapped in LG3, but only one SSR showed polymorphism between the two elite lines 97103 and Sugarlee. The reason for this is unknown, and may worth further investigation.

Evaluating the utility of core set SSRs

In order to assess the utility of the core set SSR markers, we conducted clustering analysis with the 23 SSRs on 100 additional commercial cultivars and elite breeding lines of watermelon (Table 2). A UPGMA dendrogram was constructed for all 117 entries which

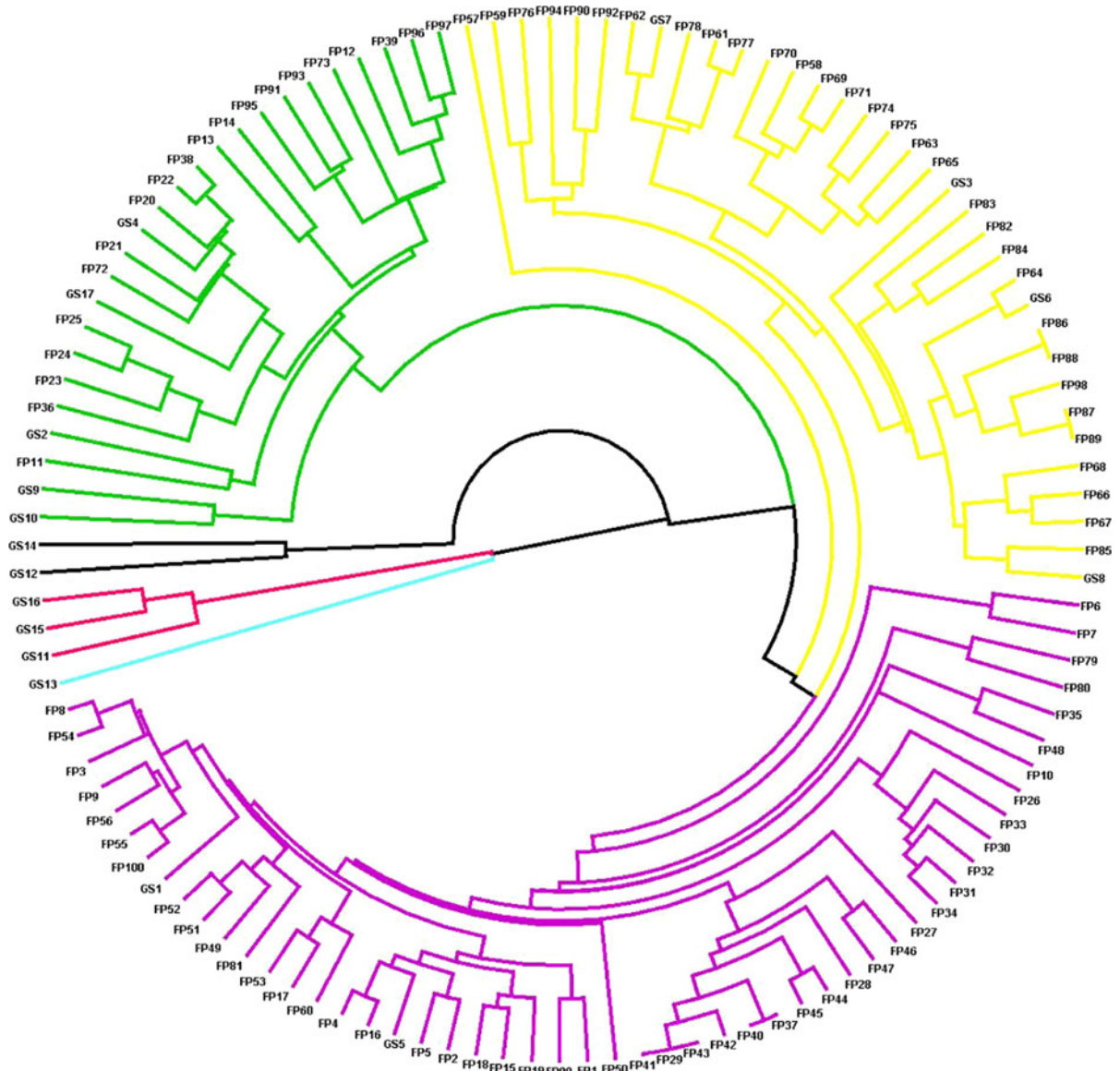


Fig. 2 UPGMA phenogram of 117 watermelon accessions (17 from Table 1 and 100 from Table 2) based on the 23 core set SSR markers. The 117 test accessions were in six distinct clusters. Color codes blue *C. colocynthis*; red *C. lanatus* var.

citroides; black wild type of *C. lanatus* var. *lanatus*; green American ecotype or its derivatives of *C. lanatus* var. *lanatus*; yellow the intermediate ecotype; pink East Asian ecotype or its derivatives of *C. lanatus* var. *lanatus*. (Color figure online)

is shown in Fig. 2. The resulting dendrogram had six distinct clusters, which belonged, respectively, to *C. colocynthis* (blue color), *C. l.* var. *citroides* (red color); wild watermelon *C. l.* var. *lanatus* (black color); American ecotype or its derivatives of *C. l.* var. *lanatus* (25 entries, green color), East Asia ecotype or its derivatives (51 accessions, pink color), and lastly a hybrid or intermediate ecotype (yellow color). The 35

accessions in the hybrid/intermediate ecotype were mostly the offspring of crosses between the American and East Asian ecotypes. This observation suggested that the accessions belonging to the American and East Asian ecotypes appeared to have relatively wider genetic distances from each other.

Some morphologically very similar watermelon lines could be differentiated with this core set SSR

markers. For example, GS1 (97103) and FP3 (98R) had the coefficient of similarity of 90%, but their morphology and growth habits were almost identical. On the other hand, some accessions, such as FP29 (Fu Feng), FP41 (Fu948-2) and FP43 (T2) shared all the alleles detected by the 23 core set SSR markers. These accessions also exhibited similar morphology and growth habits and have been previously speculated to be synonymous. In total, nine such accessions (7.7%) showed no genetic differences detected by this core set SSRs, all of which happened to be elite lines developed by NERCV.

The genetic variations in watermelon between East Asian and American ecotypes were rarely explored previously. SNP and core set SSR data from the present study clearly demonstrated a high level of genetic variation between the two ecotypes. Our SNP analysis revealed that the SNP frequency among lines of East Asian ecotype were at 0.25–0.36 SNP per kilobases (kbp), which was 0.44–0.47/kbp within American ecotype lines (data not shown). The coefficient of similarity among East Asian ecotype lines was 65–90%, which was 63–88% within American ecotype genotypes. These observations indicated that American ecotype watermelon lines had a higher level of genetic diversity than that of the East Asian ecotype ones. This may be explained by the origin of the accessions tested. The American ecotype lines were collected (Zhang et al. 2010) from different places while the East Asian ecotype lines were derived from limited parental materials with high degree of inbreeding over a long period of time. Some pre-selected lines from a few crosses were exclusively clustered with the local ones, possibly related to maintaining the quality and characteristic of the old varieties. The results also indicated the possibility to use the American ecotype watermelons to enhance the genetic diversity in Chinese breeding programs.

Conclusion

From whole genome sequencing and high resolution genetic mapping, we identified a core set of 23 highly informative and representative, evenly distributed SSR markers in watermelon, which should have wide use in germplasm management, variety identification, parental material selection and genetic pool assessment in watermelon breeding.

Acknowledgments This work was supported by funds from Beijing Municipal Science & Technology Commission, China (D08070500690803, D111100001311002, KJCX201101010, Z09090501040902 and 5100001); The Ministry of Science and Technology of China (2009BADB8B02, 2010DFB33740, 30972015, 31171980 and 2010AA10A107), and the Ministry of Agriculture of China (CARS-26).

References

- Appleby N, Edwards D, Batley J (2009) New technologies for ultra-high throughput genotyping in plants. In: Somers DJ et al (eds) *Methods in molecular biology*. Plant Genomics, Humana Press, pp 19–39
- Bisognin DA (2002) Origin and evolution of cultivated cucurbits. *Ciência Rural Santa Maria* 32(5):715–723
- Burkill HM (1985) *The useful plants of West Tropical Africa*, vol 1, 2nd edn. Richmond, Surrey, UK, Royal Botanic Gardens, Kew
- Che KP, XU Y, Liang CY, Gong GY, Weng ML, Zhang HY, Jin DM, Wang B (2003) AFLP fingerprint and SCAR marker of watermelon core collection. *Acta Bot Sinica* 45:731–735
- Coryell VH, Jessen H, Schupp JM, Webb D, Keim P (1999) Allele-specific hybridization markers for soybean. *Theor Appl Genet* 98:690–696
- Crall JM, Alstrom GW (1983) ‘Sugarlee’ watermelon. *HortScience* 18:496–497
- Dane F, Lang P (2004) Sequence variation at cpDNA regions of watermelon and related species: implications for the evolution of *Citrullus* haplotypes. *Am J Bot* 91:1922–1929
- Ebana K, Kojima Y, Fukuoka S, Nagamine T, Kawase M (2008) Development of mini core collection of Japanese rice landrace. *Breed Sci* 58:281–291
- Federico LIL, Voort AV, Osborn TC (2008) Development of a set of public SSR markers derived from genomic sequence of a rapid cycling *Brassica oleracea* L genotype. *Theor Appl Genet* 117:977–985
- Guerra-Sanz JM (2002) *Citrullus* simple sequence repeats markers from sequence databases. *Mol Ecol Notes* 2:223–225
- Harris KR, Wechter WP, Lanini B, Vivoda E, Levi A (2008) In search of markers linked to Fusarium wilt race 1 resistance in watermelon. *HortScience* 43:1238
- Huang S, Li R, Zhang Z, Li L, Gu X, Fan W, Lucas WJ, Wang X, Xie B, Ni P, Ren Y, Zhu H et al (2009) The genome of the cucumber, *Cucumis sativus* L. *Nat Genet* 41:1275–1281
- Huson DH, Richter DC, Rausch C, DeZulian T, Franz M, Rupp R (2007) Dendroscope: an interactive viewer for large phylogenetic trees. *BMC Bioinform* 8:460
- Ibrahim AA, Mohammad AB, Haseeb AK, Ahmad HAIF, Ali AAIH, Ali HB, Mohammad AIS, Mohammad S (2010) A brief review of molecular techniques to assess plant diversity. *Int J Mol Sci* 11:2079–2096
- Jarret RL, Merrick LC, Holms T, Evans J, Aradhya MK (1997) Simple sequence repeats in watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. *Genome* 40:433–441
- Jeffrey C (2001) Cucurbitaceae. In: Hanelt P (ed) *Mansfeld’s encyclopedia of agricultural and horticultural crops*, vol 3. Springer, pp 1510–1557
- Krishna GK, Zhang J, Burrow M, Pittman R, Delikostadinov S, Lu Y, Puppala N (2004) Genetic diversity analysis in

- valencia peanut (*Arachis hypogaea* L.) using microsatellite markers. *Cell Mol Biol Lett* 9:685–697
- Levi A, Thomas CE (1999) An improved procedure for isolation of high quality DNA from watermelon and melon leaves. *Cucurbit Genet Coop Rep* 22:41–42
- Levi A, Thomas CE, Keinath AP, Wehner TC (2000) Estimation of genetic diversity among *Citrullus* accessions using RAPD markers. *Acta Hort* 510:385–390
- Levi A, Thomas CE, Keinath AP, Wehner TC (2001a) Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. *Genet Resour Crop Evol* 48:559–566
- Levi A, Thomas CE, Wehner TC, Zhang X (2001b) Low genetic diversity indicates the need to broaden the genetic base of cultivated watermelon. *HortScience* 36:1096–1101
- Levi A, Thomas CE, Newman M, Reddy OUK, Zhang X (2004) ISSR and AFLP markers sufficiently differ among American watermelon cultivars with limited genetic diversity. *J Am Soc Hort Sci* 129:553–558
- Levi A, Thomas CE, Trebitsh T, Salman A, King J, Karalius J, Newman M, Reddy OUK, Xu Y, Zhang X (2006) An extended linkage map for watermelon based on SRAP, AFLP, SSR, ISSR and RAPD markers. *J Am Soc Hort Sci* 131:393–402
- Levi A, Wechter WP, Davis AR (2009) EST-PCR markers representing watermelon fruit genes are polymorphic among watermelon heirloom cultivars sharing a narrow genetic base. *Plant Genet Resour* 7:16–32
- Levi A, Wechter wp, Harris-Shultz KR, Davis AR, Fei Z (2010) High frequency oligonucleotides in watermelon expressed sequenced tag-unigenes are useful in producing polymorphic polymerase chain reaction markers among watermelon genotypes. *J Amer Hort Sci* 135:369–378
- Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. *Theor Appl Genet* 103:455–461
- Li R, Yu C, Li Y, Lam TW, Yiu SM, Kristiansen K, Wang J (2009a) SOAP2: an improved ultrafast tool for short read alignment. *Bioinformatics* 25:1966–1967
- Li R, Li Y, Fang X, Yang H, Wang J, Kristiansen K, Wang J (2009b) SNP detection for massively parallel whole-genome resequencing. *Genome Res* 19:1124–1132
- Liu KJ, Muse SV (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21:2128–2129
- Mujaju C, Sehic J, Werlemark G, Garkava-Gustavsson L, Faith M, Nybom H (2010) Genetic diversity in watermelon (*Citrullus lanatus*) landraces from Zimbabwe revealed by RAPD and SSR markers. *Hereditas* 147:142–153
- Naito Y, Suzuki S, Iwata Y, Kuboyama T (2008) Genetic diversity and relationship analysis of peanut germplasm using SSR markers. *Breed Sci* 58:293–300
- Navot N, Zamir D (1987) Isozyme and seed protein phylogeny of the genus *Citrullus* (Cucurbitaceae). *Plant Syst Evol* 156:61–67
- Nei M (1977) F-statistics and analysis of gene diversity in subdivided populations. *Ann Hum Genet* 41:225–233
- Oliveira EJ, Pádua JG, Zucchi MI (2006) Origin, evolution and genome distribution of microsatellites. *Genet Mol Biol* 29:294–307
- Padmavathi N, Tomason YR, Jeong J, Ponniah SK, Karunathilake A, Levi A, Perumal R, Reddy UK (2010) Genetic reticulation and interrelationships among *Citrullus* species as revealed by joint analysis of shared AFLPs and species-specific SSR alleles. *Plant Genet Resour* 8:16–25
- Pavlicek A, Hrda S, Flegl J (1999) FreeTree-freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of the genus *Frenkelia*. *Folia Biologica (Praha)* 45:97–99
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
- Simmonds NW (1979) Principles of crop improvement. Longman Group Ltd. pp 277
- Smith JS, Chin CL, Shu H et al (1997) An evaluation of the utility of SSR loci as molecular markers in maize (*Zeamays* L.): comparisons with data from RFLP and pedigree. *Theor Appl Genet* 95:163–173
- Sneath PHA, Sokal RR (1973) Numerical taxonomy. Freeman & Co, San Francisco, p 573
- Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc Natl Acad Sci USA* 101:11030–11035
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*. doi:10.1093/molbev/msr121
- Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA. *Nucleic Acids Res* 17:6463
- Thies JJ, Hassell R, Kousik C, Olson S, Levi A (2010) Grafting for managing southern root-knot nematode, *Meloidogyne incognita*, in watermelon. *Plant Dis* 94:1195–1199
- Varshney RK, Thiel T, Sretenovic-Rajcic T, Baum M, Valkoun J, Guo P, Grando S, Ceccarelli S, Graner A (2008) Identification and validation of a core set of informative genic SSR and SNP markers for assaying functional diversity in barley. *Mol Breed* 22:1–13
- Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Fritjers A, Pot J, Peleman J, Kupier M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res* 23:4407–4414
- Wehner TC (1999a) Vegetable cultivar descriptions for North America, list 24, 1999. *HortScience* 34:763–806
- Wehner TC (1999b) Vegetable cultivar descriptions for North America, list 25, 1999. *HortScience* 34:957–1012
- Wehner TC (2002) Vegetable cultivar descriptions for North America, list 26, 2002. *HortScience* 37:15–78
- Williams JGK, Kubelik AR, Livak KJ, Rafalski JA (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res* 18:6531–6535
- Xie R, Li X, Chai M, Sibg L, Jia H, Wu D, Chen M, Chen K, Aranzana MJ, Gao Z (2010) Evaluation of the genetic diversity of Asian peach accessions using a selected set of SSR markers. *Sci Hort* 125:622–629
- Zhang AP, Wang XW, Zhang YL, Zhao L, Xu MJ (2008) SRAP analysis for the genetic diversity of watermelon variety resources. *Chin Agric Sci Bull* 4:115–120
- Zhang FX, Luan FS, Sheng YY (2010) Analysis of genetic diversity on different ecological watermelon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai) germplasm using SSR markers. *Ch Veg* 14:36–43