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Construction of a genetic linkage map of watermelon (*Citrullus lanatus*) using CAPS and SSR markers and QTL analysis for fruit quality traits

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ABSTRACT

A genetic map of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] was constructed using an $F_{2:3}$ segregating population consisting of 145 individuals, which were derived from a cross between a cultivated inbred line (garden female parent) from China and an American inbred line (LSW-177). The map contained 125 polymorphic markers, comprising 82 cleavage amplified polymorphic sequence (CAPS) markers and 43 simple sequence repeat (SSR) markers. The map contained 11 major and 3 minor linkage groups spanning a total length of 1,244.5 cM, with an average of 9.96 cM between markers. All of the CAPS markers were newly developed based on high-throughput re-sequencing data for the garden female parent and LSW-177. A quantitative trait locus (QTL) analysis was conducted using the composite interval mapping method to locate fruit sugar content and fruit shape traits. A total of 10 QTLs were identified; 4 for fruit sugar content and 6 for fruit shape. This map and QTL analysis will be useful for breeding to improve various economic traits in watermelon.

1. Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] originated in the arid areas of southern Africa, and is now widely cultivated worldwide (Mohr, 1986). China is the largest producer and consumer of watermelon, with a total annual production of approximately 68 million tons. Because of the economic and horticultural importance of this crop, there has been increasing interest in breeding programs to improve the fruit quality and some of the morphological characteristics of watermelon plants. Among the breeding targets, sweetness and fruit shape are those most directly related to the economic value of watermelon. The Brix level is a measure of total soluble solids content in watermelon, and is highly correlated with the sugar percentage (Hashizume et al., 2003b; MacGillivray, 1947; Maynard, 2001). In a previous study, a single QTL was identified that accounted for 19% of the variation in Brix in an inter-subspecific BC_1 population (Hashizume et al., 2003b). In 2012, the first single-nucleotide polymorphism (SNP) map for watermelon was reported; this map contained 5 QTLs for brix, 10 QTLs for fruit length, 8 QTLs for fruit width, and 5 QTLs for fruit shape (Sandlin et al., 2012). However, there are fewer reports on QTLs for soluble sugars such as fructose and sucrose and glucose content of watermelon.

Using molecular linkage genetic maps and quantitative trait locus (QTL) mapping technology, it is possible to estimate the number of QTLs in the genome. Seven linkage groups were identified in the first genetic map, which was developed using an interspecific backcross population derived from *C. lanatus* × *Citrullus colocynthis* (Navot et al., 1990). Several genetic maps were developed for watermelon over the following years (Hashizume et al., 1996; Hawkins et al., 2001; Levi et al., 2006), but most of the markers used to construct these maps were based on random polymorphisms in the genome; that is, random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), restriction fragment length polymorphism (RFLP), and amplified fragment length polymorphism (AFLP) markers. In genetic mapping experiments on watermelon, low rates of polymorphism were detected using RAPD and SSR markers, but higher rates were detected using AFLP markers. However, most of the AFLP markers were clustered on a few linkage groups, and large

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regions of the watermelon genome were left uncovered (Levi et al., 2006).

The limited amount of DNA marker and genetic mapping information have hampered watermelon breeding. With the development of high throughput sequencing technology, more genomes have been successfully sequenced, thus facilitating the development of molecular markers. In 2008, the advent of single nucleotide polymorphism (SNP) technology (Henry, 2008; Kole and Abbott, 2010) provided new tools to create genetic maps. The first SNP maps for watermelon were constructed and compared using three populations; the lengths of these genetic maps were 1,438 cM, 1,514 cM, and 1,144 cM with average marker distances of 3.8 cM, 4.2 cM, and 3.4 cM, respectively (Sandlin et al., 2012). SNP markers have increased marker resolution in melon (Deleu et al., 2009) and *Cucurbita pepo* (Esteras et al., 2012). Therefore, this technology has made high-throughput mapping a reality.

The aim of the present study was to develop SNPs based on the high-throughput platform and convert them into cleaved amplified polymorphic sequence (CAPS) markers. Then, we aimed to construct a genetic linkage map for watermelon using SSR and CAPS markers, and to map QTL associated with two important horticultural traits; soluble sugars content and fruit shape.

2. Materials and methods

2.1. Plant materials

The F_2 mapping population was derived from the cross between the garden female parent and LSW-177. The fruit produced by the garden female parent was almost round [fruit shape index (FSI):1.18], with a high Brix content (11%), while the LSW-177 fruit were elongated (FSI:1.93), with a lower Brix content (8.93%) (Davis et al., 2008). One F_1 plant and 300 F_2 plants were planted in greenhouse at the Xiangfang Experimental Agricultural Station, Northeast Agricultural University, on 20 April 2010 and 25 April 2011, respectively. One hundred and forty five F_3 families (nine plants per family) from the self-pollinated F_2 population were planted in the greenhouse on 20 April 2012. Mean values for phenotypic data for the $F_{2:3}$ family were the same as the F_2 individual phenotypic values.

2.2. Phenotyping

All fruit were harvested at 40 days after self-pollination and their morphological traits were evaluated. Fruit length (FL, in cm) and fruit width (FW, in cm) were measured using a ruler. The fruit shape index (FSI) was calculated as the ratio between fruit length and width. Total soluble solids or brix contents of fruits were evaluated using a handheld refractometer, using juice squeezed both from the central and edge parts of each fruit.

Harvested fruits were brought to the laboratory for analysis. Flesh from the center and edge portion of the fruit was stored at −80 °C until use. Frozen samples were ground to a fine powder, and 5-g portions of the powder were used for analyses. Soluble sugars (glucose, fructose, and sucrose) were extracted for 40 min in 10 ml hot 80% ethanol. After centrifugationat 12000 × *g* for 30 min at 4 °C, the supernatant was removed and diluted to 25 mL with water for further analysis (WAN et al., 2009). A UV spectrophotometer was used to measure the soluble sugars content. Fructose and sucrose contents were determined by the anthrone-sulfuric acid method, and absorbance was measured at 620 nm. Glucose content was determined by the 3, 5-dinitrosalicylic acid method, and absorbance was measured at 540 nm. The concentrations of sugars were calculated from standard curves of fructose, sucrose, and glucose processed under the same conditions (Hong et al., 2009).

Calculations of mean values and standard deviations, and trait distribution analyses and pairwise correlations were conducted with SPSS ver. 19.0.

2.3. SSR and CAPS analysis

Total DNA was isolated from the expanding leaves of parents, F_1, and F_2. We used 2-week-old plants for DNA extraction. The leaf tissue (0.2 g) was ground in liquid nitrogen and then DNA was extracted using the CTAB method (Murray and Thompson, 1980). The extracted DNA was suspended in deionized water and stored at −80 °C until use.

We obtained sequences for 1574 SSR primers (watermelon, 355 primer pairs; melon, 1219 primer pairs) from previously published references and from searches in the ICuGI database (http://www.icugi.org/) and the NCBI database (http://www.ncbi.nlm.nih.gov/dbEST). In total, 55 polymorphic SSR primers were identified. The SSR reaction mixtures contained 30 ng/μl plant genomic DNA, 15 mM Mg^{2+}, 2 pmol primers, 10 mM dNTPs, 10 × Taq buffer, and 1 unit of Taq polymerase (TaKaRa) in a total volume of 25 μl. The SSR amplification program was as follows: predenaturing at 94 °C for 5 min, then 38 cycles of denaturing at 94 °C for 1 min, renaturing at 48 °C for 1 min, and elongation at 72 °C for 90 s; followed by final elongation at 72 °C for 10 min, and then incubation at 4 °C. The SSR products were mixed with 5.5 μl loading buffer and denatured at 94 °C for 5 min, then 5 μl each sample was loaded onto a 6% denaturing polyacrylamide gel and electrophoresed in 1 × TBE electrophoresis buffer for 1 h. The gel was stained with $AgNO_3$ solution.

The development of SNPs was based on recently published genome resequencing data. The parental genomes were resequenced by the Illumina HiSeq 2000 high-throughput sequencing platform, and the output data was compared with previously published watermelon genome data (Guo et al., 2013). The raw data was assembled using BWA software, and subsequently searched to identify SNP loci using Samtools software. SNP genotyping was carried out using CAPS markers, as follows: 1000-bp sequences before and after SNP loci were selected with self-compiled perl scripts, and then CAPS loci were identified using SNP2CAPS software. The results of the bioinformatics analysis implied that there were 352,483 polymorphic SNP loci between the two parental materials, and 28,722 SNP loci could be converted into CAPS markers using 11 restriction endonucleases (*Eco*RI, *Bsa*HI, *Hind*III, *Mbo*II, *Pst*I, *Sca*I, *Bam*HI, *Mlu*I, *Asu*II, *Dra*I, and *Pvu*I). We selected 20–40CAPS loci on each chromosome, and designed PCR primers for these loci using Primer Premier 5.0 software. The CAPS reaction mixture was the same as the SSR reaction mixture, and gradient PCR was performed as follows: initial denaturation at 94 °C for 7 min, 30 cycles of 94 °C for 60 s and 60 °C for 45 s, followed by a 0.5° C drop in temperature each cycle, for 60 s at 72 °C, then 10 cycles for 20 s at 94 °C, for 20 s at 45 °C, for 60 s at 72 °C, followed by post-heating for 7 min at 72 °C. The reaction mixture for enzyme digestion contained 5 μl PCR product, 9 μl deionized water, and 0.3 μl restriction enzyme (10 U/μL). The mixtures were incubated at 37 °C for 1–16 h. The enzyme-digested products were examined by 1% agarose gel electrophoresis in 1 × Tris-base acetic acid buffer. The gel were visualized under UV light and photographed.

2.4. Linkage analysis and QTL identification

The segregation of SSR and CAPS markers at a 1:3 ratio was tested by chi-square analysis. Segregation ratios that differed from the expected values (significant at $p = 0.05$ or less) were classified as distorted. A total of 163 scored markers (108CAPS markers and 55 SSR markers) were used to construct a linkage map using JoinMap version 4.0 (Ooijen and Voorrips, 2002; Van Ooijen, 2006). Markers