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# Genetic diversity among Chinese Hami melon and its relationship with melon germplasm of diverse origins revealed by microsatellite markers

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ABSTRACT

Thick-skinned melon called Hami melon is the most widely cultivated and exported type of melon in China, and mainly grown in Xinjiang province. Here the genetic variation of 64 melon genotypes including 43 Xinjiang Hami melon accessions was analyzed using 36 simple sequence repeat (SSR) markers yielding 145 alleles. The polymorphic information content of SSR markers ranged from 0.09 to 0.83 (average 0.45). Based on the SSR markers, the melon accessions were clustered into 2 major groups (thick and thin-skinned melons). In addition, the sub-cluster analysis based on SSR markers partitioned different botanical groups, even separating similar agronomic trait groups (Xinjiang landraces var. *ameri* and var. *inodorus*). SSR analysis showed that 4 SSR markers (CMBR150, CMCTT144, CMBR84 and CMBR12) produced polymorphic bands of different sizes between these two botanical groups. Those four molecular markers might be related to melon fruit maturing time. A considerably low level of genetic diversity was detected in Xinjiang melon accessions. Genetic distances indicated the relatively narrower genetic base but specific taxonomic status of Xinjiang landraces compared with foreign reference accessions.



## 1. Introduction

Melon (*Cucumis melo* L.; 2n = 2x = 24) as one major economic fruit crop is cultivated extensively in tropical and sub-tropical regions. The production in 2012 in China was 17.50 million tones, which accounted for 40% of global production (FAOSTAT, 2013). Chinese melons are divided into two types (thin and thick-skinned melons) depending on the thickness of fruit skin (Kong et al., 2011). The thick-skinned melon called Hami melon is mostly produced in Xinjiang in northwest China. The production of sweet Hami melon benefits from the climate conditions there, including dry climate, large fluctuation of daily temperature, strong sunshine and long sunshine duration in summer. Xinjiang Hami melon enjoys great reputation worldwide because of its unique flavor and high sugar content (Ning et al., 2014).

Xinjiang Hami melon is often classified into var. *cassaba*, var. *chandalak*, var. *ameri* and var. *inodorus* (Lin, 2010). Var. *chandalak* produces globular-shaped fruits with soft and spongy flesh. Due to the difficulty in storage and the unsuitability for long-distance transportation, this horticultural type is mainly consumed in Xinjiang and rarely exported overseas (Luan et al.,

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2008). Var. *ameri* and var. *inodorus* own a huge market share because of high quality, high sugar content, longer post-harvest storage life and suitability for transportation. Thus, these two types of thick-skinned melon are widely cultivated and exported overseas as major commercial ones in Xinjiang. Though Xinjiang is one of the secondary original centers of thick-skinned melon (McCreight et al., 2004), the studies on germplasm of melon groups from Xinjiang are lagged far behind compared with other original centers (Aierken et al., 2011). Therefore, with studies on the genetic diversity of Xinjiang landraces and their relationship with other melon accessions, we can evaluate the taxonomic status of Xinjiang melon, and reasonably predict parent selection for Hami melon breeding programs.

The genetic diversity and relationship of melon can be analyzed via many methods, including morphological traits, isozymes and molecular markers (Akashi et al., 2002; Yildiz et al., 2011; Bagheriyan et al., 2013). Some DNA markers have been used for the assessment of genetic relationships among Xinjiang Hami melon. Aierken et al. (2011) used SSR markers to study the genetic relationships among Hami melons of 24 Xinjiang landraces. Chen et al. (2010) used the combinations of 16 sequence-related amplified polymorphism (SRAP) primers to define the genetic relationships of 42 Xinjiang melon accessions. However, these markers could not clearly cluster melon groups. For instance, groups *inodorus* and *ameri* could not be separated from each other in their studies. Thus, our study expands their studies to defining variation among Xinjiang landraces and separating horticultural groups using more landraces and polymorphic SSR markers.

In this study, we analyzed 43 melon accessions from different regions of Xinjiang along with 21 reference accessions from other areas. We used a set of SSR markers to: (1) assess the genetic diversity of Xinjiang melons and their relationship with foreign melon accessions; (2) provide useful SSR markers for separating botanical groups of Xinjiang landraces; (3) provide recommendation for the exploration and utilization of thick-skinned melon germplasm resources in Hami melon breeding programs to increase genetic diversity.

## 2. Materials and methods

### 2.1. Plant materials and DNA extraction

A total of 64 accessions were used: 50 Chinese accessions (43 Xinjiang landraces) and 14 exotic accessions (Fig. S1). Details about the origins and horticultural groups of these accessions are listed in Table S1. Seeds of these accessions were provided by Xinjiang Academy of Agricultural Sciences (XAAS), China, and Xinjiang Putao Guaguo Yanjiu Center (XJPTGGYJ). These accessions were maintained as pure lines in the field at National Melon Engineering and Technology Research Center, Xinjiang (NMETRC). Finally, the selfed seeds of each accession were used. The genomic DNA was extracted from fresh leaves by CTAB method (Porebski et al., 1997) with minor modification. The purified DNA was diluted to 100 ng/μl.

### 2.2. Phenotypic evaluation

Phenotype of 64 melon accessions was evaluated in the field of NMETRC in 2011, including 8 plants in each accession. At least three plants from each accession were tested at three stages: cotyledon, flowering and fruit maturation. Phenotypic evaluation was based on standard measurements as Ma et al. (2006). A total of 14 phenotypic traits were recorded (Table S2). Among them, quantitative characteristics were rated as 0–10 and length traits were measured by a ruler. The total soluble solids (TSS) were measured using a hand refractometer (LB32T).

### 2.3. SSR analysis

A total of 172 SSR markers, according to the information of available melon and cucumber genetic maps (Diaz et al., 2011), were screened in one DNA bulk which was prepared from 5 accessions with distant genetic relationship. Thirty-six SSR markers were chosen based on their polymorphism, repeatability, and unambiguous polymerase chain reaction (PCR) products. The sequences of these SSR markers are listed in Table 1. PCR amplification was conducted in a Thermal Cycler (BIO-RAD C1000™) with the following protocol: 94 °C for 3min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s; and 72 °C 10 min. Finally, the amplified fragments were analyzed by electrophoresis on 32 cm × 38 cm × 0.04 cm 6% denaturing polyacrylamide gels.

### 2.4. Data analysis

Morphological and molecular data were analyzed to reveal the genetic relationships among 64 melon accessions. The morphological genetic diversity among melon accessions was analyzed using NTSYS pc 2.1 (Rohlf, 2000). DNA fragments at all SSR loci were scored as binary data matrix with “present” as 1 or “absent” as 0. For each accession, polymorphic bands (PB), number of effective alleles (*Ne*), observed homozygosity (*Ho*), Nei expected hetrozygosity (*He*) and Shannon diversity (*I*) were calculated using POPGENE (Yeh et al., 1997). The polymorphism information content (PIC) for each primer was evaluated as $PIC = 1 - \Sigma p^2_{ij}$ (Botstein et al., 1980). Marker Index (MI) was calculated as MI= (number of polymorphic bands) × (mean diversity index) (Powell et al., 1996).

Jaccard's coefficient was calculated using SIMQUAL to estimate the molecular genetic diversity among the 64 melon accessions. Then the UPGMA cluster analysis was visualized using a dendrogram based on this coefficient (Rohlf, 2000). The