Inheritance and Molecular Mapping of Tight-placenta Gene and Seed Traits in Chinese Hami Melon ‘Queen’

Xuefei Ning, Xianlei Wang, Zhijie Yu, Simeng Lu, and Guan Li¹

Department of Life Science and Technology, Xinjiang University, No. 14 Sheng Li Road, Urumqi 830046, China

Additional index words. *Cucumis melo* seed trait, wave seed gene, genetic linkage map, quantitative trait locus

Abstract. Hami melon ‘Queen’ (*Cucumis melo* ssp. *melo* var. *ameri* Pangalo) is the most widely cultivated and exported type of melon in Xinjiang Province, Northwest China. We previously found the unique traits of Hami melon ‘Queen’ for wave seeds and tight-placenta fruits. An analysis of the inheritance showed that these traits were controlled by two recessive genes *wave seed* (*ws*) gene and *tight-placenta* (*tp*) gene, respectively. Here, to identify these two traits and melon seed-related traits, segregation populations including BC₁ and F₂ derived from a cross between ‘Queen’ (P₁) and MR-1 (P₂) were used as mapping populations. Eighty-seven simple sequence repeat (SSR) markers were used in map construction of BC₁P₁ population, and as a result, *ws* and *tp* were identified on linkage group 1. Analysis of quantitative trait locus (QTL) referring seed traits showed that QTL *ss1.1* for seed shape (SS) and QTL *sl1.1* for seed length (SL) were located at LG 1, supported by likelihood of odds (LODs) of 15.6 and 13.4, respectively, and both linked with *ws*. Subsequently, the genetic linkage and parental re-sequence analysis were constructed for fine mapping *ws* and *tp*. Genetic analysis showed that *ws* and *tp* were located in CM3.5_scaffold00060 on LG 1, flanked by InDelchr1-3241 and InDelchr1-3233. The 80.9-kb physical distance of this region included 11 candidate genes. Among them, MELO3C023549 and MELO3C023551 could be candidates for *ws* and *tp* by sequence alignment and allelic variation survey in parental lines. MELO3C023549 was predicted to encode an MYB46-like transcription factor related to positive regulation of secondary cell wall biogenesis. MELO3C023551 was annotated to encode a cellulose synthase A (CESA) associated with cellulose biosynthetic process.

Melon (*C. melo* L.; $2n = 2x = 24$), a major economic fruit crop, is cultivated extensively in tropical and subtropical regions. Hami melon ‘Queen’ (*C. melo* ssp. *melo* var. *ameri* Pangalo) is the most widely cultivated and exported type of melon in Xinjiang Province, 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., 2014b).

Present genetic research on melon was focused on fruit size, quality traits (Diaz et al., 2014; Ramamurthy and Waters, 2015; Wang et al., 2016), plant morphology (Gao et al., 2014; Hwang et al., 2014; Zhu et al.,

2011), and mapping resistance genes to different biotic stresses (Argyris et al., 2015a; Daley, 2014; Guiuaronés et al., 2014; Li et al., 2017; Zhang et al., 2013). However, there are also many reports about various effects in melon seeds recently. Ashfak et al. (2014) found that melon seed oil consisted of moderate amount of unsaturated fatty acid and deoiled seed cake contained significant amounts of minerals (N, P, K, and Ca). Lei and Kang (2013) indicated that hexane extract from melon seed could be used as a potent alternative for controlling type 2 diabetes in *in vitro* studies. In addition, melon seed traits could also be used as botanical classification factors. A significant correlation between melon seed traits and botanical classification were reported by Sabato et al. (2015) and Tanaka et al. (2016). Based on these positive effects, the information on melon seed traits may contribute to increasing the commercial values of melons and promote the development of melon industry.

Hagiwara and Kamimura (1936) found that white seed testa color (vs. yellow) was controlled by one single dominant gene (symbol *Wt-2*) which was present in PI 414723. Périn et al. (2002) reported that pine-seed shaped (vs. flat) traits were controlled by a single recessive gene (symbol *pin*). We previously found the wave seed and tight-placenta fruit of melon ‘Queen’, which

were not found in most melon accessions. Tight-placenta fruit trait means that the placenta is uneasily separated from mesocarp at the maturing period. Specifically, ‘Queen’ melon seeds embedded into the flesh as a result of locular tissue degeneration (Fig. 1). Moreover, seeds separated from the melon ‘Queen’ placenta exhibit wave phenotype, which is also different from other melon accessions. Such unique traits of ‘Queen’ offer an opportunity to study the underlying mechanism of seed and placenta formation in melon.

To date, many linkage maps from different populations have been constructed and reported in melons (Obandouloa et al., 2009; Perchepe et al., 2005; Tomason et al., 2013; Wang et al., 2016; Yuste-Lisbona et al., 2011). Among them, the integrated melon map was constructed by Diaz et al. (2011) using a variety of molecular markers. After the genome of melon (DHL92) has been sequenced, an improved anchoring of the assembled melon genome was published (Argyris et al., 2015b; Garciaamas et al., 2012). The consensus ICuGI genetic map was also anchored to the reference genome of melon (DHL92) (Diaz et al., 2015). These studies not only facilitate comparative mapping in melon between past and new studies but also make it easier to analyze marker locus associated with target trait. With the development of next-generation sequencing techniques, linkage maps combined with whole-genome approach have been widely used to locate traits or QTLs and identify candidate genes (Hu et al., 2017; Liu et al., 2016; Natarajan et al., 2016; Tian et al., 2016; Yao et al., 2017).

In this context, tight-placenta and seed traits were studied by using genetic mapping and parent re-sequencing. Our objectives were to 1) study the inheritance of wave seed and tight-placenta fruit; 2) identify the QTL associated with seed weight (SW), seed width (SD), SL, and SS, and study the relationship between the tight-placenta trait and seed traits; and 3) fine map and predict candidate genes controlling wave seed and tight-placenta traits.

Materials and Methods

Plant materials and genomic DNA extraction. Two melon accessions, ‘Queen’ (P₁) and MR-1 (P₂), were used as parents in this study. ‘Queen’ bears wave seeds, small-width fruit cavity, and tight-placenta fruits, whereas MR-1 produces larger width fruit cavity, untight-placenta fruits, and glossy seeds (Fig. 1). These two parental lines were maintained as pure lines in the field at the National Melon Engineering and Technology Research Center (NMETRC), Xinjiang. MR-1 (female parent) were crossed with ‘Queen’ (male parent), and F₁ individuals were backcrossed with recurrent parent (female parent) to generate BC₁P₁ (consisting of 260 plants) and BC₁P₂ (consisting of 200 plants). The F₁ plants were self-pollinated to produce an F₂ population that consisted of 800 individuals. These experiments were conducted in the field of NMETRC in the Summer of 2012 and 2014. A total of 134 BC₁P₁ plants were used to map

Received for publication 5 Mar. 2018. Accepted for publication 16 Apr. 2018.

This work was supported by the Xinjiang Graduate Research and Innovation (XJGRI2014035), the National Natural Science Funding of China (31660297).

We thank National Melon Engineering and Technology Research Center, Xinjiang, China, for providing field and technical assistance for melon materials cultivating.

¹Corresponding author. E-mail: guanlixu@163.com.

Fig. 1. (A) Tight-placenta and wave seed characteristics of the line 'Queen'. (B) Untight-placenta and glossy seed characteristics of the line MR-1.

tight placenta and seed traits in the Summer of 2013 and 800 F_2 plants were used to fine map *tp* and *ws* genes in the Summer of 2015.

Genetic analysis and scoring for phenotypic traits. Phenotypic data of wave seed and tight-placenta fruit traits were collected from P_1 , P_2 , F_1 , and BC_1P_1 in 2013. The phenotypic data of F_2 , BC_1P_1 , and BC_1P_2 for wave seed and tight-placenta fruit traits were investigated in 2015. Data of wave seed and tight-placenta fruit traits were categorized as nonmetric data. Single-melon seeds were classified by visual inspection as wave seeds or glossy seeds. The surface of the wave seed is wrinkled, whereas the surface of glossy seed is flattened (Fig. 1). Similarly, single-melon fruits were scored for tight-placenta fruit trait by visual inspection. Tight-placenta fruit means that the placenta is closely integrated with mesocarp as the 'Queen'. The segregation ratios of the F_2 and BC_1 populations were analyzed with a χ^2 test using SAS software.

Data of SW, SD, SL, and SS were categorized as quantitative data. Data for each trait in the mapping population were measured using three replications. One hundred and twenty-eight BC_1P_1 individuals were used to measure SW, SL, and SD. For SW (g), a random sample of 30 seeds was taken for each individual plant. For average SL and SD (cm), a random sample of 10 seeds was quantitatively evaluated for each individual plant using ImageJ software. The ratio of SS is defined as SL divided by SD. The average value was used for the analysis. Statistical analysis of the data was performed using GraphPad Prism 6 (<http://www.graphpad.com/prism/>). The phenotypic correlation for seed size traits was obtained using the Pearson's correlation coefficient.

DNA isolation and molecular marker development. Genomic DNA was extracted

from fresh melon leaves by the cetyltrimethylammonium bromide method (Porebski et al., 1997) with minor modifications. The purified DNA was diluted to $100 \text{ ng} \cdot \mu\text{L}^{-1}$. A total of 499 SSR markers were selected from published sources (Chiba et al., 2003; Danin-Poleg et al., 2000; Diaz et al., 2011, 2015; Fernandezsilva et al., 2008; Fukino et al., 2007; Gonzalo et al., 2005; Harel-Beja et al., 2010; Ritschel et al., 2004) or from <http://www.melonomics.net>. Several new melon SSR and InDel primers were designed in the target region according to the resequencing data of two parent lines (Supplemental Tables 1 and 3). The genome of the melon accession DHL92 was downloaded from <http://www.melonomics.net> (version CM_3.5.1) and used as a reference genome. Resequencing of 'Queen' and MR-1 was performed at $\approx 20\times$ coverage over the whole genome using the Illumina HisSeq 2000 platform with the constructed paired-end sequence libraries at the Biomarker (Beijing, China). PCR amplification was performed in a thermal cycler (BIO-RAD C1000) with the following protocol: 94 °C for 3 min; 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 PCR products were separated on $32 \times 38 \times 0.04\text{-cm}^3$ 6% denaturing polyacrylamide gels and stained with silver.

Linkage map construction. According to Lander et al. (1987), the heterozygous bands or the glossy seed/untight-placenta fruit of BC_1P_1 plants were recorded as "H," the homozygous bands or the wave seed/tight-placenta fruit plants as "A," and unclear or missing data as "–." For F_2 population, the band pattern of the maternal parent ('Queen') was denoted as "A," that of the paternal parent (MR-1) as "B," and the heterozygous band as "H." The F_2 population with wave seed/tight-placenta fruit as the 'Queen' was

denoted as "A" and that with glossy seed/untight-placenta individual as the MR-1 was denoted as "HB."

Phenotypic data, along with SSR and InDel analyses, were combined for linkage analysis using QTL IciMapping V4.1 (<http://www.isbreeding.net>). Recombination units were converted into genetic distances based on Kosambi function (Kosambi, 1944). Markers were associated with minimum LODs ≥ 3.0 and maximum distance ≤ 50.0 centimorgan (cM). Finally, linkage maps were visualized using MapChart 2.2 (Voorrips, 2002). Gene prediction was performed according to the melon genome automated annotation database (<https://melonomics.net>). Gene annotation and analysis were conducted with the BLAST search on the published NR (nr database), SwissProt (Deng et al., 2006), GO (Gene ontology; Ashburner et al., 2000), COG (Clusters of orthologous groups of proteins; Tatusov et al., 2000), and KEGG databases (Kyoto encyclopedia of genes and genomes; Kanehisa et al., 2004).

QTL detection. QTLs regulating SW, SD, SL, and SS were analyzed using phenotypic score and SSR marker data from the BC_1P_1 generations. Composite interval mapping was used for QTL detection. The window size was set at 5.0 cM and the walk speed at 1.0 cM. Genome-wide significance thresholds were estimated by 1000 permutations to declare significant QTLs with a type-I error rate of 0.05 (Churchill and Doerge, 1994). Finally, QTLs were as presented by WinQTLCart 2.5 (Wang et al., 2012).

Results

Genetic analysis of wave seed and tight-placenta fruit traits. Fifteen F_1 individuals, generated by crossing MR-1 with 'Queen', showed glossy seeds and untight-placenta fruits. In the BC_1P_1 population, 63 of 134 individuals showed glossy seeds and 71 showed the wave seeds. Sixty-nine of 134 individuals showed untight-placenta fruits and 65 showed the tight-placenta fruits. Segregation ratios were confirmed to be 1:1 by χ^2 test. Wave seeds and tight-placenta plants were never observed in BC_1P_2 population. Furthermore, the 736 plants of the F_2 population segregated into a glossy-to-wave seed/untight-to-tight placenta ratio of 3:1 (Table 1). These results indicated that the wave seed and tight-placenta traits were controlled by two single recessive genes, and these two genes were named *ws* and *tp*. Interestingly, the phenotypic data suggested that the seeds of tight-placenta fruits were prone to be wave seeds. For the cosegregation data of the BC_1P_1 population, 63 individuals showed glossy seeds and untight-placenta fruits and 65 showed wave seeds and tight-placenta fruits. Six individuals showed wave seeds and untight-placenta fruits. For the cosegregation data of the F_2 population, 534 individuals showed glossy seeds and untight-placenta fruits and 188 showed wave seeds and tight-placenta fruits. Three individuals showed wave seeds and untight-placenta

fruits, whereas 11 individuals showed glossy seeds and placenta fruits. The cosegregation rates of these two traits were 96% and 98% in BC₁P₁ and F₂ populations, respectively (Table 1). Therefore, the seed wave trait was supposed to be related with the tight-placenta fruit trait.

Mapping of wave seed and tight-placenta genes. A total of 449 SSR marker primers were tested to identify the polymorphism between the parents. Eighty-seven of 449 markers amplified clear, reproducible, and specific polymorphism straps, which were used in map construction of BC₁P₁ population. According to the molecular marker data and phenotype scores, 10 polymorphic markers were identified to be linked with the two traits. The genotypes of these 10 markers and the phenotype data of the two traits from BC₁P₁ plants were used to calculate a linkage map (Supplemental Table 1).

The wave seed and tight-placenta traits were mapped on LG 1 as monogenic traits. The *tp* and *ws* genes were located at 25.7 and 33.0 cM and flanked by the markers ECM110 and CMMS22-2, respectively (Fig. 2A). The genetic distance between *tp* and *ws* gene locus was 7.3 cM. These results were in agreement with the hypothesis that the tight-placenta trait was related with the wave seed trait.

Phenotypic evaluations of seed size traits and QTL analysis. Mean value, SD, skewness, and kurtosis for seed size traits measured in 128 BC₁P₁ individuals were calculated (Table 2). Continuous distributions were observed in all four seed traits, and the phenotype data of these traits followed a normal distribution based on the values of skewness and kurtosis statistics. The frequency distribution of the BC₁P₁ individuals for each trait is shown in Supplemental Fig. 1.

Pearson's correlations for seed size traits are given in Supplemental Table 2. Significant correlations for most trait pairs were observed, except for the SL-SD and SW-SS pair in correlation analysis.

Genomic regions associated with SD, SL, SS, and SW were identified through QTL analysis, which involved 87 polymorphic SSR markers and two phenotype makers (Supplemental Fig. 2). Eight QTLs for those quantitative seed traits were located, based on a permutation test at *P* = 0.05, at the whole-genome level (Table 3), a major QTL for SS, *ss1.1*, was located at LG 1, with an LOD of 15.6, and *ss1.1* was linked with wave seed trait. Another major QTL for SL, *sl1.1* on LG 1, was also linked with wave seed trait, with an LOD of 13.4 (Fig. 3). The seed width was associated with four minor QTLs at LG 3, 5, 8, and 12, respectively. Two minor QTLs related to SW were detected on LG 1 and 6, in

Table 1. Chi-square goodness-of-fit test ratios of wave seed/tight-placenta trait in BC₁ and F₂ populations.

Populations	Total plants number	Number of glossy seed/untight-placenta individuals	Number of wave seed/tight-placenta individuals	Expected ratio	Wave seed/tight-placenta trait χ^2	Cosegregation rates of <i>ws</i> and <i>tp</i> (%)
F ₁ (2013)	15	15/15	0/0			
BC ₁ P ₁ (2013)	134	63/69	71/65	1:1	0.48/0.12	96
BC ₁ P ₂ (2013)	96	96/96	0/0			
F ₂ (2015)	736	546/536	190/200	3:1	0.61/0.17	98

$\chi^2 > \chi^2_{0.05} = 3.84$ is considered significant.

Fig. 2. Fine genetic mapping of wave seed (*ws*) and tight-placenta (*tp*) genes. (A) Linkage map of *ws* and *tp* genes constructed using the 128 BC₁P₁ populations of MR-1 and 'Queen'. The markers are shown at the right of LG 1 and the distances among them are indicated in centimorgans on the left. (B) Preliminary further mapping for *ws* and *tp* based on 96 F₂ plants. (C) Fine mapping including the *ws* and *tp* locus in the 736 F₂ plants. Numbers above the chromosome are recombinants at each interval. (D) The genomic region between InDelchr1-3241 and InDelchr1-3233 (80.9 kb) in which 11 genes were predicted.

Table 2. Descriptive statistics of phenotype data of two parents and BC₁ mapping population.

Trait ^a	Parents		BC ₁ mapping population					
	Queen	MR-1	Min.	Max.	Mean	SD	Skewness	Kurtosis
SS	2.24 ± 0.11	2.59 ± 0.23	1.87	3.05	2.48	0.28	-0.01	-0.59
SL (cm)	0.95 ± 0.05	1.21 ± 0.07	0.91	1.39	1.18	0.12	-0.23	-0.91
SD (cm)	0.43 ± 0.02	0.47 ± 0.03	0.44	0.56	0.48	0.03	0.56	-0.06
SW (g)	1.17 ± 0.07	1.01 ± 0.10	0.49	1.22	0.89	0.14	-0.22	0.45

^aSL, SD, and SW indicate seed length, width, and weight, respectively; SS indicate length-to-width ratio.

Table 3. QTLs affect seed characteristics in the BC₁P₁ segregated progeny of MR-1 and 'Queen'.^a

Traits	Quantitative trait loc	Linkage group	Locus closest to max. LOD	Position (cM)	Max. LOD	Additive
Seed shape	<i>ss1.1</i>	LG 1	Wave seed	33.0	15.6	-0.41
Seed length	<i>sl1.1</i>	LG 1	Wave seed	33.0	13.4	-0.20
Seed width	<i>sd3.1</i>	LG 3	MU4452-ECM205	74.2	2.0	0.02
	<i>sd5.1</i>	LG 5	ECM92	15.7	2.2	-0.01
	<i>sd8.1</i>	LG 8	TJ2	35.6	1.5	0.02
	<i>sd12.1</i>	LG 12	GCM206-DE1851	67.0	2.2	0.03
Seed weight	<i>sw1.1</i>	LG 1	Wave seed	33.0	2.0	-0.10
	<i>sw6.1</i>	LG 6	ECM52	104.1	1.6	0.11

^aAdditive: phenotypic variation of additive effect. LOD = likelihood of odd; cM = centimorgan.

which the *sw1.1* on LG 1 was also linked with wave seed trait, with an LOD of 2.0.

Fine genetic and physical mapping of the wave seed and tight-placenta locus. After LG 1 was identified as the target genomic region of *ws* and *tp*, new SSR markers were designed, of which 13 were polymorphic between two parental lines (Supplemental Table 1). A linkage map was developed with the 20 SSR markers (including seven markers from BC₁P₁) and 96 F₂ individuals, which is shown in Fig. 2B. The SSR markers DM0060 and DE1337 were closest to *tp* and *ws* locus, flanking the gene at genetic distances of 12.9 and 10.0 cM, respectively. The interval between the two markers was 555 kb (32,289,526–32,845,288 bp).

An enlarged mapping population comprising 736 F₂ plants was used for fine mapping. A total of 66 recombinants were identified between SSR markers DM0060 and DE1337 in the 555-kb region. To further narrow down the candidate region for *tp* and *ws* locus, 19 new InDel markers were designed by comparing the whole-genome resequencing data. Ten of these showed clear, stable, and polymorphic amplifications between the parents (Supplemental Table 3). Then, the fine linkage map was developed with 66 recombinant plants and 12 markers (two SSR and 10 InDel markers), and the number of recombinants between adjacent markers is presented in Fig. 2C. Linkage analysis revealed that the InDel markers InDelchr1-3241 and InDelchr1-3233 were closest to *tp* and *ws*, flanking the gene at genetic distances of 2.0 and 2.7 cM, which was ≈80.9-kb region.

Candidate gene prediction and annotation in the *ws* and *tp* mapping region. Genomic DNA regions between the two flanking markers InDelchr1-3241 and InDelchr1-3233 were annotated in the melon genome database (<https://melonomics.net>). In the 80.9-kb physical distance between InDelchr1-3241 and InDelchr1-3233, there were 11 predicted genes on CM3.5_scaffold00060 (Fig. 2D). Information about position and predicted

functions of each gene is shown in Supplemental Table 4. To pinpoint possible candidate genes of *ws* and *tp*, we first looked into sequence variations in 11 predicted genes by alignment of the genomic DNA sequence of MR-1 to 'Queen'. No SNP or InDel variations were found in the exon region of coding sequence.

To further determine whether there is marker–phenotype association for *ws* and *tp* genes in this 80.9-kb region, sequence alignment were conducted between 'Queen' and MR-1. A total of 15 unique SNP locus and 17 unique InDel variations in 'Queen' were identified (Supplemental Tables 5 and 6). According to gene prediction annotation, these SNP and InDel variations were located in MELO3C023548, MELO3C023549, MELO3C023550, MELO3C023551, MELO3C023554, and MELO3C023555. The InDel (3-bp insertion-Chr.1:32354792) observed in MELO3C023555 and InDel (1-bp deletion-Chr.1: 32408632) observed in MELO3C023548 caused UTR_3_PRIME and UTR_5_PRIME region variants, respectively. The SNP (nucleotide transition of G to T-Chr.1: 32392799) and InDel (8 bp deletion-Chr.1: 32393077) were identified in the upstream of MELO3C023550. Two InDels (1-bp deletion) were observed in the upstream MELO3C023554.

Most variations, including 14 SNPs and 11 InDel variations were found in MELO3C023549, which means the MELO3C023549 in 'Queen' may be different from that of other melon lines. MELO3C023549 was predicted to encode a member of transcription factor MYB similar to an MYB46-like transcription factor that appeared to be related to positive regulation of secondary cell wall biogenesis (Supplemental Table 4). One InDel (33 bp DNA insertion-Chr.1: 32382245) was detected in the intron of MELO3C023551. In the DHL92 reference genome, this gene was 6244 bp in length with 14 exons. This 33-bp insertion of 'Queen' was found in 105-bp intron region between exon 10 and exon 11 (scaffold00060: 1,260,215–1,260,320). MELO3C023551 was

annotated to encode CESA catalytic subunit 3 (UDP forming) and related to cellulose biosynthetic process. Thus, we inferred that MELO3C023549 and MELO3C023551 were likely associated with wave seed and tight-placenta mutation in melon 'Queen'.

Discussion

We previously found an interesting phenomenon that melon 'Queen' bears tight-placenta fruits and wave seeds. Genetic analysis showed that these two traits were controlled by the recessive genes *ws* and *tp*, respectively. In addition, the cosegregation rates showed that the tight-placenta trait was linked with the wave seed trait. This is the first report about *ws* and *tp* genes in melon.

A total of 449 SSR markers were screened, but only 87 SSR markers amplified polymorphism straps which were clear, reproducible, and specific. Fifteen polymorphic SSR markers were unable to amplify clear and specific polymorphism bands. The polymorphism level was 22.7% (102 of 449) between 'Queen' and MR-1 in this study. But this polymorphic ratio did not represent the polymorphism level of SSR markers between 'Queen' and MR-1 according to our other studies. MR-1 and 'Queen' were also used as parents to map QTL for downy mildew resistance (data unpublished). In that study, 537 SSR primers were tested and 168 (31.3%) were polymorphic between MR-1 and 'Queen'. This polymorphic ratio was similar to that found between AR 5 and Harukei 3 (30.8%) (Fukino et al., 2008), or polymorphism ratio between Edisto 47 and 'Queen' (30.5%) (Ning et al., 2014a). But this polymorphism level was still lower than that found between the parents used to derive the PI_PS map (49.6%) (Gonzalo et al., 2005). The lower polymorphism between 'Queen' and MR-1 can be attributed to their more similar genetic background.

Previous studies showed that the morphology traits of Cucurbitaceae seeds were quantitative traits generally controlled by multiple genes. Several QTLs associated with seed traits were detected on seven linkage groups in watermelon (Chi et al., 2017; Liu et al., 2014; Yi et al., 2014). QTL analysis of cucumber seed was concentrated on the five linkage groups (Chen et al., 2012; Wang et al., 2014). By using an F₂ population developed from melon Ano2 and K413, Wang et al. (2011) found that several QTLs on LG 2, 5, 7, 8, 9, and 10 controlled SL, SD, and SW. Six QTLs for seed-related traits were detected on LG 1, 2, 5, and 8 in melon MR-1 and M1-15 (Ye et al., 2017). In this study, one major SL QTL and one major SS QTL, both linked with wave seed trait, were detected at LOD scores of 15.6 and 13.4, respectively. This result showed that wave seed shape had a significant impact on SL and SS traits during melon 'Queen' fruit development.

A total of 66 SSR and 19 InDel markers on LG 1 were used in this study (Supplemental Tables 1 and 3). Forty-seven of 66 SSRs were selected based on previously published

Fig. 3. QTLs for seed length (SL), width, shape, and weight detected on WinQTLCart 2.5. Mapping of quantitative trait locus (QTL) correlated with seed shape in LG I also identified significant QTL for SL in this region.

SSRs. Among them, four SSR markers ECM110, CMMS22-2, DM0060, and DE1337 were found linked with *ws* and *tp* locus. ECM110 and CMMS22-2 have been mapped in the ICuGI consensus and anchored to the melon genome (Diaz et al., 2011). In addition, DM0060 and DE1337 contributed by Syn-genta have been reported with higher polymorphism information content of 0.49 and 0.64, respectively (Diaz et al., 2015). Therefore, these four SSR markers located in QTL could be used for further research of seed traits in other melon species.

The *ws* and *tp* genes were delimited to an 80.9-kb region that contained 11 genes and nine of them were annotated. MELO3C023549 and MELO3C023551 were inferred as the candidate genes for wave seed and tight-placenta mutation in melon ‘Queen’. The sequence analysis of MELO3C023549 showed no

unique SNPs or InDel variations of ‘Queen’ in CDS region, whereas most variations were found in upstream and downstream regions (Supplemental Tables 4 and 5). This MELO3C023549 was predicted to encode an MYB46-like transcription factor. *MYB46* has been reported to regulate the biosynthesis of secondary wall, directly or cooperatively with downstream target transcription factors (Kim et al., 2013; Ko et al., 2012). Overexpression of *MYB46* caused ectopic deposition of secondary walls in *Arabidopsis* (Ko et al., 2007, 2009). MELO3C023551 was annotated to encode a member of the class CESA. Many studies showed that CESAs involved in cellulose biosynthesis of plants and generation of primary and secondary cell wall biomass (Desprez et al., 2002, 2007; Liu et al., 2012; Persson et al., 2007; Taylor et al., 2003). Mutants of *CESA4*, *CESA7*, and

CESA8 caused the lack of the characteristic secondary thickening in xylem (Taylor et al., 2003; Wolf-Rüdiger et al., 2001). Mutants of *CESA2*, *CESA5*, and *CESA9* resulted in lower cellulose content, loss of cell shape uniformity, and reduced radial wall integrity in *Arabidopsis* seedcoat epidermal cells (Mendu et al., 2011a, 2011b). Thus, MELO3C023549 and MELO3C023551 may be related to the secondary cell wall formation during melon seed development. Such unique phenomenon of *ws* and *tp* may be due to unusual expression of these genes, which resulted in ectopic deposition of cellulose on second secondary cell wall processes of melon seeds. The unusual development of the seed could then send a hormonal signal to the placenta, which leads to the different placenta development. Obviously, these possible explanations need to be verified by subsequent research.

In the candidate 80.9-kb region, there is another interesting predicted gene MELO3C023556T1, which was predicted to encode the member of the class polygalacturonase (PG). PG genes were found closely related to multiple cell separation events, including both abscission and dehiscence in *Arabidopsis* (Ogawa et al., 2009). Although no unique variation was detected by resequencing in MELO3C023556T1, this annotated gene associated with cell wall biogenesis should also be studied further. Further research will be carried out to determine precisely the genes that confer the wave seed and tight-placenta traits in melon 'Queen'.

Literature Cited

- Argyris, J.M., M. Pujol, A.M. Martín-Hernández, and J. García-Mas. 2015a. Combined use of genetic and genomics resources to understand virus resistance and fruit quality traits in melon. *Physiol. Plant.* 155:4–11.
- Argyris, J.M., A. Ruizherreria, P. Madrizmasis, W. Sanseverino, J. Morata, M. Pujol, S.E. Ramosonsins, and J. Garciamas. 2015b. Use of targeted SNP selection for an improved anchoring of the melon (*Cucumis melo* L.) scaffold genome assembly. *BMC Genomics* 16:4–29.
- Ashburner, M., C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J.M. Cherry, A.P. Davis, K. Dolinski, S.S. Dwight, and J.T. Eppig. 2000. Gene ontology: Tool for the unification of biology. The Gene Ontology Consortium. *Nat. Genet.* 25: 25–29.
- Ashfak, H., M.H. Uddin, M.A. Mannan, S. Barua, and M.A. Hoque. 2014. Studies on the isolation, physico-chemical characterization and microbial activities of melon (*Cucumis melo*) seed oil. *Carcinogenesis* 8:1375–1383.
- Chen, L.L., Z.W. Qin, X.Y. Zhou, M. Xin, and T. Wu. 2012. Molecular marker and genetic analysis of cucumber seed length. *Chinese Agricultural Sci. Bul.* 28:165–170. (in chinese with English abstr.).
- Chi, Y.Y., P. Gao, Z.C. Zhu, F.S. Luan, L.I. Guiying, and Y.U. Peng. 2017. The QTL analysis of fruit and seed associated traits in watermelon based on CAPS markers. *Scientia Agricultura Sinica* 50:1282–1293. (in chinese with English abstr.).
- Chiba, N., K. Suwabe, T. Nunome, and M. Hirai. 2003. Development of microsatellite markers in melon (*Cucumis melo* L.) and their application to major cucurbit crops. *Breeding Sci.* 53:21–27.
- Churchill, G.A. and R.W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. *Genetics* 138:963–971.
- Daley, J.D. 2014. Mapping quantitative trait loci associated with *Alternaria* leaf blight resistance and elemental sulfur tolerance in melon. *Clemson Univ., Clemson, SC, Diss.*
- Danin-Poleg, Y., N. Reis, G. Tzuri, and N. Katzir. 2000. Development and characterisation of microsatellite markers in *Cucumis*. *Theor. Appl. Genet.* 102:61–72.
- Deng, Y., L.I. Jianqi, W.U. Songfeng, Y. Zhu, Y. Chen, and H.E. Fuchu. 2006. Integrated nr database in protein annotation system and its localization. *Comput. Eng.* 32:71–72.
- Desprez, T., M. Juraniec, E.F. Crowell, H. Jouy, Z. Pochylova, F. Parcy, H. Höfte, M. Gonneau, and S. Vernhettes. 2007. Organization of cellulose synthase complexes involved in primary cell wall synthesis in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 104:15572–15577.
- Desprez, T., S. Vernhettes, M. Fagard, G. Refrégier, T. Desnos, E. Aletti, N. Py, S. Pelletier, and H. Höfte. 2002. Resistance against herbicide isoxaben and cellulose deficiency caused by distinct mutations in same cellulose synthase isoform CESA6. *Plant Physiol.* 128:482–490.
- Diaz, A., M. Fergany, G. Formisano, P. Ziarsolo, J. Blanca, Z. Fei, J.E. Staub, J.E. Zalapa, H.E. Cuevas, and G. Dace. 2011. A consensus linkage map for molecular markers and quantitative trait loci associated with economically important traits in melon (*Cucumis melo* L.). *BMC Plant Biol.* 11:111–118.
- Diaz, A., J. Forment, J.M. Argyris, N. Fukino, G. Tzuri, R. Harel-Beja, N. Katzir, J. Garcia-Mas, and A.J. Monforte. 2015. Anchoring the consensus ICuGI genetic map to the melon (*Cucumis melo* L.) genome. *Mol. Breeding* 35:188–195.
- Diaz, A., B. Zarouri, M. Fergany, I. Eduardo, J.M. Alvarez, B. Picó, and A.J. Monforte. 2014. Mapping and introgression of QTL involved in fruit shape transgressive segregation into 'Piel de Sapo' melon (*Cucumis melo* L.). *PLoS One* 9:1–12.
- Fernandezsilva, I., I. Eduardo, J. Blanca, C. Esteras, B. Picó, F. Nuez, P. Arús, J. Garciamas, and A.J. Monforte. 2008. Bin mapping of genomic and EST-derived SSRs in melon (*Cucumis melo* L.). *Theor. Appl. Genet.* 118:139–150.
- Fukino, N., T. Ohara, A.J. Monforte, M. Sugiyama, Y. Sakata, M. Kunihiisa, and S. Matsumoto. 2008. Identification of QTLs for resistance to powdery mildew and SSR markers diagnostic for powdery mildew resistance genes in melon (*Cucumis melo* L.). *Theor. Appl. Genet.* 118:165–175.
- Fukino, N., Y. Sakata, M. Kunihiisa, and S. Matsumoto. 2007. Characterisation of novel simple sequence repeat (SSR) markers for melon (*Cucumis melo* L.) and their use for genotype identification. *J. Hort. Sci. Biotechnol.* 82:330–334.
- Gao, X.W., X.F. Ning, Y.M. Wang, X.L. Wang, W.L. Yan, Z.Q. Zhang, and G. Li. 2014. Fine mapping of a gene that confers palmately lobed leaf (*plf*) in melon (*Cucumis melo* L.). *Euphytica* 200:337–347.
- Garciamas, J., A. Benjak, W. Sanseverino, M. Bourgeois, G. Mir, V.M. González, E. Hénaff, F. Cámara, L. Cozzuto, and E. Lowy. 2012. The genome of melon (*Cucumis melo* L.). *Proc. Natl. Acad. Sci. USA* 109:11872–11877.
- Gonzalo, M.J., M.M.J. Oliver, A. Monfort, S.R. Dolcet, N. Katzir, P. Arus, and A.J. Monforte. 2005. Simple-sequence repeat markers used in merging linkage maps of melon (*Cucumis melo* L.). *Theor. Appl. Genet.* 110:802–811.
- Guiuaronés, C., A.J. Monforte, M. Saladié, R.X. Corréa, J. Garciamas, and A.M. Martín Hernández. 2014. The complex resistance to cucumber mosaic cucumovirus (CMV) in the melon accession PI161375 is governed by one gene and at least two quantitative trait loci. *Mol. Breeding* 34:351–362.
- Hagiwara, T. and K. Kamimura. 1936. Cross-breeding experiments in *Cucumis Melo*. Tokyo Horticultural School Publication, Tokyo, Japan.
- Harel-Beja, R., G. Tzuri, V. Portnoy, M. Lotan-Pompan, S. Lev, S. Cohen, N. Dai, L. Yeselson, A. Meir, and S.E. Libhaber. 2010. A genetic map of melon highly enriched with fruit quality QTLs and EST markers, including sugar and carotenoid metabolism genes. *Theor. Appl. Genet.* 121:511–533.
- Hu, Z., G. Deng, H. Mou, Y. Xu, L. Chen, J. Yang, and M. Zhang. 2017. A re-sequencing-based ultra-dense genetic map reveals a gummy stem blight resistance-associated gene in *Cucumis melo*. *DNA Res.* 0:1–10.
- Hwang, J., J. Oh, Z. Kim, J.E. Staub, S.M. Chung, and Y. Park. 2014. Fine genetic mapping of a locus controlling short internode length in melon (*Cucumis melo* L.). *Mol. Breeding* 34:949–961.
- Kanehisa, M., S. Goto, S. Kawashima, Y. Okuno, and M. Hattori. 2004. The KEGG resource for deciphering the genome. *Nucl. Acids Res.* 32:277–280.
- Kim, W.-C., J.-Y. Kim, J.-H. Ko, J. Kim, and K.-H. Han. 2013. Transcription factor MYB46 is an obligate component of the transcriptional regulatory complex for functional expression of secondary wall-associated cellulose synthases in *Arabidopsis thaliana*. *J. Plant Physiol.* 170:1374–1378.
- Ko, J.-H., W.-C. Kim, and K.-H. Han. 2009. Ectopic expression of MYB46 identifies transcriptional regulatory genes involved in secondary wall biosynthesis in *Arabidopsis*. *Plant J.* 60:649–665.
- Ko, J.-H., W.-C. Kim, J.-Y. Kim, S.-J. Ahn, and K.-H. Han. 2012. MYB46-mediated transcriptional regulation of secondary wall biosynthesis. *Mol. Plant* 5:961–963.
- Ko, J.-H., S.H. Yang, A.H. Park, O. Lerouxel, and K.-H. Han. 2007. ANAC012, a member of the plant-specific NAC transcription factor family, negatively regulates xylary fiber development in *Arabidopsis thaliana*. *Plant J.* 50:1035–1048.
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Ann. Eugen.* 12:172–175.
- Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174–181.
- Lei, C. and Y.H. Kang. 2013. In vitro inhibitory effect of oriental melon (*Cucumis melo* L. var. *makuwa* Makino) seed on key enzyme linked to type 2 diabetes: Assessment of anti-diabetic potential of functional food. *J. Funct. Foods* 5:981–986.
- Li, B., Y.L. Zhao, Q.L. Zhu, Z.P. Zhang, C. Fan, S. Amanullah, P. Gao, and F.S. Luan. 2017. Mapping of powdery mildew resistance genes in melon (*Cucumis melo* L.) by bulked segregant analysis. *Scientia Hort.* 220:160–167.
- Liu, C.Q., P. Gao, and F.S. Luan. 2014. Construction of a genetic linkage map and QTL analysis of fruit-associated traits in watermelon. *Scientia Agricultura Sinica* 47:2814–2829. (in chinese with English abstr.).
- Liu, X., Q. Wang, P. Chen, F. Song, M. Guan, L. Jin, Y. Wang, and C. Yang. 2012. Four novel cellulose synthase (*CESA*) genes from birch (*Betula platyphylla* Suk.) involved in primary and secondary cell wall biosynthesis. *Intl. J. Mol. Sci.* 13:12195–12212.
- Liu, X.P., C. Yang, F.Q. Han, Z.Y. Fang, L.M. Yang, M. Zhuang, H.H. Lv, Y.M. Liu, Z.S. Li, and Y.Y. Zhang. 2016. Genetics and fine mapping of a yellow-green leaf gene (*vgl-1*) in cabbage (*Brassica oleracea* var. *capitata* L.). *Mol. Breeding* 36:82–90.
- Mendu, V., J.S. Griffiths, S. Persson, J. Stork, A.B. Downie, C. Voiniciuc, G.W. Haughn, and S. Debolt. 2011a. Subfunctionalization of cellulose synthases in seed coat epidermal cells mediates secondary radial wall synthesis and mucilage attachment. *Plant Physiol.* 157:441–453.
- Mendu, V., J. Stork, D. Harris, and S. Debolt. 2011b. Cellulose synthesis in two secondary cell wall processes in a single cell type. *Plant Signal. Behav.* 6:1638–1643.

- Natarajan, S., H.T. Kim, S.K. Thamilarasan, K. Veerappan, J.I. Park, and I.S. Nou. 2016. Whole genome re-sequencing and characterization of powdery mildew disease-associated allelic variation in melon. *PLoS One* 11: e0157524–e0157543.
- Ning, X.F., X.L. Wang, X.W. Gao, Z.Q. Zhang, L.H. Zhang, W.L. Yan, and G. Li. 2014a. Inheritance and location of powdery mildew resistance gene in melon Edisto47. *Euphytica* 195:345–353.
- Ning, X.F., L.M. Xiong, X.L. Wang, X.W. Gao, Z.Q. Zhang, L. Zhong, and G. Li. 2014b. Genetic diversity among Chinese Hami melon and its relationship with melon germplasm of diverse origins revealed by microsatellite markers. *Biochem. Syst. Ecol.* 57:432–438.
- Obandouloa, J.M., I. Eduardo, A.J. Monforte, and J.P. Fernándeztrujillo. 2009. Identification of QTLs related to sugar and organic acid composition in melon using near-isogenic lines. *Scientia Hort.* 121:425–433.
- Ogawa, M., P. Kay, S. Wilson, and S.M. Swain. 2009. ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE1 (ADPG1), ADPG2, and QUARTET2 are polygalacturonases required for cell separation during reproductive development in *Arabidopsis*. *Plant Cell* 21: 216–233.
- Percheplid, L., M. Bardin, C. Dogimont, and M. Pitrat. 2005. Relationship between Loci conferring downy mildew and powdery mildew resistance in melon assessed by quantitative trait Loci mapping. *Phytopathology* 95:556–566.
- Périn, C., S. Hagen, C.V. De, N. Katzir, Y. Daninpoieg, V. Portnoy, S. Baudraccoarnas, J. Chadoeuf, C. Dogimont, and M. Pitrat. 2002. A reference map of *Cucumis melo* based on two recombinant inbred line populations. *Theor. Appl. Genet.* 104:1017–1034.
- Persson, S., A. Paredes, A. Carroll, H. Palsdottir, M. Doblin, P. Poindexter, N. Khitrov, M. Auer, and C.R. Somerville. 2007. Genetic evidence for three unique components in primary cell-wall cellulose synthase complexes in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 104:15566–15571.
- Porebski, S., L.G. Bailey, and B.R. Baum. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol. Biol. Rptr.* 15:8–15.
- Ramamurthy, R.K. and B.M. Waters. 2015. Identification of fruit quality and morphology QTLs in melon (*Cucumis melo* L.) using a population derived from flexuosus and cantalupensis botanical groups. *Euphytica* 204:163–177.
- Ritschel, P.S., T.C.D.L. Lins, R.L. Tristan, G.S.C. Buso, J.A. Buso, and M.E. Ferreira. 2004. Development of microsatellite markers from an enriched genomic library for genetic analysis of melon (*Cucumis melo* L.). *BMC Plant Biol.* 4:9–24.
- Sabato, D., C. Esteras, O. Grillo, B. Picó, and G. Bacchetta. 2015. Seeds morpho-colourimetric analysis as complementary method to molecular characterization of melon diversity. *Scientia Hort.* 192:441–452.
- Tanaka, K., C.J. Stevens, S. Iwasaki, Y. Akashi, E. Yamamoto, T.P. Dung, H. Nishida, D.Q. Fuller, and K. Kato. 2016. Seed size and chloroplast DNA of modern and ancient seeds explain the establishment of Japanese cultivated melon (*Cucumis melo* L.) by introduction and selection. *Genet. Resources Crop Evol.* 63:1237–1254.
- Tatusov, R.L., M.Y. Galperin, D.A. Natale, and E.V. Koonin. 2000. The COG database: A tool for genome-scale analysis of protein functions and evolution. *Nucl. Acids Res.* 28:33–36.
- Taylor, N.G., R.M. Howells, A.K. Huttly, K. Vickers, and S.R. Turner. 2003. Interactions among three distinct CesA proteins essential for cellulose synthesis. *Proc. Natl. Acad. Sci. USA* 100:1450–1455.
- Tian, G., H. Miao, Y. Yang, J. Zhou, H. Lu, Y. Wang, B. Xie, S. Zhang, and X. Gu. 2016. Genetic analysis and fine mapping of Watermelon mosaic virus resistance gene in cucumber. *Mol. Breeding* 36:131–142.
- Tomason, Y., P. Nimmakayala, A. Levi, and U.K. Reddy. 2013. Map-based molecular diversity, linkage disequilibrium and association mapping of fruit traits in melon. *Mol. Breeding* 31:829–841.
- Voorrips, R.E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. *J. Hered.* 93:77–78.
- Wang, M., H. Miao, S.P. Zhang, S.L. Liu, S.Y. Dong, Y. Wang, and G.U. Xing-Fang. 2014. Inheritance analysis and QTL mapping of cucumber seed size. *Scientia Agricultura Sinica* 41:63–72. (in chinese with English abstr.).
- Wang, S., C. Basten, and Z. Zeng. 2012. Windows QTL Cartographer v2.5. Department of Statistics, North Carolina State Univ., Raleigh, NC. <<http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>>.
- Wang, X.L., X.W. Gao, G. Li, H.L. Wang, S.D. Geng, F. Kang, and X.X. Nie. 2011. Construction of a melon genetic map with fruit and seed QTLs. *Hereditas* 33:1398–1408.
- Wang, Y.H., D.H. Wu, J.H. Huang, S.J. Tsao, K.K. Hwu, and H.F. Lo. 2016. Mapping quantitative trait loci for fruit traits and powdery mildew resistance in melon (*Cucumis melo*). *Bot. Stud.* 57:19–31.
- Wolf-Rüdiger, S., E. Ravit, R. Todd, D. Deborah, and S. Chris. 2001. Modifications of cellulose synthase confer resistance to isoxaben and thiazolidinone herbicides in *Arabidopsis* *Isx1* mutants. *Proc. Natl. Acad. Sci. USA* 98:10079–10086.
- Yao, Y., K. Li, H. Liu, R.W. Duncan, S. Guo, L. Xiao, and D. Du. 2017. Whole-genome re-sequencing and fine mapping of an orange petal color gene (*Bnpc1*) in spring *Brassica napus* L. to a 151-kb region. *Euphytica* 213:165–177.
- Ye, W.Z., S. Liu, H.Y. Ma, H.Y. Liu, G.Y. Li, and F.S. Luan. 2017. QTL analysis on related seed traits in melon based on CAPS markers. *Northern Hort.* 12:119–128. (in chinese with English abstr.).
- Yi, R., C. McGregor, Z. Yan, G. Gong, H. Zhang, S. Guo, H. Sun, W. Cai, Z. Jie, and X. Yong. 2014. An integrated genetic map based on four mapping populations and quantitative trait loci associated with economically important traits in watermelon (*Citrullus lanatus*). *BMC Plant Biol.* 14:1–11.
- Yuste-Lisbona, F.J., C. Capel, E. Sarria, R. Torreblanca, M.L. Gómez-Guillamón, J. Capel, R. Lozano, and A.I. López-Sesé. 2011. Genetic linkage map of melon (*Cucumis melo* L.) and localization of a major QTL for powdery mildew resistance. *Mol. Breeding* 27:181–192.
- Zhang, C., Y. Ren, S. Guo, H. Zhang, G. Gong, Y. Du, and Y. Xu. 2013. Application of comparative genomics in developing markers tightly linked to the *Pm-2F* gene for powdery mildew resistance in melon (*Cucumis melo* L.). *Euphytica* 190:157–168.
- Zhu, Z.C., M.L. Gao, P. Gao, and F.S. Luan. 2011. QTL analysis of the first fertile flower node of *Cucumis melo* L. *Acta Horticulturae Sinica* 38:1753–1760. (in chinese with English abstr.).

Supplemental Fig. 1. Frequency distribution of (A) SS (seed length-to-width ratio), (B) SL (seed length in centimeter), (C) SD (seed width in centimeters), and (D) SW (seed weight in grams) phenotypic traits for the 128 BC₁P₁ individuals as depicted by the histogram.

Supplemental Fig. 2. Positions of quantitative trait locus (QTLs) for seed traits detected in MR-1 and 'Queen' BC₁P₁ population. The markers are shown to the right of the linkage groups and distances between markers are indicated in centimorgans to the left. QTL nomenclature for traits is given as in Table 3.

Supplemental Table 1. Detailed primer information for SSR markers on LG 1 used in this study.

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')	Polymorphic ^z	Mapping population ^y	Location ^x	Reference
ECM85	AGGACAGCGGAGCTTTCTT	TGAAATCGAAGTCCACTCTGAA	Y	A	2,684,753	Fernandezsilva et al. (2008)
CMN53_36	TGTGGCTTGATCTATCGAG	CGTCGGCTAGAGGAGAAATG	Y	A	6,223,416	Fukino et al. (2007)
CMCT505	GACAGTAATCACTCATCAAC	GGGAATGTAAATGGATATG	Y	A/B	16,534,404	Danin-Poleg et al. (2000)
ECM58	TTGAAGCTTCTTCACTCTCTTT	CACCCCAAGAGGTTCATAA	Y	A/B	24,257,863	Fernandezsilva et al. (2008)
CMMS4-3	ACCGAAATCATAAGGAACATAAGAG	TATGAGCTGTGTGTGTATGAAAC	Y	A/B	29,404,115	Chiba et al. (2003)
CMN22_22	GCTTACCCAAATTTGTAGCA	TCACCTTGGGCTCCTTATG	Y	A/B	29,985,351	Fukino et al. (2007)
CMBR039	CTGTATTGCCACGTGTC	GGCAAAAGAAAGGAAGAGTGA	Y	A	—	Ritschel et al. (2004)
ECM110	CTTCAATCGCTAACCCCTCA	CTGGAACGATCGGAATAGGA	Y	A/B	32,156,026	Fernandezsilva et al. (2008)
CMMS22_2	CGTTATACAAGATAGATAGAGAG	TTCAACTAATCCCCAAGACAACAA	Y	A/B	34,557,604	Chiba et al. (2003)
CMCTN4	AAAACAAAAGCTCTCCACGA	CTTCTCTTATATGCTACG	Y	A/B	34,684,010	Gonzalo et al. (2005)
CMCA145	GAGGAAGGCAGAAACCAAG	GCTACTTTGTGGTGGTGG	Y	B	19,894,752	Danin-Poleg et al. (2000)
ECM173	CCACAGTACGTTCGAGTGA	CCCAACAACAGATCTCAATACCC	Y	B	27,191,104	Fernandezsilva et al. (2008)
CMCTN53	CCACATTGATGAAATCTT	CATTTATAGCTTATCTTCCG	Y	B	29,166,129	Gonzalo et al. (2005)
DE1337	CTTCACTCTCTCGCAGAC	ATAGACCTAGTCGCCCTCC	Y	B/C	32,289,526	Diaz et al. (2015)
DM0060	AAACAGAGGCAGGAATC	TTTGTGGATAAGAAATTGC	Y	B/C	32,845,288	Diaz et al. (2015)
ECM191	TCAAGATCATGTTCCGAGTTG	AAGAAGACAAGCAAGCAAAAGG	Y	B	33,329,706	Fernandezsilva et al. (2008)
DE1173	AACCCCAAAATGAGAAAC	TAGTTGGAATGCCACCC	Y	B	33,775,819	Diaz et al. (2015)
DM0200	AAAGGATGAGCAAAATGAAG	GACTTGTATCCAGCTTTGC	N	—	32,195,250	Syngenta
CMTTAAN244	CTAAAGCTCTAAATGAAATCG	CAAGCCTAAAGAGATTCC	N	—	32,320,896	Diaz et al. (2015)
OAMG18	GTGCAAGCATTTTCCATGAAG	CTTGGCAATTTCCAGCTGAA	N	—	32,522,479	Cohen et al., ARO in preparation
DM0417	GCCCATGAACTAGGAAAC	AAAGGCAATTAATGAAAGG	N	—	33,407,837	Syngenta
CMMS35_3	CGGAGAAGAGCAAGGGTTTAAAGA	ATTCTAGTTTATCATCTCTTCTC	N	—	33,741,899	Diaz et al. (2015)
CMBR152	CCTCAACTCACTGCCCCIT	AAACCATCAACCCAGCAAG	N	—	—	Ritschel et al. (2004)
CMN07_70	TAATAAATACGTCCTCGG	ATACAATGTCAATGCGAG	N	—	—	Fukino et al. (2007)
DM0339	ATGACCATGATGACTCGC	AGGCCGCAATTTGATCTG	N	—	34,234,505	Syngenta
CMN07_32	GAGGATTGAACGAGGAG	GGACAGACACATCAACTGCC	N	—	331,187	Harel-Beja et al. (2010)
ECM163	TTTTCCACCCCAATCAATTA	TGGAACCTTCAACGTTAGAGAA	N	—	552,384	Fukino et al. (2007)
ECM230	CCCCATCGTAAGATGATGAA	TTGAAGAAAGAAAGTAAGGGAGA	N	—	283,841	Fernandezsilva et al. (2008)
CMATN240	CTTTTCGTCACACGTTG	GAACACACACAAAATGAG	N	—	1,110,536	Fernandezsilva et al. (2008)
CMBR135	TGTTTGGTGTGTTGATTGCTCTTC	GCTCTGCTGGGAGTGTAAAT	N	—	1,110,444	Harel-Beja et al. (2010)
CMBR147	gCTTCAATgATgTgATAAgA	CgTCACAAATgCTTATgAgA	N	—	2,432,615	Ritschel et al. (2004)
CMN23-44	CAAGAGACAAACAAATATGGGG	TAGAGGCTAAAGCTCTTTGGGTT	N	—	—	Diaz et al. (2011)
CMCTN86	GTGACAGTTATCAAGGATGC	AAGGGAATGCATGTGGAC	N	—	—	Fukino et al. (2007)
CMGAN92	GAGAGAGAGAGAGAGATG	GGTTGGGTACTCCGAGTTA	N	—	4,396,154	Gonzalo et al. (2005)
CMN04_16	TTGGTTTCATTGTTTCCCC	GCTTCCTTAATTGTTGATGA	N	—	15,346,621	Gonzalo et al. (2005)
CMMS27-1	TCCATGAATTTATCGGGACTACCA	TTGCCTCATTAATCAACTGTATTTC	N	—	18,576,438	Fukino et al. (2007)
ECM233	CTGGAGGAGGTGGAGTAGCA	TTTAGGCAACAAACCGATACA	N	—	—	Chiba et al. (2003)
ECM139	AACTAGGCATGTGCAGTTGTC	AAAAGCTTATCAAAATGAAGATTGAG	N	—	7,747,122	Fernandezsilva et al. (2008)
ECM199	GCGAAAGGAGGTTAGATTGCG	TTATACCCATTTCCACCTCA	N	—	4,202,020	Fernandezsilva et al. (2008)
TJ26	GGAGATTGGTGTCTCTCTC	CAAAACGCAAAATGTACCAAA	N	—	26,554,226	Fernandezsilva et al. (2008)
MU3752	TCTAATGGAAACCTCTCCG	TCGCAATTGTAGATCCGAAA	N	—	29,489,696	Gonzalo et al. (2005)
CMN61-14	TGCAGGATCAAGAAATCAAGTTC	ACGAACCTCCGGCATAATCAC	N	—	31,394,527	Diaz et al. (2011)
TJ27	AAGCGGAACAAGTCATCTC	CAAAAGCATCAATTGCTTGAA	N	—	—	Fukino et al. (2007)
GCM168	TGTGACGAAATTAATGAAAT	ATTGTACCTTGTGTTCACT	N	—	31,917,475	Gonzalo et al. (2005)
ECM138	AAATGAAACCTCTCGGCAAG	AAAACCTTCTTGGCCCTGT	N	—	33,525,254	Gonzalo et al. (2005)
ECM92	TCAGACTCCATTTCAGAGCTA	CAAGGAGCTCTCCCAATTAT	N	—	34,982,361	Fernandezsilva et al. (2008)
SSRchr1-3030	CAACATGCTCTGGCTAACA	AATGGACACACGCCAAAG	N	—	35,251,971	Fernandezsilva et al. (2008)
SSRchr1-3033	TTTCATTATCCACCAACCA	TCGATATTCTAACTCAATCCAACTC	N	—	30,305,708	This study
SSRchr1-3080	TGTTTCAACAGCATAGTTTGGG	TCCTTGTTCAACTCTGGTGC	N	—	30,331,810	This study
SSRchr1-3112	TGCCACACAGATATAAACAACG	TTGGTGGTAGATGCAAGTCA	Y	B	30,803,039	This study
SSRchr1-3175	TGAAGACGAGGATAATCTCAACA	ATGCAGCCTGTGAGAGGAAT	N	—	31,128,515	This study
					31,759,111	This study

(Continued on next page)

Supplemental Table 1. (Continued) Detailed primer information for SSR markers on LG 1 used in this study.

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')	Polymorphic ^z	Mapping population ^y	Location ^x	Reference
SSRchr1-3187	GTGGGACAGGAGGACAAAA	CAGTACCGATCCATCACCT	N	—	31,873,687	This study
SSRchr1-3200	GAAGGAATTATAGAGAAATGGGCA	CCACCCAAACCCAAAAAGTAA	N	—	32,000,133	This study
SSRchr1-3212	TTCAGTTTAACGCCTCTGA	CAATAGGGGTCTTGGTTTG	N	—	32,118,595	This study
SSRchr1-3221	TGGACACACAGACCAACCTC	GGAGATTTACCCATCGAGGC	Y	B	32,213,234	This study
SSRchr1-3229	TTTTTAACGATGGACTTGGC	TAACACCATGAAAGCAAGCG	N	—	32,290,570	This study
SSRchr1-3240	GACGAAGGACTGAAGCGAAC	TCTTGAAAGCAATGTGCCA	N	—	32,408,473	This study
SSRchr1-3251	CATTTTGTGAGGCTTCC	AATTTGGAATCTCAATCAGCA	N	—	32,510,089	This study
SSRchr1-3276	TGCCATTGTTGAGGTTTGA	CCTTCCTTCTCGTTGTCA	N	—	32,763,276	This study
SSRchr1-3281	AAAAGTTAAACAATGGGAAAAA	GCGAGCGGTAGATTTTCATT	N	—	32,817,946	This study
SSRchr1-3295	CGAAAAACAAGAAAAAGGCAA	CTTTGCTGTTGCAAGTA	Y	B	32,952,229	This study
SSRchr1-3307	ACTTTGAATCCAGCACCTG	AGAGGAGGCAGTTTCTTGC	Y	B	33,073,420	This study
SSRchr1-3318	CACCAGGCGTAGGTTGAT	CGCCAAAACGTACAAAAGAA	Y	B	33,186,754	This study
SSRchr1-3326	CTTGGATTCTACCAAGCCA	TGTTGGCTGTTCTCTCCTT	N	—	33,265,077	This study
SSRchr1-3337	TTGATGGACTACGTCAGAGA	TGGAGTTTCCTTTGTCCAC	Y	B	33,376,692	This study

^zThe polymorphic of SSR markers between 'Queen' and MR-1 in pseudochromosome chr 1 (LG 1 in linkage map). Y = polymorphism; N = no polymorphism.

^yThe polymorphic SSR markers were used in different mapping population. A = BC₁P₁ population; B = F₂ population; C = 66 recombinants.

^xThe location of designed SSR markers in melon chromosome 1.

Supplemental Table 2. Pearson's correlation analyses for seed size traits.

Trait	SS	SL	SD	SW
SS		0.873**	-0.400**	0.071
SL	0.873**		0.091	0.187*
SD	-0.400**	0.091		0.215*
SW	0.071	0.187*	0.215*	

*, **Significant at the 0.05 and 0.01 levels, respectively.

SL, SD, and SW indicate seed length, width, and weight, respectively; SS indicates length-to-width ratio.

Supplemental Table 3. Information of InDel markers designed for fine mapping; all the markers located on CM3.5_scaffold00060 of the melon genome.

Marker name	Location	Forward primer (5'-3')	Reverse primer (5'-3')	Mapping population ^a
InDelchr1-3232	32,327,022	ATAACTCACTGCTTGGAT	TAGTGTCTATTGTCATCG	C
InDelchr1-3233	32,333,269	TTTGCTAGGACTGTTGAA	TGTGATGTTGGTGGTAAG	C
InDelchr1-3240	32,409,633	TACGAAGGTAAACTCACA	GTCCTCAACTCATAACAT	C
InDelchr1-3241	32,414,205	CTGAACCGATTATTGACG	AAGTTTGCTCCGACTGC	C
InDelchr1-3242	32,425,848	AACAACCTTGAGCACCTAA	GACGACATTTCAACCTTT	C
InDelchr1-3250	32,503,971	ACATTCAACCAGATAAAA	TAATATGAGATAAGGGAA	C
InDelchr1-3259	32,594,101	TATGCTGCTAATCAAATGTC	CATAGAAAGGGAGGGAGA	C
InDelchr1-3267	32,677,062	GATGGTAGGAAGTTTATAGGTT	TGGCAGGACTAATTCAGCAC	C
InDelchr1-3266	32,670,804	ATTAAAGGTCGTATCGTA	GAAAAAGTAAAAGTGGG	C
InDelchr1-3274	32,748,703	ATTAATCTTTGGCTTCAA	AATATCGTGGTTAGGTTT	C
InDelchr1-3228	32,289,584	TTTATGGTGATTGGGACT	CGATGGCTGATGAACTCT	—
InDelchr1-3234	32,346,404	GTTTGGAATTAGGAGAT	TGCATAGTATTGGGAGTT	—
InDelchr1-3245	32,455,202	CGGAGATGGTCTAGCAAA	CAGTGGCGAATGAATGTG	—
InDelchr1-3252	32,526,057	TATGCTGAATTGGTGAG	TTTAGTTGGTGGGCTTTA	—
InDelchr1-3261	32,610,065	ATTAGAATGAGAAACGAT	TATTTGATGTCCATTAGT	—
InDelchr1-3276	32,763,448	GAGATGGGTTTGTTTGA	CTTCCCTTCTTCGTTGTC	—
InDelchr1-3280	32,808,660	CTTAATTTCTTACCCTT	TACACCTTATACCGTTTC	—
InDelchr1-3245	32,458,540	GGCGGGTTTAGTATAGGC	GATTTGAATTGGATGAGAA	—
InDelchr1-3254	32,545,896	TACTTCAAACATACCCTT	TTTGCTGTCTATCTTTTA	—

^aThe polymorphic SSR markers were used in different mapping population. A = BC1P1 population; B = F₂ population C = 66 recombinants.

Supplemental Table 4. Results of wave seed and tight-placenta candidate gene prediction and annotation.

#Gene ID	Start position in Chr 1	Located in CM3.5_scaffold00060	Predicated gene function
MELO3C023547T1	32,412,241	1,226,928–1,228,971	—
MELO3C023548T1	32,408,683	1,230,486–1,235,007	Energy production and conversion Cellular component: mitochondrial inner membrane Biological process: ATP synthesis coupled proton transport Molecular function: proton-transporting ATP synthase activity, rotational mechanism ATP synthase delta (OSCP) subunit, mitochondrial-like
MELO3C023549T1	32,400,474	1,238,695–1,241,155	Positive regulation of secondary cell wall biogenesis Glucuronoxylan metabolic process; biological process: xylan biosynthetic process transcription factor MYB46-like
MELO3C023550T1	32,388,398	1,250,501–1,254,583	Defense mechanisms MATE efflux family protein FRD3
MELO3C023551T1	32,377,440	1,255,485–1,261,729	Cell wall/membrane/envelope biogenesis Cellular component: integral component of membrane Molecular function: cellulose synthase (UDP-forming) activity; cellulose synthase (GDP-forming) activity Biological process: cellulose biosynthetic process Cellulose synthase A catalytic subunit 3 [UDP-forming]
MELO3C023551T2	32,377,440	1,255,485–1,262,067	Cell wall/membrane/envelope biogenesis Cellulose synthase A catalytic subunit 3 [UDP-forming]
MELO3C023552T1	32,371,779	1,267,390–1,272,802	—
MELO3C023553T1	32,362,218	1,276,951–1,277,364	—
MELO3C023554T1	32,359,259	1,279,910–1,283,395	Nuclear transcription factor Y subunit A-9-like Secondary metabolite biosynthesis, transport, and catabolism Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen Carotenoid 9,10-cleavage dioxygenase 1
MELO3C023556T1	32,341,538	1,297,631–1,304,056	Cell wall/membrane/envelope biogenesis Molecular function: polygalacturonase activity Cellular component: extracellular region Glycosyl hydrolases family 28; pectate lyase superfamily protein Polygalacturonase QRT2 precursor
MELO3C023557T1	32,332,398	1,306,771–1,308,493	Lyase activity Possible lysine decarboxylase Cytokinin riboside 5'-monophosphate phosphoribohydrolase LOG1-like;
MELO3C023558T1	32,323,175	1,309,907–1,316,055	Actin-related protein 7 isoform X2 Cotyledon development, cell division
MELO3C023558T3	32,323,305	1,313,602–1,315,864	Cytoskeleton; actin-related protein 7-like

Supplemental Table 5. The SNP variations of 'Queen' and MR-1 melon accessions in candidate genes.

Pseudochromosome	Position	Reference	Queen ^z	MR-1 ^z	Effect	Transcript ID
chr1	32,392,799	G	T	G	UPSTREAM	MELO3C023550T1
chr1	32,393,585	T	A	T	DOWNSTREAM	MELO3C023549T1
chr1	32,396,366	T	C	T	DOWNSTREAM	MELO3C023549T1
chr1	32,396,738	C	T	C	DOWNSTREAM	MELO3C023549T1
chr1	32,397,666	A	G	A	DOWNSTREAM	MELO3C023549T1
chr1	32,400,851	A	G	A	UPSTREAM	MELO3C023549T1
chr1	32,400,981	C	T	C	UPSTREAM	MELO3C023549T1
chr1	32,401,057	G	A	G	UPSTREAM	MELO3C023549T1
chr1	32,401,129	G	A	G	UPSTREAM	MELO3C023549T1
chr1	32,401,269	C	T	C	UPSTREAM	MELO3C023549T1
chr1	32,401,329	G	T	G	UPSTREAM	MELO3C023549T1
chr1	32,401,380	G	A	G	UPSTREAM	MELO3C023549T1
chr1	32,401,887	C	T	C	UPSTREAM	MELO3C023549T1
chr1	32,401,903	A	G	A	UPSTREAM	MELO3C023549T1
chr1	32,401,917	T	G	T	UPSTREAM	MELO3C023549T1

^z'Queen' bears wave seeds and tight-placenta fruits, whereas MR-1 has untight-placenta fruits and glossy seeds.

Supplemental Table 6. InDel variations of 'Queen' and MR-1 melon accessions in candidate genes.

Pseudochromosome	Position	Reference	Queen ^z	MR-1 ^z	Effect	Transcript ID
chr1	32,354,792	C	CCCT	C	UTR_3_PRIME	MELO3C023555T1
chr1	32,359,355	ATT	AT	A	UPSTREAM	MELO3C023554T1
chr1	32,360,041	CTT	CT	C	UPSTREAM	MELO3C023554T1
chr1	32,382,245	G	GAGCATGTTTCAATCCTAAAGCACCTTCTTAACA	G	INTRON	MELO3C023551T1
chr1	32,393,077	TAAAAAAA	T	TAAAAAAA	UPSTREAM	MELO3C023550T1
chr1	32,393,728	A	AT	A	DOWNSTREAM	MELO3C023549T1
chr1	32,394,076	T	TATATATATA	T	DOWNSTREAM	MELO3C023549T1
chr1	32,395,917	GA	GAAAAAAA	G	DOWNSTREAM	MELO3C023549T1
chr1	32,397,548	G	GATATTTGAATAAGCAA ATAATT	G	DOWNSTREAM	MELO3C023549T1
chr1	32,397,597	T	TCTAG	T	DOWNSTREAM	MELO3C023549T1
chr1	32,397,904	GA	G	GA	DOWNSTREAM	MELO3C023549T1
chr1	32,397,928	A	AT	A	DOWNSTREAM	MELO3C023549T1
chr1	32,397,947	TA	T	TA	DOWNSTREAM	MELO3C023549T1
chr1	32,400,135	G	GTTGTTA	GTTATTA	INTRON	MELO3C023549T1
chr1	32,401,427	TC	T	TC	UPSTREAM	MELO3C023549T1
chr1	32,402,263	GAAAAAAA	GAAAAAAA	G	UPSTREAM	MELO3C023549T1
chr1	32,408,632	ATTT	ATT	A	UTR_5_PRIME	MELO3C023548T1

^z'Queen' bears wave seeds and tight-placenta fruits, whereas MR-1 has untight-placenta fruits and glossy seeds.