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

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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 loci (QTL) referring seed traits showed that QTL ssl1.1 for seed shape (SS) and QTL ss1.1 for seed length (SL) were located at LG1, 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 LG1, flanked by InDelchr1-3241 and InDelchr1-3323. 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 allele 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 α (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 sunshine duration in summer, and long sunshine duration in winter. 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; Duley, 2014; Guinaragonès 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 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) controlled by one single dominant gene (symbol W) 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 (Obandoulloa et al., 2009; Perchepied 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; Garciamas 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-sequence. 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 BC2P1 consisting of 200 plants). The F2 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...
tight placenta and seed traits in the Summer of 2013 and 800 F2 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 $BC_2P_2$ 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 $\chi^2$ 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 sequentially 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 ng·µL$^{-1}$. 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; Fernandes-Neto et al., 2008; Fusaka 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 ≥20× coverage over the whole genome using the Illumina HiSeq 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 × 38 × 0.04-cm² 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 $\geq 3.0$ and maximum distance $\leq 50.0$ centimorgan (cM). Finally, linkage maps were visualized using MapChart 2.2 (Voorrips, 2002). Gene prediction was performed according to the melon genome annotated 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 $\chi^2$ test. Wave seeds and tight-placenta plants were never observed in $BC_2P_2$ 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, 345 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.
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 \( \chi^2 \)  | Cosegregation rates of ws and tp (%) |
|-------------|----------------------|---------------------------------------------------|-------------------------------------------------|---------------|-----------------------------------------|-----------------------------------|
| F1(2013)    | 15                   | 15/15                                             | 0/0                                             | 1:1           | 0.48/0.12                               | 96                                |
| BC1P1(2013) | 134                  | 63/69                                             | 71/65                                           | 3:1           | 0.61/0.17                               | 98                                |
| BC1P2(2013) | 96                   | 96/96                                             | 0/0                                             | 1:1           | 0.48/0.12                               | 96                                |
| F2(2015)    | 736                  | 546/536                                           | 190/200                                         | 3:1           | 0.61/0.17                               | 98                                |

\( \chi^2 > \chi_{0.05} = 3.84 \) 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.
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](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).

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

| Traits | Parents | BC1 mapping population |
|--------|---------|-------------------------|
|        | Queen   | MR-1        | Min. | Max. | Mean | sd | Skewness | Kurtosis |
| Seed weight | sw1.1 | 2.24 ± 0.11 | 2.59 ± 0.23 | 1.87 | 3.05 | 2.48 | 0.28 | -0.01 | -0.59 |
|         | sd3.1   | 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 |

*SL, 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’.

| Traits | Linkage groups closest to | Max. LOD | Position (cM) | Max. LOD | Additive |
|--------|---------------------------|----------|---------------|----------|----------|
| Seed shape | ss1.1 | LG 4 | Wave seed | 33.0 | 15.6 | -0.47 |
| Seed length | sl1.1 | LG 1 | Wave seed | 33.0 | 13.4 | -0.20 |
| Seed width | sd3.1 | LG 3 | MU4542-ECM205 | 74.2 | 2.0 | 0.02 |
|          | sd5.1 | LG 5 | ECM92 | 15.7 | 2.2 | -0.01 |
|          | sd8.1 | LG 5 | ECM52 | 35.6 | 1.5 | 0.02 |
|          | sd12.1 | LG 12 | DE1337 | 67.0 | 2.2 | 0.03 |
| Seed weight | sw1.1 | LG 1 | Wave seed | 33.0 | 2.0 | -0.10 |
|          | sw6.1 | LG 6 | ECM52 | 104.1 | 1.6 | 0.11 |

*Additive: phenotypic variation of additive effect. LOD = likelihood of odd; cM = centimorgan.

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.

### Table 3. QTLs affect seed characteristics in the BC1P1 segregated progeny of MR-1 and ‘Queen’.

Most variations, including 14 SNPs and 11 InDel variations were found in MELO3C023548, MELO3C023549, MELO3C023550, MELO3C023551, MELO3C023554, and MELO3C023555. The InDel (3 bp insertion-Chr.1:32354792) observed in MELO3C023553 and InDel (1 bp deletion-Chr.1: 32408632) observed in MELO3C023548 caused UTR3_3_PRIME and UTR5_3_PRIME region variants, respectively. The SNP (nucleotide transition 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 which means the MELO3C023549 in ‘Queen’ gene may be different from that of other melon lines. MELO3C023549 was predicted 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.
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 Syngenta 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 seedcoat epidermal cells (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 CES4, CES7, and CES8 caused the lack of the characteristic secondary thickening in xylem (Taylor et al., 2003; Wolf-Rödiger et al., 2001). Mutants of CES2, CES5, and CES9 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.

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.
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 resequence 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’.

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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 BC1F1 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 | Mapping population | Location | Reference |
|-------------|------------------------|------------------------|-------------|--------------------|----------|-----------|
| ECM85       | AGGACAGGGGACGACGTTC    | TTGGTAGAGGAGACCTCG    | Y           | A                  |          |           |
| CMN53_36    | TCTGCTCACTCTCTCATG     | TGCACTGCTGCTGCTGGA    | Y           | A                  |          |           |
| CMCT505     | GACGCTGGGCTACTACTA     | GGACACGCTGCTGCTGGA    | Y           | A                  |          |           |
| CMCT58      | TTGGATGCTGCTGCTGGA     | YYGACGCTGCTGCTGGA     | Y           | A                  |          |           |
| CMS4-4      | ACGGAAATTCCTAAGGACCTA | TATGACGCTGCTGCTGGA    | Y           | A                  |          |           |
| CMN22_22    | GTCACGCTACGCTGCTGGA   | YTTCGACGCTGCTGCTGGA  | Y           | A                  |          |           |
| CMR309      | CTGATAGGCTGCTGCTGGA   | GGACACGCTGCTGCTGGA    | Y           | A                  |          |           |
| ECM110      | CCTATCACTCGCATCTCTCA  | CTGTGGCTGCTGCTGGA     | Y           | A                  |          |           |
| CMMS25_2    | GCTGGCTGCTGCTGGA      | GCTGGCTGCTGCTGGA      | Y           | A                  |          |           |
| CMCT53N5    | GAATCCCTCTCCTACGCT    | AGTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| DE1337      | CTATCCTTCCTACGCTG     | ATAGACGCTGCTGCTGGA    | Y           | A                  |          |           |
| DM0060      | AAACGAGACGACTACCT     | TTTGTGGCTGCTGCTGGA    | Y           | B                  |          |           |
| ECM191      | GAGAGCGCTACGCTGCTG    | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| DE173       | AAGCGACGACTACGCTG     | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| DM0200      | AAAAGGACGACTACGCTG    | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| CMCTAAAN244 | CTATAGGCTGCTGCTGGA   | GAATCCCTCTACGCTG     | Y           | B                  |          |           |
| OAM118      | GGACACGCTGCTGCTGGA    | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| CMMS15_3    | CGGACACGCTGCTGCTGGA   | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| CMBR152     | CCCATCTCTTCCTACGCTG   | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| CMN107_70   | CCTATCCTCTACGCTGCTG  | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| DM0339      | TAAGACGCTGCTGCTGGA    | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| CMCTCN273   | ACTGAGGCTGCTGCTGGA    | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| CMN3_32     | GAACGACGCTGCTGCTGGA  | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| ECM183      | ATGACGCTGCTGCTGGA     | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| ECM230      | GAGAGGACGACTACGCTG    | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| MATN240     | CTTCTGCTACGCTGCTGGA  | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| CMR135      | GTTGTGGCTGCTGCTGGA   | GCTGGCTGCTGCTGGA      | Y           | B                  |          |           |
| CMR147      | gCTTCAATGCTTCTACTGAA  | GCTTCAATGCTTCTACTGAA | Y           | B                  |          |           |
| CMN294      | CAGAGACGACTACGCTG     | GCTTCAATGCTTCTACTGAA | Y           | B                  |          |           |
| CMCTN86     | GTGACGCTTACGCTGCTGGA | GCTTCAATGCTTCTACTGAA | Y           | B                  |          |           |
| CMGAN92     | GAGAGGACGACTACGCTG    | GCTTCAATGCTTCTACTGAA | Y           | B                  |          |           |
| CMN14_16    | TTGGTTGCTCCTCTCTTTTA | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |
| CMS27-1     | TCTATACGCTGCTGCTGGA  | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |
| ECM233      | TTCGAGGCTGCTGCTGGA    | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |
| ECM320      | ATGACGCTGCTGCTGGA     | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |
| ECM199      | GAGAGGACGACTACGCTG    | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |
| MJZ27       | GAGAGGACGACTACGCTG    | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |
| CM632       | TCTATACGCTGCTGCTGGA  | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |
| CMN61-14    | TCTATACGCTGCTGCTGGA  | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |
| TJ26        | GAGAGGACGACTACGCTG    | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |
| MU3572      | TCTATACGCTGCTGCTGGA  | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |
| SSCH13-3030 | CACGAGGACGACTACGCTG  | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |
| SSCH13-3033 | CACGAGGACGACTACGCTG  | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |
| SSCH13-3080 | CACGAGGACGACTACGCTG  | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |
| SSCH13-3112 | CACGAGGACGACTACGCTG  | TTTGTGGCTCCTCTCTTTTA | Y           | B                  |          |           |

(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 | Mapping population | Location | Reference |
|-------------|------------------------|------------------------|-------------|--------------------|----------|-----------|
| SSRchr1-3187 | GTGGGACAGGGAGACAAAAA | CAGCTACCGATCCATCACCT | N           | —                  | 31,873,687 | This study |
| SSRchr1-3200 | GAAGGAATTATAGAGAAAATGGGCA | CCACCAAACCACAAAAAGTAACCC | N           | —                  | 32,000,133 | This study |
| SSRchr1-3212 | TTGCAATTTAACGCTCTGGA | CAATAGGGGTTGTTGTTTGTG | N           | —                  | 32,118,595 | This study |
| SSRchr1-3221 | TGGCACAGACAGCAACACCTC | GGAGATTTACCATTGAGGTCG | Y           | B                  | 32,213,234 | This study |
| SSRchr1-3229 | TTTTAACTGTTGATTTTTGGA | TAAACACCATAAGAGGCAAACG | N           | —                  | 32,290,571 | This study |
| SSRchr1-3240 | GACGAGGACTGAAGGGAAC | TCCTTGAAGGAATAATGTGCA | N           | —                  | 32,408,473 | This study |
| SSRchr1-3251 | CATTTTGTGTTTAGAGGCAATGCTCCC | AATTGGAATCTCAATCAAGCA | N           | —                  | 32,510,089 | This study |
| SSRchr1-3276 | TGCCATTGTTGAGGTTTGGAA | CTCCTTCTGCTCTTTGCGC | N           | —                  | 32,763,276 | This study |
| SSRchr1-3281 | AAAAGTTAAAAAATGGGAAAAA | GCAGACGGTAGATTTTCTATT | N           | —                  | 32,817,946 | This study |
| SSRchr1-3295 | CGAAACACAGAAAGACAGAAA | CTGTCTTTGTTGGTCAAGGTA | Y           | B                  | 32,952,229 | This study |
| SSRchr1-3307 | ACTTTGAATCCCGACCTTG | AGAGAGCCAGTTTTTCTTGC | Y           | B                  | 33,073,420 | This study |
| SSRchr1-3318 | CACCGGCGTGAGGGTATG | CACCAAAACAGTAAAGAAGAAA | Y           | B                  | 33,186,754 | This study |
| SSRchr1-3326 | CGTGGATTCTACAAAGCCAA | TGTGGCGTTGTTCTCCTTT | N           | —                  | 33,265,077 | This study |
| SSRchr1-3337 | TGGATGGAATCTCAGTGAGA | TGGAGTTCTCCTTTGCTCCAC | 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 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 |
|-----------------------|------------|--------------------------------------------------|--------------------------------------------------|--------------------|
| InDelchr1-3232        | 32,327,022 | ATAACTCAGTACCTGTAGGAT                           | TATATGTCTATGTGTAATCAG                          | C                  |
| InDelchr1-3233        | 32,323,269 | TTTCTGACTGAGCTGTAGA                            | TAGTGATGTGTGTAATGAA                           | C                  |
| InDelchr1-3240        | 32,409,633 | TACGAAAGTAAACTCAA                                | GTCCTCAACTCATAACAT                            | C                  |
| InDelchr1-3241        | 32,414,205 | CTGAACCGATTATTGACG                              | AAGTTGCTTCCGACTGAC                           | C                  |
| InDelchr1-3242        | 32,425,848 | AAACACTATGGACAACTAAA                            | GACGACATTTCAACCTTT                            | C                  |
| InDelchr1-3250        | 32,503,971 | ACATCAAACAGATAAA                                 | TAAATAGGATAGGAA                                | C                  |
| InDelchr1-3259        | 32,594,210 | TATGTGCTAATCAAATGTC                             | CATAGAAAGGAGGGAGA                             | C                  |
| InDelchr1-3267        | 32,677,062 | GATGGATAGAATTTTATGAGTT                        | TGCCAGGACTAATTCAGC                            | C                  |
| InDelchr1-3266        | 32,670,804 | ATTAATCTTTTGCTACAAAA                             | GAAATAGTAAAGGAGG                              | C                  |
| InDelchr1-3274        | 32,748,703 | ATAAATCTTTTGCTACAAAA                             | GATTTGAATATTGAGTTT                            | C                  |
| InDelchr1-3282        | 32,289,584 | TTATGCTGATAGGAGGACT                           | CGATGGCTGATGAACTT                             | —                  |
| InDelchr1-3234        | 32,346,404 | GTGGAGAATATAAGGGAGAT                          | TGCATATTTGAGGAAGTT                            | —                  |
| InDelchr1-3245        | 32,455,202 | CCCAGATTTGCTATAGAAA                             | CAGTGGCGAATGAAAGT                             | —                  |
| InDelchr1-3252        | 32,526,057 | TATGTGCTAATTTTATGAGT                           | TTAATGTTGTTGAGCTTTA                          | —                  |
| InDelchr1-3261        | 32,610,065 | ATTAAAATAGAAAGGAGGAC                          | TATTTGATGTCACAGGTT                           | —                  |
| InDelchr1-3276        | 32,763,448 | GAGTGGTTTGGATTCTTTGAGT                          | CTTTATCTTTTTCAGGTTG                           | —                  |
| InDelchr1-3280        | 32,808,660 | CTTAATTTTCTTACCCTT                             | TACACCTTATACCCGTTT                           | —                  |
| InDelchr1-3245        | 32,458,540 | GGCGGGATTTTAGTATAGGAC                          | GATTTGATTTTGGAGAGGA                           | —                  |
| InDelchr1-3254        | 32,545,896 | TACTTCAACATACCCTT                              | TTGCTGCTTACCTTTT                             | —                  |

*The 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 | 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 |
| MELO3C023548T1 | 32,408,683 | 1,230,486–1,235,007 | Glucuronoxylan metabolic process; biological process: xylan biosynthetic process transcription factor MYB46-like |
| MELO3C023549T1 | 32,400,474 | 1,238,695–1,241,155 | Energy production and conversion  
Defense mechanisms  
Cell wall/membrane/envelope biogenesis  
Cellular component: integral component of membrane  
Molecular function: ATP synthase (OSCP) subunit, mitochondrial-like |
| MELO3C023550T1 | 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 |
| MELO3C023549T1 | 32,388,398 | 1,250,501–1,254,583 | MATE efflux family protein FRD3  
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]  
Cellulose synthase A catalytic subunit 3 [UDP-forming] |
| MELO3C023551T1 | 32,377,440 | 1,255,485–1,261,729 | Nuclear transcription factor Y subunit A-9–like  
Secondary metabolite biosynthesis, transport, and catabolism oxidoreductase activity, acting on single donors with incorporation of molecular oxygenDb |  
Molecular function: polygalacturonase activity  
Cell wall/membrane/envelope biogenesis  
Cellular component: extracellular region  
Glycosyl hydrolases family 28; pectate lyase superfamily protein  
Polygalacturonase QRT2 precursor  
Lyase activity  
Possible lysine decarboxylase  
Cytokinin riboside 5’-monophosphate phosphoribohydrolase LOGI–like;  
Actin-related protein 7 isoform X2  
Cotyledon development, cell division  
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‘ | MR-1‘ | Effect | Transcript ID |
|------------------|----------|-----------|--------|--------|--------|---------------|
| chr1             | 32,392,799 | T          | G      | G      | UPSTREAM | MELO3C023550T1 |
| chr1             | 32,393,585 | T          | A      | T      | DOWNSTREAM| MELO3C023549T1 |
| chr1             | 32,396,366 | C          | T      | C      | 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      | DOWNSTREAM| 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 |

*Queen’ bears wave seeds and tight-placenta fruits, whereas MR-1 has untight-placenta fruits and glossy seeds.
| 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             | 32,372,235| G          | GAGCATGTCTCAATCCTAAAGCACTTTCTTTAACA | G | INTRON | MELO3C023555T1 |
| 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          | GATATTTGAATAAGCAATATAATT | 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          | GTTGTGA      | GTTATTA    | INTRON      | MELO3C023549T1 |
| chr1             | 32,401,427| TC         | T            | TC         | UPSTREAM    | MELO3C023549T1 |
| chr1             | 32,402,263| GAAAAAAA   | GAAAAAAA     | G          | UPSTREAM    | MELO3C023549T1 |
| chr1             | 32,408,832| ATT        | 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.