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Research Report

Rapid and Practical Molecular Marker Development for Rind Traits in Watermelon

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Abstract. A three-locus model for rind phenotypes in watermelon (*Citrullus lanatus*) was previously proposed based on genetic analysis. These three loci, *S* (foreground stripe pattern), *D* (depth of rind color), and *Dgo* (background rind color), segregate in a Mendelian manner. Whole genome sequencing of watermelon offers a new strategy for marker development in these rind phenotype-related loci. A genotype analysis using subsets of 188, 273, 287 and 113 probes was performed for the '0901', '10909', '109905' and '90509' rind trait-segregating F_2 populations, respectively. A total of 26, 34, 30 and 15 linkage groups with 175, 254, 269 and 79 probes were constructed for the '0901', '10909', '109905' and '90509' populations, respectively. The genetic order of the probes was mostly collinear with the physical order on the reference genome, except for some probes on chromosomes 1, 3 and 11. The three rind-related loci, *S*, *D*, and *Dgo* were anchored near chr6_25767 on chromosome 6, chr8_26061 on chromosome 8 and chr4_150/chr4_249 on chromosome 4, respectively. The three loci are located on different chromosomes, and the three-locus model was therefore verified through molecular genetic analysis. We suggest a rapid and practical marker development strategy that can be used not only for rind traits but also for other agriculturally important traits in watermelon and applied for conventional breeding.

Additional key words: *Citrullus lanatus*, genome, Fluidigm genotyping, linkage map, rind phenotype

Introduction

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai.) is one of the most important vegetable crops in the world, especially in Asian countries. Rind phenotypes are important traits for watermelon breeding because they affect consumer preference in the market. Genetic studies have been conducted to determine the inheritance patterns of watermelon rind traits, such as rind color, stripe patterns and fruit shape (Barham, 1956; Henderson, 1991, 1992; Kumar and Wehner, 2011; Rhodes and Dane, 1999; Rhodes and Zhang, 1995; Poole, 1944; Porter, 1933; Weetman, 1937; Wehner, 2008). Several hypotheses related to watermelon rind phenotypes have been put forth by various research groups. Weetman (1937) and Poole (1944) suggested a model in which three alleles at a single locus would account for stripe patterns and rind color (solid dark green > striped green > non-striped green or yellowish green). Moreover, Weetman (1937) found evidence suggesting that striped patterns and rind color are inherited through two loci. Kumar and Wehner (2011) hypothesized that a solid dark green rind color is also controlled by two loci. Currently, a three-locus model has been proposed in which the rind phenotype is determined by a combination of three independent loci; *S* (foreground stripe pattern), *D* (depth of rind color), and *Dgo* (background rind color) (Yang et al., 2015).

Molecular markers linked to genes encoding enzymes, flesh color, seed proteins have previously been developed (Guner and Wehner, 2003, 2004). Molecular markers for rind color and stripe patterns have also been developed (Kim et al., 2015). The wsbin6-11 marker, obtained through bulked segregant analysis, and the random amplified polymorphic DNA (BSA-RAPD) strategy can detect watermelon individuals producing fruit with jubilee-type stripes or crimson-type stripes. The whole genome sequencing (WGS) of watermelon has been completed, and resequencing has been performed (Guo et al., 2013). The reference sequence of watermelon was used to construct a genetic map and to develop molecular markers. A high-density map comprising over 10,000 markers was constructed via genotyping-by-sequencing (GBS; Ren

et al., 2015), and a male sterility gene was recently anchored to the watermelon genome using this approach, as well (Rhee et al., 2015).

Using the Fluidigm genotyping system, we analyzed the genetic backgrounds of four F_2 populations to develop molecular markers linked to three rind-related loci: *S* (foreground stripe pattern), *D* (depth of rind color), and *Dgo* (background rind color). We suggest a rapid and practical molecular marker development system for conventional watermelon molecular breeding.

Materials and Methods

Plant Materials

Previously, we constructed four rind trait-segregating F_2 populations of watermelon, '0901' (71 individuals), '10909' (75 individuals), '109905' (63 individuals) and '90509' (64 individuals), by crossing four inbred lines: '01' (standard green with stripes; *S/S d/d dgo/dgo*), '09' (standard green without stripes; *s/s d/d dgo/dgo*), '109' (standard yellow with stripes; *S/S d/d Dgo/Dgo*) and '905' (deep green with stripes; *S/S D/D dgo/dgo*). These lines were previously used for the genetic analysis of the three loci *S*, *D*, and *Dgo*, which determine the stripe and rind color phenotypes of watermelon (Yang et al., 2015). These four F_2 populations were used to anchor the three loci. An additional four inbred lines with distinct characteristics (bush-type, high lycopene content, branchless-type and yellow flesh color) were chosen in addition to the '01', '09', '109' and '905' inbred lines and used to identify reliable single nucleotide polymorphisms (SNPs) through WGS analysis.

WGS of Watermelon Lines

Genomic DNA samples from all individuals in the four F_2 populations and eight inbred lines were extracted using the NucleoSpin® Plant II (Machery-Nagel, Düren, Germany) genomic DNA extraction kit, following the manufacturer's protocol. The purity of the genomic DNA was analyzed and found to be acceptable for use in Illumina's protocol for WGS. WGS for genetic materials was performed using an Illumina HiSeq 2500 sequencer (Illumina Inc., San Diego, CA, USA) to acquire 100-bp paired-end reads.

Discovery and Filtering of SNPs for Probe Design

Bioinformatic analysis was carried out for the discovery of SNPs in the eight inbred lines. Short reads from the WGS were aligned to the reference genome, the watermelon 97103 genome v1 (Guo et al., 2013; http://www.icugi.org), using the BWA algorithm (Li and Durbin, 2009) version 0.7.10 and SAMTOOLs version 0.1.19. The GATK package (GATK 2.7-2-g6bda569), including the sequence data processing utility (IndelRealigner and BaseRecalibrator), variant discovery utility (UnifiedGenotyper) and variant evaluation and manipulation utility (VariantFiltration), was used for the filtering of SNPs. SNPs were filtered to identify those with the following characteristics: i) homozygous SNPs, ii) bi-allelic SNPs, iii) SNPs with a read-depth greater than 20 and less than 100, iv) SNPs with a polymorphic information content (PIC) value of ≥ 0.45, and v) SNPs that were evenly distributed in the watermelon genome. A total of 474 SNPs were selected and used to construct probes (SNP type assay; Fluidigm, CA, San Francisco). A total of 432 SNPs were used to synthesize probes, and 42 SNPs were rejected by the probe design algorithm supported by Fluidigm.

Genotyping Analysis and Linkage Mapping

Fluidigm genotyping was analyzed by the Foundation of Agricultural Tech, Commercialization & Transfer (FACT) using the Fluidigm® EP1™ (Fluidigm, San Francisco, CA, USA) genotyping platform. Linkage analysis was performed using raw data from the genotyping results of selected SNPs from the Fluidigm® EP1™ genotyping system. CarthaGene mapping software (Schiex and Gaspin, 1997) was applied with a LOD-score threshold of 3.0 and a maximum distance of 30 cM.

Results

Tests of Probe Polymorphism in the Four Segregating F_2 Populations

Four F_2 populations ('0901', '10909', '109905' and '90509') were used to anchor three rind-related loci: *S* (foreground stripe pattern; stripe > non-stripe), *D* (depth of rind color; deep color > standard color) and *Dgo* (background rind color; yellow > green). Based on SNP comparisons performed using WGS data from the four parental lines, we selected subsets of 188, 273, 287, and 113 out of 432 expected polymorphic probes for the '0901', '10909', '109905' and '90509' F_2 populations, respectively. The genotypes of all the individuals in the four F_2 populations were analyzed using these subsets of probes (Table 1). Dots of polymorphic probes indicating the FEM and HEX fluorescence ratios of individuals were clustered into two or three groups in a plot (Fig. 1). Most of the probes were polymorphic in the Fluidigm genotyping analysis; however, 11, 17, 11 and 14 probes were monomorphic for the '0901', '10909', '109905' and '90509' populations, respectively (Table 1).

Construction of a Linkage Map for each F_2 Population

Genotype data for the polymorphic probes were used to construct linkage maps for the four F_2 populations. The

Table 1. The numbers of expected polymorphic probes based on WGS sequence analysis, observed probes based on Fluidigm analysis, and mapped probes

Population	Number of polymorphic probes		Mapped probes
	Expected	Observed	
0901	188	177 (94.1%)	175 (93.1%)
10909	273	256 (93.8%)	254 (93.0%)
109905	287	276 (96.1%)	269 (93.7%)
90509	113	99 (87.6%)	79 (69.9%)

* The numbers in parentheses indicate the success rate relative to the number of expected polymorphic probes

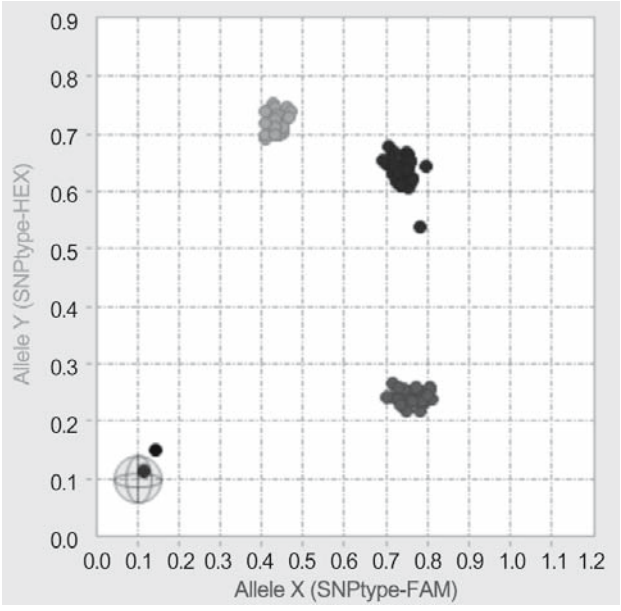

Fig. 1. Example of an SNP assay in Fluidigm analysis software (a case involving the chr_26060 probe). Dots indicate the fluorescence ratios of FAM and HEX for individuals of F_2 populations and inbred lines. Red and green clustered dots indicate homozygous genotypes of XX and YY, and blue clustered dots indicate heterozygous genotypes. Black dots indicate the negative control. The depicted case involved the chr8_26060 probe linked to the *D* locus. XX indicated the *DD* genotype (deep color), and YY indicated the *dd* genotype (standard color).

linkage maps for the '0901', '10909', '10909' and '90509' F_2 populations comprised 175, 254, 269 and 79 probes and 26, 34, 30 and 15 linkage groups, respectively (Supplementary Fig. 1). All eleven of the *C. lanatus* chromosomes were matched with at least one linkage group of each of the F_2 populations, but chromosome 6 was not matched to any linkage group in the '90509' F_2 population.

The genetic order of most probes in the linkage groups showed collinearity with the physical order of the probes in the reference genome; however, some were distorted. The genetic order of probes on chromosome 1 and chromosome 3 were moderately mismatched, and the genetic order of probes on chromosome 11 was considerably mismatched with the physical order of the reference genome sequence (Supplementary Fig. 1).

Anchoring Three Rind-related Loci that Determine Watermelon Rind Phenotypes

The *S*, *D*, and *Dgo* loci were anchored to the linkage maps of the four F_2 populations, '0901', '10909', '109905', and '90509'.

***Foreground stripe pattern (S locus)*:** Foreground stripe pattern phenotypes (present vs. absent) controlled by the *S* locus were segregated in three of the F_2 populations of '0901', '90509', and '10909'. Unfortunately, we failed to find any probes linked to the *S* locus in the '90509' and '10909' F_2 populations; however, a linkage group including the *S* locus was constructed for the '0901' F_2 population. The linkage group, comprising eight probes, was designed based on the reference genome sequence from 21,778 kb to 25,767 kb on chromosome 6 (Fig. 2). The *S* locus was linked to the end of the linkage group, near the chr6_25767 probe (Table 2). The genetic order of the probes in this linkage group indicated that the *S* locus is expected to be located beyond the 25,767 kb region of the reference genome sequence on chromosome 6.

***Depth of rind color (D locus)*:** The depth of rind color phenotype (deep color vs. standard color), controlled by the *D* locus, segregated in the F_2 populations '90509' and '109905'. A linkage group was constructed for each population containing the *D* locus. The linkage group of the '109905' F_2 population consisted of 11 probes, whereas the linkage group for the '90509' F_2 population consisted of only a single probe, chr8_26061 (Table 2). The *D* locus is closely linked to chr8_26061 in both the '109905' and '90509' F_2 populations (Fig. 2). The order of probes indicated that the *D* locus is expected to be located beyond the 26,061 kb region of the reference genome sequence on chromosome 8.

***Background rind color (Dgo locus)*:** The background rind color phenotype (green vs. yellow), conferred by the *Dgo* locus, segregated in the F_2 populations '10909' and '109905'. A linkage group was constructed for each population containing the *Dgo* locus. The linkage group '10909' comprised 6 probes designed based on the reference sequence from 249 kb to 11,500 kb, and the *Dgo* locus was linked to the chr4_249

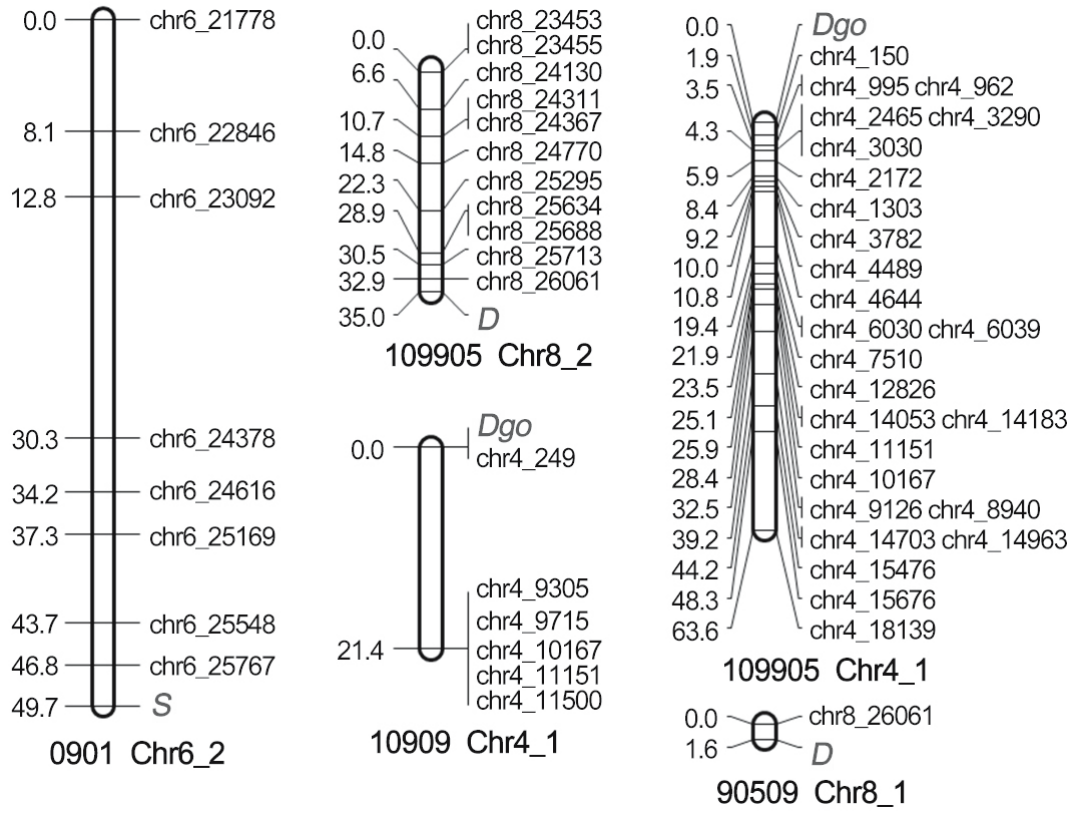

Fig. 2. Linkage maps containing three rind phenotype-related loci, *S*, *D* and *Dgo*. The *S* locus is linked to the Chr6_2 linkage group in the '0901' F_2 population. The *D* locus is linked to the chr8_2 linkage group in the '109905' F_2 population and to the Chr8_1 linkage group in the '90509' F_2 population. The *Dgo* locus is linked to the Chr4_1 linkage group in the '109905' F_2 population and to the Chr4_1 linkage group in the '10909' F_2 population.

Table 2. DNA sequences used to design four probes linked to three rind-related loci. The SNP for each allele is indicated in parenthesis

Probe	Locus	Sequence
chr4_150	*Dgo*	AAAATTTTAGAGTAGAAAAATTGTAGAGAATCAAAGTTAGAGGTTCGTTTGGTGGCCGTT**[C (yellow) / T (green)]** GAGATCCCGAGGTGTGAATCAAGGTAAGATTGACGAACTTCAATGATAGAGATTGCTTCG
chr4_249	*Dgo*	AACTTATTAGACACCAAAAATGAAAGTTCATAAACATACTAAATGCTTTTGATAATTCAA**[A (yellow) / G (green)]** AATTAAACATAAATCTCAAAGTTCAAAGACTCAACTTGCCATCGAACCATTTTGGAGTCA
chr6_25767	*S*	GTGCCACTAGACATACTTATAAATAGTCTCATTTGACCACCCTAAAAGAGAGATCTGACC**[T (non-striped) / C (striped)]** TACTTCTTTTGTGCTTAGTTGAACTTATCACTCCTTATACTAACTTAAGCATCCTACATA
chr8_26061	*D*	GAGTGAGGTAAGTGAGAAGATTCTTCACCACATTACATTACAGAAAAAGAGTGGGTTGAG**[G (deep color) / T (standard color)]** TTATTACCCATTGGAAGATGAGAGGTTCGACTGAATCGACTAAAAGCTCAAGTCGCCAAG

probe (Table 2 and Fig. 2). The linkage group '109905' comprised 26 probes designed based on the reference sequence from 150 kb to 18,139 kb, and the *Dgo* locus was linked to the chr4_150 probe (Table 2; Fig. 2). The order of the probes indicated that the *Dgo* locus is expected to be located before the 150 kb region of the reference genome sequence on chromosome 4.

Discussion

Previously, we suggested a genetic model based on the idea that the rind phenotype of watermelon is not inherited through a single locus but via three independent loci, *S*, *D*, and *Dgo*, which determine the foreground stripe pattern, depth of rind color, and background rind color, respectively (Yang et al., 2015). In this study, we successfully mapped these three loci using a Fluidigm genotyping system. The *S*, *D* and *Dgo* loci were found to be located at the end of the long arm of chromosome 6, the end of the long arm of chromosome 8, and the end of the short arm of chromosome 10, respectively. These findings indicate that the three loci are physically separated from each other. Thus, the three-locus genetic model for watermelon rind phenotypes agrees with our inheritance analysis.

The locus determining stripe pattern was previously anchored from 24,030 kb to 26,317 kb of the reference sequence of chromosome 6 (Kim et al., 2015). This locus overlaps with the expected position of the *S* locus in the present study (from 21,778 kb to 25,767 kb on chromosome 6); however, the stripe phenotypes of the plant materials differed. In our research, striped and non-striped types of watermelons were used, whereas Kim et al. used watermelons with two stripe patterns, jubilee-type watermelons and crimson-type watermelons. Furthermore, the segregation of the F_2 populations also differed among the examined plants; F_2 populations for the *S* locus segregated in a Mendelian manner, whereas F_2 populations derived from jubilee-type and crimson-type watermelons exhibited nine different phenotype patterns inherited by Quantitative trait loci (QTLs) (Kim et al., 2015; Yang et al., 2015). The striped watermelon breeding lines used in the present research were jubilee-type watermelons; therefore, the *S* locus is likely to be responsible for the presence or absence of stripes in jubilee-type watermelons, with the crimson-type stripe pattern being inherited via distinct unknown loci. The F_1 generation derived by crossing jubilee-type and crimson-type watermelons exhibited intermediate phenotypes, and six other stripe phenotypes were observed in the F_2 generation. These findings signify that both the *S* locus and unknown QTLs independently affect the stripe phenotype. We assumed that the non-striped phenotype (the *ss* genotype) is the default. When the *S* allele is present (the *SS* or *Ss* genotype), the stripe phenotype would be jubilee-type stripes; in contrast, when QTLs for the crimson type are present in watermelons with the *ss* genotype, the stripe phenotype would be crimson-type stripes. Finally, when the *S* allele and QTLs for crimson-type stripes are present in an individual, the stripe phenotype would be one of the various stripe patterns observed in the F_2 population derived from crossing jubilee-type and crimson-type watermelons.

A total of 432 probes were designed using genome sequence data from watermelon breeding lines. Of the 432 probes, 188, 273, 287, and 113 probes were expected to be polymorphic in the '0901', '10909', '109905', and '90509' F_2 populations, respectively. Therefore, the ratio of expected polymorphic probes was between 26% and 66%. We did not test the probe sets on unknown samples; however, the probe sets are expected to present high PIC values among the breeding lines. The genetic background across the breeding lines is very narrow compared with the differences between wild species and breeding lines, because genetic diversity has been reduced through domestication and modern breeding selection (Tian et al., 2009; Meyer and Purugganan, 2013). Therefore, the total number of sequence variants, such as simple sequence repeats (SSRs), SNPs, and insertions and deletions (InDels), is more limited within breeding lines than between wild species and breeding lines. On the other hand, a great deal of the sequence variation found between wild species and domesticated lines may not be valid among domesticated breeding lines; the probe sets developed based on SNPs identified between breeding lines may be rather polymorphic. Therefore, these probe sets may be useful for developing molecular markers, performing marker-assisted backcrosses (MAB) starting from domesticated breeding lines, or conducting crosses between domesticated breeding lines and wild species.

Following the development of novel sequencing technologies, many new applied marker development techniques have been introduced. GBS is a promising technique that allows the entire genome background to be scanned at a lower cost than would be required for WGS (Elshire et al., 2011; Poland and Rife, 2012). A great deal of sequence information that can be used as genotyping data can be obtained through GBS analysis of individuals in a segregating population. High-resolution maps can be constructed using this genetic information, and target gene regions can be easily narrowed down.

The Fluidigm genotyping system is a platform for mid-throughput genotyping analysis. The amount of genotype information that is obtained is limited compared to the GBS system; however, the Fluidigm system also presents advantages over the GBS system for breeding programs in terms of the analysis time. If sufficient probes with high PIC values are prepared, the genotypes of individuals in segregating populations can be analyzed in a shorter time than with GBS. Tightly linked markers (< 0.1 cM) can be developed based on the large quantities of genotype data analyzed using the GBS system; however, such markers are not essential for the early stages of a breeding program. Although the genetic distances between molecular markers are not tightly linked to the target gene, it is possible to select lines containing the target allele with high certainty. In our research, we successfully developed molecular markers with genetic distances of less than 3 cM, which was sufficient to begin marker-assisted selection for conventional breeding. Marker development strategies using NGS technologies require relatively lengthy times for library construction and sequence analysis for each population. On the other hand, mid-throughput genotyping strategies using Fluidigm require relatively short times for genotyping various populations if sufficient probes are prepared. Therefore, molecular markers linked to the target gene can be developed in a shorter time with mid-throughput genotyping systems than with NGS-based techniques.

In our research, two of the loci were found to segregate in three F_2 populations, '10909', '109905', and '90509', whereas only single locus segregated in the '0901' F_2 population. If we succeed in developing molecular markers linked to the *S*, *D*, and *Dgo* loci for the four F_2 populations, a total of seven linkage groups containing the target genes must be constructed.

However, five linkage groups were constructed in our research; therefore, the success rate of marker development was approximately 71%. It is recommended that more than two segregating populations for a single target locus should be prepared to improve the success rate.

Linkage maps of the four F_2 populations were constructed using genotype data from the Fluidigm platform, which included more than 400 probes. We compared the genetic order of the probes in our linkage maps and the physical order of the probes in the reference genome sequence. Most of the probes showed a similar order; however, syntenic relationships were broken for some probes, such as probes located after 20,000 kb on chromosome 1 and after 21,000 kb on chromosome 11, as well as a probe at 19,303 kb on chromosome 3 in the reference genome sequence. The inconsistency of these probes between linkage maps and the reference genome sequence was identical in the four F_2 populations. A high-resolution genetic map was constructed based on GBS, which also showed inconsistency between the genetic map and reference genome. Some portions of the linkage groups of chromosomes 1, 2, 4, 8 and 9 were moderately non-collinear with the reference genome, and the linkage group of chromosome 11 was markedly non-collinear with the reference genome (Reddy et al., 2014).

When scaffolds of the watermelon genome were assembled, researchers ordered the scaffolds using the watermelon linkage map built from F_8 recombinant inbred line (RIL) populations derived from a cross between the elite line '97103' (*C. lanatus* var. *lanatus*) and PI296341-FR (*C. lanatus* var. *citroides*) (Ren et al., 2012). These two parental lines belong to different subspecies, and a phylogenetic analysis between the elite line '97203' and wild species, including PI296341-FR, indicated that their relationship is distant (Guo et al., 2013). Hence, the genome structure of the domesticated subspecies and the wild subspecies could be different, which may affect the structure of the genetic map. In *Capsicum* species, translocations on chromosome 1 and chromosome 8 have been reported between domesticated *C. annuum* and wild *C. annuum* (Wu et al., 2009; Park et al., 2014). The four F_2 populations used in this study were derived from crossing a domesticated fixed breeding line, whereas the RIL population employed to order the scaffolds for genome sequence assembly was derived from crossing an elite line, '97103', and a wild species, PI296341-FR. If the genome structure of '97103' differs from that of PI296341-FR, the linkage map built using RILs derived from the cross between '97103' and PI296341-FR may show discordance with the linkage map derived from the cross between the domesticated breeding lines used in the present study. Therefore, the synteny between the linkage maps of the four F_2 populations and the reference genome sequence may be broken. Furthermore, the genetic region in which synteny is broken may be responsible for differences that have evolved between the subspecies *C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides*.

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Literature Cited

Barham WS (1956) A study of the Royal golden watermelon with emphasis on the inheritance of the chlorotic condition characteristic of this variety. Proc Am Soc Hortic Sci 67:487-489

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping -by-sequencing (GBS) approach for high diversity species. PLoS One 6:e19379

Guner N, Wehner TC (2003) Gene list for watermelon. Rep Cucurbit Genet Coop. 26:76-92

Guner N, Wehner TC (2004) The genes of watermelon. Hortic Sci 39:1175-1182

Guo S JZ, Sun H, Salse J, Lucas WJ, Zhang H, Zhang Y, Mao L, Ren Y, Wang Z (2013) The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. Nat Genet 45:51-58

Henderson WR (1991) Gene list for watermelon. Rep Cucurbit Genet Coop 14:129-138

Henderson WR (1992) Corrigenda to 1991 watermelon gene list (CGC14:129-138). Rep Cucurbit Genet Coop 15:110

Kim H, Han D, Kang J, Choi Y, Levi A, Lee GP, Park Y (2015) Sequence-characterized amplified polymorphism markers for selecting rind stripe pattern in watermelon (*Citrullus lanatus* L.). Hortic Environ Biotechnol 56:341-349

Kumar R, Wehner TC (2011) Discovery of second gene for solid dark green versus light green rind pattern in watermelon. J Hered 102:489-493

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754-1760

Meyer RS, Purugganan MD (2013) Evolution of crop species: genetics of domestication and diversification. Nat Rev Genet 14:840-852

Park SW, Jung JK, Choi EA, Kwon JK, Kang JH, Jahn M, Kang BC (2014) An EST-based linkage map reveals chromosomal translocation in *Capsicum*. Mol Breed 34:963-975.

Poland JA, Rife TW (2012) Genotyping-by-sequencing for plant breeding and genetics. Plant Gen 5:92-102

Poole CF (1944) Genetics of cultivated cucurbits. J Hered 35:122-128

Porter DR (1933) Watermelon breeding. Hilgardia 7:585-624

Reddy UK, Nimmakayala P, Levi A, Abburi VL, Saminathan T, Tomason YR, Vajja G, Reddy R, Abburi L (2014) High-resolution genetic map for understanding the effect of genome-wide recombination rate on nucleotide diversity in watermelon. G3 (Bethesda) 4:2219-2230

Ren R, Ray R, Li P, Xu J, Zhang M, Liu G, Yao X, Kilian A, Yang X (2015) Construction of a high-density DArTseq SNP-based genetic map and identification of genomic regions with segregation distortion in a genetic population derived from a cross between

feral and cultivated-type watermelon. Mol Genet Genomics 290:1457-1470

Ren Y, Zhao H, Kou Q, Jiang J, Guo S, Zhang H, Hou W, Zou X, Sun H (2012) A high resolution genetic map anchoring scaffolds of the sequenced watermelon genome. PLoS ONE 7:e29453

Rhee S, Han B, Jang YJ, Sim TY, Lee GP (2015) Construction of a genetic linkage map using a frame set of simple sequence repeat and high-resolution melting markers for watermelon (*Citrullus* spp.). Hortic Environ Biotechnol 56:669-676

Rhodes B, Dane F (1999) Gene list for watermelon. Rep Cucurbit Genet Coop 22:61-77

Rhodes B, Zhang X (1995) Gene list for watermelon. Rep Cucurbit Genet Coop 18:69-84

Schiex T, Gaspin C (1997) CARTHAGENE: constructing and joining maximum likelihood genetic maps. Proc Int Conf Intell Syst Mol Biol 5:258-267

Tian F, Stevens NM, Buckler ES IV (2009) Tracking footprints of maize domestication and evidence for a massive selective sweep on chromosome 10. Proc Natl Acad Sci USA 106 (Suppl 1):9979-9986

Weetman LM (1937) Inheritance and correlation of shape, size, and color in watermelon, *Citrullus vulgaris* Schrad. Iowa Agric Exp Stn Res Bull 228:224-256

Wehner TC (2008) Watermelons. *In* J Prohens, F Nuez, eds, Handbook of Plant Breeding. Vegetables Vol. I: Asteraceae, Brassicaceae, Chenopodiaceae, and Cucurbitaceae. Springer Verlag, New York. USA, pp 381-418

Wu F, Eannetta NT, Xu Y, Durrett R, Mazourek M, Jahn MM, Tanksley SD (2009) A COSII genetic map of the pepper genome provides a detailed picture of synteny with tomato and new insights into recent chromosome evolution in the genus *capsicum.* Theor Appl Genet 118:1279-1293

Yang H, Park S, Park Y, Lee GP, Kang S, Kim YK (2015) Linkage analysis of the three loci determining rind color and stripe pattern in watermelon. Korean J Hortic Sci Technol 33:559-565