

An Extended Genetic Linkage Map for Watermelon Based on a Testcross and a BC₂F₂ Population

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ABSTRACT

There is limited genetic mapping data useful for breeding programs of watermelon. Introgression lines should be a useful tool for genetic studies and genetic enhancement of watermelon cultivars. In this study, we used an advanced recombinant population (BC₂F₂) to identify and map chromosomal segments of the wild watermelon *Citrullus lanatus* var. *citroides* that were incorporated in the genome of the watermelon cultivar Crimson Sweet (*Citrullus lanatus* var. *lanatus*). An advanced recombinant population (BC₂F₂) was constructed using a United States Plant Introduction (PI) 494817 (*C. lanatus* var. *citroides*) (known to have moderate resistance to bacterial fruit blotch) as a donor parent, and the elite watermelon cultivar Crimson Sweet (*C. lanatus* var. *lanatus*) as the recurrent parent. The genetic linkage map consists of 272 markers, including 89 sequence-related amplified polymorphism (SRAP), 72 targeted region amplification polymorphism (TRAP), and 111 high frequency oligonucleotide-targeting active gene (HFO-TAG) markers. The 272 markers were assembled into 51 linkage groups, covering a total genetic distance of 2162 cM, with an average genetic distance of 7.9 cM between markers. Also, we expended the genetic linkage map for watermelon derived from a testcross population {Griffin 14113 [*C. lanatus* var. *citroide* (L.H. Bailey) Mansf.] x watermelon cultivar New Hampshire Midget (*C. lanatus* var. *lanatus*)} x PI 386015 [*C. colocynthis* (L.) Schrad.]. The genetic linkage map based on the test cross population consists of 558 markers that cover a genetic distance of 2760.8 cM. This linkage map consists of 41 linkage group, including 10 large linkage groups (ranging from 102 - 240 cM), nine intermediate size linkage groups (ranging from 62 - 93 cM), and 22 small linkage groups (ranging from 2 - 56 cM). Comparative mapping between these two linkage maps identified high consensus in 25 HFO-TAG markers and one TRAP marker that represent 8 linkage groups in the BC₂F₂ population and 9 linkage groups in the testcross population. These results indicate that HFO-TAG markers should be useful in comparative mapping. The extended genetic maps and the genetic population in this study should be useful in breeding programs using marker assisted selection and should serve as a platform for further development of introgression lines for watermelon.

Keywords: Testcross, Recombinant Population, SRAP, TRAP, HFO-TAG, DNA Markers, Genetic Diversity, Comparative Mapping

1. Introduction

Watermelon is an important vegetable crop worldwide. It belongs to the xerophytes genus *Citrullus* Schrad. ex Eckl. et Zeyh. Diverse populations of this genus grow freely in southern Africa which is considered to be its centre of origin. The genus *Citrullus* comprises four known diploid ($n = 11$) species. Among them is the annual *Citrullus lanatus* (Thunb.) Matsum et Nakai. This species is indigenous to the arid sandy regions of southern Africa [1]. It is considered the progenitor of the red, sweet cultivated

watermelon (*C. lanatus* var. *lanatus*) and the Tsamma watermelon (*C. lanatus* var. *citroides*) (also known as the citron or cow melon) [2,3].

Previous studies [4] indicated that North American heirloom cultivars share a narrow genetic base, while a wide genetic distance exists between the heirlooms (*C. lanatus* var. *lanatus*) and the related subspecies *C. lanatus* var. *citroides*, sharing less than 60% of their alleles [5]. A major objective of the watermelon industry is to develop breeding lines and cultivars with enhanced re-

sistance to diseases and pests. A number of PIs of *C. lanatus* var. *citroides* have been shown to contain resistance to major diseases and pests of watermelon, including resistance to gummy stem blight [6], Fusarium wilt [7], and root-knot nematodes [8]. Also, many of the *C. lanatus* var. *citroides* PIs have more plant vigor and a larger crown size and plant density than watermelon cultivars. Although the *C. lanatus* var. *citroides* fruits have white or green flesh and thick rind, they should serve as a useful source for alleles that have been lost during many years of domestication of the red, sweet cultivated watermelon. It is easy to make crosses between watermelon cultivars (*C. lanatus* var. *lanatus*) and *C. lanatus* var. *citroides* PIs. However, introgression of favorable alleles from the wild watermelon into cultivars is difficult because many favorable alleles are closely linked to undesired fruit traits.

Because of this wide genetic distance [4,5,9], F₂ genetic populations derived from crosses between watermelon cultivars (*C. lanatus* var. *lanatus*) and *C. lanatus* var. *citroides* PIs showed strong segregation distortion for most alleles [9-13]. Segregation distortion is common in wide crosses due to differences in chromosome structure (meiotic drive) [11], and may produce quasi (non-representative) genetic linkage among molecular-based markers [12-14]. Thus, classical genetic models in F₂ populations derived from a cross between *C. lanatus* var. *citroides* PIs and cultivated watermelon (*C. lanatus* var. *lanatus*) may need further evaluation because of possible segregation distortion for most alleles.

The genetic linkage maps that we have constructed for watermelon are derived from a backcross [9] or a testcross population [12,13]. These maps have been useful in identifying and mapping disease or pest resistance genes [15-17]. However, because of the distorted segregation for a large number of alleles, they have limited use in conventional breeding programs and in identifying quantitative trait loci (QTLs).

We have recently begun a study to identify markers for resistance to bacterial fruit blotch, an important seed-borne bacterial disease in watermelon. Bacterial fruit blotch, caused by the phytopathogenic bacterium *Acidovorax avenae* subsp. *citrulli* has been documented in many countries world-wide. This seed-borne pathogen can infect plants at any growth stage, but is typically seen at the seedling stage or at the mature fruit stage [18]. The first reported outbreak in the United States was in a commercial watermelon field in Florida in 1989 [18,19]. In addition to watermelon, this pathogen can cause severe disease on most cucurbits [18,20]. In recent years there have been several reports of new outbreaks of bacterial fruit blotch in the U.S., many of which are associated with watermelon [21].

Only limited chemical options exist for addressing this disease, none of which are very effective [20,21]. With such limitations in management of this disease, a source of genetic resistance (currently no cucurbit cultivar has resistance to this disease) in watermelon would be extremely beneficial to the industry. A screen of over 1,300 *Citrullus* spp. and *Praecitrullus fistulosa* accessions from the U.S. germplasm collection yielded 5 PIs with moderate levels of resistance to this disease. One of these PIs was a *C. lanatus* var. *citroides* PI 494817 [22]. To date, PI 494817 is the only known source of resistance to this disease. Studies indicate that this resistance might be a result of a complex mode of inheritance that involved several genes [23]. In order to better understand this resistance at the genetic level, we developed a F₂ population of *C. lanatus* var. *citroides* PI 494817 × *C. lanatus* var. *lanatus* watermelon cultivar Crimson Sweet for a mapping and marker study. The results of that study were inconclusive due to the wide genetic distance between PI 494817 and Crimson Sweet [4,5,9] and possibly due to distorted segregation in such wide crosses [13]. Further germplasm evaluation and genetic studies are needed to determine the mode of inheritance of resistance to bacterial fruit blotch in watermelon.

To overcome these limitations in genetic mapping we generated advanced recombinant populations (BC₂F₂ or BC₃F₂) for mapping of genetic regions that are being incorporated from *C. lanatus* var. *citroides* PIs into watermelon cultivars. These populations should exhibit normal segregation ratios for a large number of markers, as was suggested in genetic improvement studies of barley [24]. These advanced recombinant populations can be useful for genetic mapping and the identification of chromosomal segments that were introgressed from the genome of a *C. lanatus* var. *citroides* PI into the genome of a watermelon cultivar.

Eshed and Zamir [25,26] showed that introgression line populations can be useful for genetic mapping of quantitative trait loci (QTLs) and for enhancing elite varieties with exotic alleles that were lost during domestication. They proposed to develop introgression lines (ILs) by repeated backcrossing, thus introgressing chromosomal segments from an exotic wild accession into the background of an elite variety (recurrent parent). The resulting introgression lines should facilitate the dissection of quantitative traits into Mendelian factors [27]. Sets of introgression lines have been developed for different crops, including tomato [28], lettuce [29], wheat [30], soybean [31], and *Brassica oleracea* [32]. However, there is no report on the development of introgression lines in watermelon.

Our long term objective is to expand the genetic diversity in cultivated watermelon by establishing a set of lines

into which chromosomal segments containing useful disease resistance from *C. lanatus* var. *citroides* have been introgressed into the genome of watermelon cultivars (*C. lanatus* var. *lanatus*). These populations will be used in future studies of both phenotypic traits associated with the wild watermelons as well as with disease and pest resistance transferred from the wild watermelons into watermelon cultivars.

In this study, we examined 1) an advanced recombinant genetic population (BC₂F₂) for genetic mapping of watermelon, 2) if chromosomal segments of *C. lanatus* var. *citroides* PI 494817 (known to have moderate resistance to bacterial fruit blotch) [20,22,23] that were incorporated into the genomic background of the watermelon cultivar Crimson Sweet (*C. lanatus* var. *lanatus*) could be mapped and be used for construction of introgression lines. Also, we expanded the genetic linkage map for watermelon derived from a testcross population {plant accession Griffin 14113 [*C. lanatus* var. *citroide* (L. H. Bailey) Mansf.] x watermelon cultivar New Hampshire Midget (*C. lanatus* var. *lanatus*)} x PI 386015 [*C. colocynthis* (L.) Schrad.] [12,13] and performed comparative mapping between the two maps using high frequency oligonucleotide-targeting active genes (HFO-TAG) markers [33] and targeted region amplified polymorphism (TRAP) markers [34].

2. Materials and Methods

Watermelon cv. Crimson Sweet and plant introduction PI 494817 were used to create a BC₂F₂ population at the Mid-Florida Research and Education Center, Apopka, FL. Hopkins and Thompson [22] determined that PI 494817 (*C. lanatus* var. *citroides*) contains resistance to bacterial fruit blotch (BFB). The most resistant PI 494817 plant was self-pollinated and the progeny were re-evaluated for resistance to BFB in two successive generations. An F₃ plant containing resistance to BFB was selected and was crossed with 'Crimson Sweet', from which an F₂ population was produced, progeny were evaluated and the most resistant plant was backcrossed to the cultivar. A BC₁F₂ population was produced by self-pollination of one plant and was evaluated for BFB resistance. The most resistant plant was selected and backcrossed with 'Crimson Sweet'. A similar scheme was applied in subsequent generations to produce BC₂F₂ and BC₃F₂ populations (as shown in **Figure 1**).

Plant material: Young leaves (10 g) were collected from each BC₂F₂ plant, and stored at -80°C.

Isolation of DNA: To avoid co-isolation of polysaccharides, polyphenols, and other secondary compounds that damage DNA, we used an improved procedure for isolation of DNA from young leaves of watermelon [35].

SRAP and TRAP analysis: Ninety-eight SRAP primer

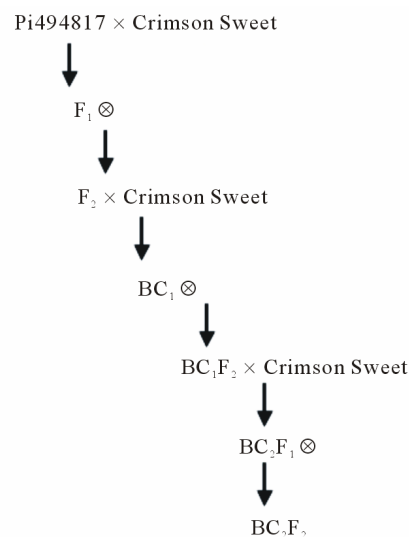


Figure 1. Breeding scheme for producing the BC₂F₂ population used for genetic mapping in this study.

combinations (**Table 1**) were tested with the donor parent PI 494817, the recurrent parent 'Crimson Sweet' and the 94 BC₂F₂ progeny. Fragment analysis was performed on capillary system CEQ8800 DNA Genetic Fragment Analysis System (Beckman Coulter, Fullerton, CA). The forward primers were labeled with one of 3 WellRED dyes (D2, D3, or D4) (Prologo, Boulder, CO). To confirm correct dye balance, the amount of dye-labeled primer added to the PCR cocktail was optimized for each primer. Each 10 µL reaction contained 1X PCR buffer (Promega, Madison, Wis.), 2.5 mM MgCl₂, 200 µM dNTPs (Sigma, St. Louis, MO), 30 ng of reverse primer (IDT, Coraville, IA), 0.25 U Taq DNA polymerase (Promega, Madison, Wis.) and 25 ng of DNA. In addition, WellRED dye-labeled forward primers were added in the following amounts: 0.1 µM for a D4 primer; 0.2 µM for a D3 primer; or 0.2 µM for a D2 primer. Samples were subjected to the following thermal profile for amplification in a PCR thermocycler (PTC 200, MJ Research): 5 min of DNA denaturing at 94°C, five cycles of three steps: 1 min of denaturing at 94°C, 1 min of annealing at 35°C and 2 min of elongation at 72°C. In the following 30 cycles the annealing temperature was increased to 50°C, with a final elongation step of 5 min at 72°C [13,33]. The PCR products were analyzed using the CEQ8800 (Beckman Coulter, Fullerton, CA) by pooling 0.35 µL of the D4 reaction, 0.45 µL of the D3 reaction, 0.55 µL of the D2 reaction, 0.28 µL of D1 size standard, and 30 µL of de-ionized formamide in a 96 well PCR plate. Samples were run on the CEQ8800 using the fragment 3 parameters and analyzed using the built-in fragment analysis software provided with the system. Fragments that ranged in size of 75 - 400 bp could be scored with high

confidence.

HFO-TAG Analysis: Thirty-six HFO-TAG primers (**Ta-**

ble 2) were used in this study were previously described [33].

Table 1. Sequence related amplified polymorphism (SRAP) forward (FP) and reverse primers (RP) used in this study.

FLP	Primer sequence 5' to 3'	RUP	Primer sequence 5' to 3'
Me1	TGAGTCCAAACCGGATA	ba1	GTCGAGCTGCCAATTATA
Me2	TGAGTCCAAACCGGAGC	ba2	GTCGAGCTGCCAATTAAT
Me3	TGAGTCCAAACCGGAAT	ba3	GTCGAGCTGCCAATTTGC
Me4	TGAGTCCAAACCGGACC	ba4	GTCGAGCTGCCAATTTTT
Me5	TGAGTCCAAACCGGAAG	ba5	GTCGAGCTGCCAATTAATA
Ab1	AGTGATTCAACCGGAGA	ba6	GTCGAGCTGCCAATTAAC
Ab2	AGTGATTCAACCGGATA	ba7	GTCGAGCTGCCAATTAAG
Ab3	AGTGATTCAACCGGAGC	ba8	GTCGAGCTGCCAATTATT
Wm1	ATTTTAGCAGCCGGGTA	ba9	GTCGAGCTGCCAATTATC
Wm2	ATTTTAGCAGCCGGAGC	ba10	GTCGAGCTGCCAATTATG
Wm3	ATTTTAGCAGCCGGGTT	ba11	GTCGAGCTGCCAATTTGA
Wm4	ATTTTAGCAGCCGGGCA	ba12	GTCGAGCTGCCAATTTGT
Wm5	ATTTTAGCAGCCGGGCA	em1	GACTGCGTACGAATTAAT
Cl1	ATAGATACGCCCGGATA	em2	GACTGCGTACGAATTTGC
Cl2	ATAGATACGCCCGGAGC	em3	GACTGCGTACGAATTGAC
Cl3	ATAGATACGCCCGGACA	em4	GACTGCGTACGAATTTGA
Cl4	ATAGATACGCCCGGATG	em5	GACTGCGTACGAATTAAC
Cl5	ATAGATACGCCCGGAGT	em6	GACTGCGTACGAATTGCA
		mw1	GGAGGTCGAAAATTGTA
		mw2	GGAGGTCGAAAATTGTC
		mw3	GGAGGTCGAAAATTGTT
		mw4	GGAGGTCGAAAATTGAG
		mw5	GGAGGTCGAAAATTGCT
		lc1	CCATCTAGGCAATTTCGA
		lc2	CCATCTAGGCAATTGGT
		lc3	CCATCTAGGCAATTGGG
		lc4	CCATCTAGGCAATTAGG
		lc5	CCATCTAGGCAATTTGC
		nw1	ACAATGGCGGAATTGGT
		nw2	ACAATGGCGGAATTTCGA
		nw3	ACAATGGCGGAATTTGG
		nw4	ACAATGGCGGAATTAGG
		nw5	ACAATGGCGGAATTTGC
		aw1	TGAAAGAGGGAATTGGC
		aw2	TGAAAGAGGGAATTGGG
		aw3	TGAAAGAGGGAATTTCGA
		aw4	TGAAAGAGGGAATTGGT
		aw5	TGAAAGAGGGAATTCCG
		se1	GAGAGAGAGAAATTGGC
		se2	GAGAGAGAGAAATTCCG
		se3	GAGAGAGAGAAATTGCG
		re1	CTCTCTCTCTAATTGGG
		re2	CTCTCTCTCTAATTGGC
		re3	CTCTCTCTCTAATTGCC

Table 2. The 36 HFO-TAG (high frequency oligonucleotides-targeting active genes) primers used in this study. The number of nucleotide bases for each primer (B), the GC content (0.875 = 87.5% and 1 = 100%) for each primer, the melting temperature (T_m) (°C), and the annealing temperature (T_a) (°C) used in PCR. In addition, the total number of fragments (TF) that were produced by each of the HFO-TAG primers and the number of fragments (MF) that could be mapped with BC₂F₂ population.

Primer	Oligos	B (no.)	GC Content	T _m	T _a	TF	MF
				(°C)	(°C)	(no.)	(no.)
HFO-14	GCGGCGGA	8	0.875	38.4	45	6	4
HFO-20	GGCGGCGA	8	0.875	38.4	45	9	9
HFO-23	ACGGCGGC	8	0.875	39.1	45	16	12
HFO-31	CGCCGCCA	8	0.875	39.1	45	5	3
HFO-37	CGGCGCCG	8	1	40.9	45	1	1
HFO-44	CGCCGGCG	8	1	40.9	45	5	5
HFO-49	GCGGCGGT	8	0.875	39.1	45	7	2
HFO-50	ACCGCCGC	8	0.875	39.1	45	2	1
HFO-55	CCGCCGCT	8	0.875	38.3	45	2	2
HFO-56	AGCGGCGG	8	0.875	38.3	45	8	7
HFO-60	TCGGCGGC	8	0.875	38.4	45	10	7
HFO-61	GCCGCCGA	8	0.875	38.4	45	1	1
HFO-66	CCGCCGGA	8	0.875	36.7	45	4	1
HFO-67	GCCGCTGC	8	0.875	36.8	45	9	4
HFO-68	GCAGCGGC	8	0.875	36.8	45	3	0
HFO-71	CCACCGCCG	9	0.889	42.4	45	5	3
HFO-72	CGGCGGTGG	9	0.889	42.4	45	2	1
HFO-75	CCGCCGGC	8	1	41.9	45	2	0
HFO-76	GCCGGCGG	8	1	41.9	45	1	1
HFO-77	CCTCCGCCG	9	0.889	41.2	45	3	1
HFO-78	CGGCGGAGG	9	0.889	41.2	45	5	3
HFO-100	CGGCGGCT	8	0.875	38.3	45	1	0
HFO-105	AGCCGCCG	8	0.875	38.3	45	10	3
HFO-117	TGCGGCGG	8	0.875	39.1	45	4	2
HFO-116	CCGCCGCA	8	0.875	39.1	45	2	2
HFO-120	AGGCGGCG	8	0.875	38.3	45	10	9
HFO-132	GGCCGCCG	8	1	41.9	45	4	2
HFO-152	TCCACCGCC	9	0.778	38.2	45	6	3
HFO-153	GGCGGTGGA	9	0.778	38.2	45	3	2
HFO-154	TGCCGCCG	8	0.875	39.1	45	9	3
HFO-158	CGGCGGCA	8	0.875	39.1	45	4	3
HFO-175	CCACCGCCA	9	0.778	38.9	45	3	2
HFO-179	ACGCCGCC	8	0.875	39.1	45	6	1
HFO-182	GGCGGCGT	8	0.875	39.1	45	13	7
HFO-184	CGGCAGCG	8	0.875	36.3	45	5	2
HFO-187	GGGCGGCG	8	1	41.9	45	9	8

Marker nomenclature: The SRAP markers were designated by combining the forward (first) and reverse primers (second) with the marker size (third) (**Table 1**). For example: the SRAP marker Wm41c5-183c (on linkage group 1) was produced by the forward primer Wm4 and by the reverse primer 1c5 (**Table 1**) and has a 183 bp size. It is unique to the recurrent parent “Crimson Sweet” and therefore was designated as “c” on the right end. The TRAP markers were designated by combining the forward (first) and reverse primers (second) with the marker size (third) (**Table 3**). For example the TRAP marker Me3-1C05-1-116 (on linkage group2) was produced by the forward primer Me3 and the reverse primer 1C05-1, and had the size of 122 bp and unique to the donor parent PI 494817. The HFO-TAG markers were designated as HFO and the size of marker they produced (**Table 2**).

Marker scoring: Markers that were unique to one of the parents, PI 494817 or “Crimson Sweet”, and were present in the BC₂ parent, and were segregating in a ratio of 1:1 could be scored for mapping in the BC₂F₂ population (**Table 4, Figure 2**). The SRAP and HFO-TAG markers were scored using the built-in fragment analysis software (provided with the Beckman CEQ8800 system).

Linkage analysis of markers in the BC₂F₂ population: Linkage analysis was performed with the JoinMap 3.0 software (Kyazma B.V., Netherlands) [36] using default options. All markers were analyzed for linkage, and recombination fractions were converted into map distances

(centimorgans, cM) with the Kosambi function. High threshold LOD scores of 7.0 - 10.0 were used for final mapping of linkage groups following removal of loci showing weak or suspect linkages. Segregation ratio distortion was evaluated with a χ^2 test for goodness of fit based on the segregation expectations of 1:1 for markers in an F₂ population [36].

Linkage analysis of markers in a testcross population: As with the BC₂F₂ population, linkage analysis was performed with the JoinMap 3.0 software (Kyazma B.V., Netherlands) [36] with default options. All markers were analyzed for linkage, and recombination fractions were converted into map distances (centimorgans, cM) with the Kosambi function. High threshold LOD scores of 7.0 - 10.0 were used for final mapping of linkage groups following removal of loci showing weak or suspect linkages. Segregation ratio distortion was evaluated with a χ^2 test for goodness of fit based on the segregation expectations of 1:1 for markers in an F₂ population [36]. Here we used the linkage mapping data that already exist for 360 markers [13] and experimented with additional markers, including HFO-TAG [26] and (TRAP) markers [34] using the testcross progeny to expand the existing genetic map [13].

Comparative mapping between the testcross and the BC₂F₂ genetic linkage maps:

A comparative genetic mapping was conducted using the JoinMap 3.0 software with the regression mapping

Table 3. The target region amplification polymorphism (TRAP) primer pairs. Each primer pair is a combination of an SRAP (forward) and a watermelon EST (reverse) primer, MP is the number of TRAP markers that could be mapped on the linkage map.

SRAP-EST	SRAP Forward Primer	EST Reverse Primer	MP
Primer Combination			
Me1-1C05-1	5' TGAGTCCAAACCGGATA	5' GCAAAGGAATAAATCTTCCTCCA	4
Me1-1E05-1	5' TGAGTCCAAACCGGATA	5' AGCAACACCAAACGCACAGA	7
Me1-1D08-1	5' TGAGTCCAAACCGGATA	5' TTCCTTCCAATTTCATCATTCA	7
Me5-1D08-1	5' TGAGTCCAAACCGGAAG	5' TTCCTTCCAATTTCATCATTCA	4
Me5-1C05-1	5' TGAGTCCAAACCGGAAG	5' GCAAAGGAATAAATCTTCCTCCA	7
Me3-1C05-1	5' TGAGTCCAAACCGGAAT	5' GCAAAGGAATAAATCTTCCTCCA	6
Me3-1D08-1	5' TGAGTCCAAACCGGAAT	5' TTCCTTCCAATTTCATCATTCA	11
Me1-2A12-1	5' TGAGTCCAAACCGGATA	5' GAAACAAACCTAAGGTGCAAAAA	2
Me1-1H11-1	5' TGAGTCCAAACCGGATA	5' TGGGTAATCCACCTGGCAAA	2
Me5-1H11-1	5' TGAGTCCAAACCGGAAG	5' TGGGTAATCCACCTGGCAAA	3
Me3-1H11-1	5' TGAGTCCAAACCGGAAT	5' TGGGTAATCCACCTGGCAAA	4
Wm2-1-A10-2	5' ATTTTAGCAGCCGGAGC	5' GAGTTTCTCTCTTTCTTGATGTTTCG	4
Wm3-1-A10-2	5' ATTTTAGCAGCCGGGTT	5' GAGTTTCTCTCTTTCTTGATGTTTCG	3
Me5-1E05-1	5' TGAGTCCAAACCGGAAG	5' AGCAACACCAAACGCACAGA	8

Table 4. The linkage groups that were mapped using the BC₂F₂ population. The linkage groups include high frequency oligonucleotide-targeting active gene (HFO-TAG) markers, sequence related amplified polymorphism (SRAP) markers, and targeted region amplification polymorphism (TRAP) markers. The total number of markers (TM) in each linkage group ranging from 2 to 40, and the genetic distance covered by each linkage group is ranging from 0 to 169 cM.

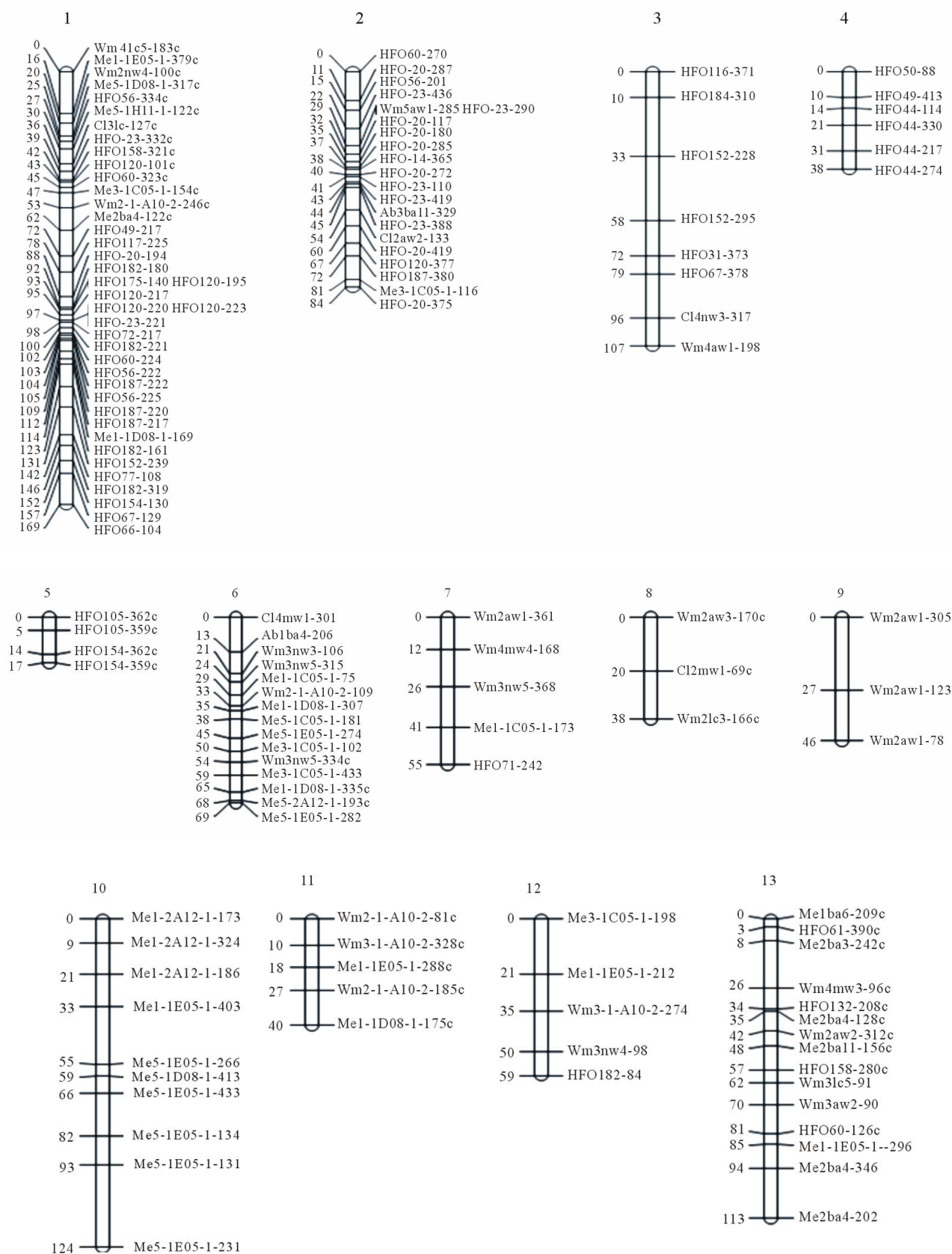
Group	HFO	SRAP	TRAP	TM	LG Distance	Group	HFO	SRAP	TRAP	TM	LG Distance
1	30	4	6	40	169 cM	27	0	2	0	2	25 cM
2	17	3	1	21	84 cM	28	0	2	0	2	26 cM
3	6	2	0	8	107 cM	29	2	1	0	3	26 cM
4	6	0	0	6	38 cM	30	0	0	3	3	47 cM
5	4	0	0	4	17 cM	31	0	2	0	2	9 cM
6	0	5	10	15	69 cM	32	3	0	0	3	13 cM
7	1	3	1	5	55 cM	33	0	3	0	3	56 cM
8	0	3	0	3	38 cM	34	0	3	0	3	56 cM
9	0	3	0	3	46 cM	35	0	1	2	3	36 cM
10	0	0	10	10	124 cM	36	3	0	0	3	5 cM
11	0	0	5	5	40 cM	37	0	0	2	2	23 cM
12	1	1	3	5	59 cM	38	0	0	2	2	11 cM
13	4	10	1	15	113 cM	39	2	0	0	2	22 cM
14	2	6	5	13	73 cM	40	2	0	0	2	8 cM
15	5	5	3	13	61 cM	41	0	2	0	2	20 cM
16	3	4	1	8	80 cM	42	0	2	0	2	10 cM
17	5	0	0	5	16 cM	43	1	0	1	2	16 cM
18	0	2	2	4	42 cM	44	0	1	1	2	20 cM
19	0	3	1	4	54 cM	45	0	2	0	2	31 cM
20	0	3	4	7	73 cM	46	0	1	1	2	23 cM
21	6	0	0	6	96 cM	47	0	2	0	2	31 cM
22	0	4	2	6	51 cM	48	1	0	1	2	21 cM
23	2	0	0	2	8 cM	49	0	0	2	2	25 cM
24	1	1	0	2	27 cM	50	2	0	0	2	0 cM
25	0	3	0	3	36 cM	51	2	0	0	2	0 cM
26	0	0	2	2	26 cM						
Total							111	89	72	272	2,162 cM

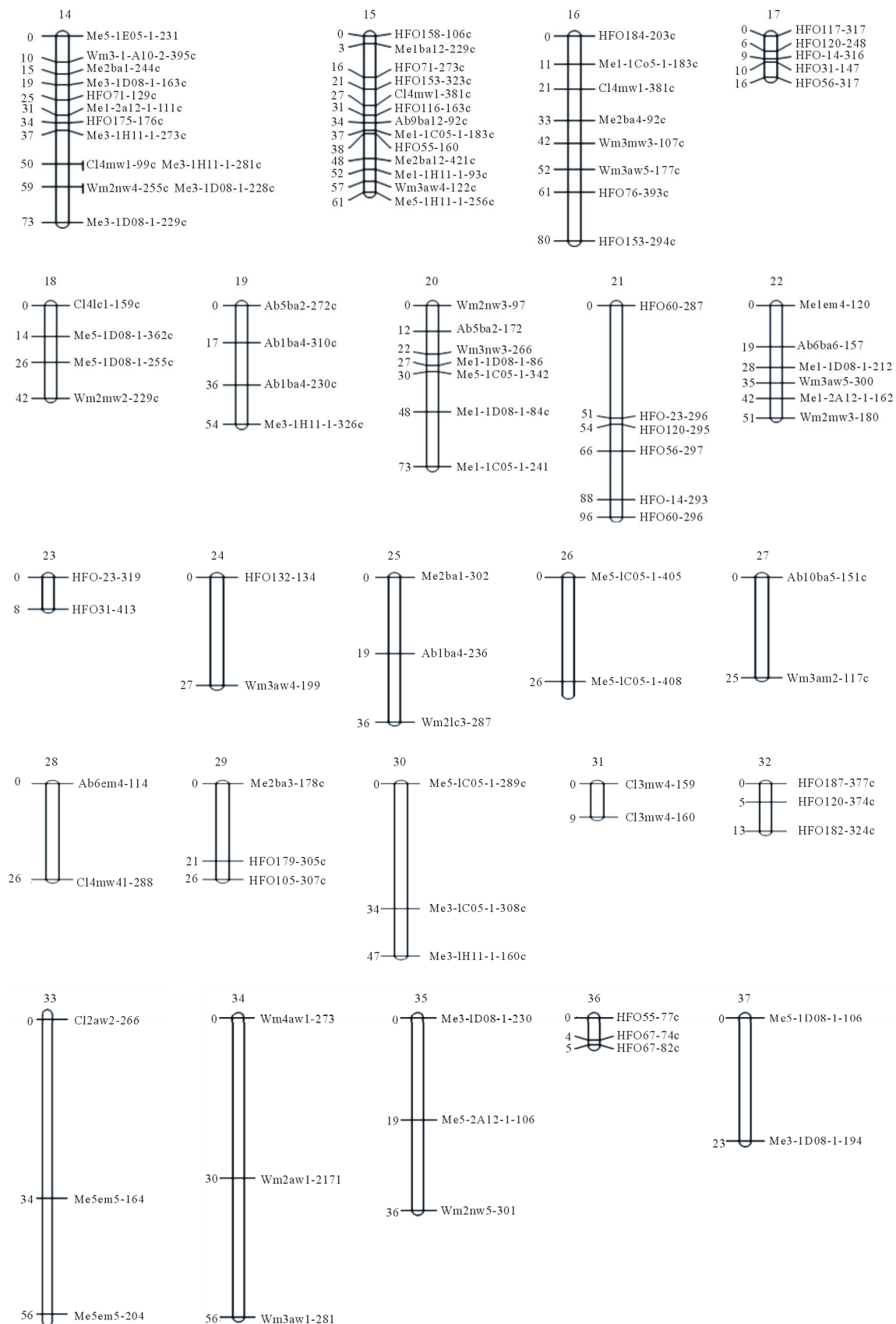
algorithm and a stringent log of the odds (LOD) score threshold of 7 - 10.

3. Results and Discussion

Polymorphism and mapping efficiency of markers using the BC₂F₂ mapping population: The SRAP, TRAP, and HFO-TAG primers produced high number of polymorphic markers that complemented each other in the assembly of linkage groups. The 73 SRAP primer combinations (**Table 1**) produced 198 polymorphic markers (2.7 markers per primer), the 14 TRAP primer combina-

tions (**Table 2**) produced 104 polymorphic markers (8 markers per primer), while the 36 HFO-TAG primers (**Table 3**) produced 195 polymorphic markers (5.4 markers per primer). Of the 497 polymorphic markers generated, one-hundred and twenty-three markers (24.7%) had distorted segregation deviating from the expected 3:1 ratio, and were not included in the mapping analysis. This number of markers with distorted segregation is still relatively high, but considerably lower than from the number of such markers found in our mapping study using an F₂ population [PI 296341 (*C. lanatus* var. *citroides*)





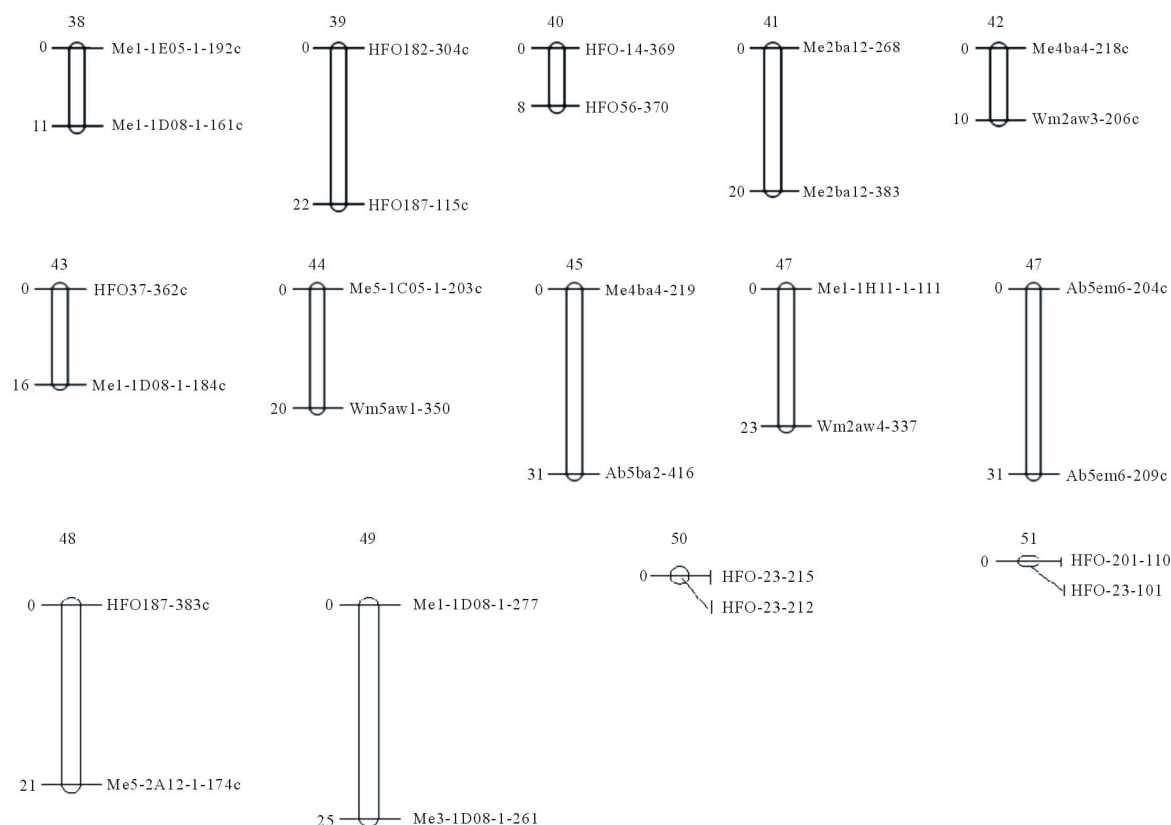


Figure 2. A genetic linkage map for watermelon derived from the BC₂F₂ population [United States Plant Introduction (PI) 494817 (donor parent), and the watermelon cultivar Crimson Sweet (recurrent parent)] with 51 linkage groups, covering a total genetic distance of 2162 cM.

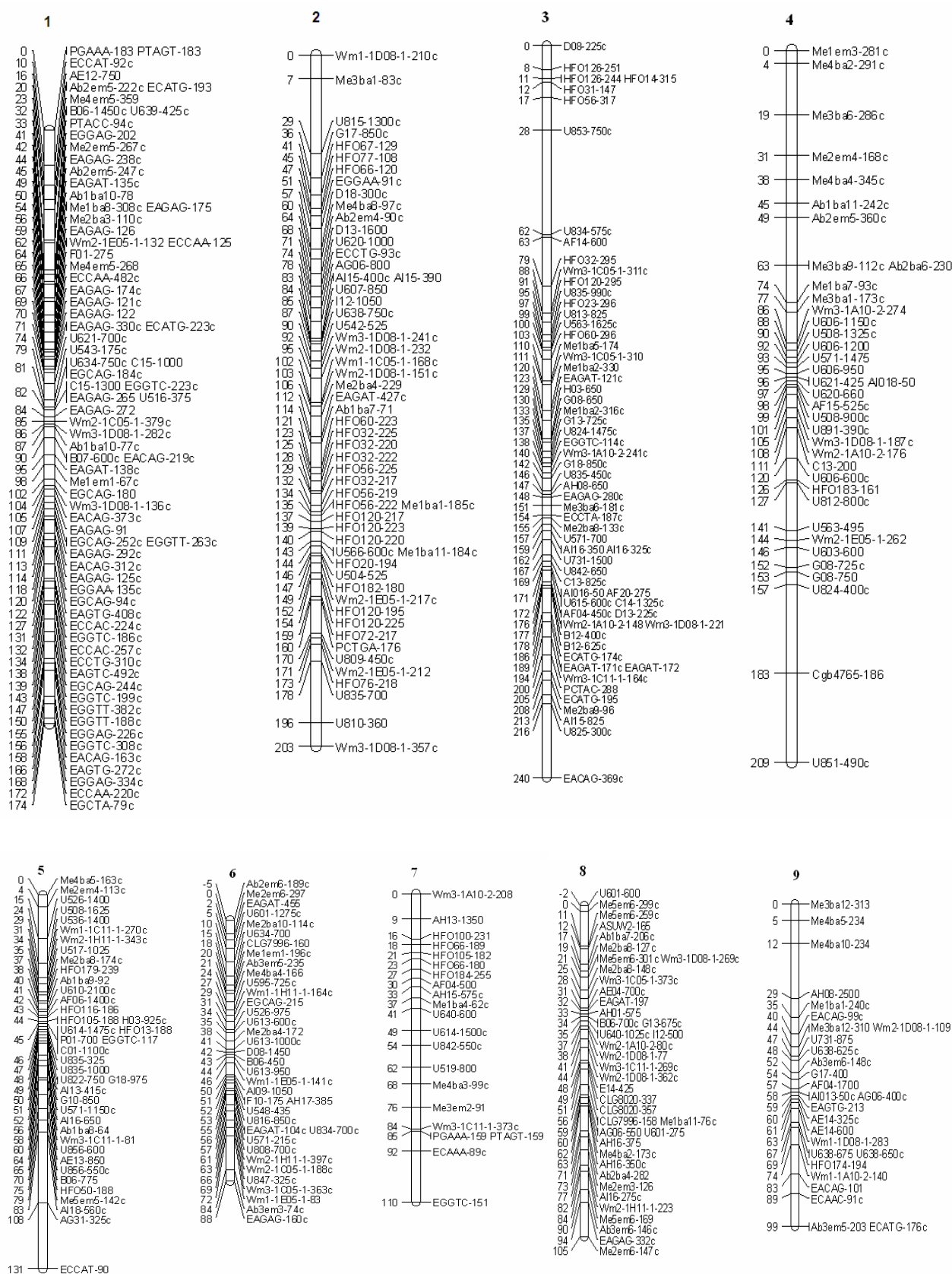
x New Hampshire Midget (*Citrullus lanatus* var. *lanatus*)). In that study, most (over 90%) of the RAPD or AFLP markers were skewed from the expected 3:1 ratio (Levi; unpublished data). In addition, the number of the skewed markers in the present study is less than that seen in the testcross mapping population {plant accession Griffin 14113 [*C. lanatus* var. *citroides* (L.H. Bailey) Mansf.] x the watermelon cultivar New Hampshire Midget (*C. lanatus* var. *lanatus*)} x PI 386015 [*C. colocynthis* (L.) Schrad.]}, where 67.7% of the AFLP and 53.8% of SRAP markers were skewed from the expected 1:1 ratio [13]. It is possible that following two backcrosses and selection for a genotype showing no infection of bacterial fruit blotch in the F₂ and in BC₁F₂ populations, some of the chromosomal regions that cause segregation distortion were excluded, resulting in a normal (Mendelian) segregation ratio for a larger number of alleles in the BC₂F₂ population. Xing *et al.* [37] showed that a quantitative trait locus (*qSSP7*) exhibiting distorted segregation in an F₂ population, had a normal segregation ratio in a BC₂F₂ population, useful for genetic mapping of rice.

Of the remaining 389 polymorphic markers that were included in the mapping analysis, 272 (70.4%) could be

mapped with high confidence (**Figure 2**). The map consists of 89 SRAP, 111 HFO-TAG (**Table 2**) and 72 TRAP markers (**Table 3**) that were assembled into 51 linkage groups that cover a total genetic distance of 2,162 cM, with an average genetic distance of 7.9 cM between markers. (**Table 4, Figure 2**). However, in contrast with our previous studies using a BC₁ or testcross populations which produced large linkage groups (133.5 - 264.5 cM) [9,12,13], most linkage groups derived from the BC₂F₂ population were relatively small (0 - 59 cM). Of the 51 linkage groups, only one was classified as a large linkage groups (Linkage group 1, having 40 markers covering a genetic distance of 169 cM; **Table 4, Figure 2**), while ten linkage groups had an intermediate size (each contains 6-21 markers, covering a genetic distance of 61 - 124 cM; **Table 4, Figure 2**). The other 40 linkage groups were relatively small (each contains 2 - 6 markers, covering a genetic distance of 0 - 59 cM). The prevalence of small linkage groups here may reflect the possible introgression of chromosomal segments from PI 494817 into the genomic background of Crimson Sweet, as was shown in other studies using BC₂F₂ or BC₃F₂ populations for the development of introgression lines [25,31,38].

Table 5. The linkage groups that were mapped in this study using the Testcross population. The linkage groups include high frequency oligonucleotide-targeting active gene (HFO-TAG) markers, , and targeted region amplification polymorphism (TRAP) markers, sequence related amplified polymorphism (SRAP) markers, amplified fragment length polymorphism (AFLP) markers, randomly amplified polymorphic DNA (RAPD) markers, and simple sequence repeat (SSR) markers. The total number of markers (TM) in each linkage group ranging from 2 to 40, and the genetic distance covered by each linkage group is ranging from 0 to 169 cM.

Group	HFO	TRAP	SRAP	AFLP	RAPD	CLG/Cgb	ASUW	1584	TM	LG Distance	
1	0	4	10	50	11	0	0	0	75	174.1	cM
2	20	8	7	4	17	0	0	0	56	203.3	cM
3	0	6	8	4	18	1	0	0	37	87.8	cM
4	5	2	3	4	7	0	0	0	21	109.6	cM
5	0	7	13	2	13	3	1	0	39	104.6	cM
6	1	3	7	5	11	0	0	0	27	98.7	cM
7	0	0	3	0	3	0	0	0	6	62.4	cM
8	1	1	2	0	1	0	0	0	5	61.9	cM
9	9	6	6	10	30	0	0	0	61	240.1	cM
10	5	3	6	2	24	0	0	0	40	130.8	cM
11	1	4	11	0	20	1	0	0	37	209.2	cM
12	3	3	4	3	13	1	0	0	27	102.3	cM
13	0	0	3	0	4	1	2	1	11	79.5	cM
14	2	0	2	1	5	0	0	0	10	84.7	cM
15	0	0	3	0	6	0	0	0	9	88.9	cM
16	0	1	3	1	1	0	0	0	6	92.8	cM
17	0	1	2	0	0	2	0	0	5	55.8	cM
18	2	2	0	0	1	0	0	0	5	20.5	cM
19	0	0	0	2	0	0	2	0	4	20.0	cM
20	3	3	3	0	4	1	1	0	15	91.3	cM
21	3	1	2	1	1	0	0	0	8	89.6	cM
22	0	1	4	1	0	0	0	0	6	75.9	cM
23	0	2	2	0	0	0	0	0	4	47.0	cM
24	0	0	1	0	2	0	0	0	3	41.0	cM
25	0	0	2	0	0	0	0	0	2	35.3	cM
26	0	0	3	0	0	0	0	0	3	33.9	cM
27	0	0	0	2	0	0	0	0	2	32.8	cM
28	0	0	0	2	0	0	0	0	2	21.9	cM
29	0	0	1	0	1	0	0	0	2	19.9	cM
30	0	0	0	2	0	0	0	0	2	12.9	cM
31	0	0	2	0	0	0	0	0	2	14.2	cM
32	0	0	0	2	0	0	0	0	2	38.3	cM
33	0	0	0	1	3	0	0	0	4	38.4	cM
34	0	0	2	0	0	0	0	0	2	33.3	cM
35	0	0	1	3	0	0	0	0	4	22.0	cM
36	2	0	0	0	0	0	0	0	2	20.6	cM
37	1	0	0	0	1	0	0	0	2	19.6	cM
38	0	0	2	0	0	0	0	0	2	18.7	cM
39	0	2	1	0	1	0	0	0	4	17.0	cM
40	0	0	2	0	0	0	0	0	2	5.9	cM
41	0	0	0	2	0	0	0	0	2	4.4	cM
Total	58	60	121	104	198	10	6	1	558	2760.8	cM



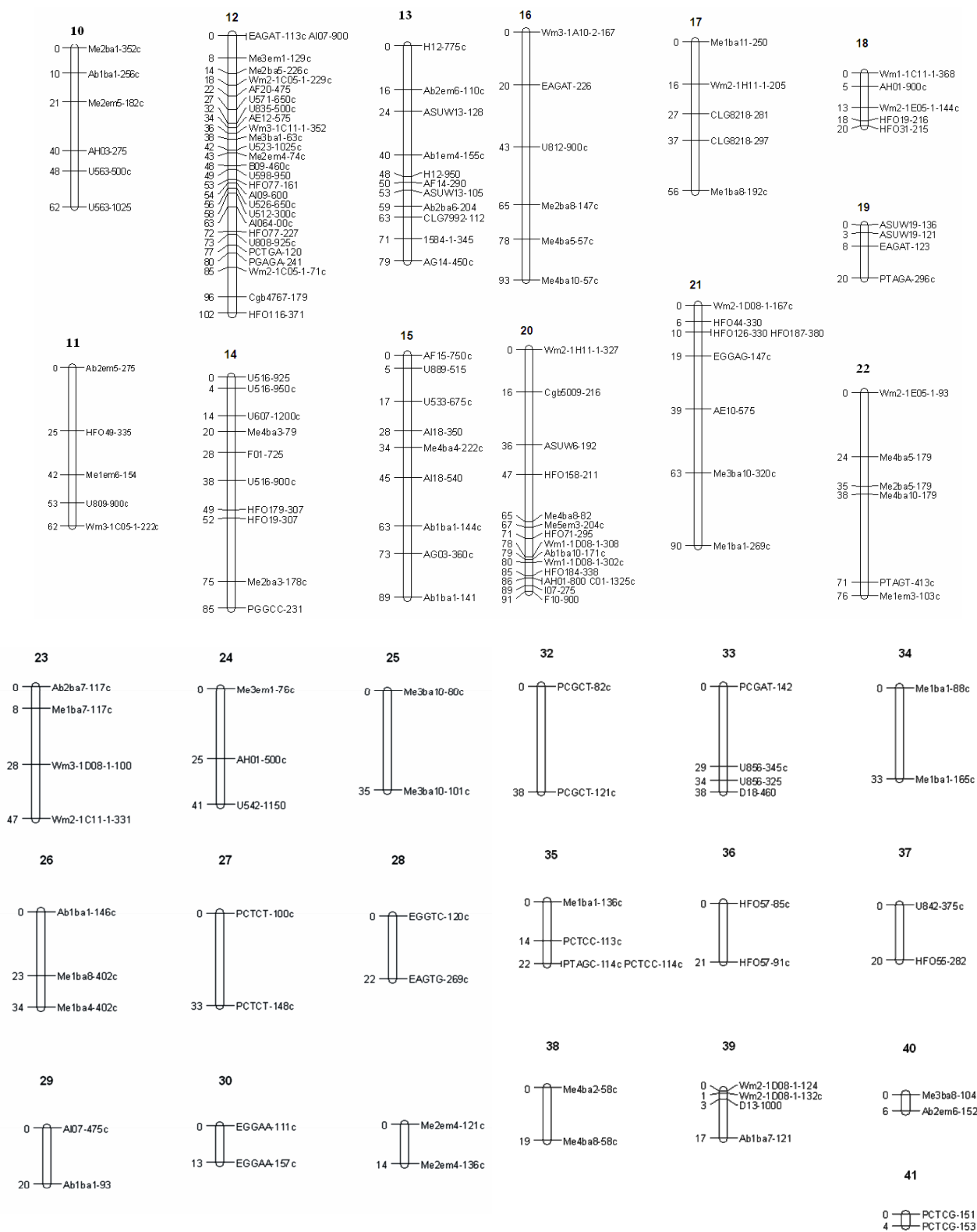
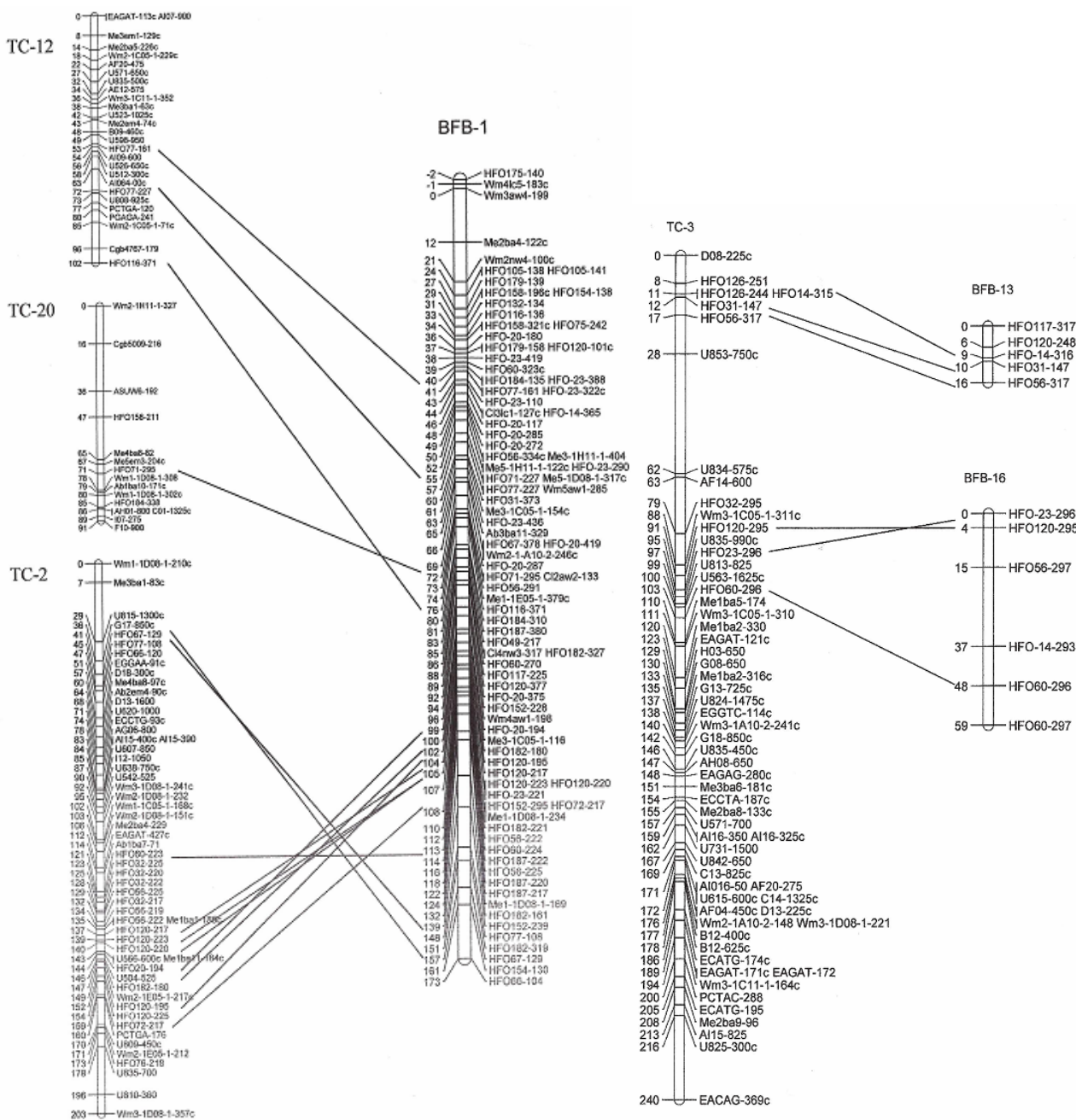


Figure 3. An extended genetic linkage map for watermelon derived from a testcross population {Griffin 14113 [*C. lanatus* var. *citroide* (L.H. Bailey) Mansf.] x watermelon cultivar New Hampshire Midget (*C. lanatus* var. *lanatus*)} x PI 386015 [*C. colocynthis* (L.) Schrad.]. The map contains 41 linkage groups with 543 makers covering a genetic distance of 2100 cm with an average distance of 4.6 cM between two markers.

Of the 274 markers that were mapped here, 176 were from the donor parent PI 494817, while the other 98 markers were from the recurrent parent "Crimson Sweet". Most linkage groups contained markers representing one parent, but not the other. Linkage groups 2, 3, 4, 7, 9, 10, 12, 17, 20, 21, 22, 23, 24, 25, 26, 28, 31, 33, 34, 35, 37, 40, 41, 45, 46, 49, 50 and 51 contained markers that represent the donor parent (PI 494817) only (Figure 2). On the other hand, linkage groups 5, 8, 11, 13, 14, 15, 16, 18, 19, 27, 29, 30, 32, 36, 38, 39, 42, 43, 47, and 48 contain markers that represent the recurrent parent (Crimson

Sweet) only (Figure 2). Linkage groups 1, 6, and 44 contain markers from both the donor and recurrent parent. The markers from the donor parent tended to group together, separated from those of the recurrent parent (as shown for linkage groups 1 and 6) (Figure 2). This clustering pattern, where markers from each individual parent tend to group unto themselves, indicates introgression of chromosomal segments from the donor into the recurrent parent (as indicated by Eshed and Zamir) [25,31].

Expanding the genetic linkage map based on a test-cross population:



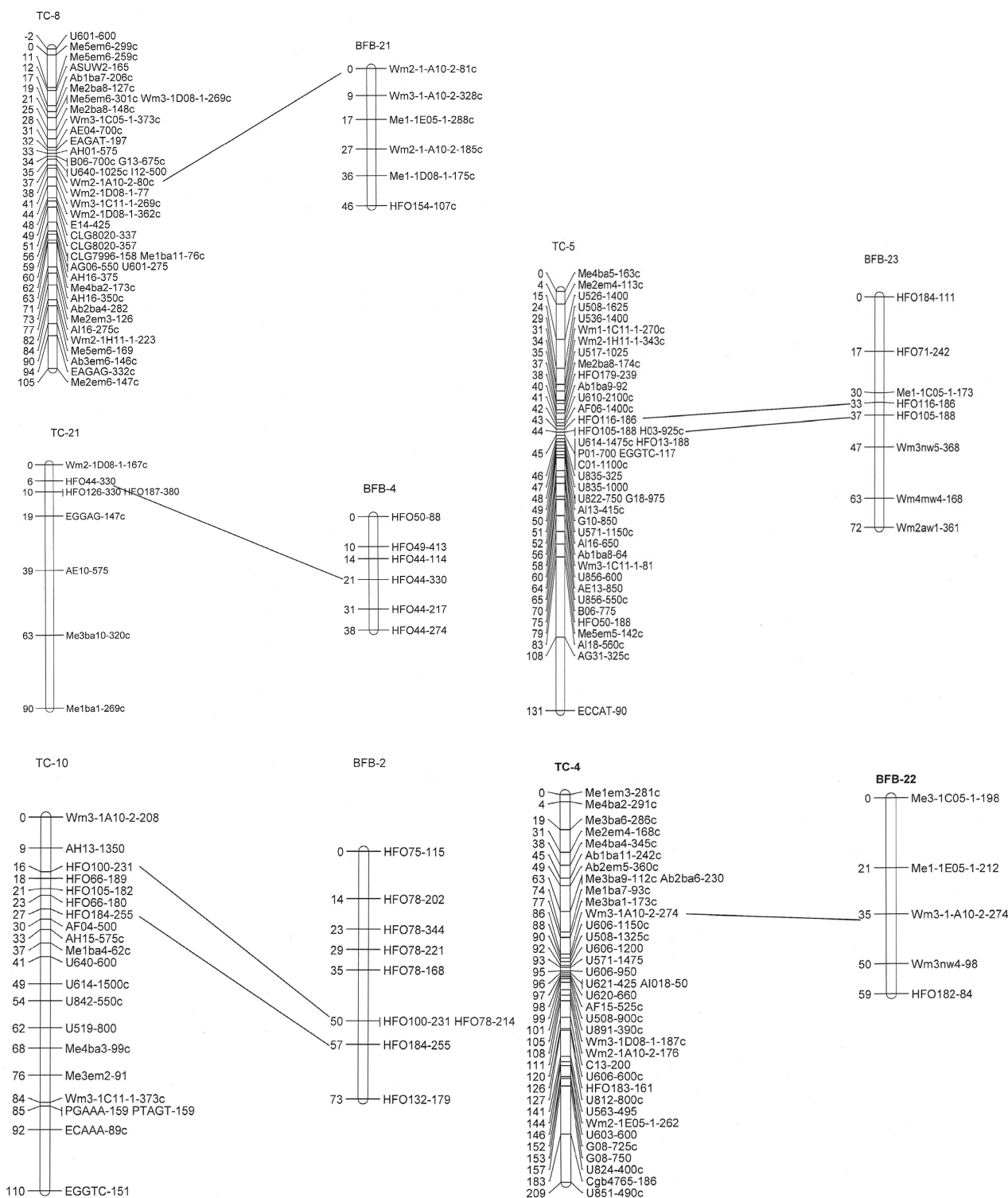


Figure 4. Comparative mapping between the linkage groups of the testcross map (Figure 3) and the BC₂F₂ genetic map (Figure 2), showing consensus in marker genetic mapping.

The testcross population used in previous genetic mapping studies [12,13,15,16] has been used here as a reference genetic map. Here the genetic linkage map was ex-

panded from 363 DNA markers [13] to 558 DNA markers (Table 5, Figure 3). This linkage map consists of 41 linkage group, including 10 large linkage groups (rang-

ing from 102 - 240 cM), nine intermediate size linkage groups (ranging from 62 - 93 cM), and 22 small linkage groups (ranging from 2 - 56 cM). It should be noted that a relatively large genetic distance exists between markers among most of the small linkage groups (Table 5, Figure 3). Thus, the linkage groups may represent chromosomal regions with high recombination frequencies.

Comparative mapping between the testcross and the BC₂F₂ genetic maps: The testcross and the BC₂F₂ population are derived from different genotypes representing wide genotypic and phenotypic diversity [13]. Here, the analysis identified 26 markers shared between the genetic maps derived from the testcross and the BC₂F₂ populations. These markers represent 9 linkage groups in the testcross and 8 linkage groups in the BC₂F₂ map (Figure 4). It is notable that 25 of the 26 markers shared between the two linkage maps are HFO-TAG markers and one is a TRAP marker (Figure 4). These results indicate that the HFO-TAG markers which represent conserved regions associated with active genes [33] should be useful in comparative mapping using genetic populations derived from different genotypes.

There are several linkage maps for watermelon including a map based on an F₂ population (both parents are cultivated accessions; *C. lanatus* var. *lanatus*), constructed by Hashizume *et al.* [39] in Japan, and a map based on a testcross population that consists of 363 DNA markers (including RAPD, AFLP, SRAP and SSR markers) [33]. Xu *et al.* [40] constructed a dense genetic linkage map for watermelon that consists mostly of SSR and EST-SSR markers. This map is derived from an F₂S₈ recombinant inbred line (RIL) population of PI 296341 (*C. lanatus* var. *citroides*) x Chinese elite line 97103 (*C. lanatus* var. *lanatus*) [41]. Additional studies are needed to merge between the genetic maps constructed for watermelon.

Although our results with the advanced recombinant BC₂F₂ population for identifying genes that confer moderate resistance to bacterial fruit blotch are not conclusive, this population should be useful for further development of introgression lines for watermelon. This BC₂F₂ population should also prove useful for identifying and incorporating resistance genes that exist in wild watermelon [15,16] into cultivated watermelon. Plant breeding programs for different crop plants have been using introgression lines for incorporating resistance genes from wild progenitors into cultivated types [38].

The linkage groups identified in this study should serve as a platform for further genetic mapping and development of introgression lines for watermelon. Several genetic populations (BC₁F₂; BC₂F₂; BC₂F₂ and BC₃F₃) derived from a cross between PI 494817 and 'Crimson Sweet' were developed in this project. These populations

are being used for further analysis and for development of introgression lines. These lines should be useful for expanding the narrow genetic base that exists among North American watermelon cultivars, and facilitate further analysis and identification of genes that confer disease resistance or control fruit quality in watermelon [15,16,42].

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