Theor Appl Genet (2002) 105:555–563
DOI 10.1007/s00122-001-0860-6

A. Levi · C.E. Thomas · T. Joobeur · X. Zhang
A. Davis

A genetic linkage map for watermelon derived from a testcross population: (*Citrullus lanatus* var. *citroides* × *C. lanatus* var. *lanatus*) × *Citrullus colocynthis*

Received: 31 August 2001 / Accepted: 8 November 2001 / Published online: 6 August 2002

Abstract A genetic linkage map was constructed for watermelon using a testcross population [Plant Accession Griffin 14113 (*Citrullus lanatus* var. *citroides*) × New Hampshire Midget (NHM; *C. lanatus* var. *lanatus*)] × U.S. Plant Introduction (PI) 386015 (*Citrullus colocynthis*). The map contains 141 randomly amplified polymorphic DNA (RAPD) markers produced by 78 primers, 27 inter-simple sequence repeat (ISSR) markers produced by 17 primers, and a sequence-characterized amplified region (SCAR) marker that was previously reported as linked (1.6 cM) to race-1 Fusarium wilt [incited by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *niveum* (E.F.Sm.) W.C. Synder & H.N. Hans] resistance in watermelon. The map consists of 25 linkage groups. Among them are a large linkage group that contains 22 markers covering a mapping distance of 225.6 cM and six large groups each with 10–20 markers covering a mapping distance of 68.8 to 110.8 cM. There are five additional linkage groups consisting of 3–7 markers per group, each covering a mapping distance of 36.5 to 57.2 cM. The 13 remaining linkage groups are small, each consisting of 2–11 markers covering a mapping distance of 3.5–29.9 cM. The entire map covers a total distance of 1,166.2 cM with an average distance of 8.1 cM between two markers. This map is useful for the further development of markers linked to disease resistance and watermelon fruit qualities.

Keywords Genetic mapping · SCAR · ISSR · RAPD · Fusarium wilt

Communicated by A.L. Kahler

A. Levi (✉) · C.E. Thomas
USDA, ARS, U.S. Vegetable Laboratory,
2875 Savannah Highway, Charleston, SC 29414, USA
e-mail: alevi@awod.com

T. Joobeur
Department of Plant Pathology,
North Carolina State University, Raleigh,
NC 27695–7609, USA

X. Zhang
Syngenta Seeds, Incorporated,
21435 Road 98, Woodland, CA 95695, USA

A. Davis
USDA, ARS, P.O. Box 159, Lane OK 74555, USA

Introduction

Watermelon accounts for 2% of the world area devoted to vegetable crops (FAO 1995). In the U.S. watermelon production has increased from 1.2 M tons in 1980 to 3.7 M tons in 1999, with a farm value of $287 million (U.S. Department of Agriculture, Agricultural Statistics 2000). Continued genetic improvement of this important crop is necessary, particularly for disease and pest resistance.

High-density genetic maps are useful in breeding programs for a large number of crop plants. They are also useful for positional cloning of genes and elucidating the genetic basis of complex traits that do not display Mendelian segregation (Lee 1995). Extensive genetic linkage maps have been constructed for major cucurbit species such as melon (Baudracco-Arnas and Pitrat 1996; Wang et al. 1997; Brotman et al. 2000; Dogimont et al. 2000; Oliver et al. 2000; Perin et al. 2000) and cucumber (Park et al. 2000; Staub and Serquen 2000). However, only a few small linkage maps have been constructed for watermelon (Navot and Zamir 1986; Navot et al. 1990; Hashizume et al. 1996; Hawkins et al. 2001). Many markers are still required for the construction of a dense map that can be used extensively in watermelon breeding programs and to isolate genes that control fruit quality or confer resistance to diseases and pests.

Cultivated watermelon is *Citrullus lanatus* var. *lanatus* (Thunb.) Matsum & Nakai. This species also includes the preserving melon (*C. lanatus* var. *citroides*) (Whitaker and Bemis 1976). Watermelon grows in tropical and subtropical climates worldwide, and is one of four known diploid (n = 11; Shimotsuma 1963) species of the xerophytic genus *Citrullus* Schrad. ex Eckl. & Zeyh. (Whitaker and Davis 1962; Jeffrey 1975; Whitaker

and Bemis 1976). The three other species in the genus *Citrullus* are the perennial bittergourd *Citrullus colocynthis* (L.) Schrad., the perennial wild species *Citrullus ecirrhosus* Cogn., and the annual wild species *Citrullus rehmii* De Winter. *C. colocynthis* grows on sandy soils throughout northern Africa, southwestern Asia and the Mediterranean (Whitaker and Davis 1962; Jeffrey 1975; Whitaker and Bemis 1976), while *C. ecirrhosus* and *C. rehmii* are endemic to the desert regions of Namibia. (Meeuse 1962; De Winter 1990).

Most watermelon cultivars developed in North America during the last 2 centuries have a narrow genetic background that may contribute to their elevated susceptibility to diseases and pests (Levi et al. 2000, 2001a). Among them, Fusarium wilt [incited by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *niveum* (E.F. Sm.) W.C. Synder & H.N. Hans] and gummy stem blight (incited by *Didymella bryoniae*) are considered the most destructive diseases of watermelon (Sowell and Pointer 1962; Netzer and Martyn 1989).

The U.S. Plant Accession Griffin 14113 has unique characteristics and is designated at the USDA Plant Genetic Resources Conservation Unit, Griffin, Ga., as *C. ecirrhosus*. It is vigorous and in the greenhouse produces a female flower for every 3–4 male flowers (16-h day light with an average mid-day intensity of 4×10^4 μE). It has small round fruits with a gray light-green speckled rind, green-white flesh, and many green olive-brown seeds, speckled with black spots and stripes (400–550 seeds per fruit). It has a period of 60 days from seed germination to fruit maturity. These traits can be useful in genetic studies with New Hampshire Midget (NHM). This watermelon cultivar produces a female flower for every 7–12 male flowers (under the same greenhouse conditions for Griffin 14113). It has an oval-shaped fruit with red flesh, gray rind, black seeds (200–350 seeds per fruit), and a period of 80 days from seed germination to fruit maturity. Although Griffin 14113 is a unique watermelon accession designated as *C. ecrrihosus*, it readily crosses with *C. lanatus* accessions and watermelon cultivars. Also, our analysis using RAPD markers indicates that it belongs to *C. lanatus* var. *citroides* (unpublished data). In contrast with Griffin 14113, all *C. colocynthis* accessions examined did not cross readily with watermelon cultivars (*C. lanatus*), and had distinct RAPD patterns that resulted in low similarity values (38.9% genetic similarity) with *C. lanatus* (Levi et al. 2000, 2001a). Due to these facts, Griffin 14113 could not be considered a distinct species, as *C. colocynthis* appears to be, but rather as *C. lanatus* var. *citroides*. Genetic similarity analysis (Nei and Li 1979) based on 253 RAPD fragments (produced by 32 primers) resulted in a 53.0% genetic similarity value between Griffin 14113 and NHM. This genetic distance should be sufficient for the production of polymorphic markers useful in the construction of a genetic linkage map employing a population derived from a cross between these two watermelon types.

Navot and Zamir (1987) consider *C. lanatus* var. *citroides* as the ancestor of *C. lanatus* var. *lanatus*. These two subspecies share many RAPD patterns. However, analysis using RAPD markers showed that higher genetic diversity exists among accessions of *C. lanatus* var. *citroides* (Levi et al. 2000, 2001a). Using SSR markers, Jarret et al. (1997) found a higher genetic diversity among accessions of *C. lanatus* var. *citroides* as compared with accessions of *C. lanatus* var. *lanatus*. A preliminary screening detected a sufficient number of RAPD markers unique to *C. lanatus* var. *citroides* that could be mapped in an F_2 or in a BC_1 population. However, cultivated watermelon had fewer markers that could be mapped with high confidence in such populations. Hawkins et al. (2001) constructed two linkage groups (26 RAPD markers covering a mapping distance of 112.9 cM) and five linkage groups (13 RAPD markers covering a mapping distance of 139 cM), using an F_2 and an F_3 population, respectively. These populations are derived from a cross between PI 296341-FR (*C. lanatus* var. *citroides*; resistant to races 1 and 2 of *F. oxysporum* f. sp. *niveum*) (Netzer and Martyn 1989; Martyn and Netzer 1991) and NHM (*C. lanatus* var. *lanatus*; Fusarium wilt susceptible). In that study most of the mapped markers were from PI 296341-FR. Additionally, 47.5% of the markers in the F_2 population and 48% of the markers in the F_3 population distortedly segregated from the expected 3:1 and 5:3 segregation ratios, respectively. In an additional map constructed for watermelon using a BC_1 population [(PI 296341-FR × NHM) × NHM], 46 (25.7%) of the 179 markers analyzed showed segregation patterns skewed away from the expected 1:1 ratio (at $P = 0.05$) (Levi et al. 2001b). Also, a large number of markers in that map were closely linked (0–2.7 cM), indicating the presence of chromosomal regions with low recombination events. To reduce the effect of a common genetic background on marker resolution and segregation as observed by Hawkins et al. (2001), and to examine whether chromosomal regions with low recombination events also occur among watermelon genotypes derived from wide crosses, a testcross population was constructed.

This paper describes a genetic linkage map for watermelon derived from a testcross population {[Griffin 14113 (*C. lanatus* var. *citroides*) × NHM (*C. lanatus* var. *lanatus*)] × U.S. Plant Introduction 386015 (*C. colocynthis*)}. The linkage map consists of RAPD and ISSR markers present in the wild accession (Griffin 14113) or in the cultivated watermelon (NHM).

Materials and methods

Plant material

Parental plants that included Griffin 14113 (provided by R. Jarret, USDA Plant Genetic Resources Conservation Unit, Griffin, Ga.), NHM, their F1 hybrid plant, and the testcross parent plant (PI 386015) were grown in the greenhouse together with 88 plants of the testcross progeny [(Griffin 14113 × NHM) × PI 386015]. Four weeks after germination young leaves (10 g) were collected from each 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 (Levi and Thomas 1999).

DNA amplification conditions and gel-electrophoresis

Ten-decamer oligonucleotides were purchased from the University of British Columbia, Biotechnology Center (British Columbia, Canada), and were used for PCR amplification as described by Levi et al. (1993) and by Rowland and Levi (1994) (see Table 1). RAPD reactions were in a 25-µl reaction buffer containing 20 µM of NaCl, 50 mM of Tris-HCl pH 9, 1% Triton-X-100, 0.01% gelatin, 1.6 mM of $MgCl_2$, 200 µM each of dATP, dCTP, dGTP and dTTP (Sigma), 0.2 µM of primer, seven units of *Taq* DNA Polymerase (Promega, Madison, Wis., supplied in storage buffer A), and 25 ng of template DNA. Amplification reactions were carried out for 45 cycles in a 'PTC-200 Thermocycler' (MJ Research, Watertown, Mass.), programmed for 60 s for DNA to denature at 92 °C, 70 s for DNA annealing at 48 °C, and 120 s for DNA transcription at 72 °C. ISSR primers with 15–20 decamer oligonucleotides were purchased from the University of British Columbia (primer # 800–899). The amplification conditions for ISSR primers were the same as for the RAPD primers, except for the DNA annealing temperature optimized for each primer (Table 2). DNA amplification conditions for a 700-bp marker band (designated as *P01-700*), using SCAR primers 5′GTAGCACTCCAACATTTATTCTAATTC and 5′GTAGCACTCCCAACTCATACAAAT (designed by Xu et al. 1999), were the same as for the RAPD and the ISSR primers, except for the DNA annealing temperature that was set at 62 °C. Amplification products were separated by electrophoresis in 1.4% agarose gels in 0.5 × Tris borate buffer (Sambrook et al. 1989). The gels were stained with 0.5 µg/ml of ethidium bromide solution for 30 min and de-stained for 15 min in distilled water. DNA fragments were visualized under UV light and photographed using a still video system (Gel Doc 2000, Bio-Rad, Hercules, Calif.). The molecular weights of the amplification products were calculated using the 100-bp or 1-kb plus DNA ladder standards (Gibco BRL, Gaithersburg, Md.). Amplification reactions with each primer were repeated twice to confirm marker reproducibility among the parents and the individuals in the testcross population.

Marker scoring

Markers that were unique to one of the parents, Griffin 14113 or NHM, and were present in the F1 plant but missing in the testcross parent (PI 386015), could be scored for mapping in the testcross population (Fig. 1).

Linkage analysis of markers in the testcross population

Data were analyzed using Mapmaker version 3.0 (Whitehead Institute, Cambridge, Mass.; Lander et al. 1987; Lincoln et al. 1992). Markers were first grouped using a minimum LOD score of 4.0 and a maximum recombination value (θ) of 0.25. For each linkage group, markers were ordered by using the "Order" command with a minimum LOD score of 4.0 and a maximum recombination value (θ) of 0.30. Markers ordered with low confidence were placed using the "Try" command. The ordered marker sequences were confirmed using the "Ripple" command. Linkage maps were generated with the "Map" command using the "Kosambi" map function. The "Error-Detection" command was used to identify possible marker scoring errors, and putative errors were re-tested. Chi-square tests were performed to check whether individual markers segregated randomly.

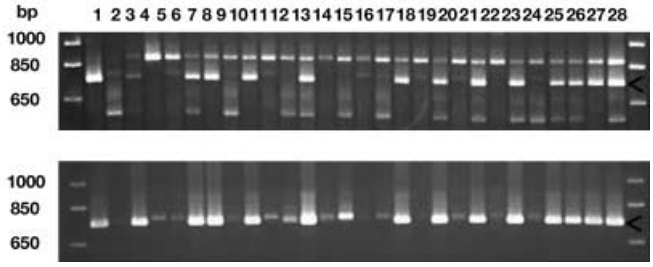

Fig. 1 Marker *P01-700* (700 bp band) produced by RAPD primer P01 (upper gel; 1.4% agarose), and by its corresponding SCAR primers (lower gel. *P01-700* is present in Griffin 14113 (lane 1) and in the F1 Hybrid (lane 3), missing in NHM (lane 2) and in the testcross parent PI 386015 (lane 4), and is segregating among the testcross progeny (lanes 5-28). In addition to *P01-700*, the SCAR primers produced a low intensity band (730 bp) in PI 386015 (lane 4) and in the testcross progeny (lanes 5-28). The molecular size markers in the external lanes ar of the '1 KB Plus DNA ladder' (GibcoBRL, Bethesda, MD).

Marker nomenclature

The RAPD or ISSR markers were designated by their serial number and their size. For example, the 500-bp marker produced by primer AW-07 (Operon), which is unique to Griffin 14113, was designated as AW07-500; and the 250-bp marker produced by primer No. 101 (University of British Columbia), which is unique to NHM, was designated as 101–250C.

Results and discussion

Marker screening

Three hundred and thirty six 10-mer RAPD primers, with 60 to 90% Guanine-Cytosine (GC) content, and 70 ISSR primers were screened in amplification reactions against the parents Griffin 14113 and NHM, their F1 hybrid, and the testcross parent PI 386015. Of these, 243 RAPD and 57 ISSR primers resulted in DNA amplification, producing 1–14 RAPD bands (0.1–3 kb) (Fig. 1), and 1–8 ISSR bands (0.1–2.6 kb) per PCR reaction. Eighty six RAPD primers and 21 ISSR primers produced 168 and 37 distinct bands, respectively. These distinct bands were uniquely present in one of the donor parents (Griffin 14113 or NHM) and in the F1 hybrid, but absent in the testcross parent PI 386015 (Tables 1 and 2, Fig. 2). Of these distinct bands, 104 and 101 were unique to Griffin 14113 and NHM, respectively. All RAPD and SSR marker bands in the present study were highly reproducible from experiment to experiment (Fig. 1), allowing accurate mapping of these markers.

Map construction

Of the 104 markers unique to Griffin 14113, 94 (90.4%) could be placed on a linkage group. Of the 101 markers unique to NHM, 75 (74.2%) could be placed on a linkage group. Twenty six of the 168 RAPD markers and ten of the 37 ISSR markers analyzed could not be ordered

Table 1 The nucleotide sequences of RAPD primers and the number of markers produced by each primer used in the mapping analysis. The size (bp) of each marker that could not be mapped (unmapped markers), and the size of markers skewed towards Griffin 14113 (SG), or towards the cultivar NHM (SC)

Primer	Sequence	Number of markers	Unmapped markers	SG	SC	Primer	Sequence	Number of markers	Unmapped markers	SG	SC
B-06[a]	TGCTCTGCCC	4	1,450c			AH-16	CAAGGTGGGT	3	325c	325c	
B-07	GGTGACGCAG	1				AH-17	CAGTGGGGAG	1			
B-09	TGGGGGACTC	1				AI-01	GGCATCGGCT	3			
B-12	CCTTGACGCA	2				AI-06	TGCCGCACTT	1			400c
C-01	TTCGAGCCAG	2	1,325c,1,100		1,325c	AI-07	ACGAGCATGG	2	900,475c		
C-13	AAGCCTCGTC	2				AI-09	TCGCTGGTGT	2			
C-14	TGCGTGCTTG	1	1,325c			AI-13	ACGCTGCGAC	1			
C-15	GACGGATCAG	2				AI-15	GACACAGCCC	3			400c,390
D-08	GTGTGCCCCA	2	225c		225c	AI-16	AAGGCACGAG	4	275c		
D-13	GGGGTGACGA	3		1,000		AI-18	TCGCGGAACC	3		350	
D-18	GAGAGCCAAC	2				504[b]	ACCGTGCGTC	1			
E-14	TGCGGCTGAG	1				508	CGGGGCGGAA	3			
F-01	ACGGATCCTG	2			725	512	GGGTGGACAT	1			
F-10	GGAAGCTTGG	2		900		516	AGCGCCGACG	4	925		
G-08	TCACGTCCAC	3				517	GGTCGCAGCT	1			
G-10	AGGGCCGTCT	1				519	ACCGGACACT	1			
G-13	CTCTCCGCCA	2				523	ACAGGCAGAC	1			
G-17	ACGACCGACA	2		400		526	AACGGGCACC	3			1,400,650c
G-18	GGCTCATGTG	2				533	GCATCTACGC	1		675c	
H-03	AGACGTCCAC	2				536	GCCCCTCGTC	1			
H-12	ACGCGCATGT	2	775c	775c		542	CCCATGGCCC	2			525
I-07	CAGCGACAAG	1		275		543	CGCTTCGGGT	1			
I-12	AGAGGGCACA	2			1,050	548	GTACATGGGC	1			
P01	GTAGCACTCC	1				563	CGCCGCTCCT	4	1,625c,495	1,625c	495
AE-04	CCAGCACTTC	1				566	CCACATGCGA	1			
AE-10	CTGAAGCGCA	1	575			571	GCGCGGCACT	5			
AE-12	CCGAGCAATC	2				595	GTCACCGCGC	1			
AE-13	TGTGGACTGG	1		850		598	ACGGGCGCTC	2	1500	1,500	
AE-14	GAGAGGCTCC	2				601	CCGCCCACTG	3	600	600	
AE-15	TGCCTGGACC	1	600c			603	ACCCACCGCG	1	600		
AF-04	TTGCGGCTGA	3		500		606	CGGTCGGCCA	4			600c
AF-06	CCGCAGTCTG	2				607	AGTGTCGTCG	2	1,200c	850	1,200c
AF-14	GGTGCGCACT	2				610	TTTGCCGCCC	1			
AF-15	CACGAACCTC	2				613	TGCACCCACG	3	950		
AF-20	CTCCGCACAG	2	475			614	GTAGTCTCGC	2			
AG-03	TGCGGGAGTG	2	1,325c,360c	360		615	CGTCGAGCGG	1			
AG-06	GGTGGCCAAG	3			800	620	TTGCGCCCGG	2			
AG-14	CTCTCGGCGA	1	450c			621	GTCTGCGCTA	2			
AH-01	TCCGCAACCA	4	900c	900c,800,500c		634	CCGTACACGC	2	700		
AH-03	GGTTACTGCC	1				638	GCGGTGACTA	4		625c	
AH-08	TTCCCGTGCC	2				639	ATCGAGCACC	1			
AH-13	TGAGTCCGCA	1				640	CGTGGGGCCT	2			
AH-15	CTACAGCGAG	1				731	CCCACACCAC	2		875	

[a] Primers are from OperonTechnologies
[b] Primers are from the University of British Columbia

after grouping (Table 1), indicating that the present map does not cover all parts of the genome. According to Perin et al. (2000), at least 526 markers (with a maximum distance of 5 cM) are required to saturate 95% of a 1,600 cM linkage map of melon (*Cucumis melo*). This cucurbit species has a genome size comparable to that of watermelon with a haploid genome size of 4.54×10^8 bp, and 4.25×10^8 bp, respectively (Arumuganathan and Earle 1991).

The present map consists of 25 linkage groups. Among them are a large linkage group that contains 22 markers, covering a mapping distance of 225.6 cM (Group I; Table 3, Fig. 2); and six large groups, each with 10–20 markers, covering a mapping distance of 68.8 to 110.8 cM (Groups II–VII; Table 3, Fig. 2). There are five additional linkage groups each consisting of 3 to 7 markers covering a mapping distance of 36.5 to 57.2 cM (Groups VIII–XII; Table 3, Fig. 2). The 13 remaining are small linkage groups, each consisting of 2–11 markers covering a mapping distance of 3.5–29.9 cM (Groups XIII–XXV; Table 3, Fig. 2). The map covers a total distance of 1,166.2 cM with an aver-

Table 2 The nucleotide sequences of SSR-anchored primers, the optimal annealing temperature, and the number of markers produced by each primer used in the mapping analysis

Primer	Sequence	Annealing temperature (C)	Number of markers	Unmapped markers[a]	SG[b]	SC[c]
808	AGAGAGAGAGAGAGAGC	59	2			925c
809	GAGGAGAGAGAGAGAGG	59	2	900c		
810	GAGAGAGAGAGAGAGAT	53	1			
812	GAGAGAGAGAGAGAGAA	53	2	900c,800c		
813	CTCTCTCTCTCTCTCTT	53	1			
815	CTCTCTCTCTCTCTCTG	48	1			
816	CACACACACACACACAT	54	2	975		
822	TCTCTCTCTCTCTCTCA	54	1			
824	TCTCTCTCTCTCTCTCG	54	2			
825	ACACACACACACACACT	54	1			
826	ACACACACACACACACC	62	1	1,150		
829	TGTGTGTGTGTGTGTGC	62	1	500		
834	AGAGAGAGAGAGAGAGYT	59	2			
835	AGAGAGAGAGAGAGAGYC	59	6	990c	990c	
842	GAGAGAGAGAGAGAGAYG	59	3	375c		
847	CACACACACACACACAAGC	62	1		325c	
851	GTGTGTGTGTGTGTGTCTG	62	1	490c		
853	TCTCTCTCTCTCTCTCAGT	54	1	750c		
856	ACACACACACACACACCTA	62	4			
889	AGTCGTAGTACACACACACACAC	62	1			
891	ACTACGACTTGTGTGTGTGTGTG	62	1			

[a] Size (bp) of each marker that could not be mapped
[b] Size of markers skewed towards Griffin 14113 (SG)
[c] Size of markers skewed towards the cultivar NHM (SC)

age distance of 8.1 cM between markers (Table 3, Fig. 2). The high number of linkage groups in the present map is partly a result of using a minimum LOD score of 4.0 and a maximum recombination value (θ) of 0.25 in grouping the markers. These stringent linkage-analysis criteria were used to avoid any possible mapping errors, and in merging this map with other maps constructed for watermelon. Using 62 markers Hashizume et al. (1996) were able to construct 11 linkage groups (524 cM), corresponding to the chromosome number of watermelon (Shimotsuma 1963). However, a more extensive map for watermelon, consisting of 155 markers, revealed ten large linkage groups (9–19 markers) and seven small linkage groups (2–4 markers), covering a mapping distance of 1,295 cM (Levi et al. 2001b).

In the present map, 21 of the 25 linkage groups, including all major linkage groups, consisted of markers from both donor parents (Griffin 14113 and NHM), while a few small linkage groups (XIV, XX, XXII and XXV) consisted only of markers from Griffin 14113 (Fig. 2). As in the other map recently constructed for watermelon (Levi et al. 2001b), all seven major linkage groups (I–VII) and four of the smaller linkage groups (XI, XIII, XV and XVI) possessed regions with low recombination events (0–2.3 cM) between markers (Fig. 2). These might be chromosomal regions near a centromere. Fewer recombination events occur in the vicinity of a centromere than in regions distant from it (Mather 1936, 1939; Dimitrov and Georgieva 1994). However, variability in crossing-over frequency may also be a result of heterochromatin properties (Mather 1939). The highly heterochromatic short arm of chromosome 10 of rice shows a lower recombination frequency than the euchromatic long arm of this chromosome (Cheng et al. 2001). Dense regions with low recombination events are also indicated in the map constructed for cucumber (Park et al. 2000; Bradeen et al. 2001). Further studies are needed to determine the nature and location of these regions in the watermelon genome. In contrast with the large linkage groups, most small linkage groups (with the exception of linkage groups XI, XIII, XV and

Table 3 Marker distribution among linkage groups

Linkage group	Number of markers	Length (cM)	Average distance (cM)	Number of skewed markers
I	22	225.6	10.74	1[a], 1[b]
II	20	110.8	5.83	1[b]
III	11	80.8	8.08	3[b]
IV	10	78.9	8.77	5[a]
V	16	78	5.2	1[b]
VI	10	73.4	8.16	1[a]
VII	11	68.8	6.88	3[a]
VIII	7	57.2	9.53	1[b]
IX	5	46.2	11.55	2[b]
X	3	46.2	23.1	–
XI	8	44.8	6.4	–
XII	3	36.5	18.25	1[a]
XIII	4	29.9	9.97	–
XIV	4	29.8	9.93	4[b]
XV	11	28.2	2.82	–
XVI	3	26	13	–
XVII	3	24.2	12.1	–
XVIII	2	17.3	17.3	–
XIX	2	17.3	17.3	1[b]
XX	2	14.2	14.2	1[b]
XXI	3	11.1	5.55	–
XXII	3	8.6	4.3	–
XXIII	2	5.3	5.3	–
XXIV	2	3.6	3.6	–
XXV	2	3.5	3.5	–
Total	169	1,166.2	8.1[c]	29

[a] Markers skewed towards NHM
[b] Markers skewed towards GRF 14113
[c] Average distance between two markers

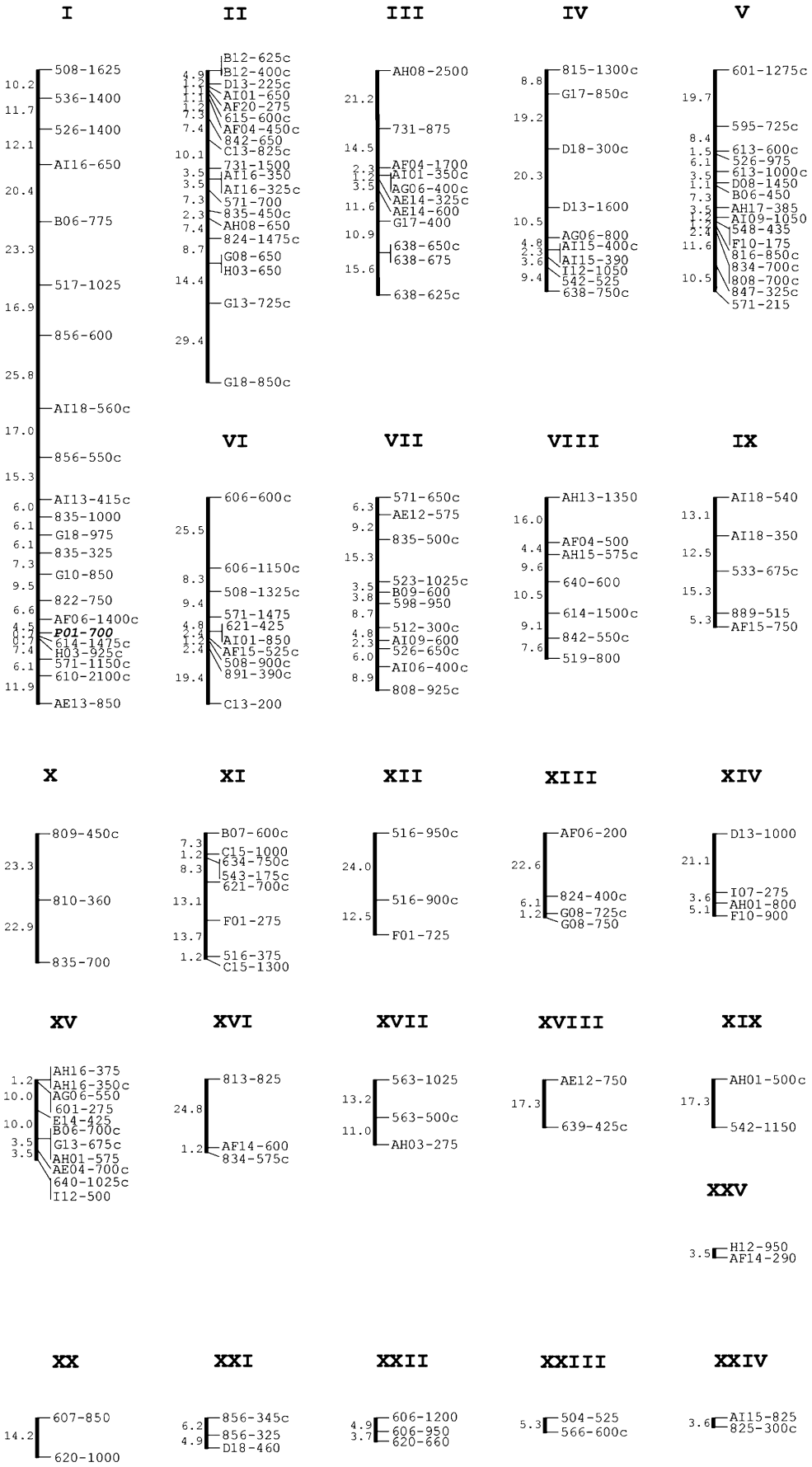

Fig. 2 A genetic linkage map for watermelon derived from a testcross population (Griffin 14113 × NHM) × PI 386015. Marker names are at the right, while distances between adjacent markers (in cM) are at the left of each linkage group

XVI) consisted of genetic distances greater than 3.4 cM between markers (Table 3, Fig. 2), indicating that they may represent euochromatic regions distant from a centromere.

Of the 27 mapped ISSR markers 21 (77.8%) were in close vicinity or clustered on a few linkage groups (I, II, V, VII, X, XVI and XXI). These groups contained 5, 3, 4, 2, 3, 2 and 2 ISSR markers, respectively. Each of linkage groups IV, VI, VIII, IX and XXIV contained one ISSR marker (Fig. 2). These results indicate that simple sequence repeats are prevalent in the watermelon genome. Jarret et al. (1997) isolated 96 SSR-bearing clones from the watermelon genome, and reported that the dinucleotide repeats $(CT)_n$ and $(GA)_n$ accounted for 82% of the SSRs that were sequenced in that study. Of the 21 ISSR primers used in the present study, five consisted of $(CT)_n$ and seven consisted of $(GA)_n$ dinucleotide repeats, while six primers consisted of $(CA)_n$ and three primers consisted of $(GT)_n$ dinucleotide repeats (Table 2). In the initial screening, ISSR primers that consisted of $(AT)_n$, $(GTT)_n$, $(GGAT)_n$ or $(GCAT)_n$ did not result in any fragment amplification. ISSR markers have been mainly used in genetic diversity studies (Levi and Rowland 1997; Prevost and Wilkenson 1999; Joshi et al. 2000). However, a few recent studies employed ISSR markers in genetic mapping of crop plants (Barcaccia et al. 2000; Santra et al. 2000; Casasoli et al. 2001). Like the RAPD markers, the ISSR markers in the present study are highly reproducible from experiment to experiment following optimization of their annealing temperature (Table 2).

Although the present map is not saturated, it can be useful for identifying important genes that affect fruit qualities and disease or pest resistance. Marker *P01-700* has been reported as linked (1.6 cM) to the Fusarium wilt race-1 resistance gene in PI 296341-FR (Xu et al. 1999, 2000). In the present study, *P01-700* has been highly reproducible using either the RAPD primer P01 (Table 1) or the SCAR primers designed by Xu et al. (1999). *P01-700* is present in Griffin 14113 and in the F1 plant, but not in NHM or in PI 386015 (Fig. 1), and is mapped on linkage group I where it is closely linked to two RAPD markers (Fig. 2). These two markers may also be useful for pursuing the Fusarium wilt race-1 resistance gene in watermelon (Zhang and Rhodes 1993).

Marker segregation

The number of markers with skewed segregation in the present testcross population was not as high (18%) as in the maps constructed for watermelon using an F_2 and an F_3 population (47.5% and 48% skewed markers, respectively; Hawkins et al. 2001), and as in the map constructed for watermelon using a BC_1 population (25.7% skewed markers; Levi et al. 2001b). Of the 205 markers analyzed, 37 showed skewed segregation patterns from the expected 1:1 ratio at $P = 0.05$. Of these, 22 markers skewed towards Griffin 14113, while 15 skewed towards NHM. Of the 22 markers skewed towards Griffin 14113, seven (AG03-360C, H12-775C, AH16-325C, 563-1625C, 598-1500, 601-600 and 835-990C) could not be mapped. One skewed marker was assigned to each of linkage groups I (AE13-850), II (AI16-325C), V (847-325C), VIII (AF04-500), XIX (AH01-500) and XX (607-850). Three markers (731–875, G17-1400 and 638-625C) were assigned to linkage group III, two markers (AI18-350 and 533–675C) were assigned to linkage group IX, and four markers (D13-1000, I07–275, AH01-800 and F10-900) were assigned to linkage group XIV (Tables 1, 2 and 3, Fig. 2). Of the 15 markers skewed towards NHM, four (D08-225, C01-1325C, 563-495 and 607-1200C) could not be mapped. One marker was assigned to each of linkage groups I (AE13-850), II (AI16-325C), V (847-325C), VIII (AF04-500), XIX (AH01-500) and XX (607-850). Three markers (731-875, G17-1400 and 638-625C) were assigned to linkage group III, two markers (AI18-350 and 533-675C) to linkage group IX, and four markers (D13-1000, I07-275, AH01-800 and F10-900) to linkage group XIV. Linkage group XIV consisted only of these four skewed markers (Tables 1, 2 and 3, Fig. 2). Most of the skewed markers were clustered in their respective linkage group (Fig. 2). Skewed segregation resulting from meiotic drive has been reported in an increasing number of crop species, particularly in mapping populations derived from interspecific or intergeneric crosses (Zamir and Tadmor 1986; Weeden et al. 1989; Durham et al. 1992). Meiotic drive is prevalent in crop plants, and plays a dominant part in plant genome evolution (Buckler et al. 1999). Thus, in these population types it may not be sufficient to determine the genetic mode of a trait through phenotypic observations, but by mapping gene loci and determining their segregation pattern.

To facilitate the construction and integration of linkage maps of watermelon, RAPD and ISSR markers in this study will be converted to SCAR markers and to RFLP probes. These markers will be used in integrating the present map with the other maps recently constructed for watermelon (Xu et al. 2000; Hawkins et al. 2001; Levi et al. 2001b). Also, an F_2 population derived from Griffin 14113 and NHM has been constructed. This population will be used for the construction of F_3 families, and for mapping gene loci that affect fruit quality and days to fruit maturity. Extensive conservation of linkage relationships has been reported in various crops (Gale and Devos 1998). Extensive maps were constructed for melon (Baudracco-Arnas and Pitrat 1996; Wang et al. 1997; Brotman et al. 2000; Dogimont et al. 2000; Oliver et al. 2000; Perin et al. 2000), and for cucumber (Park et al. 2000; Staub and Serquen 2000; Bradeen et al. 2001). Comparing genetic maps of watermelon with maps constructed for these cucurbit species may speed up the mapping and isolation of important genes in this important crop.

Acknowledgments The authors gratefully acknowledge the technical assistance of Laura Pence.

References

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:211–215

Barcaccia G, Albertini E, Rosellini D, Tavoletti S, Veronesi F (2000) Inheritance and mapping of 2n-egg production in diploid alfalfa. Genome 43:528–37

Baudracco-Arnas S, Pitrat M (1996) A genetic map of melon (*Cucumis melo* L.) with RFLP, RAPD, isozyme, disease resistance and morphological markers. Theor Appl Genet 93:57–64

Bradeen JM, Staub JE, Wye C, Antonise R, Peleman J (2001) Towards an expanded and integrated linkage map of cucumber (*Cucumis sativus* L.). Genome 44:1–9

Brotman Y, Silberstein L, Kovalski I, Klinger J, Thompson G, Katzir N, Perel Treves R (2000) Linkage groups of *Cucumis melo*, including resistance gene homologues and known genes. Acta Hort 510:441–448

Buckler ES 4th, Phelps-Durr TL, Buckler CS, Dawe RK, Doebley JF, Holtsford TP (1999) Meiotic drive of chromosomal knobs reshaped the maize genome. Genetics 153:415–426

Casasoli M, Mattioni C, Cherubini M, Villani F (2001) A genetic linkage map of European chestnut (*Castena sativa* Mill.) based on RAPD, ISSR and isozyme markers. Theor Appl Genet 102:1190–1199

Cheng Z, Presting GG, Buell CR, Wing RA, Jiang J (2001) High-resolution pachytene chromosome mapping of bacterial artificial chromosomes anchored by genetic markers reveals the centromere location and the distribution of genetic recombination along chromosome 10 of rice. Genetics 157:1749–1757

De Winter B (1990) A new species of *Citrullus* (Benincaseae) from the Namib desert, Namibia. Bothalia 20:209–211

Dimitrov B, Georgieva V (1994) Comparative analysis of sister-chromatid exchanges in plant and human chromosomes. Mutat Res 304:187–192

Dogimont C, Leconte L, Perin C, Thabuis A, Lacoq H, Pitrat M (2000) Identification of QTLs contributing to resistance to different strains of cucumber mosaic cucumovirus in melon. Acta Hort 510:391–398

Durham RE, Liou PC, Gmitter Jr FG, Moore GA (1992) Linkage of restriction length polymorphisms and isozymes in *Citrus*. Theor Appl Genet 84:39–48

FAO (1995) Production year book for 1994. No. 48. Food and Agricultural Organization of the United Nations, Rome

Gale MD, Devos KM (1998) Plant comparative genetics after ten years. Science 282:656–659

Hashizume T, Skimamoto I, Harushima Y, Yui M, Sato T, Imai T, Hirai M (1996) Construction of a linkage map for watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] using random amplified polymorphic DNA (RAPD). Euphytica 90:265–273

Hawkins LK, Dane F, Kubisiak TL, Rhodes BB, Jarret RL (2001) Linkage mapping in a watermelon population segregating for fusarium wilt resistance. J Am Soc Hort Sci 126:344–350

Jarret RL, Merrick LC, Holms T, Evans J, Aradhya MK (1997) Simple sequence repeats in watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. Genome 40:433–441

Jeffrey C (1975) Further notes on Cucurbitaceae. III. Some African taxa. Kew Bull 30:475–493

Joshi SP, Gupta VS, Aggarwal RK, Ranjekar PK, Brar DS (2000) Genetic diversity and phylogenetic relationship as revealed by inter-simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. Theor Appl Gent 100:1311–1320

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps with experimental and natural populations. Genomics 1:174–181

Lee M (1995) DNA markers and plant breeding programs. Adv Agron 55:265–344

Levi A, Rowland LJ (1997) Identifying blueberry cultivars and evaluating their genetic relationships using randomly amplified polymorphic DNA (RAPD) and simple sequence repeat-(SSR-) anchored primers. J Am Soc Hort Sci 122:74–48

Levi A, Thomas CE (1999) An improved procedure for isolation of high quality DNA from watermelon and melon leaves. Cucurbit Genet Coop Rep 22:41–42

Levi A, Rowland LJ, Hartung JS (1993) Production of reliable randomly amplified polymorphic DNA (RAPD) markers from DNA of woody plants. HortScience 28:1188–1190

Levi A, Thomas CE, Keinath AP, Wehner TC (2000) Estimation of genetic diversity among *Citrullus* accessions using RAPD markers. Acta Hort 510:385–390

Levi A, Thomas CE, Keinath AP, Wehner TC (2001a) Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. Genet Res Crop Evol 48:559–566

Levi A, Thomas CE, Zhang X, Joobeur T, Dean RA, Wehner TC, Carle BR (2001b) A genetic linkage map for watermelon based on Randomly Amplified polymorphic DNA (RAPD) markers. J Am Soc Hort Sci 126:730–737

Lincoln S, Daly M, Lander E (1992) Constructing genetic maps with MAPMAKER/EXP 3.0. 3rd edn. Whitehead Institute Technical Report

Martyn RD, Netzer D (1991) Resistance to races 0, 1, and 2 of Fusarium wilt of watermelon in *Citrullus* sp. PI-296341-FR. HortScience 26:429–432

Mather K (1936) The determination of position in crossing-over. I. *Drosophila melanogaster*. J Genet 33:207–235

Mather K (1939) Crossing-over and heterochromatin in the X chromosome of *Drosophila melanogaster*. Genetics 24:413–435

Meeuse AD (1962) The *Cucurbitaceae* of Southern Africa. Bothalia 8:1–111

Navot N, Zamir D (1986) Linkage relationships of 19 protein-coding genes in watermelon. Theor Appl Genet 72:274–278

Navot N, Zamir D (1987) Isozyme and seed protein phylogeny of the genus *Citrullus* (Cucrbitaceae). Plant Syst Evol 156:61–67

Navot N, Sarfatti M, Zamir D (1990) Linkage relationships of genes affecting bitterness and flesh color in watermelon. J Hered 81:162–165

Nei M, Li W (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Netzer D, Martyn RD (1989) PI 296341, a source of resistance in watermelon to race 2 of *Fusarium oxysporum* f. sp. *niveum*. Plant Dis 73:518

Oliver M, Garcia-Mas J, Morales M, Dolcet-Sanjuan R, Carmen de Vincente M, Gomez H, van Leewen H, Monfort A, Puigdomenech P, Arus P (2000) The Spanish melon genome project: construction of a saturated genetic map. Acta Hort 510:375–378

Park YH, Sensoy S, Wye C, Antonise R, Peleman J, Havey MJ (2000) A genetic map of cucumber composed of RAPDs, RFLPs, AFLPs, and loci conditioning resistance to papaya ringspot and zucchini yellow mosaic viruses. Genome 43: 1003–1010

Perin C, Hagen L, Dogimont C, de Cunto V, Lecomte L, Pitrat M (2000) Construction of a reference map of melon. Acta Hort 510:367–374

Prevost A, Wilkenson MJ (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor Appl Genet 98:107–112

Rowland LJ, Levi A (1994) RAPD-based genetic linkage map of blueberry derived from a cross between diploid species (*Vaccinium darrowi* and *V. elliottii*) Theor Appl Genet 87:863–868

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Santra DK, Tekeoglu M, Ratnaparkhe M, Kaiser WJ, Muehlbauer FJ (2000) Identification and mapping of QTLs conferring resistance to ascochyta blight in chickpea. Crop Sci 40:1606–1612

Shimotsuma M (1963) Cytogenetic and evolutionary studies in the genus *Citrullus*. Seiken Jiho 15:24–34

Sowell G Jr, Pointer GR (1962) Gummy stem blight resistance of introduced watermelons. Plant Dis Rep 46:883–885

Staub JE, Serquen FC (2000) Towards an integrated linkage map of cucumber: map merging. Acta Hort 510:357–366

U.S. Department of Agriculture (2000) Agricultural statistics. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

Wang YH, Thomas CE, Dean RA (1997) A genetic map of melon (*Cucumis melo* L.) based on amplified fragment length polymorphism (AFLP) markers. Theor Appl Genet 95:791–798

Weeden NF (1989) Application of isozymes in plant breeding. In: Janick J (ed) Plant breeding reviews, vol 6. Timber Press, Portland, Oregon, pp 11–54

Whitaker TW, Davis GN (1962) Cucurbits botany, cultivation and utilization. Interscience Publishers, Incorporated, New York

Whitaker TW, Bemis WB (1976) Cucurbits. In: Simmonds NW (ed) Evolution of crop plants. Longman, London, pp 64–69

Xu Y, Ouyang XX, Zhang HY, Wang YJ (1999) Identification of molecular markers linked to race-1 Fusarium wilt resistance gene in watermelon wild germplasm PI 296341. Acta Bot Sinica 41:952–955

Xu Y, Zhang HY, Kang GB, Wang YJ, Chen H (2000) Studies of molecular marker-assisted selection for resistance to Fusarium wilt in watermelon (*Citrullus lanatus*) breeding. Acta Genet Sinica 27:151–157

Zamir D, Tadmor Y (1986) Unequal segregation of nuclear genes in plants. Bot Gaz 147:355–358

Zhang XP, Rhodes BB (1993) Inheritance of resistance to races 0, 1 and 2 of *Fusarium oxysporum* f. sp. *niveum* in watermelon (*Citrullus* sp. PI 296341). Cucurbit Genet Coop Rep 16:77–78