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Linkage Mapping in a watermelon Population Segregating for Fusarium Wilt Resistance

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ABSTRACT. **Isozyme, randomly amplified polymorphic DNA (RAPD), and simple sequence repeats (SSR) markers were used to generate a linkage map in an F_2 and F_3 watermelon [*Citrullus lanatus* (Thumb.) Matsum. & Nakai] population derived from a cross between the fusarium wilt (*Fusarium oxysporum* f. sp. *niveum*) susceptible 'New Hampshire Midget' and resistant PI 296341-*FR*. A 112.9 cM RAPD-based map consisting of 26 markers spanning two linkage groups was generated with F_2 data. With F_3 data, a 139 cM RAPD-based map consisting of 13 markers covering five linkage groups was constructed. Isozyme and SSR markers were unlinked. About 40% to 48% of the RAPD markers were significantly skewed from expected Mendelian segregation ratios in both generations. Bulked segregant analysis and single-factor analysis of variance were employed to identify RAPD markers linked to fusarium wilt caused by races 1 and 2 of *F. oxysporum* f. sp. *niveum*. Current linkage estimates between the resistance trait and the marker loci were too large for effective use in a marker-assisted selection program.**

The diploid watermelon (*Citrullus lanatus*; 2n = 2x = 22; Shimotsuma, 1963) suffers from a number of serious fungal, bacterial, and viral diseases that reduce yield and quality (Bruton, 1998; Nagel et al., 1992). One of the most economically important of these is fusarium wilt caused by the soilbome fungus *Fusarium oxysporum* f.sp. *niveum* (FON) (Martyn, 1996; Purseglove, 1987). FON has been separated into three pathogenic races: 0, 1, and 2 (Martyn, 1987; Netzer, 1976). Control of this disease relies primarily on use of resistant cultivars and crop rotation. While many commercial cultivars have resistance to races 0 and 1 of the pathogen. the more aggressive race 2 overcomes all cultivars and has great potential for spread in watermelon production areas in the southeastern United States because it can be seedborne (Bruton, 1998; Hopkins et al., 1992; Martyn and Netzer. 1991). Resistance to the various races has been identified in plant accessions from Africa (Dane et al., 1998; Martyn, 1987). A single dominant gene, designated *Fo-1*, confers resistance to race 1 of FON in watermelon (Netzer and Weintall, 1980), while resistance to race 2 in PI 296341-*FR* (*Citrullus lanatus* var. *citroides*) is thought to be conferred by a recessive gene with interactions with some minor genes (Martyn and Netzer. I99 1; Zhang and Rhodes, 1993).

Variations among the cultivated watenneion are low with respect to isozymes and economically important characteristics and this has hindered construction of a detailed genetic map (Hashizume et al., 1996; Zhang et al.. 1994). In a survey of 26 allozymic loci in 550 cultivated watermelon accessions, very little variation was found, but significant divergence was detected between cultivated and wild *Citrullus* Schrad. sp. forms (Navot and Zamir, 1987). Navot et al. (1990) constructed an isozyme-based map containing seven linkage groups spanning 354 cM using a backcross population derived from *C. lanatus x C. colocynthis* (L.) Schrad. Hashizume et al. (1996) constructed a 524 cM linkage map spanning 11 linkage groups in a backcross population of a cultivated Japanese C. *lanatus* line and a wild African form. Other cucurbit genomes have been studied more extensively than the *Citrullus* genome. Genetic linkage maps have been created using molecular, isozymic. morphological, and disease resistance markers in intraspecific cucumber (*Cucumis sativus* L.) populations (Kennard et al., 1994; Meglic and Staub, 1996), and in intra- and interspecific melon (*Cucumis melo* L.) populations (Danin-Poleg et al., 1998; Oliver et al., 1998; Wang et al., 1997).

Highlysaturatedgeneticmapsfacilitate identificationofgenes controlling both qualitative and quantitative traits of interest (Wang et al., 1998). Molecular markers linked to disease resistance would accelerate the time-honored, though time-consuming method of artificial inoculation, by easing the process of screening large numbers of individual plants to evaluate the introgression of resistance (Wechter et al., 1995). Near-isogenic lines (NILs) and various pooling strategies have a great potential for rapid characterization of a trait of interest (Weising et al., 1995). Bulked segregant analysis (BSA) can be used to simulate a pair of NILs by pooling DNA from a population segregating for a specific trait (Wang et al., 1998). BSA has been used to identify markers linked to genes for disease resistance e.g. fusarium wilt resistance in melon (*Cucumis melo*, Wechter et al., 1995; 1998).

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Therefore, the objectives of this research were to 1) identify and characterize polymorphic molecular markers in an F_2 population as well as F_3 lines derived from a cross between the susceptible 'New Hampshire Midget' (NHM) (*C. lanatus* var. *lanatus*) and the resistant PI 296341-*FR* (*C. lanatus* var. *citroides*), 2)use these markers to construct a genetic linkage map. and 3) examine the association between the inheritance of specific molecular marker alleles and resistance to race 1 and 2 of FON.

Materials and Methods

PLANT MATERIAL. The susceptible 'New Hampshire Midget (female parent) and the resistant PI 296341-*FR* (Martyn and Netzer, 1991) were used as parents. Individual F_1 and F_2 plants were advanced to produce F_3 lines.

FUSARIUM WILT SCREENING ASSAY. American type culture collection (ATCC) strains of the pathogen Race I (ATCC 18467; Armstrong and Armstrong, 1978; Biles and Martyn, 1989) and Race2 (ATCC 62939; Martyn, 1987)), were used to screen NHM, PI 296341-*FR*, F,, and 72 F_3 lines. Following DNA extraction, eight, 2-week-old seedlings from each line per race were inoculated by root dip in an inoculum of 1×10^6 microconidia/mL (Martyn. 1987). The plants were evaluated under greenhouse conditions without supplemental lighting. Individual plants were rated on a scale of I to 5 with 1 = healthy, no evidence of wilt over a 3-week period, 2 = beginning signs of wilt, 3 = slightly wiilted and stunted plants, 4 = wilted and stunted, and 5 = heavily wilted and death of the seedlings (Dane et al., 1998). Disease ratings were conducted over 3 weeks. Crown (stem-root junction) sections of selected plants, both susceptible and resistant, were surface sterilized and plated on quarter-strength potato dextrose agar to verify FON infection (Wechter et al., 1995).

ISOZYME ANALYSIS. Cotyledonary tissue from four of the eight individuals from each of 72 F_3 lines (125 mg/line) was bulked and homogenized by hand in a pre-chilled mortar and pestle with 500 mL phosphate extraction buffer (HyPure, Isolab, Perkin Elmer, Branchburg, N.J.). Crude extracts were microcentrifuged for 10 min at 14,000 g_n in a microcentrifuge (Eppendorf54 I 5C; Brinkman Instruments, Westbury. N.Y.). Supernatant (8.5 mL per sample) was electrophoresed on a Hypure agarose horizontal isoelectric focusing gel (FS-5080, pH 4-5) using the Multiphor Electrophoresis System (Amersham Pharmacia Biotech, Piscateway. NJ.). Samples were assayed for acid phosphatase [ACP, Enzyme Commission (EC) 3 1.3.2], aconitase (AK; EC 4.2.1.3), alcohol dehydrogenase (ADH; EC I. 1. 1. 1), diaphorase (DIA; EC 1.6.99), esterase (EST; EC 3.1.1), glutamate-oxaloacetate transaminase (GOT; EC 2.6.1. 1), isocitrate dehydrogenase (IDH; EC 1.1.1.42), malate dehydrogenase (MDH; EC 1.1. I .37), malic enzyme (ME; EC 1.1.1.40), peroxidase (PRX; EC 1.11.1.7), 6-phosphoglucanate dehydrogenase (6-PGD; EC I. 1. I .49), phosphohexose isomerase (PHI; EC 5.3.1.9), shikimic acid dehydrogenase (SKD; EC 1.1.1.25), superoxide dismutase (SOD; EC 1.15.1.1), and triose phosphate isomerase (TPI; EC 5.3.1.1). Gels were stained as described by Wendel and Weeden (1989).

DNA ISOLATION. DNA was extracted from 100 mg freeze-dried leaf material from 98 F_2 watermelon plants, and from 500 mg of fresh cotyledon tissue from 2-week-old seedlings of PI 296341-*FR*, NHM, F_1, and four individuals from each of72 F_3 lines using a modified CTAB extraction technique (Wagner et al., 1992) with additional purification steps (Kubisiak et al., 1997), or using the Phytopure plant DNA extraction kit (Amersham Pharmacia Biotech.).

BULKED SEGREGANT ANALYSIS. Disease resistance rating means were calculated for each F_3 line for each race of the pathogen. Data were subjected to analysis ofvariance (ANOVA) using Proc GLM of SAS and means were separated by Duncan's multiple range test (SAS Inst., Inc., 1996). Two bulked DNA samples were prepared for each race of the pathogen. one resistant bulk(R) and one susceptible bulk (S). Bulked samples consisted of equal volumes ofstandardized DNA (25 $ng \cdot mL^{-1}$) from 10 resistant and 10 susceptible lines, respectively.

RAPD ANALYSIS. To identify segregating polymorphisms, one hundred fourteen 10-mer primers from Operon Technologies (Alameda, Calif.) and the University of British Columbia (Vancouver. British Columbia, Canada), along with five 12-mer primers identified by Hashizume et al., (1996) were screened on a panel of DNAs consisting of NHM, PI 29634 1 -*FR*, F_1, and 5 F_2 progenies. Primers that amplified bands that were polymorphic between NHM and PI 29634 1 -*FR*, present in the F,, and present in at least one F_2 progeny were classified as potentially useful markers.

In the F_3 population. three hundred twenty nine IO-mer primers from Operon Technologies and from the University of British Columbia were screened for their ability to detect polymorphisms between NHM and PI 29634 1 -*FR* as well as the R and S bulks. Potentially useful polymorphisms were further characterized on each of the individuals from the F_3 lines composing the respective bulks.

Polymerase chain reaction (PCR) was performed in a 25 µL volume containing 10 mM Tris-HCl, 3.0 mM $MgCl_2$, 10 mM KCl (pH 8.3), 0.2 mM of each nucleotide (Perkin Elmer), 5 pmol 10-mer or 12-mer oligonucleotide primers, IO ng genomic DNA and 1 unit of *Amplitaq* polymerase (Perkin Elmer). The thermocyclers were programmed as follows: initial denaturation at 94 °C for 10 min. 35 cycles of 94 °C for 45 s, 36 °C for 45 s, and 72 °C for 2 min, followed by a final extension at 72 °C for 5 min. Amplification products were electrophoresed on 1.2% agarose gels and detected by staining with ethidium bromide. The gels were photographed under ultraviolet light with Polaroid film 667 (Polaroid Corporation, Cambridge, M.A.). Lambda (h) DNA digested with *Pst*I was used as a molecularsizemarker. Each band was named by the primer used and its size in basepairs (bp): OPF 16_{1600} was the 1600 bp band amplified by Operon primer F 16.

SSR ANALYSIS. A total of 107 simple sequence repeats (SSR) primers from Jarret et al. (1997. and unpublished results) and Katzir et al. (1996) were screened against the parents and the F, to detect polymorphisms. Analysis was performed on four individuals bulked from each of 72 F_3 lines. PCR was performed in a 20 µL volume containing 10 mM Tris-HCl, 3.0 mM $MgCl_2$, 10 mM KCI (pH 8.3), 0.2 mM of each nucleotide (Perkin Elmer), 1 µM of each primer, I .6% bovine serum albumin, 50 ng genomic DNA and I unit of *Amplitaq* polymerase (Perkin Elmer). Thermocyclers were programmed as follows: initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for l min, 55 °C for I min and 72 °C for 2 min, followed by a final extension at 72 °C for 2 min. The amplified products were separated on a 0.4 mm thick, 6% denaturing polyacrylamide gel. For molecular weight size determinations, 25 bp and/or 100 bp DNA ladders (GibcoBRL) were used. The gel was silver-stained using the method of Bassam et al. (1991).

LINKAGE ANALYSIS. Each RAPD band was tested for goodness of fit to the expected 3: I segregation ratio in the F_2 or 5:3 ratio in the F_3 by chi-square analysis ($P < 0.01$). Those markers experiencing segregation distortion were excluded from linkage analy-

Table 1. Mean fusarium wilt race I disease resistance ratings in F, lines derived from a cross between fusarium wilt-susceptible 'New Hampshire Midget' x resistant PI 296341 -*FR*.

F, lines	Mean disease rating	
F;-2	5.0 a"	Susceptible
F,-I I, F_3-126	4.5 ab	Susceptible
F_3-30, F_3-74	4.3 abc	Susceptible
NHM, F_3-43, F_3-86, F_3-95	4.1 abcd	Susceptible
F_3-123, F_3-15, F_3-59, F_3-67, F,-48, F,-33, F_3-105	3.9 abcde	Susceptible
F_3-52, F_3-41, F_3-23, F_3-5, F_3-51	3.7 bcde	Intermediate
F,-54. F_3-9, F_3-80, F_3-39, F_3-7 I. F,-6 1. F,-79. F_3-121	3.6 bcdef	Intermediate
F_3-107, F_3-60, F_3-25, F_3-I 13, F,-98, F_3-I I I	3.5 bcdefg	Intermediate
F,-20. F_3-14. F_3-16, F,-62. F_3-I 16, F,-29. F;-89. F,-76	3.3 bcdefgh	Intermediate
F_3-6, F_3-I17, F;-70, F_3-128, F_3-IO, F_3-45 , F_3-83 . F_3-97	3.1 cdefgh	Intermediate
F,-I IO, F,-46. F_3-36, F_3-77	2.9 defghi	Intermediate
F_3-101, F_3-55	2.6 efghij	Intermediate
F_3-82, F_3-99, F,-85, F_3-1 19	2.4 fghijk	Resistant
F_3-49, F_3-120, F_3-IO2	2.3 ghijk	Resistant
F_3-8 1, F;-38. F_3-100, F_3-88	2. I hijk	Resistant
F_3-19, PI 296341-*FR*	1.7 jk	Resistant
F_1	1.4 k	Resistant

'Mean separation within column by Duncan's multiple range test, $P < 0.05$.

Table 2. Mean fusarium race 2 disease resistance ratings in F_3 lines derived from a cross between fusarium wilt-susceptible 'New Hampshire Midget' x resistant PI 296341-*FR*.

F, lines	Mean disease rating	
NHM, F_3-2	5.0 a"	Susceptible
F_3-52	4.8 ab	Susceptible
F,-43, F,-55	4.5 abc	Susceptible
F_3-45, F_3-37, F_3-I1, F_3-59, F_3-61, F,-9, F,-74	4.4 abcd	Susceptible
F_3-29, F_3-62, F;-80, F_3-23, F_3-68	4.3 abcde	Susceptible
F_3-5, F,-25. F_3-86	4.1 abcdef	Intermediate
F,-16. F_3-79, F_3-71, F_3-15, F_3-42	4.0 abcdefg	Intermediate
F_3-5 1, F_3-48, F,-60	3.8 abcdefgh	Intermediate
F_3-95, F_3-100, F,-98. F_3-54, F_3-6, F,-33, F_3-46	3.6 abcdefghi	Intermediate
F_3-120, F_3-116	3.5 bcdefghi	Intermediate
F_3-I 17, F_3-67, F,-39, F_3-10, F_3-99, F_3-111	3.3 bcdefghij	Intermediate
F,-I IO, F_3-105, F_3-101, F,-20. F_3-41, F_3-97, F_3-128, F_3-70, F_3-81	3. I cdefghij	Intermediate
F_3-121, F,-126. F_3-77, F_3-14, F_3-107, F_3-76, F_3-102, F_3-119, F,-38	3.0 defghij	Intermediate
F_3-88, F_3-85, F_3-83	2.9 efghij	Intermediate
F_3-30, F_3-89, F_3-123, F_3-82	2.7 fghij	Resistant
F;-49. F_3-I 13	2.6 ghij	Resistant
F_3-17, F,-36, F_3-19	2.5 hij	Resistant
PI 29634 I -*FR*	2.3 ij	Resistant
F_1	2.0 j	Resistant

'Mean separation within column by Duncan's multiple range test, $P < 0.05$.

ses. The mapping software packages Joinmap v. 2.0 (Stam, 1993; Stam and Van Ooijen, 1995) and MAPMAKER/EXP v. 3.0 (Lander et al., 1987) were used for linkage analysis. Marker orders were determined using MAPMAKER/EXP with LOD $\leq$ 4.0 and recombination fraction (θ) = 0.3. Map distances between linked markers were estimated using the Kosambi mapping function (Kosambi, 1944). The software program DrawMap v. 0.9 (Ooijen, 1992) was used to draw the genetic linkage maps.

Association with resistance. The degree of association between the molecular marker loci and disease resistance data was investigated using single-factor ANOVA (SAS Inst., Inc., 1996), in which the individual marker genotypes were used as class variables. Four individuals per line from each of the susceptible and resistant lines, based on results of Duncan's multiple range test were included in the analyses. A fairly liberal threshold ($P < 0.05$) was chosen in an attempt to lower the type II error rate.

Results

Fusarium wilt resistance assay. Disease rating scores for NHM, PI 29634 I -*FR*, F,, and F_1 lines, are presented in Tables 1 and 2, and indicate resistance is controlled by dominant gene action because disease rating scores of the F_1 were not significantly different from that of PI 296341-*FR*. Based on mean separation, any F_3 line with a disease rating $\geq$3.9 for race 1 was considered susceptible, while any F_3 line with disease rating 12.4 was considered resistant. All other lines were considered as intermediate (Table I). Based on this analysis, 15 F_3 lines were

Table 3. Genetic linkage map generated from markers segregating among F_2 and F_3 individuals derived from a cross between the fusarium wilt-susceptible 'New Hampshire Midget' x fusarium wilt-resistant PI 296341-*FR*.

Generation	Linkage group	No of markers	Map distance (cM)	Spacing (cM)
F_2	1	2	14.0	14.0
	2	24	98.9	4.1
	Unlinked	42		
	Total	68	112.9	
F_3	1	2	26. 1	26. 1
	2	5	39.6	8.2
	3	2	20.7	20.7
	4	2	19.6	19.6
	5	2	23.0	23.0
	Unlinked	18		
	Total	31	*139.0*	

resistant, 41 intermediate. and 12 susceptible to race 1, and this ratio does not significantly differ from the expected 1:2: 1 segregation ratio ($\chi^2 = 3.19$, df = 2, $P > 0.05$). These findings are consistent with the hypothesis of a single dominant gene controlling resistance to race 1 of FON. Similarly, any line with a disease rating 24.3 was considered susceptible to race 2 and rating ≤ 2.6 as resistant. For race 2, there were 9 resistant, 47 intermediate, and 16 susceptible F_3 lines ($\chi^2 = 8.03$, df = 2, $P < 0.01$), which differs significantly from the 1:2: 1 ratio that would be expected if this trait were controlled by a single dominant gene. F_3 lines with resistance or susceptibility to FON 1 in general showed resistance or susceptibility. respectively, to FON 2 (Tables 1 and 2).

Mendelian segregation ratios

Isozymes. Bulked F_3 samples were found to be monomorphic for the following enzymes: ME. IDH, GOT, ACP, SKD, and MDH. Banding patterns were vague when the gels were stained for 6-PGD, PHI, AK, TPI, ADH, DIA, and SOD. Staining for the enzymes esterase (EST) and peroxidase (PRX) yielded one polymorphic peroxidase locus (PRX-I) and two polymorphic EST loci in this population and Mendelian segregation analysis indicated that the peroxidase ($\chi^2_{Prx\text{-}1} = 1.3$, $P > 0.05$) and esterase loci ($\chi^2_{Est\text{-}3} = 1.52$; $\chi^2_{Est\text{-}4} = 1.15$, $P > 0.05$) fit a 3:1 ratio,

Rapd analysis. Of the one hundred fourteen 10-merRAPD primers screened, 64% revealed at least one polymorphic band between the parents, NHM and PI 296341-*FR*. Two polymorphic bands were on average detected per primer. A total 52.5% of the markers fit the expected 3:1 Mendelian segregation ratio ($P > 0.01$).

Of the three hundred twenty nine IO-mer primers'used in the BSA screen, 70 showed monomorphic patterns, 117 showed polymorphism between the parents but were monomorphic in the bulks, and 3 1 amplified at least one band that was polymorphic between NHM and PI 296341-*FR* as well as the bulks. In total, 60 potentially informative markers were identified, which were tested on four individuals from each line that composed the bulks,

Ssr analysis. Of 107 SSR primers screened against the parents and the F_1, more than half generated no amplification product, 40 were monomorphic and live generated polymorphisms (CL2-070, CL1-006, CLI-06. CLI-20, and CL2-23) under the described reaction conditions. The seven SSR primers specific for *Cucumis* L. sp (Katzir et al., 1996) generated no amplification product in this *Citrullus* population. All markers ($\chi^2_{CL2\text{-}070} = 1.15$, df = 1; $\chi^2_{CL1\text{-}006} = 0.46$, df = 2; $\chi^2_{CL1\text{-}06} = 1.64$, df = 2; $\chi^2_{CL1\text{-}20} = 0.11$, df = 2; $\chi^2_{CL2\text{-}23} = 0.13$, df = 2) exhibited the expected 3:1 (band absence versus presence, df = 1) or 1:2:1 segregation ratio (df = 2) in this population ($P > 0.05$).

Linkage mapping. Using only the markers that tit the expected Mendelian segregation ratios. at a LOD of 4.0 and a recombination fraction of 0.3, two linkage groups were detected in the F_2 using 98 individuals. Group 1 contained two markers and group 2 contained 24 markers (Table 3; Fig. 1). The isozyme and SSR markers were unlinked. A high percentage (64%) of the RAPD markers were unlinked. In an attempt to increase genomic coverage and determine whether any specific regions of the genome were experiencing segregation distortion, distorted markers were mapped one marker at a time (Kubisiak et al., 1997), and found to cluster on linkage group 2.

The linkage map in the F_3 consisting of live linkage groups containing two to five markers each was generated using the primers that were capable of detecting differences in the bulked samples. Four individuals selected from each of 28 F_3 lines,

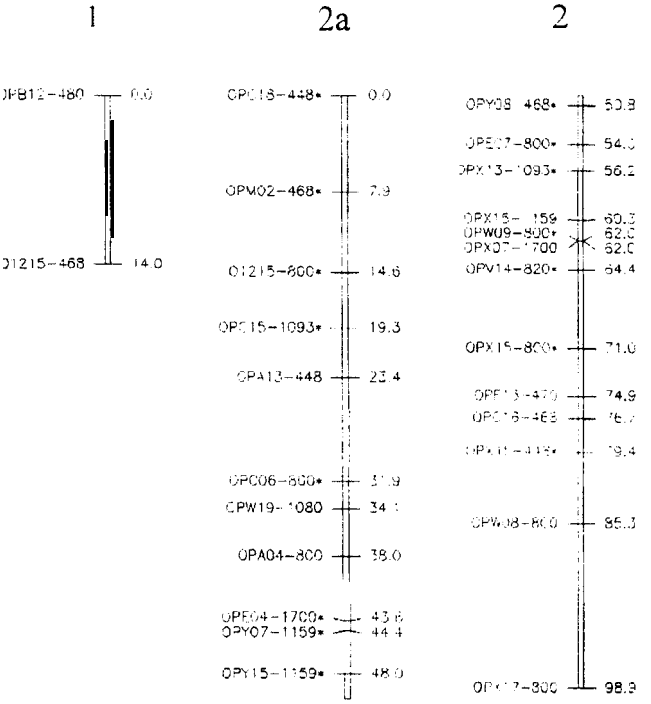

Fig. 1. Linkage map constructed using RAPD marker in F_2 watermelon population derived from a cross between the fusarium wilt-susceptible 'New Hampshire Midget' x fusarium wilt-resistant PI 296341-*FR* (LOD = 4). The marker names are given on the left and the position of markers in Kosambi cM is indicated on the right of each linkage group. with * indicating framework marker loci.

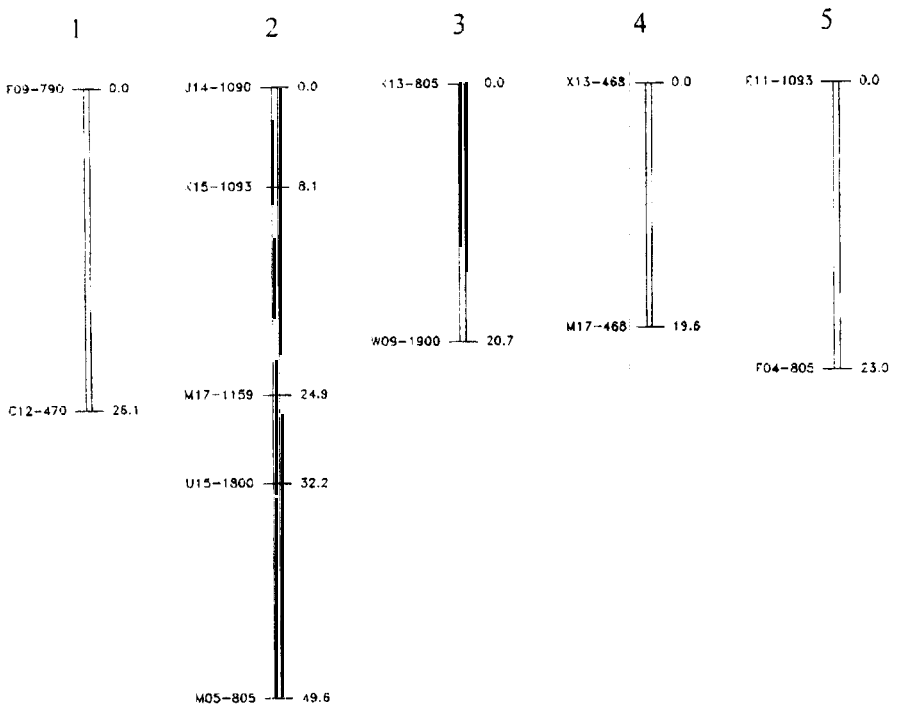

Fig. 2. Linkage map constructed using RAPD markers in an F_3 watermelon population derived from a cross between the fusarium wilt-susceptible 'New Hampshire Midget' x fusarium wilt-resistant PI 296341-*FR* (LOD = 4). The marker names are given on the left and the position ofmarkers in cM is indicated on the right of each linkage group.

chosen for theirresponse to the fusarium wilt pathogen, were used to generate this map to verify clustering of markers into one linkage group. Sixty polymorphic bands were detected from these 3 1 primers and used for mapping 1 12 F_3 individuals. Fifty-two percent ofthemarkers exhibited the expected 5:3 segregation ratio in the F_3, and 32.8% of the RAPD markers were unlinked. Distorted markers were mapped one marker at a time and clustered on linkage group 2. JoinMap and MapMaker produced similar linkages and map orders.

Several RAPD markers segregated in both the F_2 and the F_3 populations. The marker loci $OPY15_{1159}$, $OPX13_{1093}$, and $OPX\ 1\ 5_{800}$ segregated in Mendelian fashion in the F_2, but showed segregation distortion in the F_3 The markers mapped to linkage group 2 in the F_2 population (Fig. I), and when mapped one at a time, mapped to regions between markers $X\ 15\text{-}_{1093}$ and $M17\text{-}_{159}$ on linkage group 2 in the F_3 population (Fig. 2).

STATISTICAL ANALYSIS. From the individuals selected from the resistant and susceptible lines for races 1 and 2, several markers were putatively linked to FON 1 and FON 2 resistance by single-factor ANOVA. The resistance trait-marker linkages suggested by single-factor ANOVA were examined by recombination fraction of the resistant and susceptible individuals and marker absence and presence (Table 4). The minimum recombination fraction calculated was 0.33 between race 2 and marker $C12_{470}$. This level of recombination is too great to be advantageous in marker-assisted selection. Visual inspection of band presence and/or absence versus the disease-resistant phenotype confirmed that there was very little to no linkage between the trait and the marker locus.

Discussion

A molecular marker must generate reliable polymorphisms, be relatively simple to perform and be capable of processing a large number of samples per unit of time in order to be useful for marker assisted selection. Where associations between the marker locus and the trait of interest are tight (<5 cM), marker analysis can be used to increase gain from selection because the frequency

Table 4. Occurrence of marker and resistance phenotype in F_3 population derived from a cross between the fusarium wilt-susceptible 'New Hampshire Midget' x resistant PI 296341-*FR*.

		Single factor ANOVA					Observed frequencies"						
Trait/marker	χ^2	$P > F^z$	R^{2y}	Linkage group	Parents' NHM	PI 296341-*FR*	A U	Ar	aR	ar	Al	aI	θ
FON1					S	R							
$X13_{1093}$	3.33"'	0.018	5.68	Unlinked	C	A	17	15	23	4	30	9	0.64
$X13_{468}$	3.33"'	0.014	5.93	4	A	C	26	7	19	10	27	12	0.42
$M17_{468}$	1.32^{NS}	0.03 1	5.46	4	A	C	14	11	8	14	26	12	0.40
FON2					S	R							
$B12_{468}$	0.02^{NS}	0.012	5.28	Unlinked	A	C	8	3	10	0	55	6	0.62
$W19_{1080}$	24.61'	0.006	8.97	Unlinked	A	C	IO	2	11	6	62	9	0.45
$X13_{448}$	17.93*	0.028	4.85	Unlinked	A	C	4	8	11	6	62	9	0.45
$C12_{470}$	1.62^{NS}	0.018	6.10	I	'A	C	6	4	4	10	41	26	0.33

[z]Significance levels determined by F-test based on the single factor ANOVA for each pairwise comparison of trait and marker locus.
[y]Percentage of phenotypic variation explained by the marker locus based on single-factor ANOVA of marker-trait association.
[x]A = band absent, C = band present, R = resistant, and S = susceptible.
"AR = marker present. resistant; Ar = marker present. susceptible; aR marker absent, resistant; ar = marker absent. susceptible: Al = marker present. intermediate: aI = tnarker absent. intermediate.
'Recombination fraction.
[NS,*]Nonsignificant or significant at $P < 0.05$.

ofrecombination between the marker and the gene is low (Meglic and Staub, 1996). Sixty-four percent of the RAPD primers and 11.1% of the SSR primers evaluated in this study were able to detect polymorphisms between NHM and PI 296341 -*FR* under the conditions described. Most of the RAPD markers in linkage group 2 (90%) were derived from PI 296341-*FR* and absent in NHM. None of the *Cucumis* specific SSRs defined by Katziret al. (1996) were able to amplify any regions in this mapping population. Similarly Wang et al. (1997) found that only one of the seven *Cucumis* SSRs was able to detect polymorphisms in a C. *melo* population.

In the F_2 52.5% of the markers followed the expected 3: 1 or 1:2: 1 Mendelian segregation ratio, and in the F_3 population 52% of the markers followed the expected 5:3 segregation ratio. This level of distortion is higher than that reported for other species, although distortions are common in interspecific and intersubspecific populations (Causse et al., 1994). In a number of genera including *Cucumis* (Kennard et al., 1994; Perin et al., 1998), *Lens* Mill., *Capsicum* L.. *and Lycopersicon* Mill. (Zamir and Tadmor, 1986), the proportion of loci deviating from expected Mendelian segregation ratios is higher in interspecific crosses than in intraspecific crosses. These aberrant segregations may be due to either structural genes or cryptic structural differences between the parents or due to artifactual nongenetic variation, organellar bands or epigenetic interactions (Soltis et al., 1998).

Hashizume et al. (1996) using different *Citrullus* accessions. was able to construct a map covering 11 linkage groups with 2 to 10 markers each in a backcross population with greater coverage of the genome using 12-mer RAPD primers than has been presented herein. The markers generated by 12-mer RAPD markers in the population investigated in this report are all unlinked. Wang et al. (1997) used a backcross C. *melo* population that was segregating for numerous traits including resistance to *Fusarium oxysporum* f. sp. *melonis*. The markers generated by 6 RAPD primers used in that map were randomly distributed among the 20 linkage groups detected and were unlinked to each other in the *C. melo* population. These same RAPD markers were all unlinked in this *Citrullus* population.

Identification of a marker linked to FON 1 or FON 2 resistance should facilitate the introgression of disease resistance from PI 296341-*FR* into cultivated watermelon. Only loose linkages were detected by three markers to race 1, and four markers to race 2, even though 329 RAPD primers were tested. The markers identified by single-factor ANOVA as linked to resistance to either race 1 or race 2 were all too loosely linked to be advantageous in marker-assistedselection. Identificationofmarkers linked tightly to resistance to either race of FON may have been impeded by lack of high levels ofresistance in the F_3 lines and use ofrelatively small sample sizes. Several primers have been identified that are linked to the *Fon* 2 fusarium wilt resistance gene in melon (*Cucumis melo*): UBC 596, $_{0kb}$ (Wechter et al., 1995; 1998); $OPE07_{1.3kb}$, and OPG 17, $_{0kb}$ (Baudracco-Arnas and Pitrat, 1996). In this population, the bands amplified by primer UBC 596 were not clear, a fragment similar in size to $OPE07_{1.3kb}$ was monomorphic, and primer OPG1 7 did not generate amplification products.

There were several linkage groups that consisted of only two markers and it is clear that more markers are necessary to fill gaps in linkage groups, to integrate linkage groups and cover the entire genome. Over 50% of RAPD markers and all SSR markers were unlinked. Dominance impairs utility of RAPD markers due to difficulty of detecting linkage between markers in the repulsion phase (Weeden, 1994), however little polymorphism was detected using codominant *Citrullus* specific SSR primers. Wang et al. (1997) found amplified fragment length polymorphisms to be more efficient at mapping the melon genome than RAPD markers or SSRs. Construction of a detailed genetic map within *Citrullus* will require not only an interspecific cross but other strategies such as use of watermelon expressed sequence tags (Rhodes and Dane, 1999).

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