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Inheritance and linkage analysis of resistance to zucchini yellow mosaic virus, watermelon mosaic virus, papaya ringspot virus and powdery mildew in melon

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Abstract

A melon (*Cucumis melo* L.) breeding line derived from PI 414723 is resistant to three potyviruses, watermelon mosaic virus (WMV), zucchini yellow mosaic virus (ZYMV), papaya ringspot virus (PRSV), and to powdery mildew (PM). The inheritance and linkage relationships of these four resistances were studied in a segregating F2 population and derived F3 families from a cross between cultivar Top Mark and the resistant breeding line. Dominant monogenic inheritance of all four resistances was observed. We report that line 414723-4S3, which was initially selected as a source of ZYMV and WMV resistance, is also a source of dominant monogenic resistances to PRSV and PM race 1. We also report on genetic linkage (significant departure from independent segregation, $\chi^2 = 58.1$, $p \ll 0.0001$) between resistance to WMV and ZYMV. The map distance between these loci was estimated to be 7.5 cm. The genes for resistance to PM and PRSV segregated independently from each other, and from ZYMV and WMV resistance.

Abbreviations: PM – powdery mildew; PRSV – papaya ringspot virus; WMV – watermelon mosaic virus; ZYMV – zucchini yellow mosaic virus

Introduction

Most commercial melon varieties are susceptible to a number of fungal and viral pathogens. Potyviruses form the largest and most economically important group of plant viruses (Riechmann et al., 1992). Severe losses in melon production areas due to potyvirus infection, including watermelon mosaic virus (WMV), zucchini yellow mosaic virus (ZYMV) and papaya ringspot virus (PRSV) have been reported (Davis & Mizuki, 1987; Provvidenti & Gonsalves, 1984). Infected melon plants may show vine decline, reduced or absent yield and fruit quality defects including non-uniform shape, small size, discoloration and poor flavor. Among the fungal diseases affecting melons, powdery mildew (PM), caused by the two pathogens *Sphaerotheca fuliginea* [Schlech. ex Fr] Poll. and *Erysiphe cichoraceum*, is very important in all melon growing countries (Sitterly, 1978). The use of commercial varieties with resistance to these pathogens would stabilize yield and improve fruit quality in melon production areas worldwide.

The Indian accession PI 414723 (McCreight et al., 1992) possesses multiple disease and pest resistances. Resistances to WMV (Moyer et al. 1985; Gilbert et al., 1994; formerly designated WMV-2) and to ZYMV (Pitrat & Lecoq, 1984) in breeding lines selected from this accession were reported to be conferred by the single dominant genes, *Wmv* and *Zym*, respectively. In contrast, Danin-Poleg et al. (1997) reported oligogenic

inheritance for ZYMV resistance in PI 414723. Webb (1979) and Pitrat & Lecoq (1983) reported resistance to PRSV (formerly called WMV-1) in the melon accessions PI 180280 and PI 180283, and showed that the resistances from these two sources are allelic. PI 414723 also harbored a PRSV resistance gene that segregated independently from *Zym* (Pitrat & Lecoq, 1984). The inheritance of resistance to different PM races, originating from several melon accessions (WMR 29, PI 78374, PI 124111, PI 124112, cultivar Nantais Oblong) was reported (Epinat et al., 1993; Kenigsbuch & Cohen, 1992), and allelism between the different genes identified in these sources was tested. Breeding lines derived from PI 414723 were previously shown to possess a recessive gene for resistance to PM race 1, and dominant genes for resistance to race 2 (McCreight et al., 1987). The line we used in this study (414723-4S3) is resistant to both PM races 1 and 2.

It is not uncommon for breeders to identify a single genotype that serves as a source of resistance for a number of very different diseases, although the genetic basis of this observation has not been established. The objective of this study was to confirm the inheritance of resistance from this source, and examine the linkage relationships between these genes. Such data would be important for breeding, and for understanding the organization of resistance genes in key germplasm resources.

Materials and Methods

Genetic material

Cucumis melo 'UC Top Mark' (Zink & Gulber, 1987) served as the susceptible parent. Breeding line 414723-4 S3, derived from a single plant of the respective PI by three self-pollinations and selection for WMV resistance, served as the source of the different resistances. F1 individuals ('Top Mark' × 414723-4 S3) were self-pollinated, and F2 individuals were grown in the greenhouse and selfed. F3 families, 48 to 63 families for each test, were used to study the inheritance and evaluate linkage relationships between resistances to WMV, ZYMV, PRSV and PM race 1. A sample of 12–16 plants per F3 family was evaluated by inoculation with the respective pathogen under greenhouse conditions. Linkage analysis was performed using the χ^2 test; the standard error of the recombination fraction was calculated according to Allard (1956).

Viral isolates and inoculation procedures

Viral isolates WMV NY 62–76, ZYMV-CT and PRSV-W Morocco were kindly provided by Dr R. Provvidenti, Cornell University, Geneva, NY and maintained on *Cucurbita pepo* 'PMR Caserta'. WMV was maintained on *Phaseolus vulgaris* 'BT-2'. Inoculum from each virus was prepared by grinding systemically infected tissue in a blender for one minute with 200 ml of phosphate buffer, pH = 8.8 (0.1M KH_2PO_4 and 0.1M Na_2PO_4). The inoculum was filtered through a double layer of cheesecloth and kept on ice. Seedlings at the first true leaf stage were dusted with carborundum and inoculated by gentle rubbing with a pestle dipped in the viral inoculum. A second inoculation was performed a week later to ensure uniform infection. Inoculated plants were kept in a plastic greenhouse at about 25 °C with supplemental light. Visual scoring was done twice, at the 4 to 5 true leaf stage, and at the 8 to 9 leaf stage. All inoculated plants were grown until after anthesis (usually until fruit set) and observed daily. Almost all infected plants, regardless of genotype, developed moderate to severe symptoms on the inoculated leaves, although there were families, or plants within a family, that remained free of symptoms even after the second inoculation. Plants were therefore considered 'resistant' or 'recovering' (R) if viral symptoms were absent from younger leaves, and susceptible (S) when symptoms were continuously severe. A very low number of individual plants that were dead at the time of first evaluation were assumed to be killed by non-viral factors and were eliminated from the experiment.

Powdery mildew inoculation

Sphaerotheca fuliginea race 1 inoculum was collected by rinsing, with distilled H_2O, infected leaves of squash plants (*Cucurbita pepo*) that had supported abundant sporulation of the pathogen, and the race was identified at Cornell during the seasons of these experiments (Tom Zitter, personal communication). The sporangial suspension was filtered through a double layer of cheesecloth, and sprayed on young seedlings at the 2nd or 3rd true leaf stage, utilizing a plastic hand-sprayer. High relative humidity in the greenhouse (at 26 °C) following inoculation was necessary to ensure adequate disease development. Plants were scored 10 to 20 days post inoculation and classified either as resistant (R; minimal to no apparent fungal development) or susceptible (S; profuse sporulation).

Serological procedures

Double-antibody sandwich ELISA was used (Clark & Adams, 1977) to detect WMV and ZYMV coat protein in the 7th-9th leaf above the inoculation site. Antisera raised against ZYMV-CT and PRSV-W were produced by D. Gonsalves, NYSAES, Cornell University, Geneva; the WMV antiserum was from H.A. Scott, University of Arkansas. Sap was extracted by grinding leaves with 750 μl of Phosphate Buffer Saline containing 0.5% Tween 20 (PBS-T). The extracted sap was diluted 1:1 with PBS-T, mixed by vortex and centrifuged at 10 000 rpm for 10 min in a microcentrifuge. A 100 μl aliquot of the supernatant was added to microtiter plates, previously coated with immunoglobulin raised against purified ZYMV or WMV virions, and incubated for 12–16 h at 4 °C or 3–4 h at 37 °C. A second antibody conjugated to alkaline phosphatase, kindly provided by D. Gonsalves, Cornell University, Geneva, was added, and the plates were incubated as before. Phosphatase substrate (Sigma) was used for developing the reactions at room temperature and absorbance values (405 nm) were monitored using a Bio-Rad model 2550 EIA reader. The Elisa threshold signal was calculated as the mean of the moc-inoculated control samples plus three standard deviations.

Results

Reaction of parental lines and their progeny to the four pathogens

Papaya ringspot virus (PRSV) incited severe foliar mosaic and plant stunting on susceptible (S) plants. Systemic spread of symptoms was not observed on resistant plants, however interveinal mottling on inoculated leaves resulting from local infection was observed. Powdery mildew race 1 infection resulted in abundant sporulation on the upper and lower surfaces of the leaves and on stems and petioles of susceptible (S) individuals. Resistant plants showed very reduced or no fungal growth.

In the ZYMV screen, susceptible plants developed continuous, severe systemic and local infection, including mosaic and/or chlorosis. Symptoms spread rapidly through the vascular tissue resulting in growth retardation. Most of the plants scored as 'resistant' (R) showed severe systemic infection in the first two or three leaves above the inoculation site, and then complete recovery. In addition to the 'recovering' phenotype, the F3 segregants occasionally included plants that were completely asymptomatic. Such heterogeneity has been reported previously, and may indicate the presence of additional modifier genes that segregate in the progeny; alternatively, some plants may have escaped infection. In addition, environmental effects often complicate scoring of potyvirus resistance in melon (Danin-Poleg et al., 1997). The agronomic value of resistance or recovery derived from this genetic source should probably be tested in the future under a range of environmental conditions. Our experiment was performed under carefully controlled greenhouse conditions. Since, after recovery, approximately 3/4 of the plants were scored as resistant both phenotypically and by ELISA, showing the absence of viral antigen in the new leaves, a single major gene appeared to control the phenotypic difference between recovery and non-recovery.

In the WMV screen, susceptible plants (S) showed continuous, severe systemic infections, correlated with detection of high levels of viral antigen by ELISA. Symptoms usually included leaf mottling, tip stunting, growth retardation, reduction of the number of female flowers and occasional collapse of vines. Plants scored as resistant (R) showed severe systemic infections in the first one to four leaves above the inoculation site and then complete recovery, correlated with absence of viral particles from newly-grown leaves, in the ELISA test (Gilbert et al., 1994). Plants of the parental lines and segregating F3 progenies were heterogeneous with respect to the recovery phenotype (i.e., the number of infected leaves preceding recovery). In addition, completely asymptomatic plants were also occasionally observed. Mock-inoculated and uninoculated parental plants remained free of symptoms and viral antigen throughout the experiment.

Monogenic inheritance of four disease resistances

Inheritance of resistances to WMV, ZYMV, PRSV and PM race-1 were determined in a segregating progeny from a cross between two breeding lines, Top Mark, a variety which is susceptible to these diseases, and 414723-4S3, carrying multiple disease resistances. We analyzed 63 F_3 families obtained by self-pollinating F2 progeny individuals. A set of plants from each F3 family was inoculated with each pathogen, in order to determine whether that family was uniformly resistant (indicating that the respective F2 parent plant was homozygous resistant), uniformly susceptible (indicating

Table 1. Segregation of resistances to four melon diseases in an F2 population (Top Mark × 414723-4S3) – χ^2 test according to a single dominant gene model

Genotypic class[1]	ZYMV		WMV		PRSV		PM-1	
	obs	exp	obs	exp	obs	exp	obs	exp
RR	14	15.75	13	15.5	9	12	13	14
Rr	34	31.5	37	31	26	24	27	28
rr	15	15.75	12	15.5	13	12	16	14
Total tested	63		62		48		56	
d.f.	2		2		2		2	
χ^2	0.43		2.35		1.00		0.39	
P for independence	0.81		0.31		0.61		0.82	

[1] Genotypes of F2 individuals were inferred from the segregation of their F3 progeny.

an F2 homosygous susceptible parent), or segregating consistent with a ratio of 3 resistant: 1 susceptible, indicating that the parent had been heterozygous. The segregation of all genes was consistent with a ratio of 1 resistant: 2 heterozygous: 1 susceptible plant in the F2 generation, indicating monogenic dominant inheritance of all four resistances (Table 1).

Test of linkage between four resistance genes

Having screened the same F3 families with different pathogens, we examined the possible linkage relationships between the four resistances. All six pair-wise combinations of genes were subjected to a χ^2 test for independent segregation of the traits (Table 2). ZYMV and WMV resistances exhibited significant co-segregation (probability of independent segregation 10^{-13}). The map distance between them was calculated as 7.5 cm ($7.0\% \pm 2.2\%$ recombinant fraction). All other gene pairs segregated independently, indicating lack of linkage.

Discussion

As a source of resistance to WMV and ZYMV, line 414723-4S3 did not provide a high degree of resistance, such as immunity to viral infection or complete absence of symptoms. Following infection with the strains used, both the resistant and the susceptible parents, as well as F_3 plants, developed symptoms 14 to 21 days post inoculation, but the resistant plants underwent complete recovery, producing asymptomatic new growth.

Our previous study (Gilbert et al., 1994) established that WMV resistance from *Cucumis melo* PI 414723 is inherited as a single dominant gene, *Wmv.* Pitrat & Lecoq (1984) identified a single dominant gene, *Zym*, in the same PI. The major objective of the present study was to confirm the inheritance and assess possible linkage between resistances to three potyviruses, WMV, ZYMV and PRSV, and the fungal disease powdery mildew caused by *Sphaerotheca fuliginea* race 1, in breeding lines derived from PI 414723. Consequently, we show that a single line derived from this accession can serve as a source of dominant monogenic resistance to PRSV as well as to PM-1. Another novel finding of this study is the linkage (7.5 cm) between the WMV and ZYMV resistances. Although not extremely tight, this linkage between two resistance loci can be exploited in breeding by screening breeding material with both viruses, either together or sequentially, and to reduce the number of plants necessary to recover desirable genotypes with both resistances.

Co-segregation of resistance to two or more potyviruses has been previously reported in several crops including pepper, pea and bean (Grube et al., 2000; Fisher & Kyle, 1994; Kyle & Dickson, 1988; Provvidenti, 1991). In cucumber (*Cucumis sativus* L.) linkage between a recessive gene for WMV resistance, and a gene for ZYMV resistance was reported in the TMG-1 line (Wai & Grumet, 1995). More recently, the same cucumber line was further characterized and reported to be resistant to PRSV (Wai et al., 1997; Kabelka & Grumet, 1997). Host genes conferring resistance to different potyviruses may be tightly linked due to their evolution as gene clusters,

Table 2. Chi-square test for independence of segregation between pairs of the four resistances analyzed in this study

Genotypic classes	ZYMV & WMV		ZYMV & PRSV		WMV & PRSV		PM-1 & ZYMV		PM-1 & WMV		PM-1 & PRSV	
	obs[1]	exp	obs	exp	obs	exp	obs	exp	obs	exp	obs	exp
RR & RR	13	3.875	1	3	1	3	4	3.5	3	3.5	3	2.5
Rr & RR	0	7.75	7	6	7	6	7	7	6	7	2	5
rr & RR	0	3.875	2	3	2	3	3	3.5	3	3.5	2	2.5
RR & Rr	0	7.75	8	6	7	6	5	7	7	7	6	5
Rr & Rr	33	15.5	13	12	15	12	16	14	17	14	13	10
rr & Rr	5	7.75	5	6	7	6	8	7	9	7	4	5
RR & rr	1	3.875	1	3	1	3	4	3.5	3	3.5	1	2.5
Rr & rr	1	7.75	6	6	4	6	4	7	4	7	4	5
rr & rr	10	3.875	5	3	4	3	5	3.5	4	3.5	5	2.5
Total tested	62		48		48		56		56		40	
d.f.	8		8		8		8		8		8	
χ^2 Value	58.1		5.42		5.25		3.14		2.92		6.90	
P for independence	10^{-13}		0.71		0.73		0.93		0.94		0.55	

[1] Observed numbers of individuals of the different genotypic combinations are shown next to the numbers expected according to a model of two unlinked loci. Expected ratios (top to bottom) are: 1:2:1:2:4:2:1:2:1.

and it has also been suggested that one gene may protect against multiple viruses. For example, a single *Cucurbita moschata* gene conferred resistance to both WMV and ZYMV (Gilbert-Albertini et al., 1993), and a cucumber gene protected from both ZYMV and PRSV (Kabelka & Grumet, 1997). Our data indicates linkage between resistances to WMV and ZYMV, and independent segregation of these from PRSV and from PM-1 resistances.

In a recent study, Danin-Poleg et al. (1997) reported that ZYMV resistance conferred by *C. melo* PI 414723 is inherited as three complementary, dominant genes in a cross with the melon cultivar Dulce. These results are not consistent with the present report and with those of Pitrat & Lecoq (1984). The discrepancy could be explained by a difference in the virulence of the ZYMV strains that were used: Danin-Poleg and co-workers used a highly virulent strain in contrast to the moderate pathotype NF used by Pitrat and Lecoq. The ZYMV-CT strain that we used is considered to be of intermediate pathogenicity. Alternatively, there may be a genetic difference between the susceptible parents that were used. In addition, the original accession, PI 414723, is very heterogeneous and different resistance genes have been reported in different lines derived from it (Pitrat & Lecoq, 1984).

Previous studies have identified two PRSV resistance genes, *Prv1* and *Prv2*, in other melon accessions (Pitrat & Lecoq, 1983; Webb, 1979). Our data are in agreement with those of Pitrat & Lecoq (1984) reporting non-linkage between *Zym* and PRSV resistance from PI 414723. Allelism between the previously reported PRSV resistances and the single dominant gene we have identified in line 414723-4S3 should be evaluated. Similarly, the PM-1 resistance that we identified in PI 414723-4S3 should be crossed to genetic stocks containing the previously characterized PM resistance genes (Bohn & Whitaker, 1964; Epinat et al., 1993; Harwood & Markarian, 1968; Kenigsbuch & Cohen, 1992). Heterogeneity of PI 414723 was reported also with respect to PM resistance genes, and a recessive gene for PM race 1 resistance, as opposed to the dominant one we identified, was reported in line 92417 that has common ancestry with PI 414723 (McCreight et al., 1987). PI 414723 is very diverged at the DNA level from cultivar Top Mark (Neuhausen, 1992; Silberstein et al., 1999); efforts to map these resistance genes using molecular markers are under way in our laboratory.

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