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Inheritance of Resistance to Watermelon Mosaic Virus in *Cucumis melo* L.

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Abstract. **Resistance to watermelon mosaic virus (WMV) was transferred by successive backcrossing with selection from *Cucumis melo* PI 414723 to three melon varieties. Levels of resistance to virus accumulation in leaf tissue were evaluated using enzyme-linked immunosorbent assay, and procedures are described to select resistant individuals efficiently and accurately in segregating populations. Resistance is controlled by a single dominant. gene designated *Wmr*. Plants that carry this gene initially develop mosaic symptoms on inoculated leaves, but eventually recover from symptoms, and low or no virus can be detected in the youngest leaves. In contrast, susceptible plants show similar symptoms initially, but remain stunted and symptomatic with reduced fruit yield and fruit quality. Co-infection with other cucurbit viruses, specifically cucumber mosaic virus, papaya ringspot virus, and zucchini yellow mosaic virus, did not overcome resistance to WMV conferred by *Wmr*.**

Among the most significant barriers to the cultivation of *Cucumis melo* in many locations, including the major production areas in the United States, is loss to viral diseases (Nameth, 1975). Watermelon mosaic virus (WMV) is considered by many growers to be one of the most damaging of the four viruses commonly infecting melons in the United States. This is because of its ubiquitous distribution and tendency to co-infect with at least one other melon virus, often cucumber mosaic virus (CMV), with synergistic interactions that intensify symptoms (Poolpol and Inouye, 1986).

WMV is a member of the potyvirus family, the largest and one of the most economically important groups of plant viruses. The virus is characterized by a monopartite single-stranded RNA genome, filamentous particles, and nonpersistent transmission by at least 38 aphid species (Purcifull et al., 1984; Ward and Shukla, 1991). Unlike many potyviruses, WMV has an extensive host range, infecting at least 160 species in 23 dicotyledonous families and causing economic losses in cucurbit and legume crops (Purcifull et al., 1984). Typical symptoms induced by WMV on susceptible melon plants include leaf mottling and mosaic, chlorosis, tip stunting or bunching, reduced fruit yield and quality, and occasional collapse of vines as fruit approaches maturity (Purcifull et al., 1984; unpublished observations).

The existence of *C. melo* genotypes in which symptoms of WMV infection became progressively less severe was reported by Moyer et al. (1985) in studies with "breeding line 91213." Line 91213 is an S_3 aphid-resistant selection from a single plant of PI 371795 that was given a separate accession number PI 414723 (McCreight et al., 1992). In this study, they did not determine the influence of resistance in this line on fruit yield, but were able to show that the resistance reduced disease incidence in the field (Gray et al., 1986). Multiple mechanisms appear to be involved in the resistance, including a restriction in the cell-to-cell movement of the virus (Gray and Moyer, 1993). The resistance, manifested as reduced accumulation of virus, could be quantified using Enzyme-linked Immunosorbent Assay (ELISA), which measures the concentration of viral antigen in the plant tissue (Gray et al., 1988; Moyer et al., 1985). Inheritance of this response to WMV infection was not determined, but in view of the suggestion of multiple mechanisms and the observation that resistance involves expression of symptoms followed by recovery, it was postulated that the genetic basis may be polygenic. Several useful characteristics have been identified in this PI in addition to WMV resistance (Gray et al., 1986; Moyer et al., 1985; Romanow et al., 1986), including zucchini yellow mosaic virus (ZYMV) resistance (Pitrat and Lecoq, 1984; Provvidenti, 1993), resistance to the melon aphid *(Aphis gossypii* Glover) (Kishaba et al., 197 1), powdery mildew *[Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll.] resistance (McCreight et al., 1987), and resistance to inoculation of CMV by *Aphis gossypii* Glover (Pitrat and Lecoq, 1980).

A search for the best sources of resistance to WMV was begun at Cornell Univ. in late 1987. A diverse group of melon accessions was inoculated with WMV and selected for ability to grow and produce fruit while standard varieties were either dead or stunted and unfruitful. Resistant plants were found in four groups of melon that are not grown commercially in the United States (Munger, 1991). These included *C. melo conomon* Mak., the oriental pickling melon, represented by 'Freeman Cucumber' (FC) (Enzie, 1943); *C. melo dudaim* Naud., collected by J.R. Wall from the wild near New Orleans, La.; PI 182938, a small inedible melon from India, probably C. *melo agrestis* Naud.; and *C. melo momordica* Roxb., a group cultivated in India, represented by PI 371795. Several resistant plants were found in PI 371795 and selections from it, including PI 414723 (McCreight et al., 1992) and PI 414723-4, previously selected for resistance to ZYMV (Provvidenti, 1993).

Based on these results, a program was initiated to transfer watermelon mosaic resistance (WMR) to commercial melons in which the main source of resistance was the F_1 progeny of PI 414723 × *C. melo conomon* FC. *Cucumis melo dudaim* was also used as a parent in crosses with 'TAMDew' (TD). All three parents were selected for WMR. The FC x 414723 hybrid was used as the main starting point because of its superior field performance when compared with any single resistant parent. Initially, it was considered that the parents of very different origin and characteristics might have contributed complementary genes for WMR, but it is more likely that the partially dominant cucumber mosaic resistance of the *conomon* parent may have been responsible for the superiority of the hybrids. PI 414723 is extremely susceptible to CMV under our field conditions, despite reported resistance to CMV transmission in similar germplasm (Pitrat and Lecoq, 1980), and is also generally very poorly adapted to New York field conditions. Successive crosses were made to four susceptible parents, and in 1990, BC_3 progenies were inoculated with WMV and grown in the field. About half of the segregants along with the susceptible controls were killed or stunted badly, suggesting a major gene for resistance, but any differences among the remaining plants were obscured by natural infection with CMV. Resistant plants initially developed symptoms of the disease, but recovered from those symptoms and produced fruit. Based on these results, a formal genetic study was initiated.

The PI 414723 used in the Cornell program for WMR is a member of the *momordica* group of *C. melo* with fruit that split at maturity and poor adaptation to northern climates, even in the greenhouse. In this and similar cases, difficulties may be encountered in evaluating the genetic basis of the character of

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interest because of confounding effects from the segregation observed in crosses between very different parents. In the present genetic study, progenies derived from (PI 414723 × FC) were evaluated, and populations were also developed where the PI was crossed with other known susceptible genotypes.

The objectives of this study were to 1) determine the genetic basis of resistance to WMV derived from PI 414723; '2) evaluate the utility of ELISA as a criterion for selection of resistance in a melon breeding program; and 3) describe. when and where virus titer decreased in resistant plants relative to susceptible segregants to accurately distinguish resistant from susceptible plants using ELISA.

Materials and Methods

Germplasm and genetic populations. The susceptible *C. melo* genotypes UC Top Mark *Fom-3* (TM) (Zink and Gubler, 1987), TD, and the Cornell breeding line CPM 339 (CPM) were used as recurrent parents. 'TAMUvalde' was also used as a susceptible parent in some studies. PI 414723-4 S_3, which was self-pollinated three times after the original single-plant selection for WMV resistance, served as the resistant parent. Parental seed was planted in a greenhouse under supplemental lights in Mar. 1991 for the production of F_1 seed. The F_2 and backcross generations were produced via controlled pollinations from F_1 and parental plants trellised in the greenhouse under 12h of supplemental light per day during late Summer and Fall 1991. To determine inheritance of resistance, parental, F_1, F_2, and reciprocal backcross populations in TM, TD, and CPM backgrounds were evaluated phenotypically for WMV resistance. Seedlings were germinated on paper towels and sown in 4 × 8 Speedling trays 2 days later to assure even stands. A fungicide drench was applied routinely to control damping off.

Inheritance study. Inocula for mechanical transmissions or blower inoculations were prepared from infected foliar tissue homogenized in 0.1 M K_2HPO_4 buffer (pH 8.8), diluted and strained through cheesecloth, and held on ice. WMV isolate NY 62-76 was maintained on *P. vulgaris* BT-2 and increased on *Cucurbita pepo* 'PMR Caserta'. The purity of viral cultures was monitored routinely with ELISA, host index tests, and evaluation of characteristic symptomatology on *C. pepo* 'Caserta', *P. vulgaris* BT-2, and several susceptible varieties of *C. melo.* For mechanical inoculation, plants at the first true leaf (TL) stage were dusted with 400-mesh Carborundum, rubbed with inoculum, and rinsed with water. Mock-inoculated and noninoculated controls were included routinely. Inoculation of larger disease screens was done using a converted electric leaf blower that sprayed inoculum mixed with Carborundum on plants at the first TL stage until water-soaking was observed in the cotyledons (Gilbert, 1992).

The first comprehensive phenotypic evaluations were made 21 days post-inoculation (d.p.i.). Expanding leaves and growing points were rated visually using a numerical system from 1 (no foliar symptoms) to 4 (severe foliar symptoms). Notes were made regarding recovery and stunting at the tips and overall plant appearance. A second rating of each plant was made 35 d.p.i. This rating also included an evaluation of lower leaves and tips.

Based on the numerical scores given in the screen, plants were considered recovering, i.e., resistant, if their score decreased significantly from older to younger tissue. All infected plants, regardless of genotype, developed mild-to-moderate symptoms on the inoculated leaves. Individual plants that were dead at the time of the first evaluation were assumed to be killed by nonviral causes, such as damping off, and were eliminated from the experiment. Strongly symptomatic plants that died between the first and second evaluations were considered to have died from viral causes. Individuals whose score changed from 4 to 3 were not considered recovered, since 3 still indicated strong symptoms; thus, only plants that improved to a rating of 1 or 2 were considered resistant in the data analysis.

Serological analyses. To evaluate the utility of ELISA as a selection tool for resistance conferred by *Wmr*, breeding material segregating for this gene was examined as follows. Two sources of WMV resistance were considered: 1) the F_1 hybrids, 88-1217A (FC = 88-577 × PI 414723-4) and 88-1217B (FC × PI 414723-4); and 2) WMR-selected *C. melo dudaim.* Each variety in the program, TM, 'TAMUvalde', CPM, and TD, was crossed to one of the F_1 hybrids. *Cucumis melo dudaim* was crossed only with TD. Breeding populations used in this study consisted of ≈ 1000 inoculated plants from the fourth and fifth backcross F_1, including one susceptible control for every 17 segregants. One hundred plants from this population were chosen randomly from the group and tagged for evaluation with ELISA, and then handled similarly to the rest of the population for selection. All of the plants were inoculated at the first TL stage using the leaf-blower method.

The first round of phenotypic selection of the breeding material was carried out 19 to 21 d.p.i. Individuals that showed some sign of viral infection, such as foliar mottling or severe stunting, followed by recovery from symptoms, were considered resistant by phenotypic evaluation. A second round of selection was undertaken 10 days later when additional plants were removed from the resistant group.

Samples (four 6-mm-diameter leaf disks/leaf) were taken from random locations on four leaves at specified positions (the second, fifth, eighth, and eleventh TL) on each experimental plant and assayed by double antibody sandwich (DAS) ELISA (Clark and Adams, 1977). Leaf disks were taken from the second and fifth TL 24 d.p.i., placed in 4-ml polypropylene tubes containing 0.5 ml of ELISA extraction buffer on ice, and frozen at –20C. Samples from the eighth and eleventh leaves were taken 33 d.p.i. and treated similarly. All samples were tested at the same time in a single assay. Severely infected individuals that did not develop enough tissue to provide at least three samples were removed from the experiment.

The standard DAS–ELISA protocol described by Clark and Adams (1977), with some modifications (McLaughlin et al., 1981), was used to analyze the tissue samples and standards. Crude WMV antiserum was obtained from H.A. Scott, Univ. of Arkansas. Immunoglobulin (Ig) was purified from antiserum using the protocol of Clark and Adams (1977). Ig was conjugated to alkaline phosphatase by a one-step gluteraldehyde-mediated reaction (Voller et al., 1976). Forty-five samples were assayed on Coming U-bottom 96-well microtiter plates (Coming, N. Y.) coated with WMV-specific Ig at a concentration of 1.0 $\mu g \cdot ml^{-1}$ in 0.05 M carbonate buffer, pH 9.6. Frozen leaf disks were homogenized in 0.5 ml of phosphate-buffered saline (pH 7.4) containing 2% polyvinyl pyrolidine (PVP-40). Two duplicate plates were run simultaneously giving two replications per sample. The anti-WMV Ig was highly specific with little cross-reaction to healthy melon sap. In general, there was variation of <10% between replications, a reasonable variance for this assay (Hewings and D'arty, 1984). To directly compare absorbance values among plates, a dilution series of purified WMV ranging from 1 to 0.0039 $\mu g \cdot ml^{-1}$ diluted in healthy *C. melo* sap was included in each ELISA. The two absorbance values (405 nm) for each sample were averaged to determine which among the 100 randomly selected plants were classified as resistant on the basis of viral antigen titer. Maximum absorbance value limits of 0.200 for individual plants with 88-1217 resistant backgrounds and 0.400 for plants with *C. melo dudaim* backgrounds were applied.

Results and Discussion

Inheritance of resistance. Inoculated PI 414723-4 S_3 parental controls uniformly developed symptoms between 16 and 20 d.p.i., and four plants in the group of six recovered (Table 1). The two plants that did not recover died from damping off, apparently unrelated to viral symptoms, a significant problem for this extremely unadapted genotype. This event points out one of the difficulties in using the wild donor parent of a characteristic as the parent in an inheritance study instead of a genotype in which the characteristic in question has been transferred to an adapted background. The susceptible parents uniformly developed strong symptoms that increased in intensity through time (Table 1).

In the hybrid populations, the number of days until recovery varied somewhat between genetic backgrounds. Clear recovery was most common in progenies involving TM and CPM by 35 d.p.i. TD backgrounds did not always show recovery by that time. This result is consistent with observations from the breeding program that resistance in 'Honey Dew', and particularly the 'TAMUvalde' background (data not shown), is especially difficult to select.

In progeny of backcrosses to resistant par-

Table 1. Reaction of melon parents and their progenies to inoculation with watermelon mosaic virus.

Populations	No. plants[z] R	S	D	Expected ratio	Goodness of fit[y]
PI 414723-4 S_3 (PI)	4	0	2		
Top Mark (TM)	0	6	0		
TAM Dew (TD)	0	6	0		
CPM 339 (CPM)	0	6	0		
(PI x TM) F_1	12	0	2	1:0	
(PI x TM) F_2	30	9	0	3:1	0.80
(PI x TM) x PI	10	0	3	1:0	
(PI x TM) x TM	8	6	0	1:1	0.70
(CPM x PI) F_1	14	0	0	1:0	
(CPM x PI) F_2	29	9	0	3:1	0.89
(CPM x PI) x PI	19	0	3	1:0	
(CPM x PI) x CPM	9	9	0	1:1	0.99
(PI x TD) F_1	10	0	3	1:0	
(TD x PI) F_2	28	13	0	3:1	0.10
(TD x PI) x PI	16	0	1	1:0	
(TD x PI) x TD	7	10	0	1:1	0.60

[z]R= recovery from WMV symptoms; S = WMV susceptibility, no recovery; D = dead due to nonviral causes.
[y]$P_{\alpha=0.05}$; df = 1.

ents and F_1 plants, where 100% resistance was expected, a few plants did not recover according to the criteria established for this study by the time the experiment was terminated, in most cases apparently due to problems other than viral disease. All ratios for the F_2 populations and backcross progenies with susceptible parents were consistent with the expected ratios of 3 resistant : 1 susceptible and 1 resistant : 1 susceptible, respectively, when analyzed in an X^2 goodness-of-fit test at the $p_{\alpha=0.05}$ level under the hypothesis of a single dominant gene (Table 1).

Typical symptoms of WMV were evident on all but 9% of the inoculated individuals in the study. This 9% was considered to have escaped infection and was removed from the study, because both resistant and susceptible individuals show initial symptoms of infection. Recovery in resistant plants inoculated at the first TL stage is first clearly apparent at about the fourth TL and can be rated by symptom appearance and recovery in the young tissue of the plants. The noninoculated control plants in each row remained free of WMV symptoms throughout the study.

Evidence from the inheritance study suggests that the resistance is controlled by a single dominant gene that is designated *Wmr* on the basis of data-presented in Table 1. This symbol has been chosen to avoid confusion with the obsolete symbol *Wmv* (Pitrat and Lecoq, 1983) for resistance to the distinct virus, WMV-1, now known as papaya ringspot virus-W, so the gene symbol *Wmv* has been redesignated *Prv* (Pitrat, 1990).

The observed segregation ratios of all three backgrounds, TM, CPM, and TD groups, closely resembled the expected ratios under the hypothesis of single-gene dominance in conferring resistance to WMV from PI 414723-4 S_3 when evaluated according to the described criteria. However, by our definition of resistance, some resistant individuals died due to poor adaptation to winter greenhouse conditions before they could be classified unambiguously.

The resistant phenotype conferred by *Wmr* is not completely free from symptoms of disease, nor free from infection by the virus. Rather, this gene confers the ability to recover from symptoms of the disease, and to limit significantly the movement of the virus into growing tissue. Presumably, these two effects are directly related. While monogenic inheritance is clearly suggested by data in several different crosses, genetic background can affect expression of a major resistance gene.

ELISA as a criterion for selection. In segregating progenies derived from crosses with PI 414723, all plants inoculated with the virus at the first TL stage developed symptoms consisting of a mild foliar mottle. By the eight to 12 TL stage, resistant plants could be distinguished on the basis of recovery from symptoms, in contrast to susceptible plants that continued to express the foliar mottle; they also showed bunching and reduced leaf expansion at the growing tips. To determine whether virus was present in these plants, leaf samples were taken and analyzed using ELISA. In all cases where the tips of the mature plants were symptomless, ELISA indicated an absence of WMV antigen in the tips of the plants. Viral antigen could be detected in the oldest leaves of some of these plants, however, and in almost all cases, clear symptoms were also evident on the older leaves. This result suggested that in WMV-resistant plants, the virus initially established infection and then either did not enter or was eliminated from the plants' actively growing tissue. These results are consistent with the studies of the inbred line 91213, which suggested that one mechanism of WMV resistance involved inhibiting cell-to-cell movement of the virus (Gray and Moyer, 1993; Gray et al., 1988).

Individual plants categorized as susceptible or resistant to WMV based on ELISA results could be discriminated immediately when average absorbance values (four leaf du$ks/lest; two replications/ELISA) were plotted as a function of leaf position, represented by leaf number (Fig. 1). All plants for which complete data were available are represented in this figure. Seven resistant plants were eliminated based on the visual assessment that pesticide injury may have mimicked viral symptoms. WMV-susceptible plants contained up to 20 times more viral antigen than those of resistant segregants, when resistance was derived from the hybrid (88- 1217) between PI 414723 and *C. melo conomon* FC. When resistance was derived only from *C. melo dudaim* in the TD background, susceptible segregants had about five times the level of viral antigen relative to their resistant counterparts (data not shown). The absorbance values of all the plants from both sources of resistance that remained classified as resistant after three rounds of phenotypic selection were ≈ 0.05 times those of the susceptible plants. Therefore, plants selected as resistant by ELISA uniformly had been rated phenotypically resistant. Various factors can mimic viral symptoms in pheno-

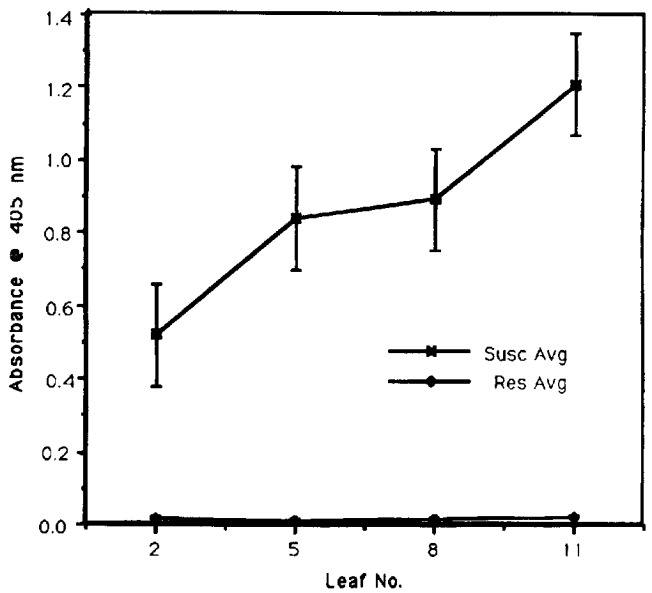

Fig. 1. Relationship between leaf location on *Cucumis melo* seedlings and ELISA absorbance values, Susceptible average: Plot of average absorbance values of all watermelon mosaic virus-susceptible plants determined by ELISA for which four data points were available (n = 53). Resistant average: Plot of average absorbance values for all individuals selected as watermelon mosaic virus-resistant using ELISA (n = 12), Standard error bars are given.

typic screens, particularly when the most apparent early symptom is a mild foliar mottle, as is the case for WMV. This experiment illustrates a key advantage for using ELISA in screening breeding material, namely increased accuracy that allows all resistant plants to be retained, thus providing a larger population in which further selection can be performed for other characteristics, such as horticultural type and yield.

Previous studies with a closely related line, 91213, also noted significantly lower levels of WMV when compared to susceptible varieties (Gray and Moyer, 1993; Gray et al., 1988; Moyer et al., 1985). Our work confirms results obtained with this sub-line, and elucidates the genetic basis for this phenotype. Because the mechanism of resistance suppresses viral antigen accumulation in inoculated resistant segregants, and resistance is readily transferable via conventional breeding techniques, individuals that carry the resistance gene can be selected easily with serology. The results of this study clearly demonstrate that ELISA can provide a definitive criterion in the selection for WMV resistance. In contrast to phenotypic evaluations based on the disease effects on the plant, ELISA screening also can give information about resistance to the pathogen.

Our results suggest that ELISA screening can be applied earlier than phenotypic screening, and with more accurate results. This method reduces time and space required to distinguish plants carrying resistance, allowing available resources to be devoted to smaller populations of resistant plants sooner. Thus, larger populations can be screened initially, because susceptible segregants can be discarded before seedlings must be transplanted out of seedling trays. The method is easily applied to large populations and requires little specialized equipment.

In our breeding program, emphasis is on developing multiple virus resistance. Selection based on ELISA provides an additional advantage because the simplest way to identify individual plants with multiple resistance is to inoculate with mixtures of CMV, WMV, and ZYMV. A plant that is resistant to WMV, but heterozygous for another resistance and therefore symptomatic, may still be a valuable parent and could be identified as such, despite the appearance of disease symptoms. Although synergistic reactions between viruses have been observed (e.g., Poolpol and Inouye, 1986), there is no evidence that multiple viral infection overcomes the resistance conferred by *Wmr*. While differences are apparent between resistance and susceptible segregants as early as the second TL, it is preferable to sample the fifth TL or leaves above in order to maximize differences in viral titer and reduce damage to small plants.

The genetic relationship between resistance to WMV and resistance to the other viruses observed in PI 414723 was not investigated in this study, i.e., whether there is any linkage between dominant resistance to WMV and dominant resistance to PRSV or ZYMV. Results from the breeding program are inconclusive, but it does appear that families selected for WMR or resistance to ZYMV are more often resistant to the other virus than would be expected under independent assortment. In some cucurbit species, cultivated types are largely lacking in resistance, while wild types frequently carry resistance to several viruses, as reported by Provvidenti et al. (1978).

PI 414723 was evaluated using restriction fragment length polymorphism and was identified as the most different from the cultivated types included in a study by Neuhausen (1992). Populations derived from this parent could offer sufficient polymorphism to generate a molecular map of *C. melo* where linkages to a number of important disease and insect resistances could be identified.

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