Monogenic Dominant Resistance to Gummy Stem Blight in Two Melon (*Cucumis melo*) Accessions

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ABSTRACT

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Two melon (*Cucumis melo* L.) accessions, plant introduction (PI) 157082 and PI 511890, reported to be resistant to gummy stem blight, a disease incited by the fungus *Didymella bryoniae*, were crossed with a susceptible parent to determine the inheritance of resistance. Resistance in both accessions is due to a single dominant gene, based on analysis of F_1 , F_2 , and backcross populations. Additionally, PI 157082 was crossed with PI 140471, the other source of resistance identified to date, to examine the genetic relationship of resistance found in these two sources. The frequency of susceptible individuals from the (PI 157082 $\times$ 140471) F_2 population was consistent with a 15:1 resistant:susceptible ratio, indicating that PIs 140471 and 157082 possess different resistance genes.

Additional keywords: cucurbits, disease resistance

Gummy stem blight (GSB) of cucurbits is incited by the fungus *Didymella bryoniae* (Auersw.) Rehm (synonyms: *Myco-sphaerella citrullina* (C. O. Sm.) Gross. and *M. melonis* (Pass.) Chiu & J. C. Walker) and its anamorph *Phoma cucurbitacearum* (Fr.:Fr.) Sacc. (synonyms: *Ascochyta cucumis* Fautrey & Roum. and *A. citrullina* (F. Chester) C. O. Sm.) (13). The disease is most common in tropical and sub-tropical areas of the world, but it is also a serious disease of cucurbits in the United States (15). In the southeastern states, GSB is the most destructive disease of watermelon (*Citrullus lanatus*) and cucumber (*Cucumis sativus*), (1,18) and also causes serious losses on melon (*C. melo*). The disease has increased in importance in recent years as effective chemical and genetic control of other cucurbit diseases has become available (14).

Symptoms of GSB include circular, tan to dark-brown spots on leaves that may

enlarge under favorable conditions to cover the leaf. On cotyledons and stems of young plants, circular black or tan lesions may be evident. Water-soaked areas may develop on hypocotyls and leaves. Cankers may appear in stem cortical tissue that produce a typical brown, gummy exudate and may girdle the stem, resulting in plant death (14).

Control practices include the use of fungicide-treated seed and a minimum 2-year crop rotation (15). Although satisfactory chemical control can be achieved with fungicides (1), resistance to benzimidazole products has been reported (2,4,5,15). Several sources of GSB resistance in wild *C. melo* accessions from the United States Department of Agriculture (USDA) National Plant Germplasm System (NPGS) have been previously reported (6,16,17,20). A recent study that employed both greenhouse and field evaluations yielded several new sources of resistance to GSB among the 800 accessions examined. Levels of resistance identified in this study were equal to or greater than any previously reported (20). Although genetic resistance was identified in *C. melo* in the 1960s and efforts to incorporate resistance into cultivated melon have been ongoing (7–10), no commercial varieties with adequate levels of resistance are currently available (15). Thus, there is still a need to incorporate higher levels of genetic resistance in melon varieties.

The first objective of this study was to determine the mode of inheritance of GSB resistance found in two highly resistant *C. melo* plant introductions (PI), USDA PIs 157082 and 511890, identified by Zhang et

al. (20). The second objective was to determine the genetic relationship between these sources of resistance and PI 140471, a wild melon previously described as having GSB resistance (17) controlled by a single dominant gene (12). Due to problems with producing seed of the necessary crosses with PI 511890, only the relationship between PI 157082 and PI 140471 was determined in this study.

MATERIALS AND METHODS

Germ plasm and population development. *C. melo* PI 157082 and PI 511890 obtained from the USDA NPGS at Ames, Iowa were crossed with a susceptible genotype, 'Cornell ZPPM 339', with each other, and with PI 140471, to determine the mode of inheritance of GSB resistance. Cv. Cornell ZPPM 339 is a cantaloupe breeding line, well-adapted to greenhouse and field conditions, and monoecious, which eliminates the need for emasculation when used as a female parent. Controlled pollinations were carried out in the field and greenhouse to generate reciprocal F_1 , F_2 , and backcross (BC) progenies. Due to poor adaptation of PI 511890 to conditions in Ithaca, New York and extreme susceptibility to powdery mildew, we were unable to produce adequate seed for testing from PI 157082 $\times$ PI 511890 and reciprocal crosses and PI 511890 $\times$ PI 140471 and reciprocal crosses in either the greenhouse or the field. To confirm previously published results regarding genetics of resistance for PI 140471, we also crossed PI 140471 with the cv. Mainstream, released by the United States Vegetable Laboratory in Charleston, South Carolina (11) and developed reciprocal F_1 , F_2 , and BC progenies. Cv. Mainstream was bred under unprotected conditions in South Carolina, and some tolerance to the disease is observed under some field conditions, but it is rated susceptible in inoculated tests (20).

For field plantings in Ithaca, New York, 4-week-old seedlings from the greenhouse were acclimated for 3 days in cold frames outside the greenhouse before transplanting to beds covered with black plastic mulch. Plots consisted of 12 plants, with 2 plants per hill. Rows were spaced 2.1 m apart and hills were 0.6 m apart within the rows. Prior to transplanting, field plots were plowed and disked once, then broadcast fertilized with 225 Kg of 10-20-20 fertilizer. At transplant, approximately 500 ml

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of a starter fertilizer (9-45-15) was applied at a rate of 7 g/liter for each hill.

Resistance evaluations. To assure uniform stands for resistance evaluations, seeds were germinated on paper towels held at 28°C for 48 h, and seedlings were transplanted to Todd planter flats (4 by 8 cells; Hummert International, Earth City, MO) filled with Peatlite mix (1:1 peat moss:vermiculite). Two susceptible control plants each of 'Top Mark' and 'Honeydew Greenflesh' were planted randomly in each flat to monitor inoculation efficacy and test severity. Seedlings were held in a greenhouse at 24°C. All evaluations for genetic studies were carried out in the greenhouse essentially as described by Zhang et al. (20) using NY1, a highly virulent *D. bryoniae* isolate collected in the field in Onondaga, New York (3).

Data collection and analyses. Disease severity on stems of individual plants was recorded using a modified version of the procedure by Zhang et al. (20). Instead of a single reading at 7 days after inoculation, a second reading was also made at 21 days after inoculation. A 1 to 5 scale was used where 1 = no damage; 2 = a single lesion 1 to 10 mm long or coalesced lesions 1 to 20 mm, no girdling; 3 = lesions 21 to 80 mm, girdling of the stem, or both; 4 = withered stem; and 5 = dead seedling (19). The numbers of individuals falling into resistant and susceptible classes were tallied, and observed segregation ratios in F₂ and BC populations were tested for goodness-of-fit using the chi-square statistic.

RESULTS

Parental reactions. Disease scores for parental lines inoculated with the GSB pathogen are summarized in Table 1. There was a clear and consistent difference in stem disease ratings at 21 days after inoculation between the resistant and susceptible parents in all tests. This defined the categories which were then used to group individuals from segregating populations. All plants of the resistant parents showed disease scores of 1 or 2. Almost all (96%) plants of PI 140471, 75% of PI 157082, and 70% of PI 511890 had scores of 1. The susceptible parent ZPPM 339 showed only disease scores between 3 and

5 (Table 1); 97% of these seedlings had either withered stems or were dead (scores of 4 and 5).

Inheritance of resistance in PI 157082.

The reciprocal F₁ progenies between PI 157082 and ZPPM 339 showed uniformly high resistance, indistinguishable from the resistant parent, and consistent with dominant inheritance with no cytoplasmic (maternal) factors involved. The F₂ population segregated consistent with a 3:1 resistant:susceptible ratio (Table 2), supporting the hypothesis that resistance in this PI is controlled by a single dominant gene. Plants from the BC population derived from the resistant parent were uniformly resistant, and the BC derived from the susceptible parent segregated consistent with a 1:1 resistant:susceptible ratio (Table 2), which further supports monogenic dominant inheritance of gummy stem blight resistance from PI 157082.

Relationship of dominant resistance from PI 157082 to the *Mc* gene from PI 140471.

To determine whether the dominant resistance allele in PI 157082 is related to the *Mc* locus previously identified by Prasad and Norton (12), PI 157082 was crossed with PI 140471. We first confirmed that PI 140471 indeed carries a single dominant gene by producing and screening F₁, F₂, and resistant and susceptible BC progenies with *C. melo* cv. Mainstream (*data not shown*). All plants from the F₁ population obtained by crossing PI 140471 and PI 157082 were uniformly highly resistant, with disease scores of 1 (Table 2). The F₂ plants segregated consistent with a 15:1 resistant:susceptible ratio (Table 2), supporting the hypothesis that GSB resistance in PI 157082 is controlled by a resistance allele at a locus that is unlinked to the *Mc* locus. Moreover, most of the plants in the susceptible category were dead (60%) or had severe stem infections (26%), while

the majority (67%) of the resistant F₂ plants had scores of 1.

Inheritance of resistance in PI 511890.

Reciprocal F₁ populations of PI 511890 and ZPPM 339 were highly resistant and their reactions did not differ, suggesting that GSB resistance from this source is also dominant, and that no cytoplasmic (maternal) factors are involved in its expression. F₂ plants segregated consistent with a 3:1 resistant:susceptible ratio (Table 2), indicating this source also possesses monogenic dominant resistance to GSB. As expected under this hypothesis, plants from the resistant BC were uniformly resistant, while those from the susceptible BC segregated consistent with a 1:1 resistant:susceptible ratio (Table 2).

DISCUSSION

Previously, a single dominant gene (*Mc*) in PI 140471 was reported (12) and has been used in the development of several varieties and breeding lines (7–10). In a recent study, however, releases originating from PI 140471 showed intermediate resistance or were rated as susceptible in greenhouse and field screens (20). Zhang et al. (20) suggested that this failure to recover parental levels of resistance in cultivated breeding types might be the result of interactions of the *Mc* gene with different genetic backgrounds. Alternatively, the resistance conferred by this gene may not be sufficiently high to protect vines that are under extreme physiological stress due to the concentrated set of large fruit (20).

Results from our genetic studies, using a different susceptible parent and a very reliable, severe screening method, confirmed the previous report of a single gene conferring resistance to GSB in PI 140471 (*data not shown*). Similarly, GSB resistance in PIs 157082 and 511890 also appears to be controlled by a single dominant

Table 2. Response of *Cucumis melo* parental genotypes and intercrossed populations to inoculation with *Didymella bryoniae*

Pedigree ^b	Generation ^c	Number of plants ^a		Expected ratio (R:S)	P _{α=0.05}
		R	S		
ZM	P	1	30	0:1	...
471	P	24	0	1:0	...
082	P	20	0	1:0	...
890	P	26	0	1:0	...
082 × ZM	F ₁	31	4	1:0	...
ZM × 082	F ₁	14	2	1:0	...
082 × ZM	F ₂	84	21	3:1	0.31
(082 × ZM) × ZM	BC _s	36	35	1:1	0.92
(082 × ZM) × 082	BC _r	65	0	1:0	...
890 × ZM	F ₁	17	1	1:0	...
ZM × 890	F ₁	46	1	1:0	...
890 × ZM	F ₂	63	27	3:1	0.14
(890 × ZM) × ZM	BC _s	40	27	1:1	0.15
(890 × ZM) × 890	BC _r	54	0	1:0	...
471 × 082	F ₁	8	0	1:0	...
471 × 082	F ₂	234	23	15:1	0.13

^a R = resistant, S = susceptible.

^b ZM = ZPPM 339; 471 = PI 140471; 082 = PI 157082; and 890 = PI 511890.

^c P = parent; BC_r and BC_s = backcross to resistant and susceptible parents, respectively.

Table 1. Stem disease severity scores for melon genotypes used as parents in this study 21 days after inoculation with *Didymella bryoniae*

Parents	Disease index ^a	Rating
ZPPM 339	4.3 (± 0.51)	Susceptible
PI 140471	1.1 (± 0.20)	Resistant
PI 157082	1.3 (± 0.57)	Resistant
PI 511890	1.3 (± 0.47)	Resistant

^a Mean ± standard deviation of severity scores based on a 1 to 5 scale (1 = no damage; 2 = a single lesion 1 to 10 mm long or coalesced lesions 1 to 20 mm, no girdling; 3 = lesion 21 to 80 mm, girdling of the stem, or both; 4 = withered stem; and 5 = dead seedling).

gene but, due to lack of availability of the necessary populations, we were unable to establish whether these two PIs share the same gene for resistance or contain two distinct resistance genes.

We were, however, able to generate the necessary populations to determine the relationship between the *Mc* locus in PI 140471 and the resistance gene from PI 157082. Our results clearly establish that these two accessions contain distinct resistance genes at unlinked loci. Preliminary results from our breeding program indicate that the combination of these two genes may yield higher levels of resistance than either gene alone (J. P. Jantz and M. K. Jahn, *unpublished results*).

We have not conducted a survey of *D. bryoniae* isolates on these two new sources of resistance, so we do not know if these genetic resources differ from PI 140471 with regard to fungal isolate specificity. We do know from previous research (3) and from additional studies (T. L. Zuniga and T. A. Zitter, *unpublished results*) that race-specificity does not exist in cucurbit species including cucumber (19), although one previous report indicated that in melon resistance from PI 140471 may not be consistently expressed in the field (16). When combined in a single genotype, diverse sources of resistance may increase both the level of resistance and the breadth of protection, augmenting chemical disease control approaches which are currently judged inadequate (1,4), especially when

environmental conditions are conducive to disease development (1).

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