Resistance to Gummy Stem Blight in Melon (*Cucumis melo* L.) Germplasm and Inheritance of Resistance from Plant Introductions 157076, 420145, and 323498

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Abstract. Gummy stem blight incited by the fungus *Didymella bryoniae* is a major disease of melons worldwide. The objectives of the present study were to critically evaluate melon (*Cucumis melo* L.) germplasm for resistance to *D. bryoniae* and to characterize the genetics of resistance in the resistant accessions. Two hundred sources of germplasm (plant introduction accessions, cultivars, breeding lines, landraces, and wild relatives) were screened against a single highly virulent isolate (IS25) of *D. bryoniae* in a plastic tunnel. The genetics of resistance to *D. bryoniae* was studied in three crosses between plant introductions 157076, 420145, and 323498, resistant parents that were fairly adapted (flowering, fruiting, powdery mildew tolerance) to Nanjing conditions, and plant introductions 268227, 136170, and NSL 30032 susceptible parents, respectively. Six populations of each cross (susceptible parent, resistant parent, F₁, F₂, the two reciprocal backcrosses) were analyzed for their responses to *D. bryoniae*. Seedlings in both studies were inoculated with a spore suspension (5×10^5 spores/mL⁻¹) of *D. bryoniae* at the four to six true-leaf stages and assessed for leaf and stem damage at 7, 14, and 21 d postinoculation. Results of germplasm screening indicated most germplasms reported as resistant elsewhere were confirmed resistant under our conditions. However, some plant introductions identified as highly resistant elsewhere were susceptible under our conditions, the most interesting being plant introduction 482399. This plant introduction that was considered resistant was highly susceptible in our study. We also identified other sources of resistance not reported previously, for example, JF1; a wild *Cucumis* from the highlands of Kenya was rated highly resistant. Analysis of segregation of F₁, F₂, and backcross generations of the three crosses indicated that each of the three plant introductions carry a single dominant gene for resistance to the *D. bryoniae*.

Melon production is severely constrained by several soil-borne disease pathogens. Of these pathogens, *Didymella bryoniae* (Auerw), Rehm that causes gummy stem blight (GSB) is one of the most destructive resulting in substantial economic losses (Bruton, 1998; Crosby et al., 2002; Frantz and Jahn, 2004; McCreight, 2002; Wako et al., 2002). The pathogen attacks and inflicts heavy losses in quality and yield in several other genera of the cucurbitaceae worldwide, e.g., squash (*Cucurbita* L.) (Zitter and Kyle, 1992), cucumber (*Cucumis* L.) in the United States (Gusmini and Wehner, 2002; St. Amand and Wehner, 1991) and on water-

melon (*Citrullus* Neck.) (Keinath et al., 1995). The disease has also been reported in Europe (Frantz and Jahn, 2004; van Steekelenburg, 1985), Asia (Wako et al., 2002), and elsewhere (Bruton, 1998).

Although chemical control has had great success, repeated use of fungicides is not advisable as a long-term solution as a result of the negative impact of pesticides in the environment. Moreover, development of resistance to some systemic benzimidazole fungicides in *D. bryoniae* from several cucurbit production areas has been reported (Kato et al., 1984; Keinath and Zitter, 1998; Malathroki and Vokalounakis, 1983). Crop rotations are only partially effective; wind and other agents can easily bring in new inoculum to start new infections (Tullu et al., 2002). This illustrates the necessity to use host resistance in conjunction with good cultural practices and judicious use of fungicides. Use of resistant cultivars is the most strategic, environmentally friendly, accepted, and economic means

of GSB management (Vokalounakis, 1993; 1995; Wehner and St. Amand, 1993).

Several greenhouse, plastic tunnel, and field evaluations were previously conducted to identify sources of resistance to *D. bryoniae*. A limited amount of resistance was reported in some cultigens. Sowell et al. (1966) tested over 1000 accessions of melon and reported resistance in plant introduction 140471 to be near immunity. On the contrary, McGrath et al. (1993) contends no sufficient resistance to GSB is available in melon. Furthermore, resistance in plant introduction 140471 has recently been described as variable (Tsutsumi and da Silva, 2004) or not effective at all (Takada, 1983). Several other sources of resistance to gummy stem blight in melon have been reported over the years (McGrath et al., 1993; Sakata et al., 2000; Sowell, 1981; Takada, 1983; Tsutsumi and da Silva, 2004; Zhang et al., 1997). Zhang et al. (1997) found plant introductions 157076, 157080, 157081, 157082, 157084, 482393, 482398, 482399, 482402, 482403, 482408, 255478, and 511890 to be as good as plant introduction 140471.

Most GSB-resistant melon varieties and breeding lines released to date derive resistance from plant introduction 140471 (McGrath et al., 1993; Norton, 1971, 1972; Norton and Cosper, 1989; Norton et al., 1985; Sowell, 1981). However, plant introduction 140471 has failed to provide sufficient resistance (e.g., Sitterly and Keinath, 1996; Zhang et al., 1997). Moreover, plant introduction 140471 was been reported to be ineffective against a Japanese isolate of *D. bryoniae* (Sakata et al., 2000; Takada, 1983). Zhang et al. (1997) also reported resistance derived from plant introduction 140471 appears to diminish when deployed into commercial, large-fruited varieties. There is still interest in developing melon varieties with higher GSB resistance than existing ones; hence, new sources of resistance should be sought and validated. New sources of resistance will be useful in melon production areas where existing sources of resistance are not effective.

Information on the genetics of resistance to the GSB pathogen in most melon cultigens reported as resistant is limited (Pitrat et al., 1998). Two genetic systems have been proposed, monogenic-dominant and monogenic-recessive. One dominant gene (*Mc*) confers high resistance to GSB in plant introduction 140471 (Prasad and Norton, 1967; Sowell et al., 1966); another gene, *Mc-2*, controlling an intermediate level of resistance in breeding lines C-1 and C-8, was reported by Prasad and Norton (1967). Frantz and Jahn (2004) confirmed the monogenic-dominant status of *Mc* in plant introduction 140471. Plant introduction 157082 and 511890 were both reported to carry monogenic-dominant genes for GSB resistance, whereas resistance in plant introduction 482399 was monogenic-recessive (Frantz and Jahn, 2004; Zuniga et al., 1999). Frantz and Jahn (2004) reported a monogenic dominant gene for GSB resistance in plant introduction 482398. The genes

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in plant introductions 140471, 157082, 511890, 482498, and 482399 were renamed *Gsb-1*, *Gsb-2*, *Gsb-3*, *Gsb-4*, and *gsb-5*, respectively (Frantz and Jahn, 2004). The five genes are independent of each other (Frantz and Jahn, 2004). Resistance in a sixth accession, Jmu-15 (*C. melo* var. *agrestis*), is conferred by incomplete dominant gene(s) (Wako et al., 2002). However, allelism in this accession has not been verified nor has the gene(s) been named. The genetics of resistance and allelic relationships of most melon plant introductions reported as resistant to GSB is still unknown, incomplete, or has not been verified, e.g., in plant introductions 296345, 266935, 436533, 157076, and others. Knowledge of the genetic basis of resistance to *D. bryoniae* is essential for the efficient development of resistant melon cultivars. The evaluation, reevaluation, crossing, and backcrossing processes can be time-consuming and tedious. Often because the genetics of resistance of resistant genotypes are unknown, the introgression process becomes one of trial and error. There is need, therefore, to determine the mode of inheritance to the GSB pathogen in other sources and possibly establish if these sources of resistance share the same genetic factors.

The objectives of the present study were: 1) to critically examine the resistance of melon germplasm to *D. bryoniae* and 2) to characterize the genetics of resistance in plant introductions 157076, 420145, and 323498. These plant introductions were confirmed to be highly resistant to *D. bryoniae* and are fairly well adapted to Nanjing conditions.

Materials and Methods

Germplasm for gummy stem blight screening. Two hundred accessions originating from 42 countries were chosen based on a diversity of characteristics for screening. Accessions included landraces and commercial varieties of melo, reticulatus, muskmelon, inodorus, conomon, and *agrestis* groups; wild cucumis species; and other cucurbits, mainly watermelon (*Citrullus* spp.). Test accessions consisted of those reported elsewhere as resistant/susceptible to *D. bryoniae* and those of unknown reaction (including a new collection, e.g., JF1). Seed of the accessions were obtained from North Central Regional Center for Genetic resources Preservation, Ames, Iowa. Other accessions were donated by Ming-Zhu Wu (China), Liz Makokha-Wolukau (Kenya), or from market/farm collections in Nanjing, China.

Population development for genetic analysis of resistance. The genetics of resistance to *D. bryoniae* in melon was studied using three F_2 populations of crosses between plant introduction 268227 (a susceptible parent) and plant introduction 157076 (a resistant parent); between plant introduction 136170 (susceptible parent) and plant introduction 420145 (resistant parent); and between NSL 30032 (susceptible) and plant introduction 323498 (resistant), F_1 hybrids,

reciprocal backcrosses, and the parents. Plant introduction 157076 is an accession from China, whereas plant introduction 268227 is a highly susceptible accession from Iran. Plant introduction 420145 is a resistant accession from Japan, whereas the highly susceptible plant introduction 136170 is from Italy. Similarly, plant introduction 323498 is originally from China, whereas the susceptible parent is a commercial cultivar ('Top Mark') from the United States. All crosses were made by controlled pollination in a plastic tunnel, Nanjing, China. The North Central Regional Center for Genetic resources Preservation, Iowa, provided all seeds for the parents used in this study.

Inoculum preparation. The inoculum was prepared based on the method of Kwon et al. (1997) with modifications. Spore isolates of *D. bryoniae* were obtained from diseased melon plant materials in plastic tunnels in Qi ling, Nanjing, China. The isolate, named IS25, was increased on potato dextrose agar (e.g., Gusmini et al., 2003; Kwon et al., 1997) in petri plates (150 cm $\times$ 30 cm) using mycelial plug inoculation. Petri plates were incubated at 25 °C in the dark for 7 d followed by ultraviolet irradiation (40 w, 12 h·d⁻¹) for 4 d at the same temperature. These conditions were conducive for the formation of spore-producing pycnidia. Inoculum was prepared by flooding the plates with 5 to 10 mL of acidified distilled water and scraping the surface of the agar with an L-shaped glass rod. The solution was acidified to pH 4.0 using lactic acid containing 20 drops per L of Tween-20 as surfactant. Low pH and surfactant increase spore discharge from pycnidia, prevent spore agglutination, and aid adhesion of spores to leaf and stem surfaces, respectively. The liquid from each plate was filtered through four layers of cheesecloth to remove mycelia, pycnidia, and dislodged agar. The spore suspension was adjusted to $\approx 5 \times 10^5$ spores·mL⁻¹ using a hemacytometer.

Plant culture. Resistance evaluation in the plastic house was conducted according to Zhang et al. (1997) with slight modifications. Briefly, seeds were wrapped in muslin cloth, surface-sterilized in warm water at 50 °C for 15 min, transferred to water at room temperature for 4 h, and then incubated at 28 °C for 2 d after draining off excess water. Uniformly germinated seedlings were transplanted into 12 cm $\times$ 13-cm plastic pots filled with sand, soil, and vermiculite at the ratio of 1:1:1. For the screening study, six to seven seedlings were planted in each replication with two replications per accession. Resistant and susceptible accessions were placed randomly among the pots as disease severity checks and to aid in disease spread. Seedlings were maintained in the plastic tunnel at 27 °C $\pm$ 2. Plants were fertilized weekly with a dilute solution of soluble N-P-K fertilizer, 1N-1.5P-1.0K per 10 L of water.

Inoculation. Plants at the four to six true-leaf stage (3–4 weeks old) were sprayed with spore suspension to near runoff. Inoculation was done in the morning or late evening. Plants were watered 1 d before inoculation

and 3 d after inoculation to encourage uniform disease development and spread of the inoculum. Additionally, plants were fine-misted on day 2 postinoculation to provide free water on the leaf surfaces. After inoculation, plants were enclosed in a tight plastic tent within the plastic tunnel to maintain high relative humidity (92%). The clear plastic tent was removed 3 d after inoculation. A dry and wet bulb thermometer was suspended inside the tent to monitor the level of humidity. For the GSB screening experiment, plants were reinoculated 7 d after the first inoculation to ensure there were no escapees or false-positives.

Data collection, analysis and interpretation. Disease ratings were scored according to the methods described in Zhang et al. (1997) and Zuniga et al. (1999) with slight modifications. Visual ratings on leaf and stem were scored at 7, 14, and 21 d post-inoculation on the following scales: leaf, 1 = 0% of leaf area affected, 2 = $\geq 25\%$ leaf area affected, 3 = $\geq 25\% \leq 50\%$ leaf area affected, 4 = $\geq 50\% \leq 75\%$ leaf area affected, 5 = $\geq 75\% \leq 100\%$ leaf area affected; stem: 1 = no damage, 2 = single lesion 1 to 10 mm long or composite lesions 1 to 20 mm long, stem not girdled, 3 = lesion 21 to 80 mm long or girdling of stem, 4 = stem withered, and 5 = seedling dead. For the screening study, analysis of variance on disease score ratings was done using GLM procedures of SAS (SAS Institute, Cary, N.C.). Phenotypic correlations between leaf and stem resistance were also calculated using CORR procedure of SAS, and the coefficient of simple correlation (r) was determined.

For the genetics of resistance, only stem score ratings were analyzed. Classification of segregating progenies is most reliable when based on stem damage ratings in which resistant individuals score 1 or 2 and susceptible plants score 3 to 5 (Zhang et al., 1997; Zuniga et al., 1999). The number of individuals falling in resistant (stem damage rating 1–2) and susceptible (stem damage ratings 3–5) categories was determined in of each segregating population. The resulting ratios were tested for goodness-of-fit using χ^2 analysis.

Results

Screening for resistance to gummy stem blight. We ranked the accessions for GSB resistance based on the mean of three ratings of two replications for leaf and stem (Table 1). Data (Table 1) indicate most germplasm reported highly resistant, including plant introduction 140471, were consistently resistant under our conditions. Other plant introductions previously reported resistant (Zhang et al., 1997) and also highly rated resistant in our study included plant introductions 157076 (which was entered two times), 157082, 323498, and 436534. All the wild relatives were rated highly resistant with a rating of 1.0 for stem and 1.0 to 1.5 for leaf. A new wild accession, JF1 (*Cucumis* spp.) collected from the highlands of Kenya was

Table 1. Foliar and stem disease indices for *Cucumis melo* L. (unless specified) germplasm in response to *D. bryoniae* inoculation from a plastic house screen.

Accession	Origin	Hort ^z group	Disease rating ^y	
			Leaf	Stem
PI	Kenya	<i>Cucumis</i>	1.0	1.0
PI 364472	S. Africa	<i>C. zeyheri</i>	1.0	1.0
PI 255478	Korea	melo	1.1	1.0
JF1	Kenya	<i>Cucumis</i>	1.1	1.0
Jinguan	China	Thins ^w	1.2	1.0
PI 420147	Japan	melo	1.2	1.0
Ames 26709	USA	texanus	1.2	1.0
PI 299571	S. Africa	<i>C. africanus</i>	1.2	1.0
PI 196477	Brazil	<i>C. anguria</i>	1.2	1.0
PI 618834	China	melo	1.3	1.0
Ames 26695	Mexico	texanus	1.3	1.0
PI200819	Myanmar	melo	1.3	1.0
M2K	Kenya	<i>C. lanatus</i>	1.4	1.0
PI 299570	S. Africa	<i>Cucumis</i>	1.4	1.0
PI 196477	Brazil	<i>C. anguria</i>	1.5	1.0
Ames 26737	USA	texanus	1.5	1.0
PI 482409	Zimbabwe	melo	1.5	1.0
PI 482402	Zimbabwe	melo	1.5	1.0
PI 164797	India	melo	1.5	1.0
<i>C. figarei</i>	Kenya	<i>Cucumis</i>	1.5	1.0
PI 436534	Senegal	melo	1.6	1.0
PI 157076	China	melo	2.0	1.0
XinmiZa9	China	Thicks ^v	1.1	1.1
PI 200819	Myanmar	melo	1.3	1.1
Ames 26698	USA	texanus	1.3	1.1
Ames 26729	USA	texanus	1.3	1.1
PI 321004	Taiwan	melo	1.4	1.1
Ames 26707	USA	melo	1.4	1.1
L8K	Kenya	<i>C. lanatus</i>	1.6	1.1
PI 183311	India	melo	1.7	1.1
PI 420177	Japan	melo	1.7	1.1
PI 266933	Japan	melo	1.7	1.1
PI 140471	USA	melo	1.8	1.1
PI 470253	Indonesia	melo	1.2	1.2
PI 185111	Ghana	melo	1.5	1.2
NJG6	China	Thins ^w	1.5	1.2
PI 500362	Zambia	melo	1.6	1.2
PI 420145	Japan	melo	1.8	1.2
PI 489689	Mexico	melo	2.2	1.2
PI 511890	Mexico	melo	1.4	1.3
M3SA	S. Africa	<i>C. lanatus</i>	1.5	1.3
PI 435345	Mexico	melo	1.7	1.3
NJG1	China	Thins ^w	1.8	1.3
M1K	Kenya	<i>C. lanatus</i>	2.5	1.3
PI 292190	S. Africa	<i>C. metuliferus</i>	1.5	1.4
PI 505601	Zambia	melo	1.6	1.4
PI 500600	Zambia	melo	1.7	1.4
PI 505599	Zambia	melo	1.8	1.4
PI 403994	Colombia	melo	1.8	1.4
PI 292190	S. Africa	<i>C. metuliferus</i>	1.5	1.4
PI 157070	China	melo	2.1	1.4
PI 438680	Mexico	melo	2.2	1.4
PI 157071	China	melo	1.6	1.5
PI 323499	China	melo	1.9	1.5
PI 420150	China	conomon	1.9	1.5
PI 157076	China	melo	2.0	1.5
PI 420149	USA	conomon	2.2	1.5
PI 618832	China	melo	1.5	1.6
L4K	Kenya	muskmelon	1.8	1.6
PI 323498	China	melo	2.0	1.6
PI 161375	Korea	melo	2.2	1.6
PI 482410	Zimbabwe	melo	2.0	1.7
PI 532829	China	melo	2.0	1.8
PI 157082	China	melo	2.0	1.8
PI 244713	USA	melo	2.3	1.8
L5K	Kenya	<i>C. lanatus</i>	3.0	1.8
PI 481479	Zimbabwe	melo	3.2	1.8
PI 295341	Australia	melo	2.2	1.9
PI 505602	Zambia	melo	2.4	1.9
PI 482424	Zimbabwe	melo	2.5	1.9
PI 420176	Japan	melo	2.3	2.0
PI 183228	Egypt	melo	2.4	2.0

(Continued on next page)

also rated 1.0. Some accessions rated highly resistant previously in the United States and elsewhere (e.g., Zhang et al., 1997) were not resistant under our conditions, the most interesting being plant introduction 482399 from Zimbabwe. Other accessions that behaved similarly were plant introductions 190554 (Iraq), 183221 (Egypt), and 183226 (Egypt). On the other hand, some accessions reported as highly susceptible were rated highly resistant in our study, e.g., plant introductions 532829 (China), 185111 (Ghana), 200819 (Myanmar), and 500362 (Zambia). Most of the Chinese commercial germplasm were highly susceptible, e.g., 'Huanghemi' (stem rating 4.63), 'Jiashi' (stem rating 3.62), and 'Lubaoshi' (stem rating 3.6). However, 'Jinguan', a thin skin melon, and 'Xin mi Za9', a hemitype melon, were rated highly resistant, with stem scores of 1.0 and 1.12, respectively. The landrace collections from farms, NJG1 and NJG6, were highly resistant, having mean stem scores of 1.3 and 1.2, respectively. Most of the commercial melon (*C. melo*) and watermelon (*Citrullus lanatus*) accessions from Kenya and South Africa were rated highly to moderately resistant (stem rating 1.0–2.25), except L7K (3.08), a muskmelon type melon from Kenya, and M4SA (2.89), a watermelon from South Africa, were susceptible. No resistance was observed in *C. melo* var. flexuosus and inodorus accessions in our study (Table 1). Generally leaf resistances were slightly higher than stem resistances, but leaf and stem resistance were highly correlated ($r = 0.97$, $P_{\infty} = 0.05$) (Table 1). However, in a few cases, some of the germplasm showed leaf resistance that was at par with stem resistance or higher like in the case of plant introductions 614174, 255478, and XinminZa9 (Table 1).

Genetics of resistance. Several of the highly resistant *C. melo* plant introductions (from previous work and our data) that appeared well adapted to our conditions (flowering, fruiting, powdery mildew tolerance) were crossed with susceptible parents to generate F₁, F₂, and reciprocal backcross populations to characterize their genetics of resistance. The most adaptable of these and with potential for breeding into commercial cultivars were plant introductions 157076, 420145, and 323498. Plant introductions 157076 and 323498 are of local Chinese origin and could have useful genes of breeding value.

The susceptible and resistant checks (Table 2) responded consistently after inoculation with the *D. bryoniae* spore suspension. The susceptible checks, e.g., 'Huanghemi', developed leaf and stem lesions uniformly within 6 to 7 d of inoculation, whereas plants of plant introduction 196477, a resistant wild *Cucumis* from Brazil, were uniformly rated highly resistant. This confirmed the virulence of isolate IS25 and showed the screening procedure was sensitive and valid.

Inheritance of resistance to *Didymella bryoniae* in plant introduction 157076. The

Table 1. (continued) Foliar and stem disease indices for *Cucumis melo* L. (unless specified) germplasm in response to *D. bryoniae* inoculation from a plastic house screen.

Accession	Origin	Hort ^z group	Disease rating ^y	
			Leaf	Stem
PI 505603	Zambia	melo	2.6	2.0
PI 476332	Russian Fed. ^x	melo	2.8	2.1
PI 266949	France	muskmelon	3.4	2.1
L3K	Kenya	<i>C. lanatus</i>	2.1	2.2
PI 482394	Zimbabwe	melo	2.6	2.2
PI 436533	Senegal	melo	2.1	2.3
PI 381773	India	melo	2.6	2.3
PI 200816	Myanmar	melo	2.7	2.3
L6K	Kenya	muskmelon	2.8	2.3
PI 512438	Spain	melo	2.9	2.3
PI 266945	Japan	muskmelon	3.0	2.3
PI 614174	India	agrestis	1.9	2.4
PI 164493	India	melo	2.5	2.4
Ames 8578	USA	melo	2.6	2.4
PI 164364	Pakistan	melo	2.5	2.5
PI 255946	Germany	melo	3.2	2.5
PI 614494	India	agrestis	2.8	2.6
PI 482397	Zimbabwe	melo	2.8	2.6
PI 482395	Zimbabwe	melo	2.8	2.6
PI 123501	India	melo	3.0	2.6
NJG7	China	NA ^u	2.4	2.7
PI 255950	Greece	muskmelon	3.2	2.7
PI 818837	China	melo	2.9	2.8
Xiaojinli	China	Thicks ^w	3.3	2.8
M4SA	S. Africa	<i>C. lanatus</i>	2.7	2.9
PI 211116	Israel	melo	3.1	2.9
PI 436532	Senegal	melo	2.6	3.0
Yindi	China	NA ^u	2.8	3.0
PI 234607	S. Africa	melo	3.3	3.0
PI 262169	France	melo	3.4	3.0
PI 169329	Turkey	melo	3.5	3.0
PI 318653	USA	melo	3.6	3.0
Ames 13330	Spain	melo	4.1	3.0
Ames 18738	USA	melo	4.3	3.0
Lubaoshi	China	Thicks ^v	2.4	3.1
PI 282448	S. Africa	melo	2.9	3.1
Ames 13312	Spain	melo	3.1	3.1
Ames 20219	USA	melo	3.3	3.1
L7K	Kenya	muskmelon	3.4	3.1
PI 164487	India	melo	3.5	3.1
PI 614189	India	melo	3.2	3.2
PI 614196	India	melo	3.3	3.2
PI 512539	Spain	melo	3.0	3.3
PI 304745	El Salvador	melo	3.4	3.3
PI 268227	Iran	melo	3.6	3.3
PI 183223	Egypt	melo	3.8	3.3
PI 438686	Mexico	melo	3.8	3.3
Ames	13259	melo	4.1	3.3
PI 512538	Spain	melo	3.4	3.4
PI 193495	Ethiopia	melo	3.5	3.4
PI 251778	Former USSR	melo	3.5	3.4
PI 525116	Egypt	melo	3.7	3.4
Ames 13325	Spain	melo	3.7	3.4
PI 136229	Canada	melo	3.8	3.4
NSL 30032	USA	melo	3.1	3.5
Ames 2831	USA	melo	3.5	3.5
PI 438685	Mexico	melo	3.4	3.6
PI 183222	Egypt	melo	3.5	3.6
Jiashi	China	Thicks ^v	3.6	3.6
PI 482399	Zimbabwe	melo	3.8	3.6
PI 507873	Hungary	melo	4.0	3.7
PI 385966	Kenya	melo	4.0	3.8
PI 124112	India	melo	4.1	3.8
PI 183221	Egypt	melo	4.1	3.8
PI 255952	USA	muskmelon	4.1	3.8
PI 136170	Italy	melo	4.2	3.8
PI 525125	Egypt	melo	4.3	3.8
PI 255949	Greece	inodorus	4.8	3.8
PI 525149	Egypt	flexuosus	3.6	3.9
Ames 2829	USA	melo	4.1	3.7
PI 255953	USA	muskmelon	4.1	3.7
PI 164723	India	melo	4.1	3.7

(Continued on next page)

parents (plant introduction 268227, plant introduction 157076), the F₁, the plant introduction 268227 × plant introduction 157076, and the backcrosses were evaluated and analyzed for response to *D. bryoniae* inoculation (Table 3) to determine the inheritance of resistance. All the plants of plant introduction 268227, the susceptible parent, developed GSB symptoms uniformly and most of them died within 2 weeks of inoculation. On the other hand, plant introduction 157076 was completely resistant with a mean stem rating of 1.3 except for plant number 1, which had superficial lesions and later died. This plant bordered another GSB screening experiment and may have received a higher dose of inoculum than the rest of the plants. All the 64 (plant introduction 268227 × plant introduction 157076) F₁ plants had an average rating of 1.4 except plant number 59, which, like in the case of plant number 1 of plant introduction 157076, was rated 3 for similar reasons described previously. F₂ segregation agreed with three resistant to one susceptible ratio, consistent with a monogenic dominant mode of resistance. The backcross to the susceptible parent (plant introduction 268227 × plant introduction 157076) × plant introduction 268227 segregated with a one resistant to one susceptible ratio, whereas the backcross to the resistant parent [(plant introduction 268227 × plant introduction 157076) × plant introduction 268227] segregated one resistant to zero susceptible.

Inheritance of resistance to Didymella bryoniae in plant introduction 420145. The responses of F₁, F₂, and backcross plants of plant introduction 136170 × plant introduction 420145 were analyzed and results are presented in Table 3. The F₁ plants were all resistant, whereas the F₂ plants segregated consistent with the three resistant to one susceptible ratio, indicating a dominant gene. The backcross population (plant introduction 136170 × plant introduction 420145) × plant introduction 136170 segregated one resistant to one susceptible, whereas the backcross population (plant introduction 136170 × plant introduction 420145) × plant introduction 420145 segregated one resistant to zero susceptible, further suggesting a single dominant gene is involved. Overall, the data indicate that resistance to GSB in accession plant introduction 420145 is conditioned by a single dominant gene.

Inheritance of Didymella bryoniae resistance in plant introduction 323498. All the F₁ plants from plant introduction 323498 × NSL 30032 cross were resistant to the *D. bryoniae* spore inoculation (Table 3). Segregation for resistance in the F₂ population revealed a three resistant to one susceptible ratio ($\chi^2 = 0.31$), whereas the backcross to the susceptible parent, NSL 30032 gave a good fit to a 1:1 ratio ($\chi^2 = 0.73$). The parents plant introduction 323498 and NSL 30032 were all uniformly resistant and susceptible respectively except plant number 11 of the resistant parent, which was rated 3; the reasons for this have been described previously. These

Table 1. (continued) Foliar and stem disease indices for *Cucumis melo* L. (unless specified) germplasm in response to *D. bryoniae* inoculation from a plastic house screen.

Accession	Origin	Hort ^z group	Disease rating ^y	
			Leaf	Stem
PI 414723	India	melo	3.8	3.8
PI 512494	Spain	melo	4.0	3.9
Ames 2830	USA	melo	3.6	4.0
PI 313970	India	melo	4.0	4.0
PI 512532	Spain	melo	4.2	4.0
PI 512540	Spain	melo	4.3	4.0
PI 439744	Iran	melo	4.3	4.0
PI 230185	Iran	melo	4.3	4.0
PI 229807	USA	melo	4.4	4.0
PI 525138	Egypt	flexuosus	4.0	4.1
PI 260651	Afghanistan	melo	4.1	4.1
PI 512535	Spain	melo	4.4	4.1
PI 512447	Spain	melo	4.4	4.1
PI 194056	China	melo	4.5	4.1
PI 183307	India	melo	4.4	4.1
PI 512508	Spain	melo	4.8	4.1
PI 183226	Egypt	melo	4.0	4.2
PI 296118	Egypt	melo	4.3	4.2
PI 197891	USA	melo	4.3	4.2
PI 181875	Syria	melo	4.4	4.2
PI 512533	Spain	melo	4.4	4.2
PI 525115	Egypt	melo	4.5	4.2
PI 324525	Afghanistan	melo	4.5	4.2
PI 175676	Turkey	melo	4.7	4.2
PI 525141	Egypt	flexuosus	4.0	4.3
PI 525144	Egypt	flexuosus	4.4	4.3
PI 124440	India	melo	4.5	4.4
PI 512448	Spain	melo	4.6	4.4
PI 174156	Turkey	melo	4.6	4.4
PI 194052	China	melo	4.6	4.4
PI 420152	Afghanistan	inodorus	4.7	4.4
PI 357791	Yugoslavia	melo	4.9	4.4
Ames 2822	USA	melo	4.4	4.5
PI 167057	Turkey	melo	4.5	4.5
PI 525146	Egypt	flexuosus	4.7	4.5
PI 418768	Afghanistan	melo	4.7	4.5
PI 512530	Spain	melo	4.8	4.5
PI 525128	Egypt	melo	4.5	4.5
PI 204304	Afghanistan	melo	4.6	4.6
PI 525135	Egypt	flexuosus	4.6	4.6
PI 435086	China	melo	4.8	4.6
Huanghemi	China	NA ^u	4.8	4.6
PI 344431	Iran	melo	4.8	4.6
PI 319214	Egypt	melo	4.6	4.7
PI 512534	Spain	melo	4.6	4.7
PI 140632	Iran	melo	4.7	4.7
PI 313976	Uzbekistan	melo	4.8	4.7
PI 124111	India	melo	4.8	4.7
Ames 13301	Spain	melo	4.6	4.8
PI 154339	China	melo	4.8	4.8
PI 255949	Greece	inodorus	4.8	4.8
PI 319218	Egypt	melo	5.0	4.8
PI 271267	Iran	melo	4.9	4.9
PI 212210	Greece	melo	5.0	4.9
PI 190554	Iraq	melo	5.0	4.9
PI 306136	Iran	melo	5.0	5.0
PI 618837	China	melo	5.0	5.0
PI 296385	Iran	melo	5.0	5.0
PI 288233	Egypt	melo	5.0	5.0

Standard deviation: leaf: 1.23, stem: 1.31.

Correlation leaf and stem at 0.01:0.970 (**).

^zHort. group = horticultural group.

^yMean of three ratings of two replications.

Visual ratings on leaves and stems were scored at 7, 14, and 21 d postinoculation on the following scale: leaf, 1 = 0% of leaf area affected, 2 = $\geq 25\%$ leaf area affected, 3 = $\geq 25\%$ –50% leaf area affected, 4 = $\geq 50\%$ –75% leaf area affected, 5 = $\geq 75\%$ –100% leaf area affected; stem 1 = no damage, 2 = single lesion 1 to 10 mm long, or composite lesions 1 to 20 mm, stem not girdled, 3 = lesion 21 to 80 mm long or girdling of stem, 4 = stem withered, and 5 = seedling dead.

^uRussian Fed. = Russian Federation.

^vthins = thin skin melon, belongs to the melo hort. group.

^wthicks = thick skin melon, belongs to the melo hort. group.

^xNA = information not available.

PI – plant introduction.

Table 2. Mean stem scores for parental genotypes and susceptible control used in this study after inoculation with *Didymella bryoniae* spores.

Parent	Mean scores ^z	Rating
Huanghemi ^y	4.6	Susceptible
PI 268227	3.7	Susceptible
PI 136170	3.8	Susceptible
NSL 30032	3.1	Susceptible
PI 157076	1.3	Resistant
PI 420145	1.1	Resistant
PI 323498	1.5	Resistant

^zMean of three ratings of two replications. Stem scores were based on a stem damage scale of 1–5, in which 1 = no damage, 2 = a single lesion 1–10 mm long or coalesced lesions 1–20 mm long with no girdling of stem, 3 = lesions 21–80 mm long, 4 = lesions or girdling of the stem, 5 = withered stem, and 5 = dead seedling.

^yHuanghemi, susceptible control.

PI = plant introduction.

observations support the hypothesis of a single dominant gene conditioning resistance to *D. bryoniae* in plant introduction 323498.

Discussion

Our study largely confirmed previous reports that some accessions are resistant or susceptible (Table 1). However, we observed extreme cases in which several plant introductions previously rated highly resistant, e.g., plant introductions 482399 (Zimbabwe), 357791 (Yugoslavia), 182226 (Egypt), were rated highly susceptible in our work. Interestingly, three other plant introductions, 482397, 482395, and 482494, also from Zimbabwe previously rated 1.0, 1.0, and 1.07 (stem ratings) were only moderately resistant, 2.55, 2.62, and 2.24, respectively, in our study. On the other extreme, we observed cases of, e.g., plant introductions 532829 (China), 500362 (Zambia), 185111 (Ghana), were rated highly resistant in our study. Differential response to *D. bryoniae* has been reported in cucumber (Van Der Meer et al., 1978; Wehner and Shetty, 2000; Wehner and St. Amand, 1993). It has been suggested that variation in response to GSB over environments may be the result of differences in fungal isolates (i.e., differences in virulence) or the result of isolate $\times$ environment $\times$ plant interactions (Song et al., 2004). Our observations support this hypothesis and validate the need for retesting germplasm found to be highly resistant in one environment under varied environments, e.g., exposing the sources of resistance to existing and new isolates over different production areas before use in breeding programs.

We observed resistance in accessions not previously reported, e.g., in ‘Jinguan’ (stem rating of 1.0), a thin skin Chinese melon, Xin miZa9 (mean stem rating of 1.12), a hemi type Chinese melon, NJG1 (stem rating 1.3), and NJG6 (stem rating 1.2). NJG1 and NJG6 are local landraces collected from farms. Landrace accessions (ecotypes) have genes for breeding value. Landraces offer great potential for breeding because they are a result of many years of adaptation, breeding

Table 3. Response of the susceptible melon (*Cucumis melo*) parents plant introductions 268227 and 136170, NSL 30032, the resistant parents plant introductions 157076, 420145, and 323498, their F₁, F₂ and reciprocal backcrosses when inoculated with *Didymella bryoniae* spores.

Pedigree ^y	Generation ^x	No. of plants ^z		Expected ratio (R:S)	P value ^w
		R	S		
PI 268227	P	0	21	0:1	—
PI 136170	P	0	20	0:1	—
NSL 30032	P	0	24	0:1	—
PI 157076	P	66	1	1:0	—
PI 420145	P	68	0	1:0	—
PI 323498	P	43	1	1:0	—
PI 268227 × PI 157076	F ₁	64	1	1:0	—
PI 268227 × PI 157076	F ₂	74	30	3:1	0.35
(227 × 076) × 227	BC _s	29	34	1:1	0.38
(227 × 076) × 076	BC _r	104	0	1:0	—
PI 136170 × PI 420145	F ₁	46	0	1:0	—
PI 136170 × PI 420145	F ₂	69	20	3:1	0.27
(170 × 145) × 170	BC _s	29	37	1:1	0.30
(170 × 145) × 145	BC _r	68	0	1:0	—
NSL 30032 × PI 323498F ₁	50	0	1:0	—	—
NSL 30032 × PI 323498F ₂	70	20	3:1	0.70	—
(032 × 498) × 032	BC _s	37	30	1:1	0.36
(032 × 498) × 498	BC _r	63	0	1:0	—

^xR = resistant, S = susceptible.

^y227 = PI 268227, 076 = PI 157076, 170 = PI 136170, 145 = PI 420145, 032 = NSL 30032, 498 = PI 323498.

^zP = parent, BC_s and BC_r = backcross to susceptible and resistant parents, respectively.

^wP value at $\alpha = 0.05$.

PI = plant introduction.

work, and a high degree of genetic diversity that is largely untapped outside where they are grown. In the present study, we also identified a wild *Cucumis* species accession collected from the wild in the highlands of Kenya. This accession, coded JF1, was rated highly resistant with a symptom stem rating of 1.1, similar to data previously reported for plant introduction 140471. We are in the process of determining its value (e.g., crossability and horticultural characteristics) in relation to *C. melo* cultigens for future work and use.

Similar to JF1, the other wild relatives (e.g., plant introductions 196477, 299571, 364472, *C. figareii*) were highly resistant both for stem and leaf scores. Our data are consistent with previous studies (Wehner and St. Amand, 1993; Zhang et al., 1997). Generally, the wild sources of resistance are not sexually compatible with cultivated melons; however, some success in interspecific hybridization in other cucurbits has been reported (Chen et al., 1997). Moreover, with advances in biotechnology, e.g., embryo rescue, protoplast fusion, wide crosses between cultivated melon and its wild relatives, may be foreseeable in the future.

Resistance in *C. melo* var. *flexuosus* and *inodorus* accessions is rarely reported. Most germplasm reported for GSB resistance are from *C. melo* var. *agrestis* (e.g., Sakata et al., 2000) or *C. melo* var. *melo* (Sowell, 1981; Sowell et al., 1966).

Stem resistance to GSB in melon has received more emphasis than leaf resistance (e.g., Frantz and Jahn, 2004; Sakata et al., 2000) probably because stem scores in different tests of a given accession are more highly correlated than leaf scores. Our observations, which need verification, were that higher leaf resistance was observed in germ-

plasms with intermediate or lower stem resistance. Higher leaf resistance coupled with stem resistance would be a definite advantage. The high correlation between leaf and stem resistance in our work is consistent with the results of Zuniga et al. (1999).

The three accessions whose genetics of resistance we characterized flowered and fruited easily under our conditions; they have moderate-sized green fruits, larger than those of 140471, and could easily be deployed into commercial germplasms. There is still a need to characterize the genetics resistance in other melon accessions reported to be resistant to the GSB pathogen and to determine if these sources share the same or carry independent genetic factors for resistance. The allelic relationships among these plant introductions have not been reported. As a result of resource limitations, we did not determine allelic relationships among these accessions and between these accessions and those already determined, e.g., plant introductions 140471 and 482398; this will form the basis of our future work. Similar to our result, Zuniga et al. (1999) also determined resistance in plant introduction 157082 was monogenic dominant. Plant introduction 157082 and plant introduction 157076 are both from China. However, Zuniga et al. (1999) went further and showed that the gene in plant introduction 157082 was not linked to that in plant introduction 140471. The gene conferring resistance in plant introduction 157082 was named Gsb-3 (Frantz and Jahn, 2004); it will be interesting to determine the allelic relationship between this gene and that in plant introduction 157076. Both genotypes are *C. melo* var. *melo*.

In conclusion, it is important to screen melon cultigens against *D. bryoniae* over varied environments to assure their univer-

sality of resistance and subsequent use in breeding programs. Equally important is the need to characterize the mode of inheritance in the sources of resistance to cut down on the costs of trial and error, crossing, backcrossing, evaluation and reevaluation, and selection from the progenies.

Literature Cited

- Bruton, B.D. 1998. Soil borne diseases in Cucurbitaceae: Pathogen virulence and host resistance, p. 143–166. In: J. McCreight (ed.). Cucurbitaceae '98—Evaluation and enhancement of Cucurbit germplasm. American Society of Horticultural Science Press, Alexandria, Va.
- Chen, J.F., J.E. Staub, Y. Toshiro, S. Issbiki, and S. Miyazaki. 1997. Successful interspecific hybridization between *Cucumis sativus* L. and *Cucumis hystris* Chakr. Euphytica. 96: 413–419.
- Crosby, K.M., M.E. Miller, and D.W. Wolff. 2002. Screening plant introductions of *Cucumis melo* for resistance to *Monosporascus cannonballus*, p. 188–191. In: D.N. Maynard (ed.). Cucurbitaceae 2002, American Society of Horticultural Science Press, Alexandria, Va.
- Frantz, J.D. and M.M. Jahn. 2004. Five independent loci each control monogenic resistance to gummy stem blight in melon (*Cucumis melo* L.). Theor. Appl. Genet. 108:1033–1038.
- Gusmini, G., T.L. Ellington, and T.C. Wehner. 2003. Mass production of gummy stem blight spores for resistance screening. Cucurbit Gen. Coop. Rpt. 26:26–30.
- Gusmini, G. and T.C. Wehner. 2002. Correlation of ratings for resistance to gummy stem blight in watermelon. In: D.N. Maynard (ed.). Cucurbitaceae 2002, p. 199–205. American Society of Horticultural Science Press, Alexandria, Va.
- Kato, T., K. Suzuki, J.K. Takahashi, and K. Kamoshita. 1984. Negatively correlated cross-resistance between benzimidazole fungicides and methyl N-(3,5-dichlorophenyl) carbamate. J. Pesticide Sci. 9:489–495.
- Keinath, A.P., F.W. Farnham, and T.A. Zitter. 1995. Morphological, pathological, and genetic differentiation of *Didymella bryoniae* and *Phoma* spp. Isolated from cucurbits. Phytopathol. 85:364–369.
- Keinath, A.P. and T.A. Zitter. 1998. Resistance to benomyl and thiophanate-methyl in *Didymella bryoniae* from South Carolina and New York. Plant Dis. 82:479–484.
- Kwon, M.K., J.R. Hong, H.J. Sun, K.Y. Sung, B.H. Cho, and K.C. Kim. 1997. Standardization of a mass production technique for pycnidiospores of *Didymella bryoniae*, gummy stem blight fungus of cucurbits. Korean J. Plant Pathol. 13:105–112.
- Malathroki, N.E. and D.J. Vokalonakis. 1983. Resistance to benzinidazole fungicides in gummy stem blight pathogen *Didymella bryoniae* on cucurbits. Plant Pathol. 32:395–399.
- McCreight, J.D. 2002. Reactions of 20 melon cultigens to powdery mildew race 2 U.S. Cucurbitaceae, p. 72–77. In: D.N. Maynard (ed.). Cucurbitaceae 2002. American Society of Horticultural Science Press, Alexandria, Va.
- McGrath, D.J., L. Vawdrey, and O. Walker. 1993. Resistance to gummy stem blight in muskmelon. HortScience 28:930–931.
- Norton, J.D. 1971. Gulfcoast—A sweet cantaloupe for the produce chain store market. Alabama Agr. Expt. Sta. Lft. 82.
- Norton, J.D. 1972. Chilton, a high quality fruit for the commercial market. Alabama Agr. Expt. Sta. Lft. 84.

- Norton, J.D. and R.D. Cospers. 1989. AC-70-154, a gummy stem blight resistant muskmelon breeding line. *HortScience* 24:709-711.
- Norton, J.D., R.D. Cospers, D.A. Smith, and K.S. Rymal. 1985. Aurora—A high quality disease resistant cantaloupe. Alabama Agr. Expt. Sta. Circ. 278.
- Pitrat, M., C. Dogimont, and M. Bardin. 1998. Resistance to fungal diseases of foliage in melon. *Cucurbitaceae*, p. 167-173. In: J. McCreight (ed.). *Cucurbitaceae '98—Evaluation and enhancement of Cucurbit germplasm*. American Society of Horticultural Science Press, Alexandria, Va.
- Prasad, K. and J.D. Norton. 1967. Inheritance of resistance to *Mycosphaerella citrullina* in muskmelon. *J. Amer. Soc. Hort. Sci.* 91:396-400.
- Sakata, Y., T. Wako, M. Sugiyama, and M. Morishita. 2000. Screening melons for resistance to gummy stem blight. *Acta Hort.* 510:171-177.
- Sitterly, W.R. and A.P. Keinath. 1996. Gummy stem blight, p. 27-28. In: T.A. Zitter, D.L. Hopkins, and C. Thomas (eds.). *Compendium of cucurbit diseases*. APS Press, St. Paul, Minn.
- Song, R., G. Gusmini, and T.C. Wehner. 2004. A summary of eleven preliminary studies of greenhouse and field testing methods for resistance to gummy stem blight in melon, p. 301-305. In: A. Lebeda and H.S. Paris (eds.). *Progress in Cucurbit Genetics and Breeding Research*. Palacky University in Olomouc, Czech Republic.
- Sowell, G., Jr. 1981. Additional sources of resistance to gummy stem blight of muskmelon. *Plant Dis.* 65:253-254.
- Sowell, G., Jr., K. Prasad, and J.D. Norton. 1966. Resistance of *Cucumis melo* introductions to *Mycosphaerella citrullina*. *Plant Dis. Rpt.* 50:661-663.
- St. Amand, P.C. and T.C. Wehner. 1991. Crop loss to 14 diseases of cucumber in North Carolina from 1983 to 1988. *Cucurbit Genet. Coop. Rpt.* 14:15-17.
- Takada, K. 1983. Breeding and characteristics of disease-resistant varieties (lines 'Ano No. 1, No. 2 and No. 3'). *Bull. Veg. Ornam. Crops Res. Series A.* 11:1-22.
- Tsutsumi, C.Y. and N. da Silva. 2004. Screening of melon populations for resistance to *Didymella bryoniae*. *Braz. Arch. Biol. Technol.* 47: 171-177.
- Tullu, A., L. Buchwaldt, T. Warkentin, B. Taran, and A. Vandenberg. 2002. Genetics of resistance to anthracnose and identification of AFLP and RAPD markers linked to the resistance gene in PI 320937 germplasm of lentil (*Lens culinaris* Mediks). *TAG.* 106: 428-434.
- Van Der Meer, Q.P., J.L. Van Bemekon, and A.C. Van Der Giessen. 1978. Gummy stem blight resistance of cucumbers (*Cucumis sativus* L.). *Euphytica* 27:661-664.
- van Steekelenburg, N.A.M. 1985. Influence of time of transmission from night to day temperature regimes in incidence of *Didymella bryoniae* and influence of disease on growth and yield of glasshouse cucumbers. *Neth. J. Plant Pathol.* 91:225-233.
- Vokalounakis, D.J. 1993. Inheritance and genetic linkage of fusarium wilt (*Fusarium oxysporium* f. sp. *cucumerinum* race 1) and scab (*Cladoporium cucumerinum*) resistance genes in cucumber (*Cucumis sativus*). *Ann. Appl. Biol.* 123:359-365.
- Vokalounakis, D.J. 1995. Inheritance and linkage of resistance in cucumber line SMR-18 to races 1 and 2 of *Fusarium oxysporium* f. sp. *cucumerinum*. *Plant Pathol.* 44:169-172.
- Wako, T., Y. Sakata, M. Sugiyama, T. Ohara, D. Ishiiji, A. Kojima, and S. Nishimura. 2002. Identification of melon accessions resistant to gummy stem blight and genetic analysis of the resistance using an efficient technique for seedling test. *Acta Hort.* 588:161-164.
- Wehner, T.C. and N.V. Shetty. 2000. Screening the cucumber germplasm collection for resistance to gummy stem blight in North Carolina field tests. *HortScience* 35:1132-1140.
- Wehner, T.C. and P.C. St. Amand. 1993. Field tests for cucumber resistance to gummy stem blight in North Carolina. *HortScience* 28: 327-329.
- Zhang, Y., M.M. Kyle, K. Anagnostou, and T.A. Zitter. 1997. Screening melon (*Cucumis melo*) for resistance to gummy stem blight in the greenhouse and field. *HortScience* 32:117-121.
- Zitter, T.A. and M.M. Kyle. 1992. Impact of powdery mildew and gummy stem blight on collapse of pumpkin (*Cucurbita pepo* L.). *Cucurb. Genet. Coop. Rpt.* 15:93-96.
- Zuniga, T.L., J.P. Jantz, T.A. Zitter, and M.M. Jahn. 1999. Monogenic dominant resistance to gummy stem blight in two melon (*Cucumis melo*) accessions. *Plant Dis.* 83: 1105-1107.