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Reaction of melon genotypes to the gummy stem blight and the downy mildew

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ABSTRACT

RESUMO

The gummy stem blight (Didymella bryoniae) and the downy mildew (Pseudoperonospora cubensis) are two foremost melon (Cucumis melo) diseases, considering their effects on yield and fruit quality. Despite the importance of such diseases, relatively few studies have been done so far on the identification of resistance sources to D. bryoniae and P. cubensis in Brazil. This work aimed at evaluating the resistance of commercial melon genotypes to the gummy stem blight and the downy mildew. Firstly, the most aggressive and representative D. bryoniae isolate was selected. Subsequently, the resistance of 86 melon genotypes to stem infection was studied upon greenhouse conditions by inoculating with the previously selected isolate. Afterwards, the resistance to mildew and leaf infection by D. bryoniae of 28 melon genotypes was evaluated in the field, under natural infection. In the greenhouse, all 86 melon genotypes were infected and showed stem infection symptoms caused by D. bryoniae four days after inoculation. Nevertheless, a significant variation on the resistance levels of the melon genotypes was found. Under field conditions and natural inoculation, genotypes Taslaki and Sary Juliabi were the most susceptible to leaf infection by D. bryoniae, significantly differing from the other genotypes. The lowest levels of susceptibility were identified in genotypes Perlita Busle S1, Valenciano Elíptico, Glaver, MR1, and 2526. All genotypes were susceptible to the downy mildew, albeit differing in susceptibility levels.

Keywords: *Cucumis melo, Didymella bryoniae, Pseudoperonospora cubensis*, genetic resistance.

Reação de genótipos de melão ao crestamento gomoso e ao míldio

O crestamento gomoso do caule (Didymella bryoniae) e o míldio (Pseudoperonospora cubensis) estão entre as principais doenças do meloeiro (Cucumis melo) ocasionando redução da produtividade e da qualidade dos frutos. Apesar da importância dessas doenças, são poucos os trabalhos envolvendo a identificação de fontes de resistência a D. bryoniae e a P. cubensis no Brasil. O objetivo deste trabalho foi avaliar a resistência de genótipos comerciais de melão a essas doenças. A partir da seleção de um isolado mais agressivo, foi estudada, em condições de casa de vegetação, a resistência de 86 genótipos de meloeiro à infecção por D. bryoniae no caule. Posteriormente, em condições de campo, foi avaliada a resistência conjunta de 28 genótipos ao crestamento gomoso e ao míldio. Em casa de vegetação, todos os 86 genótipos de meloeiro avaliados foram infectados e mostraram sintomas de crestamento no caule, quatro dias após a inoculação. Houve variação significativa nos níveis de resistência entre os genótipos avaliados. No campo, sob inóculo natural, os genótipos Taslaki e Sary Juliabi mostraram-se mais suscetíveis à infecção por D. bryoniae nas folhas, diferindo significativamente dos demais genótipos. Os menores níveis de suscetibilidade foram verificados nos genótipos Perlita Busle S1, Valenciano Elíptico, Glaver, MR1 e 2526. Na avaliação da resistência ao míldio, todos os genótipos foram considerados suscetíveis, embora tenham sido encontradas diferenças nos níveis de suscetibilidade.

Palavras-chave: *Cucumis melo, Didymella bryoniae, Pseudoperonospora cubensis,* resistência genética.

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he cultivation of melon (Cucumis L melo L.) in Brazil has a mounting economic importance due mainly to the opportunities opened by the external market. The Brazilian Northeastern region hosts over 90% of the melon fields, as well as of the national production (Santos et al., 2004). In this region, high temperatures and profuse insolation promote the development of fruits with high contents of soluble solids. In the State of Tocantins, where melon also founds favorable soil and climatic conditions, crops are expanding gradually from the current fields grown by small farmers.

In spite of the suitable environmental conditions, the lack of cultivars with resistance to the most important diseases hampers the expansion of melon cultivation in Tocantins. The gummy stem blight or stem canker, induced by the fungus Didymella bryoniae (Fuckel) Rehm, ranks among the leading melon diseases due to the setback it causes in both fruit yield and quality (Santos & Café Filho, 2006). The gummy stem blight is a common disease in tropical and subtropical areas worldwide (Sitterly & Keinath, 1996). The downy mildew, whose etiological agent is Pseudoperonospora cubensis (Berk. et

Curtis) Rostowzew, is another ordinary, but relevant disease in melon. The downy mildew may give grounds to field total losses, mainly when rainy conditions co-occur with cool temperatures (Blancard et al., 1996). According to Rego (1995), wind, rain drops, and sprinkler irrigation are responsible for Р. cubensis dissemination. Sporangia are driven far away by the wind. The pathogen infects the majority of the species in family Cucurbitaceae, both cultivated, such as watermelon (Citrullus lanatus (Thunb.) Matsum. & Nakai), and weeds. Under the Tocantins environmental conditions, the pathogen is challenging mainly in July, when the temperature falls from 35°C during the day to 25°C at night, and leaf wetting simultaneously takes place (Santos *et al.*, 2005). At present, control measures are based on fungicide spraying. However, the efficacy of chemical control is very restricted in highly susceptible cultivars and when meteorological conditions are favorable to the pathogen (Thomas, 1998).

There are few reports on the identification of sources of resistance to D. bryoniae and P. cubensis in Brazil. In an experiment carried out at the greenhouse and under plastic tunnel to evaluate the resistance to D. bryoniae in two half-sib populations derived from genotypes PI 140471 and Anô#2, both progenies were more resistant than the reference cultivar. Nevertheless, cultivar Anô#2 was considered a more adequate resistance source, since it concurrently assembles multiple disease resistance and favorable commercial characteristics (Tsutsumi & Silva, 2004). The resistance of PI 140471, a Texan native melon, is controlled by a pair of genes (Norton & Prasad, 1967). The resistance to D. bryoniae in melon cultivars Anô#1, Anô#2, and Anô#3, was introgressed from cultivar Mi-tang-ting (Takada, 1983). Other researchers have demonstrated that the moderate resistance in lines C#1 e C#8, derived from the melon cultivar Auburn Cantaloup, was controlled by an independent pair of genes. Accessions PI 157082 and PI 511890 own their resistance to the gummy stem blight to a single dominant gene (Zuniga et al., 1999). The yellow melon genotype PI 124111F, native from India, displayed resistance to all pathotypes of P. cubensis. The resistance is controlled by two complementary loci with partial dominance (Cohen & Eyal, 1987; Thomas et al., 1988).

Considering the importance of the gummy stem blight and the downy mildew in the melon agribusiness and the scarce research efforts on studying the reaction of commercial resistance sources to both diseases, the aim of this work was to evaluate the resistance of commercial melon genotypes to the gummy stem blight and the downy mildew. **Table 1.** Aggressiveness of *Didymella bryoniae* isolates to melon seedlings, cultivars Dalton and Solarking, assessed as the average length of the stem lesion, four days after inoculation (agressividade de isolados de *Didymella bryoniae* a plântulas de melão, cultivares Dalton e Solarking, avaliada de acordo com o comprimento médio da lesão no caule do hospedeiro, quatro dias após a inoculação). Gurupi, UFT, 2007.

Isolate	Lesion Class*	
	cv. Dalton	cv. Solarking
BRSb	+++	++
78	++	+
MGP	++	+
BRSc	+	+
76	+	+
BRSa	+	+
CNPH	+	+

*Scale for lesion classes (escala para classe de lesão): (-) symptonless seedlings (plântula sem sintomas), (+) seedlings with lesions shorter than 1 cm (lesão menor que 1 cm), (++) seedlings with lesions longer than 1 cm (lesão maior que 1 cm), (+++) seedlings showing damping-off (tombamento da plântula).

MATERIAL AND METHODS

Assay I: Aggressiveness of Didymella bryoniae isolates to melon seedlings - In this assay, we used seven D. bryoniae isolates. Isolates were obtained from melon plants with the gummy stem blight symptoms and conserved in PDA medium (250 g of potato + 15 g of dextrosis + 20 g of agar)at the Mycological Collections of the University of Brasília and Embrapa Vegetables. Aiming at standardizing the aggressiveness potential of the isolates, which were kept in vitro for different periods at the Collections, all isolates used in this study were inoculated in 20-day old melon seedlings, cultivar Dalton. Before inoculation, isolates were cultured in PDA medium and incubated for ten days. For inoculation, mycelium disks with 0.6-cm in diameter, taken from the inner part of the colony, were fixed to the seedling stem 3.0 cm over soil level, using a sterile pin. After inoculation, seedlings were incubated for 48 h in a moist chamber, with 12-hour photoperiod and temperature ranging from 28 to 33°C.

For studying isolate aggressiveness, we used only colonies developed out of re-isolation from the abovementioned inoculated plants. Experiments were carried out at the greenhouse, with temperatures also ranging between 28 and 33°C. Seedlings grew in 4.0-kg polyethylene pots, filled with a 3:1 mixture of vegetal mold + manure. We sowed six seeds per pot of cultivars Solarking and Dalton, thinning to the four most uniform plants. Pots were irrigated daily to keep the soil constantly moist.

To study isolate aggressiveness, sensu Andrivon (1993), the experiment was set in a completely randomized design, with seven treatments and three replications. Treatments corresponded to the following D. bryoniae isolates: Melon Brasília a (BRSa), Melon Brasília b (BRSb), Melon Brasília c (BRSc), Melon DF1 (76), Melon DF2 (78), Melon Gurupi (MGP), and CNPH. The experimental plot consisted of a 4-plant pot, with each plant taken as a subsample. Isolate aggressiveness was assessed by measuring the length of the stem lesion four days after inoculation. To select isolates, we used the methodology established by Santos (2005), according to the following scale: (-) symptomless seedlings, (+) seedlings with lesions shorter than 1 cm, (++) seedlings with lesions longer than 1 cm, (+++) seedlings showing damping-off.

Assay II: Resistance of melon genotypes to *Didymella bryoniae* stem infection - The resistance of 86 melon genotypes to *D. bryoniae* was challenged at the greenhouse, with temperatures varying from 28 to 33°C. Plants grew in 3.0 L-pots, filled with a 3:1 mixture of vegetal mold + manure.

Table 2. Resistance of melon genotypes to the stem inoculation of *Didymella bryoniae*, assessed as the average length of the stem lesion, four days after inoculation (resistência de genótipos de melão à inoculação no caule de *Didymella bryoniae*, avaliada pelo comprimento médio da lesão, quatro dias após a inoculação). Gurupi, UFT, 2007.

Genotype*	Origin	Lesion Length (cm)**
Hegykô	Hungary	1,4 c
M63A17	Spain	1,4 c
Melão Caboclo	Ceasa-DF1	1,4 c
M59CH4	Spain	1,4 c
Valenciano Elíptico	Brasília	1,4 c
Sary Juliabi	The Soviet Union	1,5 c
Hales Best Jumbo	IPB Seeds	1,5 c
Glaver	Japan	1,5 c
Amarelo	Commercial	1,5 c
F1 Helios	Cotia County - SP	1,5 c
Valenciano Amarelo	Commercial	1,5 c
PS-2 Pele de Sapo	Spain	1,5 c
2526	German	1,5 c
Sea Bolt	Japan	1,5 c
Eldorado	Embrapa Vegetables	1,5 c
MelãoTrevo	Commercial	1,5 c
Taslaki	The Soviet Union	1,5 c
Csárdaszállás	Hungary	1,5 c
Prince	USDA/USA	1,5 c
A2	Spain	1,5 c
A1	Spain	1,5 c
Nyíregyháza	Hungary	1,5 c
Dolidor F1	Netherlands	1,5 c
MR 1	USDA/USA	1,5 c
Aroma F1	Wisconsin University	1,5 c
Uc. Perlita Busle S1	Wisconsin University	1,5 c
Gold Beauty	USA	1,5 c
Caipira	Currais Novos County - RN	1,6 c
Kzil	The Soviet Union	1,6 c
IC-Kzil	The Soviet Union	1,6 c
Galileu	Comercial	1,6 c
Polpa Laranja	Brasília - DF	1,6 c
Nyíribrony	Hungary	1,6 c
Galia F1	Topseed	1,6 c
Ananás	Germany	1,6 c
Gaucho	Sakata	1,6 c
Gynox Resist. P.	Wisconsin University	1,6 c
	•	
Nagycserkesz	Hungary	1,6 c
PS-1 Pele de Sapo	Spain	1,6 c
193246	Pelotas County - RS	1,6 c
TM-002-F1	Japan	1,6 c
TM-001-F1	Japan	1,6 c
Flexuosus "M-2791"	Israel	1,6 c
Chilton	Wisconsin University	1,6 c
Cristobal	Asgrow	1,6 c
Shavit Hibrid	Israel	1,6 c
M53A2	Spain	1,6 c
193254	Canguçu County - RS	1,6 c
2534	Germany	1,7 b
Valenciano Redondo	Brasília - DF	1,7 b
Melão Caipira	Brasília - DF	1,7 b

The experiment was planned in a completely random design, with 86 treatments (Table 2), and three replications. We used isolate *D. bryoniae* BRSb, selected due to its comprehensive representation (Santos *et al.*, 2008), higher aggressiveness, and uniform results, according to the outcomes of the aggressiveness assay (Table 1). Inoculation and incubation of inoculated seedlings, as well as lesion evaluation, were performed as described for assay 1.

Assay III: Field resistance of melon genotypes to the gummy stem blight and the downy mildew - We used the 28 melon genotypes most resistant to the gummy stem blight in the greenhouse to evaluate the joint resistance to this disease and to the downy mildew in the field (Table 3). The experiment was carried out from January to March 2007, in Gurupi county, State of Tocantins, at the experimental station of the Federal University of Tocantins (11°43'45"S; 49°04'07" W, 280 m over sea level). The experimental area was previously used for growing watermelon and had a history of occurrence of both the gummy stem blight and the downy mildew. Melon plants grew under natural disease pressure, upon the existing field inoculum. The experiment was designed in completely randomized blocks, with 28 treatments and three replications. In each plot, twelve plants were assessed.

After plowing and harrowing, the experimental area was fertilized (800 kg ha⁻¹, formulation 05-25-15 of N-P-K). Seeds were sown in holes, in 1.0 x 0.5 m spacing, placing 5 seeds hole⁻¹, 2 cm deep. Each experimental plot consisted of six holes planted with the same cultivar. Thinning was carried out 30 days after emergence (DAE), leaving two plants hole⁻¹, with side fertilization performed along (200 kg ha⁻¹, formulation N-P-K 20-00-20). Weeding was carried out three times during the whole experiment, 10, 20, and 30 DAE, through hand-hoeing. Plants were rainfed, except for a two-day period without rain, when sprinkler irrigation was used with 12-h shifts and a 7.9 mm average daily depth. Metamidophos at 0.24 kg ha⁻¹, applied 20, 30, and 45 DAE, was used for controlling pests. No fungicides were sprayed.

Table ? Dart II

The gummy stem blight and the downy mildew were assessed in leaves 75 DAE, when both diseases were spread uniformly. Diseases were evaluated based on the following scale (Santos et al., 2005): 0 = healthy plant; 1 = less than 1% of affected leaf area; 3= between 1 and 5% of affected leaf area; 5= between 6 and 25% of affected leaf area: 7= between 26 and 50% of affected leaf area; 9= more than 50% of affected leaf area. In view of the co-occurrence of both diseases, symptom differentiation was based on the lesion general aspect, according to Blancard et al. (1996). The gummy stem blight symptoms were recognized as dark necrotic spots, in which the damaged tissue dries and get loose from the plants. The downy mildew symptoms were identified as angular or round spots that progress from light green to necrosis, displaying the fungi light reproductive structures in the abaxial leaf surface. To support the visual diagnosis, infected leaves were collected and examined under optical and stereoscopic microscope to confirm the presence of either pathogen. Data were submitted to analysis of variance and the means for genotypes were separated using the Scott-Knott test. We used the software ASSISTAT 7.4 beta-2007 (Silva & Azevedo, 2006).

RESULTS AND DISSCUSSION

Assay I: Aggressiveness of Didymella bryoniae isolates to melon seedlings - All isolates were pathogenic to melon seedlings, cultivars Dalton and Solarking. Nevertheless, isolates differed from each other in aggressiveness, sensu Andrivon (1993). Isolate BRSb was the most aggressive, since it induced damping-off in cultivar Dalton seedlings and caused an average stem lesion longer than 1 cm in cultivar Solarking seedlings (Table 1). This isolate belongs to the diversity group which is prevalent in Brazil (Santos et al., 2008) and may well be employed in melon breeding programs for resistance to the gummy stem blight. Cultivar Solarking was less susceptible than cultivar Dalton for three out of the seven isolates studied.

Assay II: Resistance of melon genotypes to *Didymella bryoniae* stem infection - All genotypes were infected

Genotype*	Origin	Lesion Length (cm)**
Sib do CNPH 82-038	Dessert Seed	1,7 b
Janne Canari	Petoseed	1,7 b
Honey Dew Green	Petoseed	1,7 b
W6	Wisconsin University	1,7 b
Asgrow 200230	USA	1,7 b
Deltex	Commercial	1,7 b
W 30	Japan	1,7 b
PMR-45	Petoseed	1,7 b
Karry Kiz	The Soviet Union	1,7 b
Honi Deni Hami	China	1,7 b
193249	Piratini County - RS	1,7 b
Edisto 47	Wisconsin University	1,7 b
Doublon	Commercial	1,7 b
Golden	Petoseed	1,7 b
Miskolc	Hungary	1,7 b
TR 63230	Turkey	1,7 b
Verde Comprido	Spain	1,7 b
Super Doce	Spain	1,7 b
Sul	State of Rio Grande do Sul	1,8 b
Pinonet	France	1,8 b
Attajskaja	Embrapa GR&B2	1,8 b
Glover	Japan	1,8 b
193248	Piratini County - RS	1,8 b
Margot	France	1,8 b
Marigold	USA	1,8 b
193258	Rio Grande County - RS	1,9 a
Rochedo	Asgrow	1,9 a
Koh	Japan	1,9 a
G-35	Japan	1,9 a
Gold Mine	Petoseed	1,9 a
Extra Supermercado	Brasília - DF	1,9 a
PI 532830-	China	2,0 a
Caipira da Bolívia	Bolivia	2,0 a
Ak-Kaun	The Soviet Union	2,1 a
193257	Rio Grande County - RS	2,1 a
CV%		9,7

*Seedlings inoculated by a stem wound, 20 days after emergence (plântulas inoculadas com ferimento no caule, 20 dias após a emergência); **Means followed by the same letter in the column do not significantly differ from each other, Scott-Knott clusteting test, p<0.05 (médias seguidas da mesma letra nas colunas não diferem entre si, teste de agrupamento de Scott–Knott, p<0.05).

¹CEASA-DF = Wholesale market for Brasília-DF (Central de Abastecimento de Brasília– DF); ²Embrapa GR&B= Embrapa Genetic Resources and Biotechnology (Embrapa Recursos Genéticos e Biotecnologia).

upon stem inoculation and presented characteristic symptoms four days after inoculation. In spite of the inexistence of immunity, significant variation in susceptibility to stem infection was observed among the inoculated genotypes. Within 86 genotypes, 48 had stem lesions ranging from 1.4 to 1.6 cm; 28, from 1.7 to 1.8 cm; and 10, from 1.9 to 2.1 cm, the latter ones clustered as the most susceptible. The genotypes with the shortest stem lesions were M59CH4, Valenciano Elíptico, Melão Caboclo, M63A17, and Hegykô. The most susceptible genotypes, with the longest stem lesions, were 193257, AK-Kaun, Caipira da Bolívia, and PI 532830 (Table 2). In watermelon, Santos & Café-Filho

Table 3. Field resistance of 28 melon genotypes to the gummy blight (*Didymella bryoniae*) in leaves and to the downy mildew (*Pseudoperonospora cubensis*) (resistência em campo de 28 genótipos de melão ao crestamento gomoso (*Didymella bryoniae*) e ao míldio (*Pseudoperonospora cubensis*)). Gurupi, UFT, 2007.

Genotypes	Disease Severity*		
	Gummy blight in leaves	Downy mildew	
Perlita Busle S1	3.6 c	4.3 b	
Valenciano Elíptico	3.6 c	5.0 a	
Glaver	3.6 c	5.6 a	
MR1	3.6 c	7.6 a	
2526	3.6 c	7.6 a	
M59CH4	4.3 c	6.3 a	
Hegykô	4.3 c	6.3 a	
Aroma F1	4.3 c	4.3 b	
Melão Caboclo	4.3 c	3.6 b	
Csardaszallas	4.3 c	5.6 a	
M88CH4	4.3 c	3.6 b	
Valenciano Amarelo	4.3 c	7.6 a	
Gold Beauty	4.3 c	6.3 a	
M63A17	5.0 c	6.3 a	
F1 Helios	5.0 c	5.0 a	
P S-2 Pele de Sapo	5.0 c	6.3 a	
A2	5.6 c	6.3 a	
Hales Best Jumbo	5.6 c	5.6 a	
Amarelo	5.6 c	5.0 a	
Prince	5.6 c	3.6 b	
Nyiregyhara	5.6 c	6.3 a	
Sea Bolt	6.3 b	7.0 a	
Dolidor F1	6.3 b	5.6 a	
Melão Trevo	6.3 b	5.0 a	
A 1	7.0 b	5.6 a	
Eldorado	7.6 b	5.6 a	
Taslaki	9.0 a	3.0 b	
Sary Juliabi	9.0 a	5.6 a	
CV%	11,3	10,8	

*Prior to the application of the means separation test, raw data were tranformed to X="x + 0,5 (antes da aplicação do teste de médias, os dados originais foram transformados para X="x + 0,5). Means followed by the same letter in the column do not differ significantly from each other, Scott-Knott clustering test, p<0.05 (médias seguidas da mesma letra nas colunas não diferem significativamente entre si, teste de agrupamento de Scott-Knott, p<0,05).

(2005) also found variability in the reaction to the gummy stem blight from cultivar to cultivar. The protection against the disease given by genetic resistance, although only partial, decreases the outbreak progress rate and is valuable in association with other measures, contributing to the reduction in both the use of fungicides and production costs. Genetic resistance can also be useful to lessen chances of environmental contamination, accidents with the person in charge of spraying, and presence of chemical residues in fruits.

Assay III: Field resistance of melon genotypes to the gummy blight in leaves and the downy mildew - High precipitation occurred during the whole crop cycle, especially in the first months, when more than 500 mm of rain fell down. Monthly rain distribution, according to Metereological Station of the Universitary Campus of Gurupi (Federal University of Tocantins), was as follows: 228, 330, 151, and 21 mm, respectively from January to April 2007. Such climatic condition, particularly in the beginning of the cycle, might have favored both the gummy blight and the downy mildew,

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since high humidity contributes to dissemination of both sporangia and zoospores.

Regarding the gummy blight, genotypes Taslaki and Sary Juliabi were significantly the most susceptible to the leaf infection, while genotypes Perlita Busle S1, Valenciano Elíptico, Glaver, MR1, and 2526 were the less susceptible (Table 3). Genotypes M59CH4, Valenciano Elíptico, Melão Caboclo, M63A17, and Hegykô, which were clustered as less susceptible in relation to stem infection (Assay 2), were grouped among those genotypes considered as moderately resistant to leaf infection. The correlation between susceptibility in stem and leaves was previously reported in watermelon (Santos & Café-Filho, 2005), in which a correlation value of r = 0.77 was found between symptoms in both plant organs. The methodology of searching for resistance based on evaluating symptoms both in stem and leaves is also employed by other authors (Zhang et al., 1997; Sakata et al., 2000). Santos et al. (2004), when studying the resistance of 20 melon hybrids to the main diseases in States of Ceará and Rio Grande do Norte, observed that all genotypes were susceptible to the gummy stem blight and the downy mildew.

In spite of all genotypes being susceptible to the downy mildew, there were significant differences in susceptibility among genotypes. The highest disease severity scores came from genotypes MR1, Valenciano Amarelo, and 2526. Genotype Taslaki, for its turn, had the lowest severity score, followed by genotypes M88CH4, Prince, and Melão Caboclo (Table 3). Other authors, when studying the resistance of melon lineages to the downy mildew, found few sources of disease resistance. Cohen et al. (1995) studied the resistance of eight melon genotypes and found tolerance in lineages P22a and P6a-3.

All commercial genotypes challenged in this study were susceptible to both the gummy blight and the downy mildew. Nevertheless, there were distinct levels of susceptibility among them. The

differential reaction points to the possibility of selecting cultivars which are likely to be less severely affected among those suitable for the market. Yet, this is a partial protection. Therefore, farmers should make use also of further integrated management measures, such as crop rotation, chemical spraying, adequate season for planting, irrigation method (avoiding leaf wetting), and balanced fertilization. In addition, it is important to implement new breeding programs and to expand the existing ones aiming at developing melon genotypes with improved levels of resistance to the gummy stem blight and the downy mildew.

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