Screening *Cucumis melo* L. *agrestis* germplasm for resistance to *Monosporascus cannonballus*

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ABSTRACT

The destruction of melon roots by the fungus *Monosporascus cannonballus* causes vine decline and crop loss in south Texas and other hot regions. This investigation was carried out to screen germplasm accessions of *Cucumis melo* L. *agrestis*, along with commercial melons, for resistance to this pathogen. Field tolerant and susceptible varieties were included as checks. All lines were grown in pasteurized sand, which had been inoculated with a high level (60 CFUs/g of soil) of *M. cannonballus* mycelium from culture. After three weeks all root systems were carefully cleaned and a root damage rating was taken. Three accessions, 20608, 20747, 20826 all demonstrated high resistance or immunity to the fungus with ratings of 1. This was superior to the best commercial melon lines, 'Deltex,' and 'TXC 2015.' All other commercial materials were moderately to highly susceptible, with ratings of 3 or more.

RESUMEN

La destrucción de las raíces de melón por el hongo *Monosporascus cannonballus* ocasiona el declinamiento de la planta y pérdidas del cultivo en el sur de Texas y otras regiones cálidas. Esta investigación se efectuó para evaluar germoplama de *Cucumis melo* L. *agrestis*, así como de cultivares comerciales en lo referente a la resistencia a este patógeno. Se incluyeron variedades tolerantes y susceptibles en campo como testigos. Todas las lineas se cultivaron en arena pasteurizada, la cual había sido inoculada con un alto nivel (60 UFCs/g de suelo) de micelio cultivado de *M. Cannonballus*. Después de 3 semanas todos los sistemas radicales se limpiaron cuidadosamente y se evaluó el grado de dañó a la raíz. Tres materiales (20608, 20747 y 20826) mostraron alta resistencia o inmunidad al hongo presentando un grado de daño 1. Este grado fue superior que el de las mejores líneas comerciales de melón, 'Deltex' y 'TXC 2015'. Todos los otros materiales comerciales fueron moderadamente o altamente susceptibles con grado de daño 3 o mayor.

Additional index words: Monosporascus cannonballus, resistance, germplasm

Vine decline of melons caused by *Monosporascus cannonballus* has become increasingly severe in many intensively cultivated fields in Texas and other hot regions of the world (Martyn and Miller, 1996; Cohen et al., 2000). Failure of popular commercial varieties of melons to withstand this disease has led to crop loss and poor quality fruit, particularly when plants are stressed by other factors (Pivonia et al., 1997). Chemical control of the fungus has not been completely effective to date and is expensive in any case (Mertely et al., 1991, 1993a). The process of developing host plant resistance was initiated at the TAES in 1993 with extensive screening of commercial melon varieties (Wolff and Miller, 1999; Wolff, 1996; Mertely et al., 1993b). Initially, melon lines were screened in infested field soils. Since 1996, controlled inoculations with a virulent isolate of the fungus have been employed as well. Combinations of screening procedures have identified several sources of intermediate resistance to the pathogen but nothing has demonstrated immunity (Crosby et al., 2000; Crosby, 2000). Screening has been expanded to include hundreds of melon accessions from around the world. The next step in the process is to begin screening more distantly related, but cross-compatible members of the melon species. This paper is the first report of an experiment to screen diverse germplasm of the *agrestis* sub-species of melons for resistance to *M. cannonballus*. These are weedy, wild melons with inedible fruit, but potential sources of valuable disease resistance genes.

MATERIALS AND METHODS

Seventy four accessions of *Cucumis melo ssp. agrestis* were acquired from the National Plant Germplasm System of the USDA. These were a random assortment of *agrestis* germplasm from India. The virulent strain of *M. cannonballus*, TX 90-25, was cultured on plates of V8 agar for 14 days to allow extensive mycelial growth. Plastic 1 liter jars were filled

Table 1. Root damage ratings from *Monosporascus cannonballus* infection on various cucurbits

Entry	Average Root Rating[z]
Ames 20608 *agrestis*	1.0a[y]
Ames 20747 *agrestis*	1.0a
Ames 20826 *agrestis*	1.0a
Wax Gourd *(Benincasa hispida)*	1.0a
Ames 20608 *agrestis*	1.3ab
Ames 20852 *agrestis*	1.3ab
Ames 20640 *agrestis*	1.7abc
Ames 20690 *agrestis*	1.7abc
Ames 20692 *agrestis*	1.7abc
Ames 20600 *agrestis*	1.7abc
Ames 20772 agrestis	1 .7abc
Ames 20773 agrestis	1.7abc
Ames 20804 agrestis	1 .7abc
Ames 20430 agrestis	2.0abcd
Ames 20607 agrestis	2.0abcd
'TXC 2015' melon	2.0abcd
'Deltex' melon	2.0abcd
'Straight 8' cucumber	2.0abcd
Ames 20635 *agrestis*	2.3bcde
Ames 20584 *agrestis*	2.7cdef
Ames 20606 *agrestis*	2.7cdef
'Primo' melon	3.0defg
Ames 20612 *agrestis*	3.3efgh
Ames 20636 *agrestis*	3.7fgh
Ames 20693 *agrestis*	3.7fgh
'Ovation' melon	4.0gh
'Mainpak' melon	4.0gh
'Morning Ice' melon	4.3h

[z]Inoculated roots were rated on a scale of 1 to 5: 1= no apparent necrosis, healthy roots; 2= slight necrosis of fine roots, few tan lesions; 3= slight necrosis of all roots, moderate tan lesions; 4= severe necrosis of all roots, few remaining fine roots, extensive tan lesions; 5= only tap root remaining, necrotic and completely tan to brown.

[y]Mean separations by LSD, $P \leq 0.05$.

with a 1:1 mixture of sand and rice hulls, then autoclaved for 45 minutes at 80ºC, on five consecutive days. Twenty plugs of the mycelial growth from the petri plates were placed into each jar under a sterile flow hood. Each jar was then sealed and placed on a lab shelf at 24ºC for 30 days. Every ten days, each jar was shaken to assure thorough penetration of mycelia throughout the medium. At the end of the thirty day growth period, a small sample from each jar was plated onto PDA to observe any potential contamination of the inoculum. In addition, one gram of inoculum from each jar was placed into a sterile solution of water and glycerol. Serial dilutions of tenfold, hundredfold, thousandfold and ten thousandfold were made. Three vials of each dilution were poured onto three plates containing PDA and allowed to grow for 6 days. Colonies were counted on each plate and averaged for each separate inoculum jar to estimate colony forming units (CFUs). Each jar was then labeled with the CFU count. Fine sand was pasteurized at 70ºC for 12 hours in a steam box. The appropriate amount of inoculum to yield 60 CFUs of *M. cannonballus* per gram of soil was calculated. Black, plastic trays with 38, 15 cm deep cells were used. The drain hole of each cell was partially blocked with one cotton ball. Two-hundred grams of sand were mixed with the inoculum and placed into each 15 cm deep cell. Three replications of both control and inoculated trays were planted with the 74 *agrestis* plant introduction accessions as well as 22 other assorted cucurbits. Seeds were watered and germination observed. After germination, all seedlings received 100 ml of soluble fertilizer (Peters 9.1 N-8.7 P- 16.6 K (400 $mg*L^{-1}$), plus micronutrients). Three weeks after germination, all plants were carefully extracted from the trays. Vines were cut at soil level and fresh weight was measured. Roots were carefully submerged into a container of clean water using a fine mesh strainer to allow all sand to wash away. Clean roots were then rated on a scale of 1 to 5: 1= no apparent necrosis, healthy roots; 2= slight necrosis of fine roots, few tan lesions; 3= slight necrosis of all roots, moderate tan lesions; 4= severe necrosis of all roots, few remaining fine roots, extensive tan lesions; 5= only tap root remaining, necrotic and completely tan to brown. All data were subjected to ANOVA and mean separations by LSD, using StatGraphics Plus (Manugistics, Rockville, MD). Some examples of the healthiest and most diseased roots were scanned into the computer program Rhizo Pro 3.8 (Regent Instruments, Quebec, Canada) using an HP 4c widebed scanner (Hewlet Packard, USA). This program was used to analyze morphological characteristics such as total root area and length.

RESULTS

Twelve accessions of *agrestis* demonstrated a higher level of resistance than the best commercial types (2.0), 'Deltex,' and 'TXC 2015 '(Table 1). Three entries, Ames 20608, Ames 20747 and Ames 20826 had average root ratings of 1.0, indicating no disease. Ratings of 1.0 were not significantly different than ratings of 2.0 but were significantly different than all ratings above 2.0 (LSD, $P \leq 0.05$). Ratings of 2.0 were not significantly different than ratings of 3.0. The wax gourd also appeared to be immune or extremely resistant to *M. cannonballus* with an average root rating of 1.0. The popular commercial variety, 'Primo,' demonstrated average performance under inoculation (3.0). The other 62 accessions of *agrestis*, 2 *Cucurbita* spp. and *Lagenaria siceraria* did not demonstrate resistance superior to 'Deltex,' with average root ratings ranging from 2.5 to 5.0. The 14 other commercial melon varieties (not in table) had root ratings ranging from 4.5 to 5.0 and were all considered extremely susceptible to *M. cannonballus.*

Figure 1 demonstrates the superior condition of fine roots, after inoculation with *M. cannonballus*, of 'Ames 20608' as compared to the most resistant commercial variety 'Deltex'. Percentage of fine roots between 0 and *0.5* mm was higher for 'Ames 20608'(79%) than for 'Deltex' (73%). This was reflected in a lower average root diameter (0.047 mm) for 'Ames 20608' than for 'Deltex' (0.054).

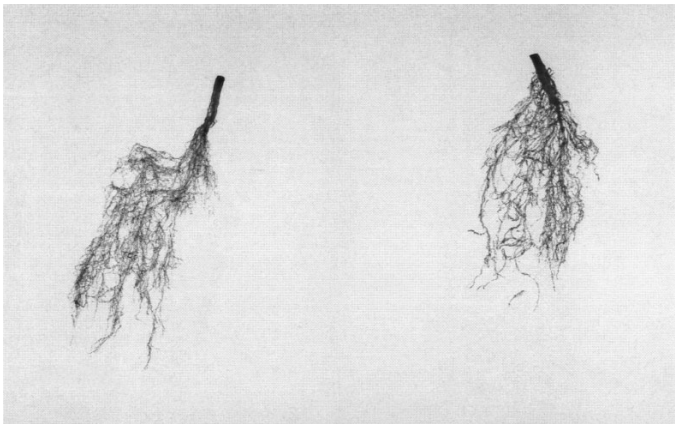

Fig. 1. *Monosporascus cannonballus* inoculated roots of 'Ames 20608' *agrestis* (left) and 'Deltex' (right), demonstrating less necrosis of fine roots on the plant introduction.

DISCUSSION

The results of this experiment provided useful, novel information about the potential of *Cucumis melo* ssp *agrestis* as a source of resistance genes against *M. cannonballus*. The possibility that a extremely high level of resistance or immunity to this pathogen exists within the melon species now appears plausible. This may be increasingly important in south Texas and other regions, where continuous melon culture has led to elevated levels of *M. cannonballus* in the soil (Mertely et. al., 1993a). The capacity of the plant to restrict damage to the fragile fine roots was demonstrated by several entries. Fig. 1 demonstrates this phenomenon in Ames 20608 as compared to one of the most resistant commercial varieties, 'Deltex.' 'Deltex' and a few other cantaloupe types have demonstrated significantly less damage to fine roots than all other commercial types in previous tests (Crosby et. al., 2000). However, this has not proven to be sufficient resistance under severe disease pressure in several locations (Cohen- personal communication). No commercial western shipper melons have been discovered to date with sufficient resistance to *M. cannonballus* (Wolff and Miller, 1998). The possibility of introducing genes from *agrestis*, which condition a higher level of resistance may be a solution. An intensive backcrossing and screening program will be required to introgress the resistance genes while eliminating undesirable characters such as small, inedible fruit. Initial crosses between cantaloupes and the three best *agrestis* accessions will provide useful information about the genetic control of this resistance. The strategy for breeding a more resistant cantaloupe can then be more efficiently designed.

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