A Technique to Screen Muskmelons for Resistance to Alternaria Leaf Blight

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ABSTRACT

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Five muskmelon cultivars were evaluated for resistance to Alternaria leaf blight in the cotyledon and first-leaf stages. Differences in resistance among cultivars were noted after cotyledons were inoculated, whereas no differences were detected when first leaves were inoculated. Cultivars with a flecking lesion or three or fewer lesions per plant were rated resistant, and those with more than three lesions per plant were rated susceptible. Corroborative field studies confirmed seedling ratings. The technique was tested on 27 muskmelon breeding lines. Breeding lines showing resistance in the cotyledon stage were also resistant in the field.

Additional key words: *Alternaria cucumerina*, *Cucumis melo*, cucurbits, plant breeding, screening techniques

Alternaria leaf blight, incited by *Alternaria cucumerina* (E. & E.) Elliot, is found throughout the world on various cucurbits (6), with muskmelon (*Cucumis melo* L.) being the most frequently reported host (7). In southern Texas, the disease is frequently masked by downy mildew (*Pseudoperonospora cubensis* (Berk. & Curt.) Rostow.) or powdery mildew (*Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll.). With the present trend of incorporating downy mildew and powdery mildew resistance into muskmelon cultivars, Alternaria leaf blight is becoming more conspicuous each year (12).

In Egypt, Prasada et al (10) found that the yield loss from a single plant was as high as 63% with early infection of *A. cucumerina* when environmental factors were favorable for disease development. Vines infected with *A. cucumerina* have low vitality and are predisposed to heat and wind damage. Death of older leaves exposes the fruit to direct sunlight and increases the probability of sun scald (8). Respiration increases and soluble solid content and sweetness of the fruit decrease from high temperatures that occur when defoliation exposes fruit to the sun (2).

Cultivars resistant to Alternaria leaf blight and adapted in southern Texas are currently unavailable. However, breeding lines with resistance to Alternaria leaf blight, downy mildew, and powdery mildew are available (5,13).

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Evaluation procedures are essential for plant breeders to identify superior resistant genotypes. Several techniques based on disease severity or incidence have been used to evaluate plants for their reactions to fungal pathogens (1); however, a rating system based on lesion number, size, or type is more objective.

The purposes of this study were to develop a seedling screening technique to identify muskmelon cultivars resistant to Alternaria leaf blight with a disease rating scale based on lesion parameters and to compare the reactions of cultivars in the cotyledon stage with field reactions.

MATERIALS AND METHODS

Five muskmelon cultivars were used for initial seedling studies. These included Perlita, Hale's Best, TAM-Uvalde, Greenflesh Honeydew, and TAM-Mayan Sweet. Seeds were planted eight per row in Speedling trays 111 × 36 cm with a cell size of 4.5 cm^2. Growing medium was a mixture of peat-perlite-vermiculite (2:1:1, v/v). Trays of plants in a greenhouse were watered twice a day with an automatic sprinkler system before inoculation.

Either the cotyledons or first leaves of seedlings were inoculated with a CO_2-powered atomizer, delivering about 0.2 ml of inoculum per plant. The isolate of *A. cucumerina* (obtained from C. E. Thomas, USDA, ARS, Weslaco, TX) was grown for 7–12 days on V-8 juice agar. Inoculum was prepared as described by Shahin and Shepard (11). Seedlings were then incubated for 18 hr at 21 ± 1.5 C and 100% relative humidity. After removal from the chambers, seedlings were grown for 5 days under fluorescent lights with an 11-hr photoperiod.

To determine the optimum number of leaf wetness hours needed for infection, seedlings were inoculated on cotyledons and first true leaves 14 and 17 days, respectively, after planting. Inoculated seedlings were placed in incubation chambers at 100% relative humidity and 21 ± 1.5 C for cotyledons and 27 ± 1.5 C for first true leaves (3). Seedlings received either 12 or 18 hr of leaf wetness and then were placed on a growth bench under fluorescent lights (7,000 lux) with an 11-hr photoperiod. After the light treatment, seedlings either were returned to the incubation chambers for an additional 13 hr of leaf wetness or left on the bench for 5 days. Seedlings that received the 13-hr repeated leaf wetness treatment were returned to the bench for 11 hr. The cycle of 11 hr of light and 13 hr of leaf wetness was continued for 5 days. Lesion number, size, and type were recorded for each seedling 5 days after inoculation.

Five muskmelon cultivars were grown in field plots at the Texas Agricultural Experiment Station (TAES) at Weslaco in 1982 and 1983. Seeds were planted on 27 March 1982 and 15 March 1983 in 16.8-m rows with 3-m alleys. Cultivars were replicated three times. Plants were inoculated with a conidial suspension of 168 conidia per milliliter on 15 June 1982 and 149 conidia per milliliter on 26 May 1983. Inoculation was with a tractor-mounted sprayer delivering 56 L of water per hectare. Leaf wetness was maintained at about 13 hr nightly with an automatic sprinkler system. Pyrazophos (280 ml/ha) and metalaxyl (930 ml/ha) were applied weekly from 14 May until 9 July to control powdery mildew and downy mildew, respectively.

Lesion number, size, and type were recorded weekly from the onset of bloom until harvest for each cultivar. Five leaves per plant and five randomly chosen plants per replicate were sampled. Leaves were collected starting five leaves basal to the vine bloom in 1982 and seven leaves basal in 1983.

In 1982, 27 breeding lines obtained from E. L. Cox (TAES, Weslaco) were tested for resistance to Alternaria leaf blight. The F_3 lines were from a cross between a green-rind casaba and a cantaloupe. Seedlings were treated as the five aforementioned cultivars, with the exception that breeding lines were grown in peat pots (3 × 3 cm) rather than in Speedling trays. Thirty-five seeds of each line were planted. After 7–10 days, 30 uniform seedlings of each line were chosen for screening.

In the field, breeding lines were planted in single 6.1-m rows, and replicates were taken within rows. Cultivation of these plots was as described for previous field studies. Lesion number and size were recorded from the onset of bloom until plant maturity. Three plants in each plot were marked and data obtained from five leaves on each plant, starting seven leaves basal to the vine bloom.

RESULTS

The mean number of lesions on cotyledons increased from one to five when the leaf wetness period was increased from 12 to 18 hr. An additional exposure to 13 hr of leaf wetness did not result in many additional lesions. The length of wetness period or repeated wetness period had little effect on numbers (n) of lesions on the first leaves ($0.7<n<2$).

Two distinct types of lesions (circular or flecking) were noted on both the cotyledons and first leaves, depending on the cultivar. Circular lesions on Perlita and Hale's Best were dark brown, light in the center, and ranged from 2 to 4 mm in diameter, whereas on TAM-Uvalde and TAM-Mayan Sweet, circular lesions ranged from 1 to 2 mm in diameter. Flecking-type lesions observed on the Greenflesh Honeydew were light colored and never greater than 1 mm in diameter.

Sections of cotyledons with each type of lesion were cleared using the techniques of Philley (9) and examined with a compound microscope. The mycelium of *A. cucumerina* grew and sporulated on cotyledons from seedlings with the light to dark brown circular lesion type (Fig. 1). Cotyledons with the light colored flecking-type lesions showed areas of collapsed cells where mycelium had penetrated.

Inoculation of cotyledons consistently resulted in more lesions than did inoculations of first leaves (Table 1). The number of lesions ranged from 1.3 to 2.7 when first leaves were inoculated and from 2.3 to 11.5 when cotyledons were inoculated. There was no significant difference among cultivars inoculated at the first-leaf stage. In the cotyledon stage, however, there was a significant difference ($P = 0.05$) among cultivars based on number of lesions. Perlita and Hale's Best had the most lesions on cotyledons.

From inoculations on cotyledons, cultivars with three or fewer lesions per plant were rated resistant and cultivars with more than three lesions per plant were rated susceptible (Table 1). Two types of lesions were associated with the resistant reaction: the flecking-type lesion on Greenflesh Honeydew and the light brown circular lesions on TAM-Mayan Sweet. When the flecking-type lesion appeared, the resistance rating was based on this character rather than lesion number. Cultivars rated as resistant in the cotyledon stage were also resistant in the field.

Average lesion numbers on cultivars in the field were similar in both 1982 and 1983. Perlita and Hale's Best showed disease symptoms 13 wk after planting. The pathogen spread more rapidly on Perlita, resulting in death of all leaves 3 wk after inital symptom expression. All leaves on Hale's Best were killed 4 wk after initial appearance of symptoms.

The other cultivars displayed significantly ($P=0.05$) fewer lesions than either Perlita or Hale's Best. Initial symptoms were observed 14 and 15 wk after planting on the TAM-Uvalde and TAM-Mayan Sweet cultivars, respectively; leaves of these two cultivars were not killed. No lesions developed on the Greenflesh Honeydew.

Lesion size was recorded after each treatment in the seedling studies and weekly in the field studies (Table 2). The largest lesions in both seedling and field studies were on Perlita and Hale's Best. The smallest lesions were recorded on the TAM-Mayan Sweet, TAM-Uvalde, and Greenflesh Honeydew cultivars.

In the seedling studies of 27 breeding lines, seven (CAS 2A-17, CAS 2A-20, CAS 2A-21, CAS 2B-9, CAS 2B-11, CAS 2B-12, and CAS 2B-16) displayed the same flecking-type lesion found on the Greenflesh Honeydew. Flecking-type lesions were considered a resistant reaction and were therefore not counted. On the remaining lines, lesion numbers ranged from an average of 0.2 to 11.5 lesions per plant (Table 3). The mean number of lesions on leaves of field-inoculated plants ranged from 0 to 54.3 (Table 3). Breeding lines ranked according to mean number of lesions on inoculated cotyledons and on leaves of field-inoculated plants were positively correlated ($r = 0.74$, $P = 0.01$).

DISCUSSION

Results of the leaf wetness studies confirm the findings of Prasada et al (10),

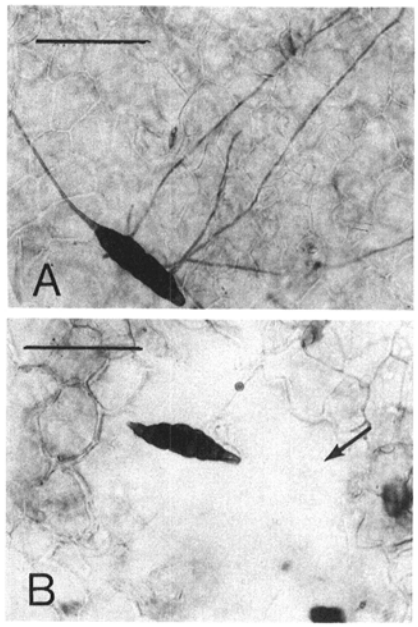

Fig. 1. Conidial germination and hyphal penetration of *Alternaria cucumerina* on cotyledons of muskmelon. **(A)** Tissue with light to dark brown lesion type and **(B)** tissue with flecking-type lesion showing collapsed host cells surrounding conidium (arrow). Scale bar = 26 μm.

Table 1. Mean number of lesions and resistance ratings on five muskmelon cultivars 5 days after inoculation with *Alternaria cucumerina* in the seedling stage and 15 wk after planting in the field[v,w]

Cultivar	Mean no. of lesions[x]		
	Cotyledon	First leaf	Field
Perlita	11.5 a (S)[y]	1.5 a (R)	17.8 a (S)
Hale's Best	5.9 b (S)	1.3 a (R)	29.2 b (S)
TAM-Uvalde	3.0 c (R)	2.7 a (R)	2.0 c (R)
TAM-Mayan Sweet	2.3 c (R)	1.7 a (R)	0.7 c (R)
Greenflesh Honeydew	4.0 c (R)[z]	2.2 a (R)[z]	0.0 c (R)

[v] Cotyledons were inoculated with 5,500 conidia per milliliter, then incubated for 18 hr at 21 C. First leaves of seedlings were inoculated with 3,000 conidia per milliliter and incubated at 27 C.
[w] Ratings based on average number of lesions on 24 seedlings per cultivar and 75 leaves per cultivar in the field.
[x] Numbers followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).
[y] S = susceptible and R = resistant.
[z] Flecking-type lesion.

Table 2. Average lesion size and resistance ratings on five muskmelon cultivars 5 days after inoculation with *Alternaria cucumerina* in the cotyledon stage and 15 wk after planting in the field[w,x]

Cultivar	Average lesion size (mm)[y]	
	Cotyledon	Field
Perlita	1.7 a (S)[z]	3.6 a (S)
Hale's Best	1.7 a (S)	3.0 a (S)
TAM-Uvalde	1.5 a (S)	1.8 b (I)
TAM-Mayan Sweet	1.0 b (R)	0.5 c (R)
Greenflesh Honeydew	1.0 b (R)	0.0 c (R)

[w] Cotyledons were inoculated with 5,500 conidia per milliliter, then incubated for 18 hr at 21 C.
[x] Ratings based on average lesion size on 24 seedlings per cultivar and 75 leaves per cultivar in cotyledon and field studies, respectively.
[y] Numbers followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).
[z] S = susceptible, R = resistant, and I = intermediate.

Table 3. Mean number of lesions on 27 muskmelon breeding lines 5 days after inoculation with *Alternaria cucumerina* in the cotyledon stage and 15 wk after planting in the field

Breeding line	Mean no. of lesions	
	Cotyledons[a]	Field[b]
CAS 2B-1	11.5	36.0
CAS 2A-9	8.3	29.2
CAS 2B-7	7.4	17.0
CAS 2B-8	7.4	26.0
CAS 2B-17	6.1	54.3
CAS 2A-1	4.6	25.8
CAS 2A-23	4.6	11.7
CAS 2B-13	4.6	14.4
CAS 2A-11	4.2	2.1
CAS 2A-16	2.3	15.7
CAS 2A-13	2.1	5.0
CAS 2A-3	2.1	21.0
CAS 2A-2	2.1	13.0
CAS 2A-12	1.9	6.9
CAS 2B-4	1.6	8.0
CAS 2A-6	1.5	21.9
CAS 2A-15	1.4	17.2
CAS 2A-7	0.6	12.4
CAS 2A-4	0.5	5.3
CAS 2A-8	0.2	4.1
CAS 2B-11	0.0[c]	5.3
CAS 2A-21	0.0[c]	2.3
CAS 2A-17	0.0[c]	1.9
CAS 2B-09	0.0[c]	1.8
CAS 2A-20	0.0[c]	1.5
CAS 2B-12	0.0[c]	0.3
CAS 2B-16	0.0[c]	0.0

[a] Mean number of lesions on 30 plants per breeding line.
[b] Mean number of lesions on 15 leaves per breeding line.
[c] Flecking-type lesions observed but not included in lesion counts.

who reported that conidial germination, infection, and lesion numbers increased with increasing time of leaf wetness treatments up to 20 hr at 100% relative humidity. The return of seedlings to incubation chambers was inconvenient and provided no significant increase in lesion numbers in the cotyledon stage. Therefore, the optimum leaf wetness period was 18 hr.

Different levels of resistance and lesion types among cultivars were identified more precisely on cotyledons than on first leaves. Inoculations of cotyledons resulted in more infections and more lesions overall. Consequently, the cotyledon stage was chosen as the optimum growth stage for screening of cultivars.

Resistance and susceptibility ratings based on average lesion size are impractical because of the time required to measure each lesion. Lesion type and number are easier to evaluate and can accurately identify resistant and susceptible cultivars.

A flecking-type lesion in the seedling stage correlated with a high resistance at maturity. Localized death of host cells invaded by a fungus has been termed hypersensitivity (4) and may be the reaction type observed in the Greenflesh Honeydew.

The breeding lines with flecking-type lesions on cotyledons in the seedling test were among those with the fewest leaf lesions in the field, supporting the decision to use lesion type as the prime criterion to identify resistant material. Screening in the cotyledon stage correlates with disease development in the field and therefore can be used as a screening tool. The most common error occurred when seedlings of breeding lines with relatively few lesions had unexpectedly high numbers of lesions on leaves in the field. It is important to note that none of the breeding lines with a high mean number of lesions (more than six) in the seedling stage were among those with a low mean number of lesions (fewer than eight) in the field. This is an important observation because a line with many lesions on the cotyledons would probably be discarded, resulting in the loss of a potential source of resistance.

The advantage of the seedling screening procedure is twofold: first, muskmelon cultivars resistant to Alternaria leaf blight can be identified within 20 days of planting; and second, numerous seedlings can be evaluated in a limited amount of space.

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