Screening Tomato Seedlings for Resistance to Bacterial Spot

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Abstract. A ‘spray-inoculation seedling screening procedure was developed for detecting resistance to Xanthomonas campestris pv. vesicatoria (Doidge) Dye, causal agent of bacterial spot of tomato (Lycopersicon esculentum Mill.). Two-week-old transplants were preconditioned under 95% humidity for 16 hours before spray inoculation and then rated for bacterial spot 2 weeks later. Resistant plants could also be distinguished from susceptible plants by marking advertisement

not practical for large-scale use. Thus, we initiated studies to identify a screening method or methods that would differentiate genotypes on a large scale with accuracy similar to field tests. A spray-inoculation seedling screening procedure was developed for differentiating genotypes with various levels of resistance derived from Hawaii 7998. This procedure and an adaption of a cotyledon-dip screening technique developed for bacterial speck resistance (Emmatty et al., 1982) were compared to field disease severity ratings.

Materials and Methods

Spray-inoculation seedling screening technique. The tomato genotypes possessing varying levels of resistance to X. c. vesicatoria used in these studies were ‘Waker’ (susceptible SI), ‘Sugar’ (S), ‘Lyconorma’ (S), ‘Campbell 28’ [partially resistant (PR)], and Hawaii 4013-3 (PR), Florida 317 and 325 [resistance (R) derived from Hawaii 7998], and Hawaii 7998 (R). Seed of the genotypes were sown in Black Beauty spent coal (Reed Minerals Div., Highland, Ind.) in flats and covered with a 0.5-cm layer of vermiculite. Seedlings were transplanted into Todd planter flats (cell size, 3.8 cm) (Speedling, Sun City, Fla.), which had been cut into thirds and filled with a soil mix composed of 1 vermiculite : 1 Canadian peat (0.03 m³ : 0.03 m³) amended with 105 g superphosphate, 200 g dolomite, and 52 g hydrated lime. One row of each genotype was randomly placed in each flat. There were five replications, with five plants of each genotype per replication. About 12 days after inoculation, plants were rated on the following scale: O = healthy; 1 = 0% to 3%; 2 = 3% to 6%; 4 = 6% to 12%; 5 = 12% to 25%; 6 = 25% to 50%; 7 = 50% to 75%; 8 = 75% to 87%; 9 = 87% to 94%; 10 = 94% to 97%; 11 = 97% to 100%; and 12 = 100% diseased tissue.

Cotyledon-dip seedling screening technique. The technique of Emmatty et al. (1982), developed for screening for bacterial speck resistance, was modified. Seed of ‘Walter’, ‘Lycononna’, ‘Sugar’, ‘Campbell 28’, Ohio 4013-3 (PR), Walter × Hawaii 7998 (S × R), Florida 317 and 325, and Hawaii 7998 were sown in spent coal and placed in the greenhouse. Ten days later, aerial portions of the plants were immersed for 15 min in 106 cfu/ml of X. c. vesicatoria/ml in a preliminary experiment and in 108 cfu/ml in seven experiments, with the inoculum prepared as described previously. Inoculated seedlings were then transplanted to Todd planter flats and placed in a growth chamber as described previously. There were five replications, with five plants of each genotype per replication.

Field disease severity ratings. In Summer 1983 and 1986, seed of ‘Walter’, ‘Campbell 28’, ‘Lyconorma’, Ohio 4013-3, and Hawaii 7998 were sown in spent coal and transplanted 10 days later to Todd planter flats with 3.8-cm cells. In Summer 1988, Florida 317 and 325 were included in addition to the lines mentioned above. Transplants were set 1 month later in the field on raised beds of EauGallie fine sand (sandy siliceous hypothermic Alfic haplaquod). The beds had been fumigated 2

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weeks earlier with 67% methyl bromide; 33% chloropicrin at 392 kg·ha\(^{-1}\) covered with white polyethylene mulch. Beds were 15 cm high, 75 cm wide, and 1.4 m from center to center. Plants were spaced 61 cm apart within rows. Genotypes were arranged in a completely randomized design with three replications and six plants per treatment. Standard fertilizer and insecticide practices were used. Plants were staked, tied, and watered by seep-age irrigation. Several weeks after transplanting, plants were inoculated by misting a suspension containing 10\(^{6}\) cfu of X. c. vesicatoria ml on the plants with a Solo (Solo Kleinmotoren, Sindelfingen, Germany) backpack sprayer in the early morning. Inoculum was prepared as described previously. Plants were visually assessed 6 weeks after transplanting for defoliation percentage in 1983 and 1986 and by the Horsfall–Barratt scale in 1988.

### Results and Discussion

#### Comparison of spray-inoculation screening and field disease severity (SSF D) on a plant-by-plant basis.

In Summer 1991, seed of Florida 7171 (S), 896007 × 896134 (R × S), Hawaii 7998 (R), six resistant parents, and six susceptible genotypes were used. Several weeks after transplanting, plants were inoculated by spraying a suspension containing 10\(^{6}\) cfu of X. c. vesicatoria/ml spray-inoculated on 2-week-old tomato plants resulted in more disease and better differentiation of genotypes than 10\(^{5}\) cfu/ml. Both 2- and 3-week-old plants provided reliable results, although 2-week-old plants could be inoculated earlier and more evenly with the bacterial suspension.

In spray-inoculation seedling screening experiments designed to compare different periods of preconditioning, all preconditioning periods, except 24 h (where \(P = 0.06\)), differentiated the genotypes (Table 1). At 16 h, resistant genotypes had significantly lower disease ratings than either susceptible genotype. Partially resistant genotypes could only be differentiated from susceptible and resistant genotypes with the 16-h preconditioning treatment, where partially resistant genotypes had higher ratings than resistant genotypes. When comparing 16-h preconditioning to no preconditioning, the nonpreconditioned (0-h) treatment resulted in more variability and escapes, and a smaller differential between ratings of susceptible and resistant genotypes. With nonpreconditioned plants, resistant genotypes were differentiated from one but not both susceptible genotypes. When plants were preconditioned for 16 h before inoculation, disease ratings were generally higher with a greater range between susceptible and resistant genotypes. When Davis and Halmos (1958) tested the effect of air moisture on the predisposition of tomato to bacterial spot on one genotype, they concluded that within the limits of their experiment, the longer the plants were exposed to 100% RH (up to 2 days before inoculation) the greater their susceptibility to the pathogen. In our experiments, the 16-h treatment resulted in better differentiation of genotypes than the 24-h treatment.

#### Statistical analyses.

Analyses of variance were performed on data and means were separated by Duncan’s multiple range test \((P \leq 0.05)\), with the exception of the SSFD experiment. F tests were done on individual experiments for both the spray-inoculation seedling screening and the cotyledon-dip method. All F values were significant \((P \leq 0.05)\), and experiments were used as replications which were presented in Tables 1 and 2. For the 1983 and 1986 field studies, defoliation percentage ratings ranged from 0% to 100% and therefore were appropriately transformed to arcsin square root for analysis (Gomez and Gomez, 1984). Phenotypic correlations were based on averages rather than individual plant ratings in all experiments with the exception of the SSFD experiment.

### Table 1. Effectiveness of spray-inoculation seedling screening procedures (preconditioning or nonconditioning period) for differentiating bacterial-spot-resistant, partially resistant, and susceptible tomato genotypes.

<table>
<thead>
<tr>
<th>Genotype/year</th>
<th>Reaction</th>
<th>Disease severity (^{a})</th>
<th>Preconditioning (^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyconorma</td>
<td>S</td>
<td>2.7 a</td>
<td>2.4 a</td>
</tr>
<tr>
<td>Walter</td>
<td>S</td>
<td>2.1 a c</td>
<td>2.8 a</td>
</tr>
<tr>
<td>Campbell 28</td>
<td>PR</td>
<td>2.3 ab</td>
<td>2.8 bc</td>
</tr>
<tr>
<td>Ohio 4013-3</td>
<td>PR</td>
<td>1.4 c</td>
<td>1.9 bc</td>
</tr>
<tr>
<td>Florida 317</td>
<td>R</td>
<td>1.3 c</td>
<td>1.2 c</td>
</tr>
<tr>
<td>Florida 325</td>
<td>R</td>
<td>1.7 bc</td>
<td>1.8 bc</td>
</tr>
<tr>
<td>Hawaii 7998</td>
<td>R</td>
<td>1.4 c</td>
<td>1.4 c</td>
</tr>
</tbody>
</table>

\(^{a}\)Disease severity ratings by Horsfall–Barratt scale.

\(^{b}\)Means within columns by Duncan’s multiple range test \((P \leq 0.05)\).

### Table 2. Disease ratings from a cotyledon-dip inoculation technique for screening tomato seedlings for resistance to bacterial spot.

<table>
<thead>
<tr>
<th>Genotype(^{c})</th>
<th>Reaction</th>
<th>Disease rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyconorma (L)</td>
<td>S</td>
<td>2.4 a</td>
</tr>
<tr>
<td>Walter (W)</td>
<td>S</td>
<td>1.9 ab</td>
</tr>
<tr>
<td>Campbell 28 (C)</td>
<td>PR</td>
<td>2.0 ab</td>
</tr>
<tr>
<td>Ohio 4013-3 (O)</td>
<td>PR</td>
<td>2.0 ab</td>
</tr>
<tr>
<td>Sugar (S)</td>
<td>S</td>
<td>1.5 bc</td>
</tr>
<tr>
<td>W X H</td>
<td>S X R</td>
<td>1.1 cd</td>
</tr>
<tr>
<td>Florida 325</td>
<td>R</td>
<td>1.1 cd</td>
</tr>
<tr>
<td>Florida 317</td>
<td>R</td>
<td>1.0 cd</td>
</tr>
<tr>
<td>Hawaii 7998</td>
<td>H</td>
<td>0.8 d</td>
</tr>
</tbody>
</table>

\(^{c}\)Number of experiments for W, C, and H = 7; for Florida 317 and 325 = 6; for L = 4; for O, S, and W X H = 3.

\(^{d}\)S = susceptible; PR = partially resistant; R = resistant. Based on field evaluation, where ratings were >5 for susceptible, intermediate for partially resistant, and ≤3 for resistant genotypes by the Horsfall–Barratt scale.

\(^{e}\)Disease rating scale: 0 = healthy; 1 = symptoms on one cotyledon; 2 = symptoms on both cotyledons; 3 = symptoms on true leaves; 4 = severe symptoms on true leaves and cotyledons; and 5 = dead.

\(^{f}\)Mean separation within columns by Duncan’s multiple range test \((P \leq 0.05)\).
kept as constant as possible from experiment to experiment to allow comparison across experiments. Lawson and Summers (1984) were able to identify lines with considerable resistance, and although they recommended field evaluations to verify results, comparisons of greenhouse and field results were not reported.

Emmatty et al. (1982) developed a useful seedling screening technique for bacterial speck. Adaptations of this method were tested for their usefulness for bacterial spot screening. In a preliminary experiment, inoculum concentration (10<sup>6</sup> or 8 × 10<sup>4</sup> cfu of X. c. vesicatoria/ml) did not significantly influence the results (data not shown). In seven cotyledon-dip tests (10<sup>6</sup> cfu X. c. vesicatoria/ml), plant death was infrequent, with the average disease severity ratings for susceptible genotypes ranging from 1.5 to 2.4 (Table 2). Resistant genotypes could be distinguished from susceptible genotypes, with the exception of ‘sugar’.

In our studies, correlations of seven cotyledon-dip experiment ratings with three field seasons were significant for only two of 21 possible field-cotyledon-dip experiment combinations. In contrast, with the spray-inoculation seedling screening procedures (preconditioning and nonpreconditioning), correlations of ratings with the same three seasons of field disease severity were significant for 35% (19 of 54) of the possible combinations for all pre- and nonpreconditioned treatments and for 49% (16 of 33) of the possible combinations of all preconditioned treatments (8-, 16-, and 24-h). These data indicate that preconditioning is superior to nonpreconditioning and more effective than the cotyledon-dip screening technique.

In Summer 1991, individual plants were evaluated by the spray-inoculation seedling screening (16-h preconditioning) method and then were placed in the field to determine susceptibility under field conditions on a plant-by-plant basis (SSFD). Correlations between seedling screening and field ratings were significant (P ≤ 0.0001), with r being 0.28 and 0.34 for the first and second field rating, respectively. Where individual seedling screening ratings were less than or equal to a Horsfall–Barratt rating of 3 (selected as resistant based on ratings of Hawaii 7998), 31% of the field-evaluated plants in the first rating were resistant and 69% were susceptible. When seedling screening ratings were >3, 10% of plants in the field were resistant and 90% were susceptible. Where plants were rated as ≤3 in the seedling screening rating, 17% were resistant and 83% were susceptible in the second field rating. Where the seedling screening rating was >3, 370 of plants were resistant and 97% were susceptible in the field. These results indicate that most susceptible plants (90% and 97% in the first and second rating, respectively) can be eliminated before field testing by discarding all plants with a rating >3. Thus, the use of the spray-inoculation seedling screening procedure can decrease space, labor, and time and therefore increase the efficiency of tomato improvement programs.

**Literature Cited**


