A Technique to Evaluate Tomatoes for Resistance to Phytophthora Root Rot in the Greenhouse

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ABSTRACT

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Tomato seedlings were grown in speedling trays and inoculated with a zoospore suspension at the first-leaf stage. Percent stand and root rot severity 7 days after inoculation was used to compare genotypes. The amount of seedling resistance was positively correlated with the degree of Phytophthora root rot in the field.

Phytophthora root rot is a major disease of tomatoes (*Lycopersicum esculentum* Mill.) in most tomato-growing areas of California. The disease can be incited by *Phytophthora capsici* Leonian or *P. parasitica* Dastur; the latter species, however, is reported to be responsible for more than 85% of Phytophthora root rot on processing tomatoes (1). There are no commercial tomato cultivars resistant to Phytophthora root rot. Management of the disease has been limited to fungicide applications to soil, seed, preplant root-dip treatments, and cultural practices (1,6). Yet severe losses may occur when poor water management is practiced and during years with excessive late-season rainfall. Development of cultivars with resistance to Phytophthora root rot would be a valuable contribution to growers and processors. Progress, however, depends on the availability of an effective technique to identify resistant germ plasm and progeny at the seedling growth stage. This paper describes a rapid, simple, and reliable greenhouse method to evaluate resistance to Phytophthora root rot in processing tomatoes.

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MATERIALS AND METHODS

Several preliminary experiments were conducted with known susceptible cultivars to establish appropriate zoospore concentrations. Only the adopted methods are described.

Isolates and inoculum preparation. An isolate from each of *P. capsici* and *P. parasitica* was obtained from naturally infected tomato plants in the Sacramento Valley of California. The isolates were pathogenic on tomato and had morphological and cultural characteristics consistent with those reported for *P. capsici* and *P. parasitica* (8,9). The isolates were maintained on potato-dextrose agar slants at 10 C and were increased on V-8 agar (5) for each experiment. Inoculum was prepared by transferring 25 disks of mycelium plus agar (10 mm in diameter) from 4- to 5-day-old cultures grown at room temperature (25 ± 2 C) to sterile plastic plates (110 × 110 × 30 mm) containing 100 ml of sterile distilled water. The plastic plates were then incubated at room temperature (25 ± 2 C) for 48 hr or until sufficient numbers of zoosporangia were produced (but no longer than 72 hr). Zoospore liberation was induced by refrigeration at 10 C for 45–60 min. Zoosporangia discharge occurred within 30 min after the plates were returned to room temperature (25 ± 2 C). The resultant zoospore suspension was decanted through two layers of cheesecloth, and zoospores were quantified with a hemacytometer. Required zoospore concentrations were made with sterile water.

Plant material. Four cultivars and one accession (LA 1312, supplied by C. M. Rick, Department of Vegetable Crops, University of California, Davis) were selected for assessment of disease reaction. The cultivars Early Pak 7 and VF 6203, susceptible to Phytophthora root rot in the field, were used as susceptible genotypes. The accession LA 1312 and the cultivars CX 8303 and CX 8201, which showed varying degrees of resistance under field conditions (S. J. Warnock, *personal communication*), were used as resistant genotypes.

Seedling preparation and inoculation procedure. Seeds of each genotype were planted separately 1 cm deep in plastic speedling trays containing a 1:2:2 (v/v) steam-sterilized mixture of soil, sand, and peat moss. Each speedling tray (50 × 30 cm) had 51 cavities (45 mm in diameter) divided in six rows (three rows of eight and three rows of nine cavities), and each row was seeded with seeds from a different genotype. First-leaf-stage seedlings were thinned to two per cavity and inoculated by placing the speedling trays in metal trays (39 × 55 × 9 cm) containing 1 L of the desired zoospore concentration. Inoculum concentrations were 1×10^3 and 3×10^3 motile zoospores per milliliter for *P. capsici* and *P. parasitica*, respectively. The tomato seedlings were kept in the zoospore suspension for 2–3 hr to ensure adequate time for zoospore encystment and attachment (4). Tomato seedlings similarly prepared, but kept in water, were used as controls. There were five speedling trays per *Phytophthora* sp. tested, each tray serving as a replicate. All seedlings were maintained on a greenhouse bench in a completely randomized block

design and watered as required. The temperature in the greenhouse ranged from 15 to 30 C. Three, 7, and 10 days after inoculation, the seedlings from each genotype were examined for post-emergence damping-off and root infection. After percent stand was recorded, the seedlings were uprooted and visually rated on a disease severity index (DSI) of 0–4, where 0 = no obvious disease symptoms, 1 = water-soaked brown lesions on taproot and/or lateral roots but no rot development, 2 = roots rotted but secondary root regeneration present, 3 = roots rotted and no secondary root regeneration present, and 4 = seedling killed. Percent stand and the DSI were then used to compare the responses of genotypes to *P. capsici* and *P. parasitica*. The entire experiment was repeated five times.

Correlation between greenhouse and field tests. Cultivars CX 8303 and VF 6203 were used to compare greenhouse experiments and field trials. The cultivars were planted in a randomized complete block design in a field with a known history of Phytophthora root rot. The percentages of plants with Phytophthora root rot obtained from field plots were used to calculate the coefficient of linear correlation between greenhouse and field tests.

RESULTS AND DISCUSSION

Symptomatology. Water-soaked brown lesions were visible on taproots and lateral roots of affected seedlings within 3 days. Postemergence damping-off occurred 3–4 days after inoculation and continued to develop for 7 days after inoculation. Generally, stems of affected seedlings turned dark and shriveled at the soil level and often had a completely decayed root system. However, in spite of a rotted taproot, most seedlings from genotypes with field resistance to Phytophthora root rot continued to grow and maintain healthy tops by regenerating an extensive number of secondary roots. Resistance to Phytophthora root rot was expressed as the ability of the genotype to regenerate secondary roots and was probably conditioned by multiple characters.

Infection of tomato seedlings by *P. capsici* or *P. parasitica* was confirmed by reisolating the fungus from symptomatic tissue and comparing it with original culture. All control plants had an extensive root system free of Phytophthora root rot.

Inoculation tests. Observations 3 days after inoculation did not permit consistent separation of susceptible and resistant genotypes. Differentiation was most successful when seedlings were observed 7 days after inoculation. Ten days after inoculation, a high percentage of resistant genotypes showed root rot symptoms, rendering it difficult to differentiate them from the susceptible genotypes. This is probably due to secondary infection through the production of zoosporangia and zoospores in each experimental pot as was observed with *P. cinnamomi* Rands on seedlings of Fraser fir (*Abies fraseri* (Pursh) Poir.) (3).

Table 1. Reactions of five tomato genotypes to *Phytophthora parasitica* and *P. capsici* 7 days after inoculation

Genotypes	*P. parasitica* DSI[x]	*P. parasitica* Stand (%)	*P. parasitica* Host reaction[y]	*P. capsici* DSI	*P. capsici* Stand (%)	*P. capsici* Host reaction
Early Pak 7	3.9 a[z]	11.0 a	S	3.9 a	6.8 a	S
VF 6203	3.4 a	29.4 a	S	3.8 a	12.8 a	S
CX 8303	2.2 b	55.1 b	MR	3.5 a	20.9 a	S
CX 8201	1.9 b	59.3 b	MR	3.6 a	19.3 a	S
LA 1312	2.3 b	51.0 b	MR	3.8 a	16.6 a	S

[x] Disease severity index determined on a scale of 0–4, where 0 = no visible symptoms, 1 = brown lesions on taproot and/or lateral roots but no rot development, 2 = roots rotted but secondary root regeneration present, 3 = roots rotted and no secondary root regeneration, and 4 = seedling killed.

[y] S = susceptible, MR = moderately resistant.

[z] Means in the same column followed by the same letter are not statistically different ($P = 0.01$) according to Duncan's multiple range test. The means are the average of five experiments, five replicates per experiment, 16 seedlings per replicate.

In all five experiments, the genotypes ranked in the same order of response to Phytophthora root rot caused by *P. parasitica*. Early Pak 7 and VF 6203 were the most susceptible cultivars, with a mean DSI of 3.9 and 3.4, respectively, on a scale of 0–4 (Table 1). LA 1312, CX 8303, and CX 8201 were the most resistant genotypes, with a mean DSI ranging from 1.9 to 2.3 (Table 1); the percent resistance to *P. parasitica* observed with each of these three genotypes was significantly different ($P = 0.01$) from that observed for Early Pak 7 or VF 6203. The genotypes LA 1312, CX 8303, and CX 8201 did not differ significantly ($P = 0.05$) among themselves for resistance to *P. parasitica* (Table 1). On the basis of these data, the reactions of the five genotypes to *P. parasitica* can be classified into two classes: moderately resistant (LA 1312, CX 8303, and CX 8201) and susceptible (Early Pak 7 and VF 6203). The five genotypes tested were equally susceptible to *P. capsici* (Table 1).

Correlation between greenhouse and field tests. Responses of cultivars CX 8303 and VF 6203 to Phytophthora root rot under field conditions were similar to those observed in greenhouse experiments. Cultivar VF 6203 remained highly susceptible to Phytophthora root rot. Cultivar CX 8303, which was moderately resistant at seedling stage, maintained this resistance. The mean percentage of plants with Phytophthora root rot symptoms, when rated at harvest, was 22.5 and 60.7 for CX 8303 and VF 6203, respectively. There was a significant ($P = 0.05$) correlation ($r = 0.77$) between greenhouse experiments and field tests. Thus, the technique described in this paper can be used to identify Phytophthora root rot-resistant tomato lines in the seedling stage. The technique is simple and rapid and permits the assessment of a large number of experimental lines and selection for resistance to Phytophthora root rot. Similar techniques have been reported to screen tobacco (*Nicotiana tabacum* L.) for resistance to *P. parasitica* var. *nicotianae* (Breda de Haan) Tucker (7) and peanut (*Arachis hypogaea* L.) for resistance to *Pythium myriotylum* Drechs. (2).

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