Inoculation Technique to Screen for Bacterial Speck Resistance of Tomatoes

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ABSTRACT

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Seedlings of susceptible tomato cultivars, inoculated by dipping the cotyledons in a suspension of *Pseudomonas syringae* pv. *tomato,* were killed within 12 days. However, the resistant cultivar Ont. 7710 exhibited only cotyledonary lesions and was not killed. These lesions resulted in slight stunting of 12% of the plants 18 days after inoculation, and the rest were healthy. This technique can be used for the automatic elimination of susceptibles in a segregating population.

Bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye & Wilkie is reported to be of increasing commercial importance in the United States and other countries (2–6). Pitblado and Kerr (3) reported that the cultivar Ont. 7710 has exhibited resistance to bacterial speck under natural and artificial inoculations. The artificial inoculation was done by spraying a bacterial suspension of *P. syringae* pv. *tomato* onto plants 10–20 cm tall. They were incubated under high relative humidity (RH) at 25 C for 6 days. Leaves of the resistant cultivar, Ont. 7710, were free of bacterial speck lesions and hence were considered to be resistant to bacterial speck. Bashan et al (1) used plants at the three-leaf stage for inoculation studies. They reported that the disease developed mainly in the plants incubated under mist after inoculation and that wounding by Carborundum increased the number of lesions per leaflet.

Our study was undertaken to develop a simpler and more efficient method of inoculation.

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MATERIALS AND METHODS

P. syringae pv. *tomato* was isolated from a naturally infected tomato field in Bowling Green during the 1979 growing season. Inoculum was made by washing 2-day-old nutrient agar cultures with distilled water. The absorbance of the

Table 1. Response of susceptible and resistant tomato seedlings to *Pseudomonas syringae* pv. *tomato* inoculation at 21 C/12.8 C (day/night) and 14-hr photoperiod

	Percentage of seedlings 12 days after incubation					
	Lesions on cotyledons only		Dead seedlings		Healthy seedlings	
Cultivars[a]	Inoc.	Not inoc.	Inoc.	Not inoc.	Inoc.	Not inoc.
Chico III	0	4	100	0	0	96
C-28	0	0	100	0	0	100
H2653	0	2	100	0	0	98
Ont. 7710	68	0	0	0	32	100

[a] Chico III, C-28, and H2653 are susceptible cultivars; Ont. 7710 is resistant.

suspension was adjusted to 5% transmittance at 650 nm using a spectrophotometer (Spectronic 20, Bausch & Lomb), giving a concentration of 7×10^8 colony-forming units per milliliter. Ten-day-old tomato seedlings at the cotyledonary stage were uprooted from the sand medium used for germination, and the aerial parts were dipped in the inoculum for 15 min. Seedlings dipped in distilled water served as controls. The plants were transplanted to presterilized moist soil, watered after 3 hr, and transferred to a growth chamber set at 21 C/12.8 C (day/night) and a 14-hr photoperiod. No special efforts were taken to maintain high RH during the incubation periods other than watering the plants twice a day.

Three susceptible tomato cultivars, Chico III, C-28, and H2653, were used. The only resistant cultivar, Ont. 7710, was used because of its high level of resistance, which originated from the cultivar Farthest North (3). The experiment was replicated four times with 12 plants; cultivars replicated in a split-plot design were the main plot and inoculation was the split plot. Although disease symptoms were evident within 4 days, readings were taken on the 12th day after inoculation.

RESULTS

The seedlings of the three susceptible cultivars were all killed within 12 days after inoculation (Table 1). Necrosis resulted from severe infection of the terminal bud and cotyledons. Black irregular spots spread rapidly, desiccating the cotyledons and stem and causing the seedlings to collapse. However, parts below the soil level (hypocotyl and roots) remained turgid without lesions. This suggests that the plants were killed from aerial rather than root infection. Ont. 7710 (the resistant cultivar) was not killed, but 68% of the seedlings showed cotyledonary lesions. These lesions were restricted and did not spread as in the case of the susceptibles. After 18 days, 12% of these plants exhibited slight stunting compared with the uninoculated plants, but the rest showed normal growth. The uninoculated control seedlings showed one or two lesions on the cotyledons in 4% of Chico III and 2% of H2653, possibly because of secondary spread from the inoculated plants. These lesions did not cause necrosis or stunting of the plants.

The same trend was observed for susceptible and resistant reaction when the experiment was repeated under greenhouse conditions at an average temperature of 18.8 C.

DISCUSSION

Necrosis of susceptible seedlings when inoculated with *P. syringae* pv. *tomato* has not been reported earlier. Necrotic reaction of susceptible seedlings to *P. syringae* pv. *tomato* makes this screening technique very efficient. In a conventional spray technique, plants at the three- to four-leaf stage must be kept at a high RH for 4–6 days for lesion development; susceptible plants must then be identified and manually removed to complete the screening. However, this dipping technique automatically eliminated the susceptibles from a segregating population, leaving only healthy plants for further selections in a breeding program.

This inoculation technique is also very simple. Many plant breeders use transplants for their breeding work. The uprooting of the seedlings for transplant production is a common practice, and speck inoculation can be combined with that operation without additional labor. The technique can also be combined with the conventional root-dip technique to screen for Verticillium wilt, Fusarium wilt, and bacterial canker resistance to achieve multiple screening in one operation.

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