

Mass screening for root rot resistance

P. capsici isolates and inoculum preparation. Three virulent isolates each from the A1 and A2 mating types of *P. capsici* were used in the mass screening and subsequent inoculation tests (Table 1). A mixture of zoospores from these isolates were used in inoculating the test plants. The zoospores were produced aseptically by transferring 10 agar plugs from the advancing portion of 5-day-old cultures (25 °C, under dark condition) of *P. capsici* in 5% (v/v) clarified V8 juice agar (Kuhajek et al., 2002) to 100 x 15 mm petri dishes (ca. 12 plates/isolate) and 10 ml of clarified V8 juice were added thereafter. After 24 h of incubation at 25 °C under dark condition, the V8 juice in each plate was replaced with 10 ml sterile mineral salt solution (MSS) (Kuhajek et al., 2002) and incubated at 20 °C, 30 cm under two fluorescent lights (cool white, 20 W, 25 °C, 35 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 24 h. The MSS from each plate were then replaced with the same volume of fresh MSS and allowed to incubate for three more days.

Zoospores from each isolate were harvested separately. To harvest the zoospores, the MSS was removed from each plate and then washed twice with 10 ml of sterile distilled water. After the second washing, ten ml of sterile distilled water was added to each plate and placed in the refrigerator (1.3 °C) for 45 min. The plates were then placed on top of a laboratory bench and monitored for zoospore release. The zoospore suspension from each petri dishes were then transferred very slowly to a 250-ml graduated cylinder and left undisturbed for five min. The upper 50 ml of the zoospore suspension was pipetted out and transferred to a 50-ml conical centrifuge. The tube was then inverted gently 2-3 times to distribute the zoospores in the suspension. One ml of the suspension was transferred to a 2-ml microcentrifuge tube with flat cap and vortexed for 90 sec to encyst the zoospores. The zoospore concentration was determined by using a hemacytometer and standardized at 5,000; 2,000 and 40,000 zoospores per ml for foliar (Alcantara and Bosland, 1994), root (Bosland and Lindsey, 1991), and stem (Sy et al., 2005) inoculations, respectively. Equal volumes of zoospore suspensions were then combined together.

Test plant preparation. Seeds from each accession were sown in plastic cells of a multipot bedding plant container (Com-Pack D806, Hummert International, St. Louis, MO). Each cell measured 6 cm x 4 cm x 5.5 cm and contained Redi Earth plug and seedling mix (Sun Gro, Bellevue, WA). A total of 6-12 seeds were planted for each accession at the rate of two seeds per cell. The cells containing the seeds were then placed in 52.3 cm x 25.9 cm x 6.1 cm plastic trays with drainage holes (F1020 flats, Hummert International, St. Louis, MO).

Inoculation. The root inoculation was done according to a previously-described procedure (Bosland and Lindsey, 1991). Briefly, two hours prior to inoculation the trays containing 14-day-old pepper seedlings were placed in water-filled trays to saturate the roots. A five ml zoospore suspension was then delivered to each cell by using an automatic dispenser (Finpipette, Vantaa, Finland) resulting in a final concentration of 10,000 zoospores per cell. The saturated condition was maintained for another 48 hrs and disease evaluation was performed 14 days after inoculation. The plants were

evaluated based on a 10-point scale (Bosland and Lindsey, 1991): 0 = no response, vigorous, healthy; 3 = brown roots, slight stunting, very small lesions on stems; 5 = brown roots, small lesions on stems, lower leaves wilted, stunted plants; 7 = brown roots, large lesions on stems, girdling, whole plant wilted, and stunted; 9 = death. Even numbers corresponded to intermediate response. A disease index value of 2 or less was considered resistant, and a value greater than 2 was susceptible.

Table 1. Isolates of *P. capsici* used for the mass screening of *Capsicum annum* accessions.

Isolate	Mating type	Source
PC-F6S1 A1	Bell pepper	(Tift County, GA)
PC-F6S3 A1	Bell pepper	(Tift County, GA)
PC-1A1 A1	Squash	(Tift County, GA)
PC-F1R3 A2	Bell pepper	(Tift County, GA)
PC-F1R6 A2	Bell pepper	(Tift County, GA)
PC-F1S12 A2	Bell pepper	(Tift County, GA)

*Root rot severity scale (Bosland and Lindsey, 1991):

0 = no response, vigorous, healthy (as in uninoculated control)

1 = slight root darkening, vigorous, healthy

3 = brown roots, slight stunting, very small lesions on stems or small water-soaked lesions at base of stem, browning of primary root

5 = brown roots, small lesions on stems, lower leaves wilted, stunted plants, partial girdling at stem base

7 = brown roots, large lesions on stems, girdling, whole plant wilted, and stunted

9 = dead plants

≤2 = R

>2 = S

(Bosland, P. W. and D. L. Lindsey 1991. A seedling screen for *Phytophthora* root rot of pepper, *Capsicum annum*. Plant Disease. 75:1048-1050.)