Techniques

A Growth Chamber Test For Measuring Phytophthora Root Rot Tolerance in Soybean Seedlings

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ABSTRACT


Soybean cultivars with high, moderate, and low tolerance to root rot caused by Phytophthora megasperma f. sp. glycinea, were grown in a growth chamber for 7 days, and then their taproots were inoculated with mycelial suspension. Six days later, elongation of the first internode and extent of tissue colonization in the hypocotyl were measured. The first internodes of the moderate- and low-tolerant lines were significantly shorter than those of uninoculated seedlings, and there were significant differences in hypocotyl tissue colonization among the three cultivars. Shortening of the first internode was not a reliable predictor of hypocotyl tissue colonization. Nonuniformity of root rot indicated that heterogeneity for tolerance may exist within cultivars, and high- or low-tolerant components can be selected for further evaluation after this nonlethal tolerance test.

Additional key words: Glycine max, resistance.

Tolerance of soybean (Glycine max [L.] Merr.) to root rot, incited by Phytophthora megasperma f. sp. glycinea Kuan and Erwin, (hereafter designated P. megasperma), is now well documented from studies in the field (1,9,11), greenhouse (11), and laboratory (2,9). In general, soybean cultivars can be classified along a continuum from high to low tolerance to P. megasperma. High-tolerant lines show little stunting and/or yield loss when roots are infected, whereas low-tolerant lines have severe plant or yield loss. Incorporation of race-specific resistance into susceptible cultivars has only a minor influence on the level of tolerance (12).

The potential for increasing tolerance in breeding lines has recently been investigated, and the genetics of this tolerance mechanism appears to be quantitative (10). Further, tolerance to P. megasperma is race nonspecific, and production of glyceollin or other fungitoxic compounds in infected roots does not account for it (5,6).

Selecting for tolerance to P. megasperma in the field requires large amounts of space and time and the availability of a field heavily infested with races virulent to all soybean lines being tested. Additional irrigation is often necessary to ensure an adequate amount of water at critical infection periods. A growth chamber test with zoospores and continually flooded soil conditions has been demonstrated (2). However, inoculum density must be controlled to accurately select tolerance levels. A laboratory procedure (9), based on cotyledon inoculation, requires less time and space, but expertise is necessary to produce zoospores and

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inoculate the cotyledons of the plants. There is a need for a
tolerance test analogous to the standard hypocotyl resistance test.

The purpose of this work was to devise a nonlethal growth
chamber test that would allow for routine screening for root
tolerance to *P. megasperma* and retention of plants for
further study.

**MATERIALS AND METHODS**

Stock cultures of race 4 of *P. megasperma* were maintained at 10
C on V-8 juice agar. To preserve isolate aggressiveness, cultures
were periodically inoculated into susceptible, low-tolerant
soybeans (7) and reisolated from rotted hypocotyls. As described
previously (6), ten-day-old cultures of *P. megasperma* on V-8 juice
agar were used for inoculum. Seeds of the cultivars Voris 295,
Sloan, and OX 20-8 (high-, moderate-, and low-tolerance, respectively)
were germinated in damp vermiculite in plastic pots
with bottom drainage in a growth chamber with 14-hr daylength
(21 klux) at 25 C. Tolerance levels of these cultivars were
determined previously by using standard field and greenhouse
methods (11). Three days after planting, the pots were watered
to excess with 25% Hoagland’s nutrient solution. On day 6, the plants
had upright cotyledons, but their primary leaves were not yet
expanding. Lateral roots had developed along the taproot from
the soil line downward to about 6 cm. Six-day-old plants were gently
lifted from the vermiculite and the roots were rinsed with tepid
water. Seedlings were arranged on slant boards (3,4,8), modified by
the use of polyester greenhouse wicking material as absorbent
backing, rather than perlite-filled bags. Boards with plants, were
irrigated with 50 ml of nutrient and maintained standing in 25% nutrient solution to a depth of 10 cm and returned to the same
growth chamber for inoculation 24 hr later. Nutrient solution was
replaced every 2 days.

On day 7 the polyester cloth covering the roots was pulled back
and a 1-cm scrape-wound was made into the stele with a single-edge
razor blade 2-cm below the hypocotyl-root junction in the area of
developing lateral roots. Two drops of inoculum from a syringe
were placed directly on the wound. Control plants received only a
scrape-wound and V-8 agar suspension. Roots were again covered
with polyester cloth and the slant-boards, maintained standing in
10-cm of nutrient solution, were returned to the growth chamber.
At inoculation, the root tips had not yet reached the level of the
nutrient solution.

Tolerance evaluation was made 6 days after inoculation. At that
time, length of the first internode was measured. Preliminary
experiments indicated that control-wounded and control-unwounded plants of the three cultivars did not significantly differ
in first-internode length. Thus, the use of control-unwounded
plants was discontinued. Taproots and hypocotyls were split
to expose rotted interior tissues and the extent of tissue colonization
upward through the taproot and into the hypocotyl was measured.

Immediately after colonization measurements, plants were
retained for propagation in the following way. The hypocotyl and

![Table 1. Comparison of first-internode length and amount of upward tissue colonization in soybean cultivars wound-inoculated with *Phytophthora megasperma* f. sp. *glycinea* (Pmg) on day 7 and measured on day 13.](image)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Disease tolerance</th>
<th>Tissue colonization (mm)</th>
<th>Pmg</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>OX 20-8</td>
<td>low</td>
<td>30^a</td>
<td>44</td>
<td>75</td>
</tr>
<tr>
<td>Sloan</td>
<td>moderate</td>
<td>21</td>
<td>52</td>
<td>75</td>
</tr>
<tr>
<td>Voris 295</td>
<td>high</td>
<td>7</td>
<td>75</td>
<td>75</td>
</tr>
</tbody>
</table>

LSD at *P* = 0.05 2.01 5.51
LSD at *P* = 0.01 3.68 7.84

^aTap roots were inoculated with a suspension of virulent race 4 of Pmg in the area of developing lateral roots approximately 20 mm below the hypocotyl-root junction.

^Values shown are the mean of three experiments *×* two replications for a total of 400 plants per cultivar tested.

**RESULTS AND DISCUSSION**

Three cultivars of soybean, susceptible to race 4 of *P. megasperma*,
were taproot inoculated at 7 days and incubated for 6
days on slant boards in a growth chamber. First-internode length
and extent of tissue colonization in the taproot were measured
(Table 1). The first internodes of all three cultivars had essentially
the same length as the first internode in the control plants.
Internode length of the inoculated, high-tolerant cultivar (Voris 295)
did not differ significantly from the control, whereas the moderate-and low-tolerant cultivars (Sloan and OX 20-8) had
significantly shorter mean internode lengths (*P* = 0.01). Tissue
colonization was significantly different (*P* = 0.01) in the three
cultivars.

Linear regression analysis was performed by using the first-internode length as a predictor of tissue colonization. The
relationship was significant only for low-tolerant OX 20-8. The *r*²
values were low, (0.0–7.3%), adjusted for degrees of freedom
indicating that measurement of the first internode in inoculated
plants would not be a useful predictor of tolerance level.

Disease progress was not uniform within cultivars. A large
percentage of plants of the high-tolerant line had no, or only slight,
rotting the hypocotyl-root junction. The low-tolerant cultivar had
a high percentage of plants that had colonization well into the
hypocotyl and occasionally into the cotyledons. The nonuniformity of colonization for each line is depicted in the
histograms of Fig 1. We have seen this same general pattern of

![Figure 1 Percentage distribution of length of tissue colonization caused by *Phytophthora megasperma* f. sp. *glycinea* on 13-day-old soybeans. The uppermost portion of the inoculation zone is at 0. The approximate location of the hypocotyl-root junction is shown by the arrow. Each histogram taken from three experiments times two replications for a total of 400 plants.](image)

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nonuniform rotting in other soybean cultivars that we have investigated (6). Because the root was scrape-wounded before inoculation, we do not believe that plants without tissue colonization were escapes due to unsuccessful infection. Additionally, plants that had colonization only to the hypocotyl-root junction appeared to contain a mechanism that retarded the advance of the fungus, perhaps similar to that described by Tooley and Grau (9).

More than 90% of the severed tops and 80% of the severed cotyledons formed new roots or shoots, respectively, and these were grown to maturity. Less than 5% of the developing plants had disease symptoms and these were successfully treated with metalaxyl. This procedure establishes single-plant lines that can be used for further genetic and disease evaluation. We are now testing these lines to determine whether we have been able to select for high- and low-tolerant components within lines.

LITERATURE CITED