Expression of Disease Reaction Types in Soybean Callus from Resistant and Susceptible Plants

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ABSTRACT

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Conditions influencing expression of disease reaction types were examined in calluses derived from soybean plants resistant (cultivar Cutler 71) or susceptible (cultivar Cutler) to race 1 of *Phytophthora megasperma* var. *sojae* (Pms). Cutler 71 calluses were colonized less than those of Cutler when both were grown on medium containing 6 or 10 mg of 2,4-D/L and incubated at 16 or 20 C prior to and following inoculation with Pms zoospores. Differences between colonization rates of Cutler and Cutler 71 calluses were greater in callus sections 5 mm thick than in thicker or thinner sections. Differences in colonization rates remained high with inoculum doses varying from 50 to 1,000 zoospores per callus section. Sections of Cutler and Cutler 71 calluses 5 mm thick were colonized equally by race 3 of Pms which is pathogenic to plants of both cultivars. No combinations of incubation temperatures, 2,4-D concentrations, sizes of calluses, or numbers of zoospores used for inoculum resulted in Cutler 71 calluses with the nearly absolute resistance to race 1 of Pms found in whole plants of that cultivar.

Plant tissue culture can provide simplified model systems for studying host-pathogen relationships (6). Helgeson et al (2) reported that the single dominant gene which confers resistance to *Phytophthora parasitica* var. *nicotianae* in tobacco plants was expressed in tobacco pith callus cultures. Resistance was expressed, however, only when hormone (2,4-D) concentrations were adjusted to yield tight callus, and expression also was influenced by incubation temperatures and amounts of inoculum (3).

Monogenic resistance to *Phytophthora megasperma* Drechs. var. *sojae* A. A. Hildeb. (Pms) in soybean (*Glycine max* [L.] Merr.) plants is characterized by a hypersensitive reaction and production of the phytoalexin, glyceollin (7,10). This host-parasite system has been employed as a model in several investigations of disease resistance mechanisms (8).

The purpose of this study was to determine if a callus culture system might be developed which would allow genotypic expression of soybean cultivars resistant or susceptible to Pms. We report conditions which influence expression of these genotypes.

MATERIALS AND METHODS

Callus cultures were obtained from two near-isogenic soybean cultivars, Cutler and Cutler 71. Cutler plants are susceptible and Cutler 71 plants are resistant to Pms, race 1. Both cultivars are susceptible to Pms, race 3. Callus cultures were grown on the B5 medium of Gamborg et al (1) solidified by addition of 12 g agar per liter. Explants were 5-mm hypocotyl sections obtained from 5-day-old seedlings grown axenically in the dark at 28 C. Explants were placed on B5 medium in petri dishes and, after 4 wk, proliferating callus tissue was isolated and transferred to fresh B5 medium. Subsequent subcultures were made by cutting a callus, which had grown in 3 wk to about 2.5 cm in diameter, into five pieces which were transferred to a single dish of fresh B5 medium. Tissues were selected for subculture only on the basis of size and age. All cultures were incubated at 28 C in the dark.

Cultures of Pms, races 1 and 3, were maintained on V8 agar at 24 C in the dark and zoospores were obtained by the method of Ho and Hickman (5). Zoospores were counted on a 0.1-ml Palmer-Maloney counting chamber and diluted with sterile distilled water; the desired numbers of zoospores were delivered to calluses in single drops from a sterile pipet.

Soybean calluses, 3–4 wk old, were transferred to fresh B5 medium in Pyrex jars (100 mm × 80 mm) with fitted lids 3 days prior to inoculation. Each jar contained five calluses, all from the same cultivar. Jars were coded and arranged randomly in a laminar-flow hood and each callus was inoculated with Pms zoospores. Zoospores also were placed on V8 agar to check for contamination.

Inoculated calluses were rated numerically according to a visual determination of amounts of colonization by Pms. Numerical ratings were: 0 = no mycelial growth; 1 = mycelium colonizing less than half of the callus; 2 = mycelium colonizing more than half, but not all, of the callus, and 3 = mycelium colonizing the entire callus. Ratings were begun 4 days after inoculation and were repeated daily for 8 days or until mycelial growth over the agar medium made it difficult to rate an individual callus.

RESULTS

Incubation temperature. Calluses of Cutler and Cutler 71 were subcultured three times on B5 medium supplemented with 6 mg of 2,4-D/L. Thirty calluses of each cultivar were incubated at 16, 20, 24, or 28 C for 24 hr, inoculated with approximately 10 zoospores of Pms race 1 per callus, and incubated for 7 days. Calluses of both cultivars, incubated at 24 or 28 C, were colonized equally but, at 16 or 20 C, those of Cutler 71 were colonized less than those of Cutler (Fig. 1); maximum differences in colonization occurred on calluses incubated at 16 C. This incubation temperature, therefore, was used in succeeding experiments.

Concentrations of 2,4-D. Thirty calluses of each cultivar were subcultured three times on B5 medium containing 2, 6, 10, or 20 mg of 2,4-D/L and incubated at 16 C for 24 hr. Each callus was inoculated with about 10 zoospores of Pms, race 1, and incubated at 16 C. Colonization of Cutler 71 calluses was significantly less than that of Cutler when they were grown on media with 6 or 10 mg of 2,4-D/L (Fig. 2); calluses grown at these concentrations were tighter and less friable than those at other concentrations. Maximum differences in colonization occurred in calluses grown on medium with 6 mg of 2,4-D/L and, therefore, that concentration was used in succeeding experiments.

Callus sizes and inoculum concentrations. Cylinders were cut with a sterile 16-mm cork borer from centers of 3-wk-old calluses of both cultivars. Cylinders were sliced perpendicular to the long axis into sections 1.0, 2.5, 5.0, and 10-mm thick which were placed in Pyrex jars containing fresh B5 medium containing 6 mg of 2,4-D/L

and incubated 4 days at 28 C. Sections were incubated 24 hr at 16 C and 20 sections of each thickness from each cultivar were inoculated with about 10 zoospores of Pms, race 1, and incubated at 16 C. Cutler 71 callus sections 5.0-mm thick were colonized less than similar Cutler calluses, but 1.0- and 2.5-mm sections from both cultivars were rapidly colonized and their mean colonization ratings were similar to those of 5-mm callus sections of Cutler (Fig. 3). Ten-millimeter sections of callus from both cultivars were colonized slowly and their mean colonization ratings were similar to those of 5-mm callus sections of Cutler 71.

The results with smaller Cutler 71 callus sections suggested that resistance could be overcome by increasing zoospore:callus ratios. To test that hypothesis, 5-mm callus sections of both cultivars each were inoculated with 50, 100, 500, or 1,000 zoospores of Pms, race 1. With increasing amounts of inoculum, colonization increased on 5-mm callus sections of both cultivars, but 5-mm callus sections from Cutler 71 always were colonized less rapidly. Thus, resistance was expressed in 5-mm sections of Cutler 71 callus even when inoculated with about 1,000 zoospores each, the highest level tested.

Pathogen races. Thirty, 5-mm callus sections from each cultivar

were each inoculated with about 10 zoospores of Pms, race 3, which is pathogenic to plants of both cultivars. Colonization of sections of both cultivars was similar to that of Cutler sections inoculated with 10 zoospores of Pms, race 1 (Fig. 4).

DISCUSSION

Cutler and Cutler 71 calluses were colonized equally by Pms, race 1, under many of the conditions tested but, under certain conditions, Cutler 71 calluses were colonized less. Under no

Fig. 3. Fungal colonization ratings of **A,** 1.0-mm; **B,** 2.5-mm; **C,** 5.0-mm; and **D,** 10-mm thick callus sections from soybean cultivars Cutler (▲—▲) and Cutler 71 (●—●) inoculated with zoospores of *Phytophthora megasperma* var. *sojae,* race 1, as a function of time in days. Mean ratings on the same days with the same letter are not significantly different, *P* = 0.05. Comparisons are between cultivars, and between section thicknesses within cultivars.
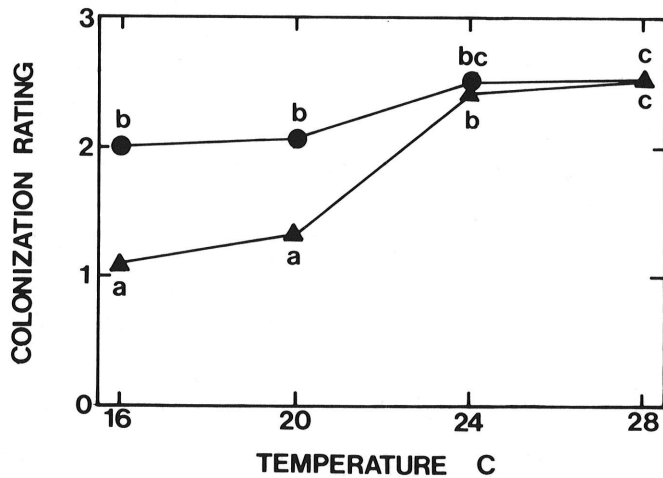

Fig. 1. Colonization of Cutler (●—●) and Cutler 71 (▲—▲) soybean calluses, incubated at various temperatures, 7 days after inoculation with *Phytophthora megasperma* var. *sojae,* race 1. Mean ratings with the same letter are not significantly different, *P*= 0.05.
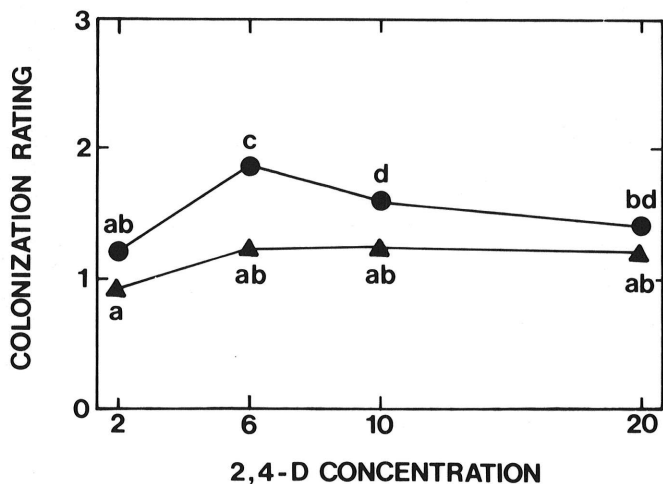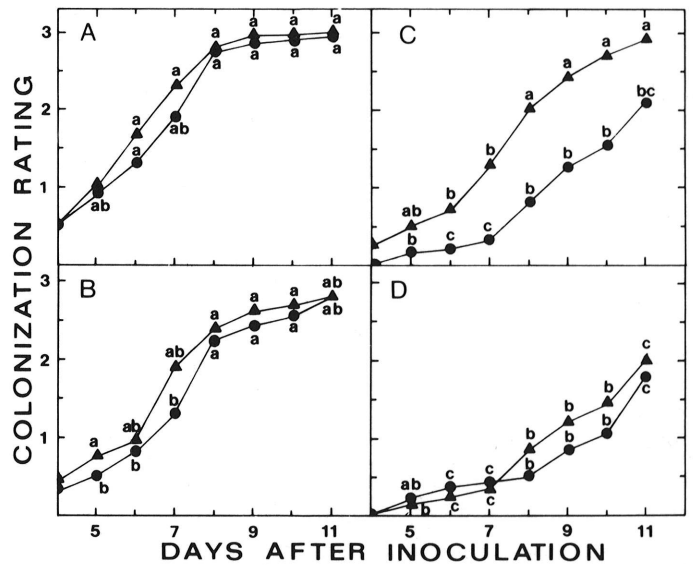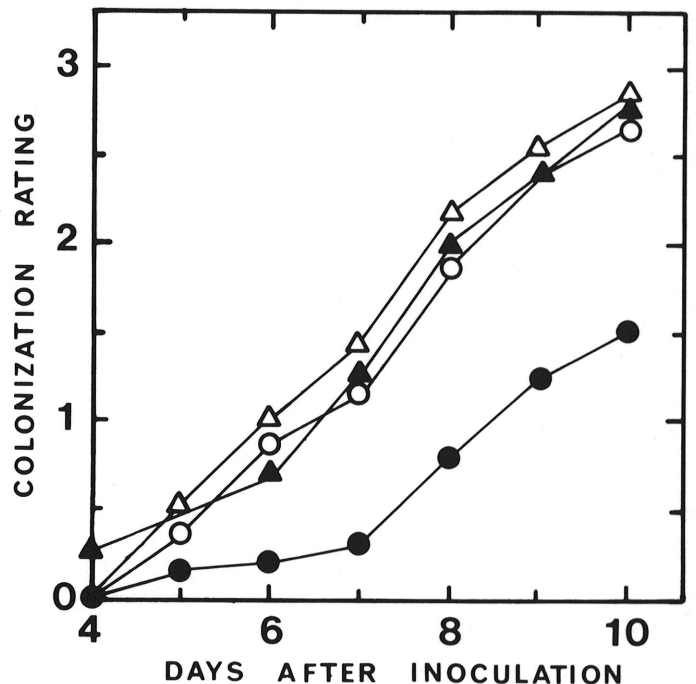

Fig. 2. Fungal colonization ratings of calluses from soybean cultivars Cutler (●—●) and Cutler 71 (▲—▲) grown on various concentrations (mg/L) of 2,4-D, 8 days after inoculation with *Phytophthora megasperma* var *sojae;* race 1. Mean ratings with the same letter are not significantly different, *P*= 0.05.

Fig. 4. Fungal colonization rates of 5-mm-thick callus sections of soybean cultivars Cutler (▲—▲) and Cutler 71 (●—●) by race 1 or of Cutler (△—△) and Cutler 71 (o—o) by race 3 of *Phytophthora megasperma* var. *sojae.*

combination of conditions were Cutler 71 calluses colonized more than Cutler calluses. This difference in colonization was attributed to expression of resistant and susceptible genotypes in soybean calluses.

Slow colonization of both Cutler and Cutler 71 calluses at 16 C by *Phytophthora megasperma* var. *sojae,* race 1, (Fig. 1), may be due, in part, to decreased growth of the fungus at that temperature (4). The effect was greater on Cutler 71, however, indicating that temperature alone was not responsible but rather that the decreased colonization was caused by a resistance mechanism in the calluses. At temperatures above 20 C, Pms, race 1, apparently overcame this resistance.

Although both cultivars generally supported more fungal growth at 2,4-D concentrations of 6 or 10 mg/L (Fig. 2) than at lower or higher concentrations, colonization was much less in Cutler 71 calluses. Ability of Cutler 71 calluses to exclude Pms, race 1, possibly resulted from greater cell density in calluses grown at these 2,4-D concentrations. Enhanced resistance in tighter calluses also was observed in the tobacco/*P. parasitica* system (3). A collective response by many cells appears to be required for expression of resistance. This theory is supported by research with the potato/*P. infestans* system in which uninfected adjacent cells were actively involved in the resistant response (9).

The concept of a minimum number of cells necessary for expression of resistance is further supported by loss of resistance in thin (1.0 or 2.5-mm) sections of Cutler 71 callus which otherwise should have expressed resistance (Fig. 3). Loss of resistance perhaps was due to reduction in the already limited number of cells that could respond collectively to infection. Since 5-mm sections of Cutler 71 callus were more resistant than Cutler even when inoculated with about 1,000 zoospores, loss of resistance in thin sections probably was not due to a competitive advantage given to the fungus because of higher fungus:callus ratios.

The apparent increase in resistance of both cultivars in 10-mm callus pieces (Fig. 3D) is a result of expressing the colonization ratings as percentages of each callus-piece colonized. Thus, large callus pieces have lower ratings than smaller callus pieces with equal amounts of colonization. Valid comparisons can be made only between similar callus-piece-sizes and not between different callus-piece-sizes of different cultivars.

Although differences in colonization indicate the occurrence of a resistant response in Cutler 71 calluses, no combinations of incubation temperatures, concentrations of 2,4-D, sizes of calluses, or numbers of zoospores used for inoculum resulted in the very high resistance found in whole plants. This may be a result of the inherent autonomy of callus cells which, even in tight callus, prevents collective participation necessary for absolute resistance. In addition, incomplete resistance may result because the conditions of cell-culture afford the pathogen advantages not found in whole plants. For instance, cells broken by rapid expansion of underlying dividing layers may provide nutrients which allow the pathogen to grow superficially on calluses. This may have been responsible for loss of resistance in thick (10-mm) sections (Fig. 3) which had upper layers of surface cells not included in preparation of thinner sections. The culture medium itself may,

in addition, provide nutrients and a carbon source for saprophytic growth of the fungus. Also, high humidity in culture vessels favors superficial growth that may in turn increase the parasitic ability of the pathogen. Apparently these advantages bias the normally balanced virulence/resistance scheme heavily in favor of the pathogen. By reducing some of these advantages, such as lowering incubation temperatures, and by promoting cellular cohesiveness, at least partial expression of genetically defined resistance is permitted in soybean calluses.

In contrast to the resistance of whole plants, there was no observable hypersensitive reaction in inoculated Cutler 71 calluses. This may be responsible for lack of absolute resistance, but the fact that partial resistance occurred without hypersensitivity may indicate a need to reevaluate the role of hypersensitivity in disease resistance in soybeans.

Resistant and susceptible reactions to Pms, races 1 and 3, were expressed with the same qualitative specificity in soybean calluses as in plants from which they were derived (Fig. 4). Qualitative similarity of disease reaction types between calluses and whole plants suggests that similar basic aspects of reaction types occur in calluses and whole plants. Callus cultures, therefore, may be useful for understanding resistance mechanisms at the cellular level.

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