

Annual Report of Progress
to the
MISSISSIPPI SOYBEAN PROMOTION BOARD
for 1999

PROJECT TITLE: Selection for Charcoal Rot Resistance in Soybean

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OBJECTIVES & SIGNIFICANT ACCOMPLISHMENTS:

1. Screened soybean seedling hypocotyl sections on shoot initiation = media containing 50% (v/v) autoclaved cell-free fungal culture filtrate = (CF-A) of *Macrophomina phaseolina* for regeneration of plants displaying = tolerance/resistance to charcoal rot (CR).

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Two soybean varieties, Tracey and Williams, were screened for = their responses on media containing cell-free, autoclaved *Macrophomina* = liquid culture filtrate (CF-A). Hypocotyl explants from both were placed = on shoot initiation media containing 50% CF-A. *Macrophomina* cultures were = initiated from cultures obtained from Dr. Sciumbato and Dr. Richard Baird. = Liquid fungal cultures were grown in fresh potato dextrose broth (PDB) = for 14 days then harvested by passing the cultures through cheesecloth, = then autoclaved. Sterile CF-A were added to double-strength (2X) soybean = shoot initiation media. To date, 548 Tracey (145 survived, 74% mortality) = and 567 Williams (202 survived, 64% mortality) hypocotyl explants were = screened via our tissue culture process. Great differences were noted = between explants grown on control media (no CF-A) and the selective media = (contained CF-A) [Figure 1 (Attachment 1)]. Some explants have survived = on media containing CF-A for up to 5 months. Others have died within 2 = months. Viable explants are being maintained on media containing 50% CF-A = and a small number of these are producing small shoots. Once large = enough, these shoots will be placed on rooting media to generate intact = soybean plants to generate seeds for future screenings.

Although it was believed that Tracey displayed some tolerance to = CR, it displayed a higher mortality rate and, in general, appeared more = susceptible than Williams [Figure 2 (Attachment 2)]. This small-scale = difference between varieties was noted on a much larger scale in Dr. = Sciumbato's screenings (33 varieties, described below).

Differences in explant responses within varieties were also noted =

when different CF-A preparations were used in screenings, even when initiated and prepared at the same time(s) [Figure 3 (Attachment 3)]. Macrophomina liquid cultures varied dramatically in color (pink, bright red, light and dark tea, dark charcoal gray...). They also, most likely, varied in the amount of CR toxin(s) present in the processed CF-A. This is most likely due to the lack of pure Macrophomina cultures, and the belief that they appear to mutate readily (Dr. Sciumbato, personal observation). What is lacking in our selection systems is a way to quantify how much CR toxin(s) we are using in our screenings. If a CR toxin could be purified and/or quantified, we would be able to standardize the amount of at least one CR toxin used in our screenings. This would create consistency between batches of CF-A utilized and should also yield more reliable results.

2. Screened seeds of numerous soybean genotypes during germination in/with water and in solutions containing different concentrations of CF-A for identification of seedlings displaying tolerance/resistance to CR. Screenings also determined each variety's overall sensitivity to predict its field response to CR.

Macrophomina cultures were isolated from diseased soybean plants and purified. Fungal cultures were grown in fresh PDB for 14 days and harvested. Liquid cultures were filtered through cheesecloth and autoclaved for 15 minutes to obtain CF-A.

Several different methods were evaluated to determine the effect of the CF-A on soybean seedlings. Seeds were germinated in test tubes, in vermiculate, and in rag dolls in the presence of the CF-A. The "rag doll" method was found to be the most reliable method.=20

Germination paper was soaked in the undiluted CF-A solution or in water (check) and 100 seed per replication were placed on the paper and folded into a "rag doll". Three replications each of the CF-A and water checks were used. The seed were then germinated at standard 70o F for one week. After germination, shoot and root lengths were determined. Each length was sorted into the following groups based on length: less than one inch (in.), 1-2 in., 2-3 in., 3-4 in., 4-5 in., 5-6 in., 6-7 in., and 7-8 in. Grouping the seedlings into several lengths based on individual root and shoot measurements is very labor intensive, but necessary to determine CR toxin effects on germination.=20

Limited equipment (ie. shakers & seed germinators) and autoclave outage restricted the number of cultivars tested. Thirty-three entries from the Mississippi Soybean Variety Trials have been evaluated. The other entries in the Trials are being run at this time and will be completed in 2-3 months. The results for the completed varieties are presented in the attached Tables [Attachments 4 (Table 1, shoot) and 5 (Table 2, root)]. The Factor column is the number of seedlings containing roots or shoots of a particular length after germination in CR toxin rag =

dolls divided by the number of control (germinated in water) seedlings = displaying the same length. A Factor of 1.0 should indicate the CR = toxin(s) had no effect on the numbers of seedlings displaying that = particular shoot or root length (same results as control). A Factor = greater than 1.0 indicated that greater numbers of seedlings exposed to CR = toxin(s) displayed that particular length. A Factor less than 1.0 = indicated that less seedlings exposed to CR toxin(s) were represented in = that particular length class compared to controls. Statistical analysis = including regression analysis and correlation coefficients will be = conducted to determine which statistical analysis will separate the = resistant, tolerant, and susceptible varieties. We will also be able to = determine if root or shoot elongation is the most important indicator of = CR toxin susceptibility. The CR toxin(s) had the greatest effect on shoot = elongation of the following varieties: FFR 665, DPX 8547, AG 4702 RR, WF = 480, Truman, USG 7499, and HBK 4890. Varieties that the CR toxin(s) had = the least amount of effect on were TVX 4589, EKXP 4900, HBK 4600, A 4402, = and TS 490. =20

We will have the major entries in the 1999 variety trial screened = before the 2000 planting season. This data will be posted on the = Internet. Among the varieties we have screened, large differences in shoot = and root elongation in the presence of the CR toxin(s) have been observed.

PUBLICATION:

Dan, Y., and N.A. Reichert. 1999. Soybean transformation and regeneration = methods. U.S. Patent No. 5,968,830, issued October 19.