THE EVALUATION OF HIGH TANNIN COTTON LINES FOR RESISTANCE TO *RHIZOCTONIA SOLANI* AND *PYTHIUM APHANIDERMATUM*

A Thesis

by

RAYMOND MATTHEW KENNETT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

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Major Subject: Plant Breeding

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Approved by:

Co-Chairs of Committee,	C. Wayne Smith
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ABSTRACT

The Evaluation of High Tannin Cotton Lines for Resistance to *Rhizoctonia solani* and *Pythium aphanidermatum.* (December 2009)

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Dr. Peggy Thaxton

Cotton seedling disease complex is caused by a number of pathogens inducing similar symptoms and can lead to thin, uneven stands that grow slowly and yield poorly. Preliminary work indicated that a set of high tannin cotton lines developed and released in 1989 by Texas A&M AgriLife Research, (Smith et al., 1990a, Smith et al., 1990b, Schuster et al., 1990) may possess increased resistance to disease. This evidence, along with additional studies that show a clear role of tannin in disease resistance, suggest that these high tannin cotton lines may prove useful in breeding for increased resistance to cotton seedling disease complex. High tannin cotton lines were screened for their resistance to *Rhizoctonia solani* and *Pythium aphanidermatum*. While no high tannin germplasm line was more resistant to R. solani than the resistant control, Tamcot SP 21, the potential for significant gains from selection was demonstrated. Fifteen high tannin lines expressed resistance to *P. aphanidermatum* equal to the resistant control, Tamcot Sphinx (El-Zik and Thaxton, 1996). This resistance was not shown to be correlated with tannin content, though it is still unclear whether or not this lack of correlation is real or due to limitations in measuring tannin in infected seedlings.

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1. INTRODUCTION AND REFERENCES

Cotton seedling disease complex (CSDC) is caused by a number of different pathogens, including *Rhizoctonia solani* (Kuhn), *Pythium sp*., *Fusarium sp*., *Thielviopsis basicola* and *Xanthomonas campestris* pv. *Malvacearum* (Pammel). These pathogens can cause a variety of symptoms including seed rot, pre-emergence damping-off, post-emergence damping-off, discolored roots, and dark lesions on the hypocotyl. Surviving plants often have weak, shallow root systems that leave plants susceptible to drought and various other stresses. This can lead to thin, uneven stands of slow growing plants that yield poorly. Seedling disease caused an estimated yield loss of 3.3% in 2002 in the United States, which corresponds to over $140,000,000 in lost farm income (Blasingame and Patel, 2003).

Of the various CSDC pathogens, it is thought that the two pathogens that cause the most damage are *R. solani* and *Pythium sp.*. *R. solani* is the asexual stage of a basidiomycete fungus, *Thanatephorus cucumeris*. It does not produce any asexual spores, or conidia, and only occasionally produces sexual spores. In nature, *R. solani* reproduces asexually and exists primarily as vegetative mycelium and or sclerotia (Ceresini, Shew and Cubeta, 1999). *R. solani* is the most common cause of post-emergence damping-off of cotton seedlings throughout the world (National Cotton Council, 2000). The pathogen causes a girdling lesion on the hypocotyl at or near soil level. Surviving plants are left weakened and often bare a canker from the lesion referred to as soreshin.

Pythium is an oomycete in the order *Peronosprales*. It tends to infect the seedling prior to or just after germination, although it is possible, though rare, for infection to occur in the hypocotyl at soil level. *Pythium* most often causes seed rot and pre-emergence damping off, though it can cause post-emergence damping-off, stunting, and chlorosis in later stages of plant

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development. Symptoms include watery, straw colored lesions and the rotting of root cortical tissue leaving the central vascular stele of the tap root intact. Oospores are the principle inoculum and also the life stage that survives unfavorable conditions (Agrios, 1997). Two of the more important *Pythium* species that cause seedling disease are *P. ultimum* (Trow) and *P. aphanidermatum* (Edson). *P. ultimum* tends to be a problem when conditions are cool while *P. aphanidermatum* is favored by warm temperatures. Both species grow best in moist soils.

Management practices designed to reduce losses to CSDC include rotation, planting only when warm, dry weather is forecasted, planting high quality seed, and treating seeds and/or soil with fungicides. Resistant cultivars are not available to manage CSDC, but this strategy could prove both economically and ecologically beneficial by reducing or eliminating the need for fungicide treatments. Since fungicide treatments usually are effective, for resistance to be a viable commercial strategy, it has to be both strong and reliable.

No cultivars have been released that are highly resistant to *Rhizoctonia* or *Pythium*. There have been several reports of low levels of resistance to both pathogens (Johnson and Palmer, 1985; Garber et al., 1991; Henard et al., 1997; Wang and Davis, 1997). Hefner (1968) reported one breeding line as having resistance to *R. solani*. Bush et al. (1978) tested 37 multi-adversity resistance (MAR) lines for resistance to CSDC and found significant differences among lines at moderate inoculum levels. Wallace et al. (1983) examined nine MAR lines and five MAR cultivars and found that three MAR lines were more resistant than the other lines tested. Despite all of this, a source of strong resistance to either pathogen has yet to be identified.

One possible source of resistance to CSDC could be cultivars with high levels of various plant defense compounds. Gossypol would be an obvious choice since it has been shown to

inhibit the growth of *Rhizoctonia*. Unfortunately concentrations in the seedling are too low to inhibit the pathogens (Puckhaber, 2002). While it has been shown that infection by *Trichoderma virens* can cause increased gossypol content and provide resistance, so far this strategy has had limited commercial use (Martin and Loper, 1999). Another possible class of plant defense compounds that could be used are condensed tannins.

Tannins are astringent, bitter-tasting plant polyphenols that bind and precipitate proteins. The term tannin originated from the leather industry and was used to describe substances that were capable of turning animal hide into leather; however, the term is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. Tannins have molecular weights ranging from 500 to over 3,000.

Tannins are considered to be the most important secondary plant compound involved in plant defense against insects and disease (Swain, 1979). Tannins have been shown to inhibit the growth of *Cladosporium caryigenum,* the causal organism of pecan scab, in vitro (Laird, 1990) and to be strongly correlated with grain mold resistance in sorghum (Menkir, 1996). Cotton cultivars released through the MAR program at Texas A&M University AgriLife Research, which were selected for resistance to several diseases, contain higher concentrations of tannins than most non-MAR lines with increased concentrations in successive MAR germplasm releases (Bell et al., 1988). This increase in tannins occurred despite no direct selection for tannins which would indicate that tannins are an important part of cotton disease resistance.

More importantly, tannin has been shown to be strongly correlated with resistance to both *Pythium* and *Rhizoctonia*. Kantar et al. (1996) showed that seed coats in *Vicia faba* cultivars containing high levels of condensed tannin presented a significant barrier to *Pythium* when

compared with low tannin cultivars. Donnelly (1983) observed that low tannin *Lespedeza cuneata* cultivars have greater susceptibility to *R. solani* when compared to high tannin cultivars and that disease severity was correlated with condensed tannin concentration.

Tannin suppresses *Rhizoctonia* through the inactivation of polygalacturonase as well as other enzymes important in pathogenesis. Hunter (1974) showed that catechin, a flavan-3-ol that is a tannin precursor, oxidized by peroxidase extracted from healthy plants, inhibited polygalacturonase activity. Un-oxidized catechin does not suppress polygalacturonase (Byrde et al., 1960). Condensed tannins in cotton are polymers of catechin and gallocatechin with lesser concentrations of epicatechin and epigallocatechin as well as other flavan-3-ols. The ratio of catechin to gallocatechin varies from 4:1 to 1:1. Concentrations of tannin are normally 10 times that of flavan-3-ols. The characteristic dark brown lesions associated with *Rhizoctonia* may be due to this oxidation of catechin and tannins (Bell et al., 1992).

Tannin concentrations increase as the plant ages from six to 12 days old and greatly increase 24 h after inoculation with *Rhizoctonia,* with greater post-infection increases coming from older plants (Hunter, 1974). When a concentration of catechin equivalent to what would be found in a 14-day old seedling is added to the growth media, growth of *Rhizoctonia* is strongly inhibited, while the concentration found in a five-day old plant has little effect (Hunter 1978), suggesting that the increase in resistance seen as the plant ages could be due to tannin production.

Further evidence of the role of tannins in seedling disease resistance, specifically resistance to seed-rot and pre-emergence damping off, is seen in the manner in which pathogens infect the seed prior to germination. Infection in almost all cases occurs through the chalaza, which unlike the rest of the seed coat, is low in tannins and is permeable. The pathogen then

infects the inner layer of the seed coat, which is also low in tannins. The nucellus, which is high in tannins, acts as a significant barrier to further infection, so much so that infection of the embryo can only take place in the end proximal to the chalaza, which contains a much lower tannin concentration (Halloin, 1982).

One possible source of higher tannin content is a set of 37 germplasm lines, referred to as high tannin (HT) lines, which were developed and released in 1989 by the Texas Agricultural Experiment Station, (Smith et al., 1990a, Smith et al., 1990b, Schuster et al., 1990). These lines were developed using accessions known to have elevated levels of condensed tannins that were collected from the Yucatan Peninsula in Mexico, Belize, and India. The lines were developed with the idea that increased levels of tannins might act as a feeding deterrent to insects, specifically bollworm (*Helicoverpa zea*). Unfortunately, either the levels of condensed tannins were too low or tannin does not act as a feeding deterrent to bollworm (Smith et al., 1992). However, several of these HT lines possessed increased resistance to bacterial blight (Chana, 1987).

2. RESEARCH OBJECTIVES

The objectives of this research were 1. evaluate 37 HT lines for their resistance to *R. solani* and *P. aphanidermatum*; 2. make individual plant selections for *R. solani* resistance within these HT lines; 3. determine the correlation between the concentration of condensed tannins in germinating seeds and levels of resistance; and 4. determine gain from selection.

3. MATERIALS AND METHODS

The HT lines used in this research were from the 37 HT lines released by Smith et al. (1990a and 1990b) and Schuster et al. (1990) having elevated levels of condensed tannins in mature leaves as indicated by HCl butanol assay. These lines were developed with the idea that an increase in tannin level might be a feeding deterrent to the bollworm complex. In addition to having been selected for high tannin levels based on the above plant analysis, they also were selected for resistance to two-spotted spider mite, *Tetranychus urticae.* Because of limited availability of seed, only 32 of the 37 lines used for the *R. solani* experiments and 36 of the 37 lines lines used for evaluation of resistance to *P. aphanidermatum*.

3.1 *R.solani* Resistance Screening

Seeds were planted in Container™ planters filled with Metromix 200™ potting soil wetted to field capacity. Seeds were grown in an incubator at 27º C for three days, followed by inoculation with 4 ml of *R. solani* (strain J1) inoculum (obtained from C. Howell, USDA-ARS at College Station, TX in 2004). Inoculum was prepared by growing a 0.5 cm diameter plug of *R. Solani* on potato dextrose agar (PDA) in a 6 cm petri dish for three days at 25º C. PDA media consisted of 15 g of PDA and 15 mg of rifampicin liter^{-1} of water. After 3 days, 100 ml of reverse osmosis (RO) water was added to each plate, followed by maceration in a blender for 30 sec. Four ml of inoculum solution was pipetted into each conetainer containing an emerged seedling and plants were moved to the greenhouse and allowed to grow for 7 days. At 10 days after planting, the number of surviving plants of each HT line was recorded, along with 'Tamcot SP21' (Bird, 1976 PI 529634) as the resistant check and TAM 96WD 18 (Thaxton et al., 2005; PI 635879) as the susceptible check. Tamcot SP21 and TAM 96WD-18 expressed 38% and 8%, respectively, resistant plants in a preliminary screening of several cultivars and represent

resistant and susceptible genotypes relative to the original set of cultivars screened (unpublished data). Seedlings of each HT line and the controls were established in November of 2004 and March, April, May, June and October of 2005. Due to insufficient greenhouse space and the physical limitations on the number of seedlings that could be inoculated in a timely fashion, each individual experiment was considered a replication of the same experiment separated in time. Twelve replications were preformed with 14 seedlings per entry per replication. Treatment means were subjected to ANOVA to determine differences. An uninoculated TAM 96WD 18 entry was included in each replication to confirm that disease symptoms did not occur in the absence of the pathogen.

An analysis of variance of plot means was used to determine if genotypes differed in their response to *R. solani*. A Bartlett's test of homogeneity indicated that variances were not equal and resistance scores were arcsin angle transformed for analysis. Means were separated using the Waller LSD at k=100, which approximates the 5% probability level and reported in the original units.

3.2 Selection for *R. solani* Resistance

Four HT lines (TAM 86 III 16, TAM 86 J 1, TAM 86 III 11, and TAM 86 III 24) that showed elevated levels of resistance in preliminary tests were subjected to a single cycle of selection for resistance to *R. solani*. Fifty seedlings of each line were inoculated as described above except with 2.5 X the concentration of inoculum used to evaluate resistance/susceptibility. Surviving seedlings were grown to maturity and seed collected for evaluation of gain from selection.

Seed from each of the selected plants (C_1), the unselected parent populations (C_0), a susceptible check (TAM 96WD 18) and a resistant check (Tamcot SP21) were grown and

inoculated. The procedure used was as described above for the original screening procedure. Resistance scores were indexed to the resistant control at 100% of the plants surviving and all other entries appropriately adjusted to standardize data from replication to replication. Two replications were completed; the first containing 30 seeds of each entry but only 20 seeds of each in the second replication. Progress from one cycle of selection was analyzed using General Linear Model procedure in SAS® with a split plot design. Pedigree was used as main plot and selected vs. unselected was used for the subplots.

3.3 *P. aphanidermatum* Resistance Screening

The HT lines were evaluated for resistance to *P. aphanidermatum*, which is considered to be of greater local importance relative to *P. ultimum*, although both are of equal importance as causal agents of seedling disease across the U.S. Cotton Belt (personal communication, C. Howell, 2006). One mycelia mat of a 10-day old culture of *P. aphanidermatum* was mixed with 100 ml of RO water and macerated in a Waring™ 12 volt two speed blender on high for 30 sec. *P. aphanidermatum* cultures were grown on 25 ml of liquid V8/cholesterol media consisting of 177 ml of V8 juice, 823 ml of water, 3 g of $CaCO_3$, 2 ml of a 0.3 g ml^{-1} cholesterol/ethanol solution and 15 mg of rifampicin. This mixture was then applied to 1 kg of a soil mixture containing 60% field soil and 40% sand (Howell, 2002). Eleven g of the soil inoculum mixture, one seed and 2 ml of RO water were added to test tubes. Tubes were placed in an incubator at 30º +/- 1 C and covered with a layer of Glad Wrap™, which allows for gas exchange but limits water loss. Seeds were allowed to grow for seven days and then the number of surviving seedlings was recorded. Four replications of 36 seeds of each of the 36 HT lines for which seed were available were performed. Percentage survival in each replication was recorded and survival rates were subjected to an analysis of variance to determine the response of genotypes to

this pathogen. Unequal variances again required transformation with arcsin angle prior to statistical analysis. Means were separated by the Fisher Protected LSD and reported in original units. These experiments were preformed between March and June of 2006.

'Tamcot Sphinx' (El-Zik and Thaxton, 1996; PI 592801) and 'Stoneville 213' (Calhoun and Bowman, 1994) were used as the resistant controls and 'SureGrow 747' (PVP no. 9800118) was used as the susceptible control. Howell (2002) reported that Tamcot Sphinx and Stoneville 213 had survival rates of 100% and 93%, respectively, when planted in soils with a naturally heavy infestation of both *P. aphanidermatum* and *P. ultimum*, while SureGrow 747 had a survival rate of only 7%. An uninoculated treatment of SureGrow 747 was planted in each replication to show that disease symptoms did not occur in the absence of the inoculum and that the seed was viable since this pathogen kills the emerging seedling almost immediately after germination with little evidence of growth.

3.4 Correlation of Tannin Concentration and Resistance to *P. aphanidermatum*

Seeds of each HT line along with Tamcot Sphinx and SureGrow 747, were allowed to germinate for 24 h on wetted paper towels at 30ºC followed by seed coat and nucellus removal since both contain larger amounts of tannins prior to germination relative to the remainder of the seed. Approximately 0.06 g of the seed (about half) was taken from the micropylar end and used for the assay. This tissue was then weighed and placed in a screw cap vial containing 5 ml of extracting solution. Extracting solution consisted of 5% HCl and 95% butanol by volume.

The tissue was ground using the flat end of a Bic pen. The closed vials were then placed in a 98º C water bath for 1 hour, and then refrigerated (approximately 3 +/-1º C) overnight. Absorbance of the supernatant at 550 nm was measured the following day using a Spectronic

20™ Colorimeter. Absorbance readings were converted to tannin content in g kg^{-1} fresh weight using the following equation:

Tannin = {[(optical density at 550 nm)(5 ml reagent per vial)(1/sample wt.)]/240} x 10 (Lege et al., 1992). Tannin levels in three seeds were measured and averaged for each of the HT lines in each of 6 replications. Data were subjected to analysis of variance to determine significant differences among entries and means were separated using Fisher's Protected LSD. Tannin concentration was correlated with *Pythium* resistance scores using PROC CORR in SAS®.

3.5 Correlation of *R. solani* Resistance with *P. aphanidermatum* Resistance

Correlation between resistance scores for both pathogens for all the HT lines was determined using PROC CORR in SAS®.

4. RESULTS AND DISCUSSION

4.1 *R. solani* Resistance Screening

All experiments were conducted in greenhouse with temperatures ranging from 25 to 30º C. The ANOVA indicated significant differences among the 34 genotypes evaluated for resistance to *R. solani* (Table 1). Eleven HT lines were not lower (p=0.05) in percentage survival than the resistant genotype, Tamcot SP21, which averaged 65% survival after 7 days following inoculation with *R. solani* (Table 2). The susceptible control, TAM 96WD-18, also was not different than the resistant control. Twenty-one HT lines were more susceptible than Tamcot SP21 but many of these were not different from the more resistant HT lines.

Insufficient greenhouse space and the time requirement to inoculate the number of seedlings chosen to represent each genotype prohibited the growing and inoculation of all replications simultaneously. Thus, replications were separated in time as separate experimental activities. The high degree of variation among replications likely was due to variation in greenhouse conditions. Despite the fact that every effort was made to ensure a consistent environment from replication to replication, a certain amount of variation in both temperature and light due to weather is inherent in any greenhouse study. Within lines there was a wide range of disease severity with plants exhibiting no symptoms of infection to severe disease symptoms within the same replication and entry. Since inoculation procedures and environment were consistent from plant to plant within any given replication, the variation within lines may suggest genetic variation in the level of resistance within these HT lines.

4.2 Selection for *R. solani* Resistance

Approximately 9% (data not shown) of the seedlings of each of the four parental HT lines survived inoculation with 2.5 X the amount of *R. solani* inoculum used in the screening process

noted above. These seedlings were transplanted to large pots and plants were grown to maturity in the greenhouse and seed collected. Survival rate within Tamcot SP 21 was similar to that of the four HT lines, while TAM 96WD-18 had 0% survival. Selection was not performed on either check.

The overall survival of unselected, C_0, plants was 58% of that of Tamcot SP21 while the survival rate of the C_1 progeny was 169% of Tamcot SP21. Standardized resistance scores for the selected lines ranged from 100% for TAM 86 J 1 to 241% for TAM 86 III 11, versus the unselected lines which ranged from 0% for TAM 86 III 11 to 150% for TAM 86 III 24. 96WD18 had a survival rate of 0% (Table 3). The ANOVA showed a significant effect of selection at a .05 level of significance (Table 4). These data suggest that while no one HT line possessed strong resistance to *R. solani,* there is selectable variation for resistance within lines. The fact that the selected lines in all cases equaled or outperformed the resistant check also suggests it is possible that these lines could potentially be useful sources of resistance to *R. solani*.

The large variation in response to inoculation among the HT lines should be noted. In the selection experiment, TAM 86III-11 exhibited 0% survival among the unselected lines yet it was not different than the resistant check in the general screening experiment noted above while the opposite was true for TAM 86III-24. Nonetheless, only one cycle of selection appears to confirm that direct and simple single plant selection for resistance to *R. solani* could result in the development of genotypes with increased resistance.

4.3 *P. aphanidermatum* Resistance Screening

The ANOVA indicated that the HT lines and the commercial controls varied in percentage survival after inoculation with *P. aphanidermatum* (Table 5).

Tamcot Sphinx, Stoneville 213 and Sure Grow 747 had survival rates of 72.2, 42.3 and 13.9%, respectively; confirming their use as resistant and susceptible controls (Table 6). The difference in survival rates of the controls compared to those previously found by Howell (2002) are likely due to differences in resistance to *P. ultimum* and *P. aphanidermatum* and due to the fact that temperature conditions used in this experiment were optimized for *P. aphanidermatum* infection rather than using a temperature suitable for both species. Howell (2002) used a temperature of 25º C while a temperature of 32º C was used for this study. This change in temperature was made based on personal communication with Dr. Howell (2006).

Resistance scores varied widely across HT germplasm lines with the least resistant line, TAM 86 DD 17, having an average score of 7% survival and significantly lower than the most resistant lines, TAM 87 N 6 and TAM 86 III 26, which had an average scores of 80% and were not different ($p=0.05$) than the most resistance control, Tamcot Sphinx (Table 6). Fourteen HT lines, TAM 87 N 6, TAM 86 III 26, TAM 87 N 7, TAM 87 N 5, TAM 86 E 8, TAM 86 III 11, TAM 86 III 8, TAM 87 M 48, TAM 86 E 9, TAM 86 DD 12, TAM 86 E 7, TAM 86 E 19, TAM 87 M 41, TAM 86 III 15, TAM 86 E 20, TAM 86 III 16, TAM 86 CC 7, TAM 86 J 1, TAM 86 III 24, TAM 86 DD 16, TAM 86 E 3, TAM 86 III 22, TAM 86 DD 11 and TAM 86 III 7, were equal to the most resistant control, Tamcot Sphinx, in resistance to *P. aphanidermatum*. Fifteen HT lines, TAM 86 III 24, TAM 86 DD 16, TAM 86 E 3, TAM 86 III 22, TAM 86 DD 11, TAM 86 III 7, TAM 87 N 4, TAM 86 E 6, TAM 86 E 14, TAM 86 CC 13, TAM 86 CC 12, TAM 86 E 4, TAM 86 CC 18, TAM 86 DD 18 and TAM 86 CC 11, were significantly better than SureGrow 747 at 14 % survival but were not different from Stoneville 213, 42% survival, in resistance to *P. aphanidermatum*. TAM 86 III 31, TAM 86 DD 17, and TAM 86 CC-17 exhibited low levels of resistance not different ($p<0.05$) than SG 747, the most susceptible check.

4.4 Correlation of Tannin Concentration and Resistance to *P. aphanidermatum*

While there was a significant effect of genotype on tannin content (Tables 7 and 8), there was not a correlation observed between tannin content and *Pythium* resistance. Pearson's coefficient of correlation between the two values was found to be -0.08623, with a p value of .617. This does not rule out a relationship between tannin content and resistance, the lack of a detectable relationship may be due to the fact that the critical period for production of tannins to induce resistance is past the first day. Also it is likely that increases in tannin production only occurs after infection has taken place. Uninfected seeds were used only because of the speed with which infected seeds are broken down by the pathogen, which makes tannin measurement difficult if not impossible.

4.5 Correlation of *R. solani* Resistance with *P. aphanidermatum* Resistance

When the resistance levels to these two pathogens among the HT genotypes used in this study are compared it appears that resistance to one pathogen is a poor predictor of resistance to the other. The Pearson's correlation between the two resistances was -0.046 with a p value of .8035. However, there were several lines that exhibit acceptable levels of resistant to both pathogens. TAM 86 CC 7, TAM 86 DD 12, TAM 86 DD 16, TAM 87 N 5, TAM 87 N 6, TAM 87 N 7, TAM 86 DD 17, TAM 86 E 3, TAM 86 III 11 and TAM 87 M 48 were not statistically different from the resistant controls for both pathogens (Tables 2 and 6). This indicates that these lines may be useful in breeding for resistance to the CSDC.

5. CONCLUSIONS

1. No HT line was more resistant to *R. solani* than the resistant control, Tamcot SP21.
2. Progeny of plants of TAM 86 J 1, TAM 86 III 11, TAM 86 III 11 and TAM 86 III 24 that survived a 2.5 X rate of *R. solani* inoculum were more resistant than their respective unselected HT parental population. This suggests that while there were no HT lines that possess strong resistance to *R. solani*, selectable variation in the level of resistance exists within certain HT lines. This variation could be due to out-crossing between lines (open pollinated seed was used for all experiments), a lack of selection for tannins in the early stages of growth in the development of the parental lines, or simply residual heterogeneity since cotton strains are rarely homozygous due to selection and maintenance procedures. These results suggest that these lines could be used as source material for efforts in breeding for greater resistance to *R. solani.*
3. Fifteen HT germplasm lines expressed resistance to *P. aphanidermatum* equal to the resistant control, Tamcot Sphinx. These lines should be useful in breeding for *P. aphanidermatum* resistance since under field conditions Sphinx has been shown to have 100% resistance to *Pythium* (Howell, 2002) and thus the HT lines may be a new source of resistance genes.
4. Under conditions of this study, seed tannin content of uninfected seeds was not correlated with *P. aphanidermatum* resistance. This does not mean that there is no relationship between tannin and level of resistance. It is possible that tannin is produced at a later developmental stage. It is also likely that tannin is produced in response to infection and is not present in large amounts prior to actual infection by the pathogen.

5. TAM 86 CC 7, TAM 86 DD 12, TAM 86 DD 16, TAM 87 N 5, TAM 87 N 6, TAM 87 N 7, TAM 86 DD 17, TAM 86 E 3, TAM 86 III 11 and TAM 87 M 48 were as resistant to *R. solani* and *P. aphanidermatum* as the resistant controls and should prove useful in breeding programs developing cotton host plant resistant germplasm or cultivars.

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APPENDIX

Table 1: Analysis of variance components for percentage survival of 32 HT[†] germplasm lines, Tamcot SP21, and TAM 96WD 18 following inoculation with *R. solani* under greenhouse conditions at College Station, TX in November 2004- October 2005.

Source of Variation	*Df*	*MS*	*F*
Genotype	33.00	364.69	2.91***
Replications	11.00	4364.48	34.82***
Error	363.00	125.33	
Total	407.00		

*** Significant at < 0.001.

[†] HT = high tannin.

Table 2: Percentage of seedling survival of 32 HT[†] germplasm lines, Tamcot SP21, and TAM 96WD-18 following inoculation with *R. solani* under greenhouse conditions at College Station, TX in 2004 and 2005.

Genotype	Percent Survival[‡]	
Tamcont SP21	65	a
TAM 87 N 4	59	ab
TAM 86 III 11	57	abc
TAM 87 M 48	57	abc
TAM 86 E 3	55	abcd
TAM 86 DD 17	52	abcde
TAM 87 N 7	51	abcdef
TAM 87 N 6	50	abcdefg
TAM 86 DD 12	50	abcdefg
TAM 87 N 5	50	abcdefgh
TAM 86 DD 16	50	abcdefgh
Tamcot 96WD18	50	abcdefgh
TAM 86 CC 7	49	abcdefgh
TAM 86 III 22	48	bcdefgh
TAM 86 CC 18	47	bcdefgh
TAM 86 CC 13	47	bcdefgh
TAM 86 E 8	46	bcdefghi
TAM 86 DD 11	45	bcdefghi
TAM 86 J 1	44	bcdefghij
TAM 87 M 41	44	bcdefghij
TAM 86 III 15	44	bcdefghij
TAM 86 E 9	43	bcdefghij
TAM 86 III 31	41	cdefghij
TAM 86 CC 17	41	cdefghij
TAM 86 CC 11	40	defghij
TAM 86 III 7	39	efghij
TAM 86 III 26	38	efghij
TAM 86 III 16	38	efghij
TAM 86 E 7	38	efghij
TAM 86 CC 12	34	ghij
TAM 86 III 24	33	hij
TAM 86 E 4	30	ij
TAM 86 E 19	28	j
TAM 86 III 8	28	j

[†] HT = high tannin

[‡] Means followed by the same letter are not different according to Waller LSD at K=100.

TABLE 3: Mean resistance scores after one cycle of selection for resistance to *R. solani.* Two replications were preformed under greenhouse conditions at College Station, TX in December 2004.

Genotype	Percent Survival[†]
TAM 86 III 16 Selected	133
TAM 86 III 16 Unselected	17
TAM 86 J 1 Selected	100
TAM 86 J 1 Unselected	67
TAM 86 III 11 Selected	242
TAM 86 III 11 Unselected	0
TAM 86 III 24 Selected	189
TAM 86 III 24 Unselected	150
Overall Selected	169
Overall Unselected	58
SP21 (resistant check)	100
WD18 (susceptible check)	0

[†] Resistance scores have been standardized with the resistant check being set to 100% and all other entries being adjusted to a percentage of that.

Table 4: ANOVA for percentage survival after one cycle of selection for resistance to *R. solani* in HT germplasm lines. Two replications were preformed under greenhouse conditions at College Station, TX in November –December of 2005.

ANOVA

Source of Variation	*Df*	*MS*	*F*
Genotype	3	7479	0.70
Replications	1	41412	3.89
Error a	3	10654	
Selection	1	47262	10.97*
Selection x genotype	3	9807	2.28
Error b	4	4310	

* significant at .05 probability level

Table 5: ANOVA for effect of entries on percentage survival after infection by *P. aphanidermtum*. Four replications were preformed in an incubator at 30º C in March through June 2006.

ANOVA

Source of Variation	*Df*	*MS*	*F*	*P-value*
Genotype	38.00	563.04	15.61***	2.8242E-30
Rep	3.00	272.35	7.55***	0.00011848
Error	114.00	36.06		

*** Significant at < 0.001

Table 6: Percentage seedling survival of 32 HT[†] germplasm lines, Tamcot Sphinx, Stoneville 213, and Suregrow 747 after infection by *P. aphanidermatum.* Four replications were preformed in an incubator at 30º C in March – June 2006.

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Genotype	Percent Survival[‡]	
TAM 86 III 26	80%	a
TAM 87 N 6	80%	a
TAM 87 N 7	76%	ab
TAM 87 N 5	74%	abc
TAM 86 E 8	74%	abc
Tamcot Sphinx	72%	abcd
TAM 86 III 11	72%	abcd
TAM 86 III 8	72%	abcd
TAM 87 M 48	70%	abcde
TAM 86 E 9	70%	abcde
TAM 86 DD 12	69%	abcde
TAM 86 E 7	69%	abcdef
TAM 86 E 19	68%	abcdefg
TAM 87 M 41	68%	abcdefg
TAM 86 III 15	67%	abcdefg
TAM 86 CC 7	67%	bcdefgh
TAM 86 E 20	67%	bcdefgh
TAM 86 III 16	67%	bcdefgh
TAM 86 J 1	65%	bcdefghi
TAM 86 III 24	64%	bcdefghi
TAM 86 DD 16	63%	cdefghij
TAM 86 E 3	60%	defghij
TAM 86 DD 11	60%	defghij
TAM 86 III 22	60%	defghij
TAM 86 III 7	59%	efghij
TAM 87 N 4	56%	fghij
TAM 86 E 6	56%	ghij
TAM 86 E 14	54%	hijkl
TAM 86 CC 13	53%	ijkl
TAM 86 CC 12	50%	jklm
TAM 86 E 4	46%	klm
TAM 86 CC 18	45%	klm
TAM 86 DD 18	44%	klm
Stoneville 213	42%	lm
TAM 86 CC 11	38%	m
TAM 86 CC 17	22%	n
Sure Grow 747	14%	no
TAM 86 II 31	13%	no
TAM 86 DD 17	7%	o

[†]HT = high tannin

[‡]Means followed by the same letter are not different according to Waller LSD at K=100.

Table 7: ANOVA of the effect of entry on average tannin content of germinated seeds of HT lines. Seeds were germinated for 24 hours prior to tannin measurement. Six replications were completed and tannin measurements were taken in September 2006.

ANOVA

Source of Variation	*df*	*MS*	*F*
Genotype	37	0.45446	1.825772**
Rep	5	0.520205	2.0899002
Error	185	0.248914	
Total	227		

Table 8: Fisher's Protected LSD for tannin content of germinated seeds of HT lines. Seeds were germinated for 24 hours prior to tannin measurement. Six replications of three seeds each were completed and tannin measurements were taken in September 2006.

Genotype	g/kg fresh weight[†]	
TAM 87 N 7	1.79	ab
Tamcot Sphinx	1.26	abcdefg
TAM 86 CC 18	1.22	bcdefg
TAM 86 III 16	1.17	bcdefg
TAM 86 DD 18	1.16	bcdefg
TAM 86 DD 12	1.13	bcdefg
TAM 86 CC 12	1.10	bcdefg
TAM 86 E 20	1.10	bcdefg
TAM 86 DD 17	1.00	bcdefg
TAM 86 DD 11	0.99	bcdefg
TAM 86 CC 13	0.98	bcdefg
TAM 86 DD 16	0.97	bcdefg
TAM 86 CC 17	0.89	bcdefgh
TAM 86 CC 7	0.89	bcdefgh
TAM 86 E 7	0.88	bcdefgh
TAM 86 III 22	0.86	bcdefghi
TAM 87 N 6	0.83	bcdefghi
TAM 86 III 26	0.83	bcdefghi
TAM 86 J 1	0.82	bcdefghi
TAM 86 CC 11	0.82	bcdefghi
TAM 86 III 15	0.81	bcdefghi
TAM 86 III 31	0.74	bcdefghi
TAM 86 E 8	0.71	bcdefghi
TAM 87 N 5	0.71	bcdefghi
TAM 87 M 48	0.71	bcdefghi
TAM 86 E 6	0.71	bcdefghi
TAM 86 E 3	0.69	bcdefghi
TAM 86 E 4	0.69	cdefghi
TAM 86 E 9	0.66	cdefghi
TAM 86 III 11	0.64	defghi
Sure Grow 747	0.62	defghi
TAM 86 E 14	0.61	defghi
TAM 87 M 41	0.60	efghi
TAM 87 N 4	0.60	efghi
TAM 86 III 7	0.58	fghi
TAM 86 III 24	0.51	ghi
TAM 86 III 8	0.38	hi
TAM 86 E 19	0.30	i

[†]Means followed by the same letter are not different according to Waller LSD at K=100.

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