1644 Identification of germplasm resistant to Thielaviopsis basicola in the USDA cotton germplasm collection

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Lines (258) from the USDA cotton collection were grown in soil that was artificially infested with *Thielaviopsis basicola* (causal agent of black root rot). A susceptible ('Paymaster 2326 RR') and a resistant (A20, *Gossypium herbaceum*) check were included in each test. Pots were grown for 25 days in a growth chamber at 19C and then the percent necrosis of the tap root was visually estimated. Lines that had levels of root necrosis similar to that of the resistant check included: TX1, TX 5, TX 15, TX 25, TX 27, TX 28, TX 29, TX 51, TX 68, TX 70, TX 83, TX 93, TX 96, TX 104, TX 111, TX 125, TX 129, TX 130, TX 140, TX 141, TX 143, TX 147, TX 151, TX 153, TX 163, TX 304, TX 320, TX 1867, and TX 2498. There were 58 lines that had an intermediate level of root necrosis and 171 lines that had levels of root necrosis similar to that of the susceptible check. Lines that tested resistant to black root rot originated from Ceara, Brazil (1), Chiapas, Mexico (2), Chiquimula, Guatamala (1), Jalapa, Guatamala (1), Jutiapa, Guatamala (8), Guatamala (3), Oaxaca, Mexico (3), Mexico (6), and St. Lucia (1).

Keywords: *Gossypium hirsutum,* seedling disease

Fungal pathogens of cotton seedlings include *Rhizoctonia solani*, *Pythium* spp., *Fusarium* spp., and *Thielaviopsis basicola*. *Thielaviopsis basicola*, the causal agent of black root rot, is primarily a post-emergence problem, where the lateral root system does not develop extensively, because of root necrosis. The fungus does not usually kill the seedlings, but does delay the development of lateral roots. Once the roots are able to slough off the necrotic root tissue, the lateral roots are able to form. The disease is most severe in weather where cool temperatures (≤ 21 C) persist (Bland et al., 1953; Rothrock, 1992). The fungus can also interact with the root-knot nematode, *Meloidogyne incognita*, in which case the damage to the roots is more extensive, and yield loss may increase (Walker et al., 1998; Walker et al., 1999; Walker et al., 2000).

Management of *T. basicola* is primarily achieved by planting under growing conditions that allow the plant to emerge and grow rapidly, or by crop rotation to nonhosts. *Thielaviopsis basicola* reproduces on a wide range of hosts (Johnson, 1916; Yarwood and Levkina, 1976), however, monocots are nonhosts. The primary infection propagule is similar to a chlamydospore, which survives well in the absence of a host**.** Therefore**,** in severe infestations, it may be necessary to rotate to nonhosts for a number of years. Chemical management with seed treatments can reduce the amount of root necrosis caused by *T. basicola* and increase yield (Kaufman and Wheeler, 1998). However, only partial control is achieved, and in moderate to severely infested fields, 100% root necrosis can occur even with fungicide seed treatments that are active against *T. basicola*. It is unlikely that a seed treatment will have sufficient systemic activity to protect a taproot. An in-furrow fungicide may be more effective in providing a zone of protection for roots, however, no fungicides have been labeled for that use in the United States.

Resistance to *T. basicola* has been identified and used in tobacco production (Haji et al., 2003). However, in most crops natural resistance has not been identified. Partial resistance was identified in *G*. *arboreum* (Wheeler et al., 1999), but no previous resistance of any strength has been found in *G*. *hirsutum*. It would greatly facilitate management of black root of cotton if there were a strong source of resistance available in *G*. *hirsutum*. The objective of this study was to screen some of the USDA cotton collection of *G*. *hirsutum* (http://www.ars-grin.gov/cgi-bin/npgs/html/tax_site_acc.pl?COT%20Gossypium%20hirsutum) for resistance to *T*. *basicola*.

METHODS AND MATERIALS

Seed was obtained from the USDA cotton germplasm collection and increased. Soil was autoclaved twice at 120C and 1.05 kg/cm^2 for 2.5 hours, before use. *Thielaviopsis basicola* was grown on carrot agar (Wheeler et al., 1999) for at least six weeks, and then the propagules were scraped off the plates and washed through a sieve with a 74-µm pore size. The inoculum density was adjusted to 500 clamydospores/cm^3 soil. Soil (110 cm^3) was added to a plastic Cone-Tainer (3.8 cm diameter, 14 cm long, Stewe & Sons, Corvallis, OR, USA), and a single seed was planted. There were 25 replications of each line planted for each test. In each test, a susceptible ('Paymaster 2326 RR', *G*. *hirsutum*) and a resistant line (A1 20, *G*. *herbaceum*) were included. Entries were arranged in a randomized complete block design and held in a growth chamber at 19C for 25 days. After 25 days, the roots were washed free of the soil and rated for percent necrosis. A total of 258 lines were screened.

Statistical analysis was done with PROC MIXED in SAS (SAS Institute, Cary, NC, USA). The fixed factor was line and replication was a random factor. The PDIFF test was used to determine if lines differed from the resistant or susceptible checks. There were an unequal number of observations between lines, since germination was very different between entries. Lines were considered different if $P \leq 0.05$, however, it is unknown what effect the uneven number of observations of each entry had on the probability level.

RESULTS AND DISCUSSIONS

Lines that had levels of root necrosis similar to that of the resistant check (A1 20, *G*. *herbaceum*) included: TX1, TX 5, TX 15, TX 25, TX 27, TX 28, TX 29, TX 51, TX 68, TX 70, TX 83, TX 93, TX 96, TX 104, TX 111, TX 125, TX 129, TX 130, TX 140, TX 141, TX 143, TX 147, TX 151, TX 153, TX 163, TX 304, TX 320, TX 1867, and TX 2498 (Table 1). Locations with a high frequency of resistant lines included Ceara, Brazil (TX 2498) and St. Lucia (TX 1867), however, only one entry was tested from each of these sites. Locations with a moderate percentage of tested lines that were resistant were Jutiapa, Guatemala (8 of 36 were rated resistant) and Guerrero, Mexico (3 out of 21 were rated resistant) (Table 1). There were 58 lines that had an intermediate level of resistance to *T*. *basicola* (Table 2) where the percent of root necrosis was significantly less than the susceptible check, but higher than the resistant check. There were 171 lines that had a level of root necrosis similar to that of the susceptible check (data not shown).

The identification of resistance to *T*. *basicola* in tetraploid cotton should greatly facilitate the development of cotton lines with good agronomic properties and resistance to black root rot. Resistance to black root rot has been found in diploid cotton (i.e. A 20, Wheeler et al., 1999), however, it has proven difficult to move the resistance into tetraploid cotton. With a starting point of resistant tetraploid cotton, it should be possible to move the resistance into cotton lines with good agronomic properties much more rapidly than when starting with resistant diploid cotton. Resistant cultivars would be an excellent alternative to current methods of control.

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Table 1. List of cultivars identified with resistance to *Thielaviopsis basicola*.

Entry	PI	# of plants rated	% Root necrosis	Rating/ Susceptible cultivar[z]	Rating/ Resistant cultivar[y]	Collection area
TX1	153981	11	40	0.44	1.22	Mexico
TX5	153987	6	33	0.37	1.02	Guerrero, Mexico
TX15	154013	18	35	0.39	1.07	Mexico
TX25	154035	4	15	0.25	0.86	Mexico
TX27	154037	16	29	0.47	1.66	Chiapas, Mexico
TX28	154038	7	27	0.44	1.56	Mexico
TX29	154040	19	29	0.48	1.68	Mexico
TX51	154071	15	21	0.27	2.26	Chiapas, Mexico
TX68	153960	5	36	0.37	1.25	Guatemala
TX70	153964	17	41	0.42	1.41	Guatemala
TX83	153972	5	66	0.72	1.08	Guatemala
TX93	163654	11	68	0.74	1.11	Jutiapa, Guatemala
TX96	163665	11	57	0.62	0.94	Jutiapa, Guatemala
TX104	163676	11	68	0.74	1.11	Jalapa, Guatemala
TX111	163639	6	63	0.68	1.03	Jutiapa, Guatemala
TX125	165329	9	70	0.76	1.15	Mexico
TX129	165282	10	74	0.80	1.21	Oaxaca, Mexico
TX130	165296	11	64	0.70	1.05	Oaxaca, Mexico
TX140	163614	3	72	0.78	1.18	Jutiapa, Guatemala
TX141	163640	13	67	0.73	1.10	Jutiapa, Guatemala
TX143	163707	9	64	0.70	1.06	Chiquimula, Guatemala
TX147	165310	11	75	0.81	1.22	Oaxaca, Mexico
TX151	163633	10	65	0.71	1.07	Jutiapa, Guatemala
TX153	163653	7	69	0.75	1.14	Jutiapa, Guatemala
TX163	163641	3	58	0.63	0.96	Jutiapa, Guatemala
TX304	165366	3	20	0.27	8.89	Guerrero, Mexico
TX320	165385	3	20	0.27	8.89	Guerrero, Mexico
TX1867	530498	4	16	0.22	7.22	St. Lucia
TX2498	607803	12	14	0.19	6.11	Ceara, Brazil

[z]The susceptible check that was used in every test was 'Paymaster 2326 RR'.

[y]The resistant check that was used in every test was A20 (*Gossypium herbaceum*).

Table 2. Identification of *Gossypium hirsutum* lines with weak resistance to *Thielaviopsis basicola*.

Entry	PI	# of plants rated	% Root necrosis	Rating/ Susceptible cultivar[z]	Rating/ Resistant cultivar[y]	Collection area
TX3	153984	20	70	0.77	2.1	Guerrero, Mexico
TX6	153988	18	78	0.86	2.4	Puebla, Mexico
TX14	154011	41	77	0.85	2.3	Oaxaca, Mexico
TX17	154022	20	36	0.59	2.1	Mexico
TX22	154029	17	39	0.64	2.3	Chiapas, Mexico
TX67	154103	8	53	0.54	1.8	Chipas, Mexico
TX71	153965	15	49	0.50	1.7	Guatemala
TX75	153968	18	51	0.53	1.8	Guatemala
TX76	153968	8	51	0.53	1.8	Guatemala
TX77	153969	10	69	0.71	2.4	Guatemala
TX78	153969	10	52	0.54	1.8	Guatemala
TX184	163642	8	57	0.67	3.6	Jutiapa, Guatemala
TX187	163723	12	60	0.71	3.7	Zacapa, Guatemala
TX198	163655	11	37	0.44	2.3	Jutiapa, Guatemala
TX206	165368	14	61	0.72	3.8	Guerrero, Mexico
TX215	163637	12	61	0.72	3.8	Jutiapa, Guatemala
TX216	163649	16	63	0.75	4.0	Jutiapa, Guatemala
TX220	163683	19	43	0.50	2.7	Jalapa, Guatemala
TX221	163706	13	56	0.66	3.5	Chiquimula, Guatemala
TX228	163672	60	54	0.64	3.4	Jalapa, Guatemala
TX236	163650	12	36	0.43	2.3	Jutiapa, Guatemala
TX243	165324	14	58	0.68	3.6	Oaxaca, Mexico
TX247	163631	13	37	0.44	2.3	Jutiapa, Guatemala
TX248	163673	2	60	0.71	3.8	Jalapa, Guatemala
TX256	165245	10	53	0.63	3.3	Oaxaca, Mexico
TX257	165253	7	55	0.65	3.5	Oaxaca, Mexico
TX259	165267	13	59	0.70	3.7	Oaxaca, Mexico
TX264	165302	10	58	0.69	3.6	Oaxaca, Mexico
TX277	165249	4	58	0.68	3.6	Oaxaca, Mexico
TX280	165292	11	46	0.63	20.6	Oaxaca, Mexico
TX281	165299	18	46	0.63	20.5	Oaxaca, Mexico
TX282	165306	16	53	0.72	23.5	Oaxaca, Mexico
TX285	165251	13	45	0.62	20.0	Oaxaca, Mexico
TX292	165230	13	45	0.62	20.2	Oaxaca, Mexico

TX295	**165252**	**13**	**46**	**0.63**	**20.3**	**Oaxaca, Mexico**
TX296	**165266**	**11**	**39**	**0.53**	**17.2**	**Oaxaca, Mexico**
TX297	**165273**	**9**	**43**	**0.59**	**19.3**	**Oaxaca, Mexico**
TX298	**165280**	**17**	**34**	**0.46**	**15.0**	**Oaxaca, Mexico**
TX301	**165301**	**18**	**47**	**0.65**	**21.0**	**Oaxaca, Mexico**
TX303	**165352**	**10**	**46**	**0.63**	**20.4**	**Oaxaca, Mexico**
TX305	**165376**	**6**	**43**	**0.58**	**18.9**	**Guerrero, Mexico**
TX311	**165370**	**4**	**35**	**0.48**	**15.6**	**Guerrero, Mexico**
TX325	**165393**	**14**	**36**	**0.49**	**16.0**	**Guerrero, Mexico**
TX1148	**273895**	**20**	**38**	**0.52**	**16.9**	**Kefa, Ethiopia**
TX1533	**530164**	**13**	**49**	**0.67**	**21.7**	**Martinique**
TX1585	**530216**	**15**	**41**	**0.57**	**18.4**	**Haiti**
TX1612	**530243**	**14**	**38**	**0.52**	**17.0**	**Guadeloupe**
TX1614	**530245**	**12**	**47**	**0.64**	**20.9**	**Guadeloupe**
TX1620	**530251**	**5**	**40**	**0.55**	**17. 8**	**Guadeloupe**
TX1824	**530455**	**15**	**24**	**0.32**	**10.5**	**Dominican Republic**
TX1841	**530472**	**4**	**45**	**0.62**	**20.0**	**Desirade, Guadeloupe**
TX1862	**530493**	**12**	**50**	**0.68**	**22.0**	**Guadeloupe**
TX2115	**478759**	**16**	**45**	**0.62**	**20.1**	**Australia**
TX2214	**501394**	**14**	**52**	**0.71**	**23.2**	**Netherlands Antilles**
TX2237	**501417**	**13**	**31**	**0.43**	**13.8**	**Jamaica**
TX2297	**501477**	**13**	**38**	**0.52**	**16.8**	**Puerto Rico**
TX2852		**3**	**43**	**0.59**	**19.3**	

[z]The susceptible check that was used in every test was 'Paymaster 2326 RR'.

[y]The resistant check that was used in every test was A20 (*Gossypium herbaceum*).