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THE EFFECT OF CHITINASE AND 1,3-B-GLUCANASE GENES IN TRANSFORMED COTTON ON DISEASES

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Abstract

Two cotton lines were transformed with a bean chitinase (ch5b) and one of the lines with β-1,3 glucanase (bg). Individuals of each transformation were tested for presence of the gene, increased and tested in a greenhouse assay for activity against *Rhizoctonia solani*. The lines with the best apparent resistance were increased and tested in three replicated field trials, which also included the original parent and commercial variety Paymaster HS-26. At sites which was predominantly affected by *Thielaviopsis basicola, Meloidogyne incognita*, and *Rotylenchulus reniformis*, there were no benefit or detriment of the transformed genes on root necrosis or nematode population density. At a site dominated by *Verticillium dahliae*, several of the transformed breeding lines to the more wilt susceptible parent became more resistant and one line became more susceptible as compared to the nontransformed parent. For more wilt resistant parent,, transformed lines either had equal or higher susceptibility to wilt than the nontransformed parent.

Introduction

Transformation of cotton has had a profound affect on weed and insect management. However, there are no commercial cotton varieties which feature transformations affecting plant diseases. The majority of cotton diseases are caused by soilborne fungi and nematodes. Diseases are typically caused by fungi with broad host ranges in which traditionally selected plant resistance has been only partially successful at best. Examples would include *Rhizoctonia solani*, *Pythium* spp., *Thielaviopsis basicola*, *Phymatotrichum omnivorum*, *Verticillium dahliae* and *Fusarium* spp. Nematodes like *Meloidogyne incognita* and *Rotylenchulus reniformis* also cause significant yield losses in cotton and > 99 % of all cotton cultivars planted are susceptible to these nematodes.

Plant resistance is usually formed by a combination of structural and chemical defenses. Some are present continuously while others are formed in response to a threat. Pathogenesis-related proteins can be induced by pathogens. Two types of pathogenesis-related proteins are chitinases and glucanases. The cell walls of many fungi contain chitin and β-D-glucans. Nematode eggs also contain chitin. When plants are infected with certain fungi, increased levels of chitinases and β-1,3-glucanases have been measured (Joosten and De Wit, 1989; Kombrink et al., 1988; Meins and Ahl. 1989). Tobacco which was transformed with a bean chitinase became more resistant to *R. solani* (Broglie et al., 1991). The objective of this study was to determine if cotton plants transformed with a bean chitinase (ch5b) and β-1,3 glucanase from *Arabadopsis* (bg2), could improve disease resistance against a number of different pathogens in field trials. Preliminary work testing these lines was conducted in a growth chamber against *R. solani,* resulting in the selection of 30 lines for seed increase and field testing. The transformation was via pollen tube pathway mediated method (Hess, 1972; Hess, 1987). No details of the transformation will be included in this work, only the testing of the selected lines.

Materials and Methods

Test sites included a field at the Texas A&M Research center located at Halfway, TX which was heavily infested with *T. basicola* and lightly infested with *Pythium* and *R. solani*; a research field located at the Texas A&M Research and Extension Center in Lubbock, which was infested with *Verticillium dahliae* and *Rotylenchulus reniformis*; and a field under a center pivot system located in Gaines county at the Western Peanut Growers Farm (WPGF) which was heavily infested with *Meloidogyne incognita*, and lightly infested with *Pythium* spp., *R. solani*, and *T. basicola*. There were two nontransformed parents ('A' = 95-T20#-1517, which was derived from ({[6-19 x (998 x 108)-4-69] x 60-611-5}-4-76) and 'B' = 95-T20#-114 which was derived from EP-PMS-Del Cerro 515) and provided by John Gannaway. There were six lines that were transformed with chitinase from parent A (ch5b4, ch5b16, ch5b26, ch5b27, ch5b30, and ch5b55). There were 12 lines which were transformed with β-1,3 glucanase from parent A (bg16, bg20, bg24, bg26, bg42, bg47, bg48, bg49, bg50, bg99, bg114, bg215). There were 12 lines which were transformed with chitinase from parent B (ch5b71, ch5b78, ch5b121, ch5b122, ch5b123-1, ch5b123-2, ch5b124, ch5b125, ch5b126, ch5b132, ch5b137, ch5b146). The variety Paymaster HS-26 was included in all tests. For all tests, there were four replications of each breeding line or variety arranged in a randomized complete block design. Seed was treated with Vitavax-PCNB (6 oz/100 lb seed) and Allegiance FL (0.75 oz/100 lb seed) (designated as VA). Row width was 40" for the Lubbock and Halfway sites, and 36" for the WPGF.

At the Halfway site, additional seed was treated with Captan 50 (3 oz/100 lb seed) for each breeding line. Seed treatment (VA versus Captan) was included as a split plot design, with breeding line as the main plot. Each single row plot was 20' long and contained 100 seed. This site was planted on 1 May and row watered a week after planting, and then at approximately monthly intervals. Stand counts were taken at weekly intervals (13, 20 and 27 days after planting). At 21 days after planting, six plants were removed and rated for % root necrosis and hypocotyl damage. The hypocotyl rating was based on a 0 to 3 scale with 0 being healthy, 1 = superficial hypocotyl lesion, 2 = sunken hypocotyl lesion, and 3 = dying plant due to hypocotyl lesion. At 30 days after planting, each row was rated for thrips by taking one leaf from each of five plants and beating it on a white cup. At that time, most plants had only 2-true leaves. Then a motorized blower was used to blow insects into a plastic bag from each row for the VA seed treatments. At harvest (22 November), both seed treatments for a given breeding line were harvested together with a two-row plot stripper. A sample from each plot was ginned to obtain % lint for each plot. Analysis of variance (ANOVA) was conducted on all collected data, after separating breeding lines into either an A or B type of plant. PM HS-26 was analyzed within both plant types. A split plot analysis was conducted for seedling disease data with breeding line as the main plot and seed treatment as the split plot, while for insect and yield data, only breeding line and replication were used in the analyses.

At the Lubbock site, five seeds were planted per foot row, into single row, 40' long plots on 2 May. Once a final stand had been achieved, 4' alleys were cut between each plot and plant stand was counted. On 26 July, 13 August, and 5 September, all plants were rated for Verticillium wilt. A rating of 0 (no disease), 1 (disease symptoms were only in the bottom ½ of the plant), or 3 (disease symptoms were in the top ½ of the plant) were given for each plot. The sum of incidence and severity ratings for a plot were then divided by the plant stand, and in the case of incidence, multiplied by 100 to give a percentage. On 20 August, each plot was sampled and assayed for reniform nematode using a modified Baermann funnel technique (Thistlethwayte, 1970). Each single row plot was machine harvested with a two-row plot stripper on 16 December, and a sample of the plot was ginned to estimate % lint. ANOVA was conducted on all data. *Rotylenchulus reniformis* population density (Rr) was transformed with $\log_{10}(Rr + 1)$.

At the WPGF site, 100 seed were planted over a 20', single row plot on 6 May. Stand counts were taken at 7, 14, and 21 days after planting. At 21 days after planting, six roots were removed per plot and rated for root necrosis and hypocotyl damage as described for the Halfway site. The test was destroyed by hail, and replanted on 4 June. The replanted plots consisted of 16' of row with four seed/ft, due to a shortage of seed. On line, bg48 was omitted due to seed shortages. Only the final plant stand was counted. On 1 October each plot was sampled for nematodes. Two assays were conducted on each sample, which included the modified Baermann funnel test to isolate second-stage juveniles of *M. incognita* and a sodium hypochlorite assay to isolate root-knot nematode eggs (Hussey and Barker, 1973). The egg assay consisted adding 2 L of water to 500 cm^3 soil and root fragments, stirring for 15 sec. and allowing to settle for 15 sec. Then the organic matter was caught on a sieve with 0.23-mm-pore sieve. The eggs were extracted with sodium hypochlorite, stained with acid fuschin and an aliquot was counted. The test was hand harvested on 13-14 November, and a subsample of each plot was ginned to estimate % lint. ANOVA was conducted on all data. *Meloidogyne incognita* population density (Mi) was transformed with $\log_{10}(Mi + 1)$.

Results

The weather after planting somewhat cool for the month following planting (Fig. 1) and dry (Fig. 2). The cool temperatures were conducive for black root rot (caused by *T. basicola*), but the dry weather was not conducive for other types of seedling disease. At the Halfway site, plant emergence was slower in general for many of the transformed lines than for the commercial variety PM HS-26 (Tables 1,2). Two chitinase lines (ch5b27 and ch5b55) and six β-1,3 glucanase lines had slower emergence at 13 days after planting than the parent line A (Table 1). However, by 20 and 27 days after planting, only ch5b27 had poorer plant stands than the parent line A (Table 1). PM HS-26 had poorer stands at 27 days after planting than any of the A-type lines. Early plant emergence was poorer for five of the chitinase transformed lines than the parent line B (ch5b71, ch5b121, ch5b123-1, ch5b124, ch5b137), and was better in one transformed line (ch5b132) (Table 2). By 27 days after planting, stands were similar across all the chitinase transformed and the parent line B, ranging from 67 to 74 % of the planted seed (Table 2). PM HS-26 had poorer stands at 27 days after planting than all the tested lines. Root necrosis was substantial at this site and probably related to the slow rate of plant maturation that was seen during the first 45 days of the growing season. There were no differences in root necrosis or hypocotyl ratings between transformed lines and the nontransformed parents (Table 1,2). Insects identified at the test site included thrips, big eyed bug, collops, damsel bug, hooded beetle, lady beetle, orius, spiders, beet army worm, fleahopper, green stinkbug, leafhopper, and Lygus. The big eyed bug had a higher density on bg49 and bg50 than on bg16, bg26, ch5b4, ch5b30, and ch5b55 (Table 1), though none of these transformed lines were different than the nontransformed parent. The chitinase transformed line ch5b126 had a higher density of spiders than the parent line B (Table 2). Two of the β-1,3 glucanase transformed lines (bg50 and bg215) had significantly greater yields than the parent line A (Table 1). Five of the chitinase transformed lines (ch5b71, ch5b122, ch5b123-2, ch5b124, and ch5b132) had significantly higher yields than the parent line B (Table 2).

At the Lubbock site, plant stand was significantly poorer for ch5b27, ch5b55, and bg99 than for the parent line A (Table 3) and in ch5b137 than the parent line B (Table 4). The temperature was somewhat warm in July and especially August (Fig. 3), which slowed down the development of wilt. Wilt incidence and severity were higher in replications 1 and 4 than in replications 2 and 3 (Table 5). The poor symptom development in the middle blocks made it more difficult to determine differences between breeding lines since there relative differences disappeared in those replications. Early disease incidence was higher for ch5b55 than for any other lines including the parent line A. This breeding line showed a higher incidence of wilt as well as severity than all other breeding lines. None of the transformed breeding lines had a lower incidence of wilt than the nontransformed parent, though numerically, ch5b26, ch5b30, bg114, and bg215 had almost a 50 % reduction in plants with wilt for much of the growing season. In terms of wilt severity for plants from parent line A, ch5b55 had the highest severity ratings in both August and September, though not significantly different than the parent A. The lowest severity ratings were found with ch5b26, PM HS-26, and ch5b30, for both August and September (Table 3). Wilt incidence and severity were similar between parent line B and PM HS-26 (Table 4). Wilt incidence and severity was higher for ch5b71 and ch5b137 than parent line B at all three evaluation periods (Table 4). The reniform nematode was a significant problem in replications 3 and 4, and barely present in replications 1 and 2 (Table 5). There were no differences between breeding lines with respect to reniform nematode population density. However, the lack of uniformity of the nematode across the test area would have made it difficult to show breeding line differences. Due to the variability in both reniform nematode density and Verticillium wilt, it is not surprising that no yield differences were found between breeding lines.

At the WPGF, plant stands at 14 days after planting were lower for breeding lines of both A and B types than for PM HS-26 (Tables 6, 7). However, by 21 days after planting, only ch5b55 had poorer emergence than PM HS-26 or line A (Table 6), and there were no differences with any of the transformed B line types and parent line B or with PM HS-26. Root-knot nematode was present at relatively high levels at this site, though there were no differences between any transformed plants and the nontransformed parents or PM HS-26. There were also no differences in yield at this site between any lines.

Discussion

The objective of this project was to determine if bean chitinase or Arabadopsis β-1,3 glucanase could increase general disease resistance in cotton. The genes were inserted using a pollen-tube mediated transformation method. While this method is simple and inexpensive to execute, it does not provide control over where the gene is inserted. There is also no control over the number of copies of the gene which are inserted. For the diseases which could be critically evaluated in 2002, it was apparent that neither gene provided any enhanced protection against *Thielaviopsis basicola* which causes black root rot. Against Verticillium wilt, some breeding lines became more susceptible to the disease. While not conclusive, due to nonuniform distribution of the wilt pathogen, it appeared that some chitinase lines did have improved disease resistance compared to the highly susceptible parent line A. However, when comparing wilt symptoms in the much more resistant parent line B, there was no improvements with transformed lines. The best of the β-1,3 glucanase lines did not match the wilt reduction numerically compared to the best of the chitinase lines. The glucanase gene in the best lines appeared to increase resistance by about ½ compared with the best of the chitinase lines (Figure 4). The lack of uniformity of the reniform nematode made it impossible to draw any conclusions with respect to enhanced resistance. However, there was clearly no difference in *M. incognita* reproduction with respect to chitinase or β-1,3 glucanase genes, and this organism was well distributed across the test area. Finally, there was no apparent impact of these genes on beneficial insects at the Halfway test site.

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Table 1. Affect of lines transformed from type "A" on seedling disease, yield and insects at Halfway, TX.

Plant[a]	% Emergence at			%	lbs of lint	Big Eyed
Line	13 DAP[b]	20 DAP	27 DAP	Necr[c]	per acre	Bug[d]
PM HS-26	52 a[e]	57 d	45 f	48 a	512 efg	0.5 ab
A	45 abc	74 bc	69 a-d	44 ab	540 c-g	0.50 ab
ch5b4	40 b-f	76 ab	72 abc	49 a	405 g	0 b
ch5b16	42 a-e	75 abc	71 a-d	46 ab	706 abc	0.75 ab
ch5b26	34 c-h	79 ab	71 abc	41 ab	671 a-e	0.5 ab
ch5b27	10 j	61 d	63 e	28 ab	675 a-e	0.5 ab
ch5b30	49 ab	79 ab	71 a-d	37 ab	475 efg	0.25 b
ch5b55	24 hi	74 bc	70 a-d	24 b	474 fg	0 b
bg16	34 c-h	76 ab	69 b-e	51 a	518 d-g	0.25 b
bg20	28 ghi	80 ab	73 abc	32 ab	656 a-e	0.25 b
bg24	31 e-i	77 ab	71 abc	34 ab	592 b-f	0.25 b
bg26	33 c-i	77 ab	72 abc	43 ab	694 a-d	0 b
bg42	32 d-i	78 ab	73 abc	37 ab	559 c-g	0.5 ab
bg47	43 a-d	78 ab	73 abc	44 ab	643 b-f	0.5 ab
bg48	27 ghi	78 ab	65 de	35 ab	648 b-f	0.5 ab
bg49	37 c-g	80 a	75 a	38 ab	715 abc	1.5 a
bg50	29 f-i	75 abc	74 ab	42 ab	836 a	1.5 a
bg99	22 j	69 c	68 cde	28 ab	585 b-g	0.25 b
bg114	42 a-e	77 ab	72 abc	48 ab	611 b-f	0.5 ab
bg215	43	77 ab	70 a-d	43 ab	752 ab	0.25 b

[a]Plant lines include Paymaster (PM) HS-26, a nontransformed parent, 'A' = 95-T20#-1517, which was derived from ({[6-19 x (998 x 108)-4-69] x 60-611-5}-4-76), and parent A transformed with chitinase (ch5b lines) or β-1,3 glucanase (bg lines).
[b]DAP is days after planting.
[c]Necr. is necrosis.
[d]Big eyed bug is *Geocoris* spp., and counts represent the number/20' row.
[e]Mean separation tests were the Waller-Duncan k-ratio t-tests ($P = 0.05$).

Table 2. Affect of lines transformed from type "B" on seedling disease, yield and insects at Halfway, TX.

Plant Lines[a]	% Emergence at 13 DAP[b]	20 DAP	27 DAP	Hypo[c] rating	lbs of lint per acre	Spiders[d]
PM HS-26	52 ae	57 e	45 c	1.0 ab	512 de	0.25 bc
B	44 bc	78 abc	71 ab	1.0 ab	510 e	0.25 bc
ch5b71	32 ef	74 bcd	67 b	0.9 ab	761 abc	0.25 bc
ch5b78	37 cde	77 abc	71 ab	1.1 ab	627 cde	0 c
ch5b121	36 def	78 ab	74 a	1.2 ab	688 b-e	0.25 bc
ch5b122	41 cd	79 ab	72 ab	0.9 ab	714 a-c	0.25 bc
ch5b123-1	34 def	74 bcd	69 ab	1.0 ab	683 b-e	0 c
ch5b123-2	52 ab	77 abc	70 ab	1.3 a	699 bcd	1.0 ab
ch5b124	29 fg	72 cd	68 ab	0.8 b	887 a	0 c
ch5b125	38 cde	81 a	68 ab	0.8 b	740 abc	0 c
ch5b126	38 cde	77 abc	70 ab	1.3 a	695 b-e	1.25 a
ch5b132	56 a	81 a	70 ab	1.1 ab	850 ab	0 c
ch5b137	24 g	74 bcd	71 ab	1.4 a	627 cde	0.75 abc
ch5b146	51 ab	71 d	68 ab	1.2 ab	581 cde	0 c

[a]Plant lines include Paymaster (PM) HS-26, a nontransformed parent, 'B' = 95-T20#-114 which was derived from EP-PMS-Del Cerro 515, and lines which derived from B and were transformed with a chitinase gene.
[b]DAP is days after planting.
[c]Hypocotyl rating was conducted on roots at 21 days after planting, with 0 = no disease, 1 = superficial lesion, 2 = sunken lesion, and 3 = plant dying from lesion.
[d]This represents the number of spiders from a 20', single row plot.
[e]Mean separation tests were the Waller-Duncan k-ratio t-tests ($P = 0.05$).

Table 3. Affect of transformed lines from type "A" on Verticillium wilt at Lubbock, TX.

Plant Lines[a]	Plants/ ft row	Incidence of wilt on 7/26	8/13	Severity[b] of wilt on 9/5	8/13	9/5	$\log_{10}$(Reniform[c]+1) per 500 cm^3 soil
PM HS-26	2.5 c-f[d]	4 b	8 b	11 b	0.21 bc	0.31 b	2.39
A	2.8 ab	8 b	20 ab	21 ab	0.54 ab	0.57 ab	2.38
ch5b4	2.9 ab	6 b	17 ab	25 ab	0.44 abc	0.74 ab	1.42
ch5b16	2.9 ab	8 b	12 b	22 ab	0.31 bc	0.64 ab	1.65
ch5b26	2.8 abc	3 b	6 b	11 b	0.15 c	0.32 b	2.10
ch5b27	2.3 f	6 b	14 b	16 ab	0.34 bc	0.41 ab	2.30
ch5b30	2.9 ab	7 b	9 b	12 b	0.22 bc	0.32 b	1.95
ch5b55	2.4 ef	16 a	29 a	31 a	0.78 a	0.88 a	1.51
bg16	2.8 abc	7 b	15 ab	22 ab	0.40 bc	0.64 ab	2.26
bg20	2.6 b-f	7 b	17 ab	27 ab	0.44 abc	0.77 ab	1.22
bg24	3.0 a	5 b	11 b	19 ab	0.28 bc	0.56 ab	2.32
bg26	2.8 abc	6 b	14 b	21 ab	0.32 bc	0.57 ab	2.09
bg42	3.0 a	8 b	16 ab	21 ab	0.40 bc	0.59 ab	1.98
bg47	2.9 ab	8 b	16 ab	22 ab	0.39 bc	0.62 ab	1.90
bg48	2.9 ab	5 b	14 b	21 ab	0.39 bc	0.61 ab	1.35
bg49	2.9 ab	7 b	12 b	25 ab	0.33 bc	0.71 ab	1.60
bg50	2.6 a-e	9 b	16 ab	24 ab	0.42 abc	0.68 ab	1.20
bg99	2.4 def	4 b	17 ab	22 ab	0.47 abc	0.64 ab	1.70
bg114	2.9 ab	4 b	11 b	17 ab	0.23 bc	0.50 ab	2.22
bg215	2.7 a-d	7 b	11 b	15 ab	0.26 bc	0.44 ab	1.71

[a]Plant lines include Paymaster (PM) HS-26, a nontransformed parent, 'A' = 95-T20#-1517, which was derived from ({[6-19 x (998 x 108)-4-69] x 60-611-5}-4-76), and parent A transformed with chitinase (ch5b lines) or β-1,3 glucanase (bg lines).
[b]Severity was calculated by rating each plant in a plot as 0 (healthy), 1 (wilt symptoms on only the bottom ½ of the plant), or 3 (wilt symptoms in the top ½ of the plant), and dividing the sum of the severity by the number of plants in a plot.
[c]The reniform nematode is *Rotylenchulus reniformis*, and was sampled on 20 August.
[d]Mean separation tests were the Waller-Duncan k-ratio t-tests ($P = 0.05$).

Table 4. Affect of Verticillium wilt on lines transformed from type "B" at Lubbock, TX.

Plant Lines[a]	Plants/ ft row	Incidence of wilt on 7/26	Incidence of wilt on 8/13	Severity[b] of wilt on 9/5	Severity[b] of wilt on 8/13	Severity[b] of wilt on 9/5	log_{10}(Reniform[c]+1) per 500 cm^3 soil
PM HS-26	2.5 bc[d]	4 bcd	8 cd	11 cd	0.21 cde	0.31 cd	2.39
B	2.8 ab	1 d	8 cd	11 cd	0.21 cde	0.31 cd	2.79
ch5b71	2.5 abc	11 ab	20 a	28 a	0.50 a	0.75 ab	3.13
ch5b78	2.9 a	6 a-d	7 d	13 bcd	0.16 de	0.35 cd	2.18
ch5b121	2.8 b	5 a-d	12 a-d	20 abc	0.32 a-d	0.58 abc	2.18
ch5b122	2.6 abc	9 abc	12 a-d	23 ab	0.30 a-e	0.61 abc	2.72
ch5b123-1	2.8 ab	7 a-d	10 bcd	13 bcd	0.23 b-e	0.36 cd	1.20
ch5b123-2	2.9 ab	10 ab	16 abc	19 abc	0.37 abc	0.51 abc	3.11
ch5b124	2.6 abc	7 a-d	11 bcd	15 bcd	0.27 b-e	0.38 cd	2.23
ch5b125	2.9 ab	7 a-d	11 bcd	22 abc	0.28 b-e	0.63 abc	2.78
ch5b126	2.8 ab	4 bcd	7 cd	16 bcd	0.19 cde	0.46 bc	0.88
ch5b132	2.7 abc	8 abc	9 cd	17 abc	0.20 cde	0.51 abc	2.25
ch5b137	2.4 c	12 a	17 ab	28 a	0.41 ab	0.80 a	1.95
ch5b146	2.9 ab	3 cd	5 d	6 d	0.11 e	0.12 d	3.27

[a]Plant lines include Paymaster (PM) HS-26, a nontransformed parent, 'B' = 95-T20#-114 which was derived from EP-PMS-Del Cerro 515, and lines which derived from B and were transformed with a chitinase gene.
[b]Severity was calculated by rating each plant in a plot as 0 (healthy), 1 (wilt symptoms on only the bottom ½ of the plant), or 3 (wilt symptoms in the top ½ of the plant), and dividing the sum of the severity by the number of plants in a plot.
[c]The reniform nematode is *Rotylenchulus reniformis*, and was sampled on 20 August.
[d]Mean separation tests were the Waller-Duncan k-ratio t-tests ($P = 0.05$).

Table 5. Affect of field position on incidence of Verticillium wilt and population density of *Rotylenchulus reniformis* (Rr), averaged across all breeding lines (33 entries).

Block	Incidence of wilt on 7/26	Incidence of wilt on 8/13	Incidence of wilt on 9/5	Log_{10}(Rr+1)/ 500 cm^3 soil
1	7 b[a]	16 a	21 b	0.81 b
2	4 c	10 b	14 c	0.43 b
3	3 c	10 b	11 c	3.39 a
4	13 a	17 a	30 a	3.60 a

[a]Mean separation tests were the Waller-Duncan k-ratio t-tests ($P = 0.05$).

Table 6. The effect of plant lines transformed from type "A" on % emergence and harvest density of *Meloidogyne incognita* (Mi) at the Western Peanut Growers Field.

Plant Lines[a]	Days after Planting 14	Days after Planting 21	Log_{10}(Mi+1)/ 500 cm^3 soil
PM HS-26	71 a[b]	74 ab	3.87
A	46 b-e	72 abc	3.93
ch5b4	27 fgh	61 abc	3.99
ch5b16	41 c-g	66 abc	4.33
ch5b26	33 e-h	73 ab	4.26
ch5b27	8 i	60 abc	3.87
ch5b30	61 ab	80 a	4.01
ch5b55	24 ghi	53 c	3.66
bg16	32 e-h	68 abc	4.13
bg20	32 e-h	70 abc	3.85
bg24	42 c-g	73 abc	4.17
bg26	44 b-f	73 ab	3.92
bg42	34 e-h	60 abc	3.93
bg47	36 d-g	71 abc	4.27
bg49	31 e-h	70 abc	3.90
bg50	46 b-e	71 abc	3.91
bg99	16 hi	59 bc	3.77
bg114	54 a-d	76 ab	4.04
bg215	55 abc	74 ab	4.12

[a]Plant lines include Paymaster (PM) HS-26, a nontransformed parent, 'A' = 95-T20#-1517, which was derived from ({[6-19 x (998 x 108)-4-69] x 60-611-5}-4-76), and parent A transformed with chitinase (ch5b lines) or β-1,3 glucanase (bg lines).
[b]Mean separation tests were the Waller-Duncan k-ratio t-tests (P = 0.05).

Table 7. The effect of plant lines transformed from type "B" on % emergence and harvest density of *Meloidogyne incognita* (Mi) at the Western Peanut Growers Field.

Plant Lines[a]	% Emergence at 14 days	Log_{10}(Mi+1)/ 500 cm^3 soil
PM HS-26	71 a[b]	3.87
B	49 b-e	3.36
ch5b71	42 c-f	3.95
ch5b78	37 d-g	4.03
ch5b121	35 efg	3.63
ch5b122	37 d-g	3.45
ch5b123-1	26 g	3.89
ch5b123-2	53 bc	3.90
ch5b124	29 fg	3.83
ch5b125	51 bcd	3.53
ch5b126	46 b-e	3.91
ch5b132	53 bc	4.00
ch5b137	31 fg	3.87
ch5b146	58 ab	3.80

[a]Plant lines include Paymaster (PM) HS-26, a non-transformed parent, 'B' = 95-T20#-114 which was derived from EP-PMS-Del Cerro 515, and lines which derived from B and were transformed with a chitinase gene.
[b]Mean separation tests were the Waller-Duncan k-ratio t-tests (P = 0.05).

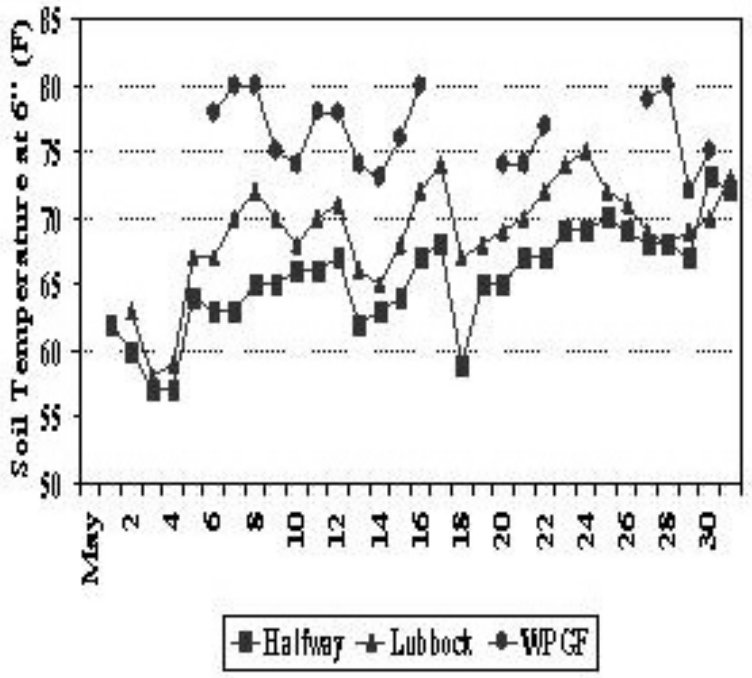

Figure 1. Soil temperature at a 6" depth during the seedling stage of 2002 for the test sites at Lubbock, Halfway, and the Western Peanut Growers Field (WPGF).

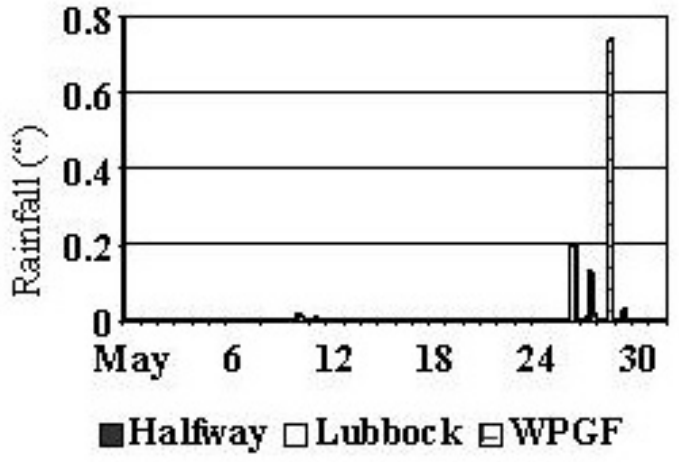

Figure 2. Rainfall during the seedling stage of 2002 for the test sites at Lubbock, Halfway, and the Western Peanut Growers Field (WPGF).

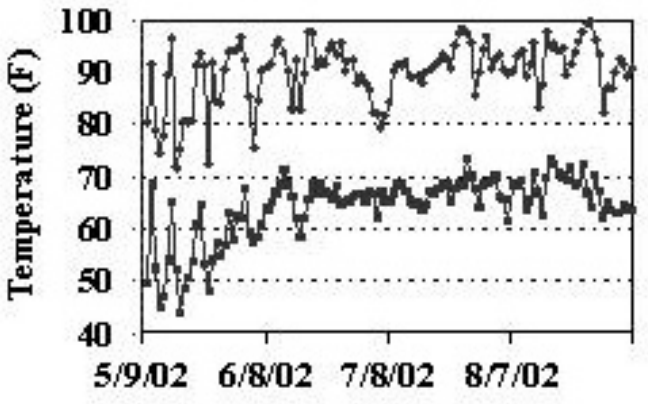

Figure 3. Air temperature (maximum and minimum) and the Lubbock Research and Cooperative Extension Center during the summer of 2002.

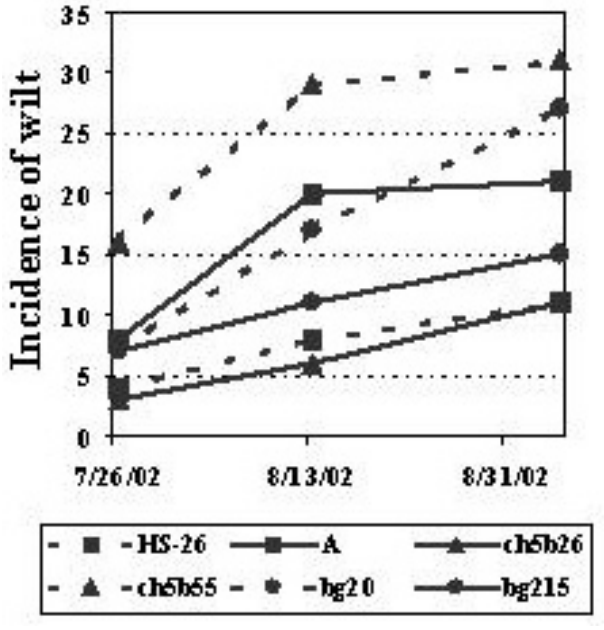

Figure 4. Verticillium wilt incidence during the 2002 growing season for Paymaster HS-26, a parent breeding line A (= 95-T20#-1517, which was derived from ({[6-19 x (998 x 108)-4-69] x 60-611-5}-4-76), and parent A transformed with chitinase (ch5b lines) or β-1,3 glucanase (bg lines) .