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Tolerance to *Rotylenchulus reniformis* and Resistance to *Meloidogyne incognita* Race 3 in High-Yielding Breeding Lines of Upland Cotton[1]

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Abstract: Field experiments in 1992 and 1994 were conducted to determine the effect of *Rotylenchulus reniformis,* reniform nematode, on lint yield and fiber quality of 10 experimental breeding lines of cotton (*Gossypium hirsutum*) in untreated plots or plots fumigated with 1,3-dichloropropene. Controls were La. RN 1032, a germplasm line possessing some resistance to *R. reniformis,* and Stoneville 453, a cultivar that is susceptible to reniform nematode. Several breeding lines produced greater lint yields than Stoneville 453 or La. RN 1032 in both fumigated and untreated plots. Average lint yield suppression due to *R. reniformis* for six of the 10 breeding lines was less than half of the 52% yield reduction sustained by Stoneville 453. In growth chamber experiments, *R. reniformis* multiplication factors for La. RN 1032 and breeding lines N222-1-91, N320-2-91, and N419-1-91 were significantly lower than on Deltapine 16 and Stoneville 453 at 6 weeks after inoculation. *R. reniformis* populations increased by more than 50-fold on all entries within 10 weeks. In growth chambers, the breeding lines N220-1-92, N222-1-91, and N320-2-91 were resistant to *Meloidogyne incognita* race 3; multiplication factors were ≤1.0 at both 6 weeks and 10 weeks after inoculation compared with 25.8 and 26.5 for Deltapine 16 at 6 and 10 weeks after inoculation, respectively, and 9.1 and 2.6 for Stoneville 453. Thus, the results indicate that significant advances have been made in developing improved cotton germplasm lines with the potential to produce higher yields in soils infested with *R. reniformis* or *M. incognita.* In addition to good yield potential, germplasm lines N222-1-91 and N320-2-91 appear to possess low levels of resistance to *R. reniformis* and a high level of resistance to *M. incognita.* This germplasm combines high yield potential with significant levels of resistance to both *R. reniformis* and *M. incognita.*

Key words: cotton, *Gossypium hirsutum, Meloidogyne incognita,* reniform nematode, resistance, root-knot nematode, *Rotylenchulus reniformis,* tolerance.

The reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira, was first identified as a parasite of cotton (*Gossypium hirsutum* L.) in 1940 (Birchfield and Jones, 1961). More than 35 years ago, *R. reniformis* was considered a potential threat to cotton production in Louisiana (Birchfield, 1962; Birchfield and Jones, 1961; Jones et al., 1959). The reniform nematode also is recognized as a pest of cotton in the Lower Rio Grande Valley (LRGV) of Texas (Birchfield et al., 1966; Heald et al., 1972; Robinson et al., 1987). Lawrence and McLean (1995) suggested that *R. reniformis* is becoming the most damaging nematode species in most cotton-producing areas of the southeastern United States. This increase in reniform nematode infestation may be the result of expanding cotton hectarage under continuous cotton production.

Typically, plants in cotton fields infested with *R. reniformis* are stunted, yield poorly, and do not respond to supplemental irrigation or fertilizer applications (Birchfield and Jones, 1961). Reniform nematode damage is difficult to diagnose in the field because symptoms of root infection are nonspecific and plants are uniformly stunted throughout the field (Veech, 1990). Reniform nematode causes reduced lint yield, boll size, lint percentage (weight of lint divided by the combined weight of seed and lint), plant growth, seed index, and fiber micronaire value (Cook and Namken, 1992; Jones et al., 1959).

Currently, the most effective strategies for managing *R. reniformis* in cotton include nematicides and rotation with nonhost crops. Cotton cultivars with reniform nematode resistance and tolerance as defined by Cook

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and Evans (1987) are currently unavailable. However, resistance to *R. reniformis* has been reported in upland cotton (Beasley and Jones, 1985), and a wild cotton species, *G. longicalyx* Hutch. & Lee, is immune (Yik and Birchfield, 1984). Four upland cotton germplasm lines, with significantly more resistance to both *R. reniformis* and *Meloidogyne incognita* race 3 (Kofoid & White) Chitwood than Deltapine 41, were released in 1988 (Jones et al., 1988). Several advanced *G. hirsutum* breeding lines recently were selected from fields of the LRGV where high *R. reniformis* populations occur (C.G. Cook and L.N. Namken, unpublished data). Our objective was to determine the response of these advanced breeding lines to *R. reniformis* and *M. incognita* race 3.

Materials and Methods

Field experiments: In 1992 and 1994, 12 cotton genotypes were evaluated at Weslaco, Texas, in a field naturally infested with *R. reniformis*. Soil type was a Hidalgo sandy clay loam (fine-loamy, mixed, hyperthermic Typic Calciustolls). No other plant-parasitic nematode species were detected in the experimental plots. The experimental design each year was a split-plot with four replications in 1992 and five replications in 1994. Main plots were either untreated or fumigated with 1,3-dichloropropene (Telone II, DowElanco, Indianapolis, IN). Subplots were the 12 cotton genotypes. Ten of the genotypes were experimental breeding lines developed by the cooperative cotton research program at Weslaco, Texas (USDA ARS and Texas Agricultural Experiment Station). Controls were Stoneville 453, a cultivar susceptible to *R. reniformis*, and La. RN 1032, a cotton germplasm line moderately resistant to both *R. reniformis* and *M. incognita* (Jones et al., 1988). Planting dates were 19 February 1992 and 3 March 1994. In the fumigated plots, 1,3-dichloropropene (190 kg a.i./ha) was applied on 18 December 1991 and 29 December 1993 with chisel equipment to a depth of 30 cm. Plots were fertilized with 45 kg N/ha as NH_4NO_3 on 9 January 1992 and 12 January 1994. Pendimethalin (*N*-[1-ethylpropyl]-3,4-dimethyl-2,6-dinitrobenzenamine) was applied for weed control as a preemergence treatment at 1.12 kg a.i./ha. Insect control followed standard practices for the LRGV. Planting included 120 acid-delinted untreated seeds of each entry in 9.1-m single-row plots, spaced 1.0 m apart. Initial and final *R. reniformis* population densities were estimated in the untreated and fumigated plots from composite soil samples (six 5-cm-diam. × 20-cm-deep cores per replication) at planting and at harvest. Nematodes were extracted with modified Baermann funnels (Robinson and Heald, 1989), and nematode population estimates were expressed as the mean across genotypes ± standard error. Sequential harvests from a 4.0-m section of each row were used to calculate lint yield. Harvest dates were 7 July, 23 July, and 10 August 1992 and 7, 14, and 27 July 1994. Fiber analyses were performed by the International Textile Center of Texas Tech University, Lubbock, Texas. Data were recorded for first lint harvest, total lint yields, lint percent, fiber length, fiber strength, and micronaire and analyzed by analysis of variance. Means were separated with Fisher's protected least significant difference (LSD) procedure.

Growth chamber experiments: The abilities of *R. reniformis* and *M. incognita* race 3 to reproduce on six experimental breeding lines were evaluated in two simultaneous experiments in a single growth chamber, which was programmed for a 14-hour day length. Chamber lamps provided 1,000 lux of incandescent light at the upper plant canopy during the first and last half hour of light, 10,000 lux mixed fluorescent and incandescent light during the second and second-to-last half hour, and 20,000 lux mixed light during the remaining 12 hours. Air temperature was 26 °C for a 1-hour period beginning at first light, followed by a linear 4-hour ramp to 30 °C, a 6-hour hold, a 3-hour ramp down to 28.5 °C, and a final 10-hour ramp back to 26 °C, ending at first light. Relative humidity was not controlled but remained above 50% most of the time. Soil and air temperatures were similar and

optimal for reproduction by both nematode species (Rebois, 1973; Van Gundy, 1985).

Inoculum of *R. reniformis* consisted of mixed vermiform stages collected from soil of greenhouse-grown tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) incubated in Baermann funnels for 12 hours before inoculation. Eggs of *M. incognita* were extracted from tomato roots with an NaOCl technique (Hussey and Barker, 1973), followed by centrifugal flotation in a 1-M sucrose solution; *M. incognita* inoculum consisted of second-stage juveniles (J2) that hatched from the eggs over a 3-day period. Nematodes of both species were >95% motile when used for inoculations.

Two seeds of each cotton entry were planted in a 500-cm^3 plastic pot filled with a 3:1:1 (v:v:v) mixture of sand (<400-µm particle size), peat, and vermiculite, supplemented with 4-g-per-kg soil pelletized limestone. Twenty-four pots were prepared for each entry. After 2 weeks, when seedlings were at the first true leaf stage, one seedling was removed from each pot. On the same day, the planting medium in each pot was infested with 1,000 J2 of *M. incognita* or 2,000 mixed vermiform stages of *R. reniformis* by injecting the appropriate nematode suspension 1 to 5 cm deep at four points 2 cm from the stem. One week later, 2,000 mixed vermiform stages of *R. reniformis* were added to the pots that had been previously infested with *R. reniformis*. Plants were watered daily and fertilized weekly with dilute liquid fertilizer (15-16-17 N-P-K and 1.0-0.2-0.1 Mg-Fe-Zn).

The *M. incognita* and *R. reniformis* pots were placed on opposite sides of the growth chamber and were separated by a splash-guard. The experimental design for both experiments was a randomized complete block with 12 replications and nine cotton entries: N220-1-91, N222-1-91, N320-2-91, N419-1-91, C224-91, C306-91, La. RN 1032, and Deltapine 16, with Stoneville 453 as the susceptible control. Each block was a plastic tray containing one pot of each entry. Thus, there were two rows of 12 trays, one with *R. reniformis* and the other with *M. incognita*, on opposite sides of the growth chamber. Six and 10 weeks after inoculation, the pots of every other tray of each row were removed and plant height, number of vegetative nodes, fresh and dry foliar weight, fresh and dry root weight, and taproot length were determined. Eggs were extracted as for initial inocula. Vermiform stages were extracted from soil with covered Baermann funnels (Robinson and Heald, 1989) that were tapped after 24 hours for *R. reniformis* and after 48 hours for *M. incognita*. Multiplication factors for each species were estimated at 6 and 10 weeks by dividing the total number of eggs and nematodes extracted per pot by the number originally introduced (1,000 for *M. incognita* and 4,000 for *R. reniformis*). Root systems from the *M. incognita* experiment were rated for severity of galling on a 0-to-5 scale, where 0 = no galls detected, 1 = galls detected on <5% of the root system, 2 = approximately 25% of the root system galled, 3 = 50% galled, 4 = 75% galled, and 5 = >95% of the root system galled.

Data for each nematode species at each harvest date were analyzed separately by a two-way analysis of variance; means were separated with Fisher's protected LSD to compare the six breeding lines, La. RN 1032, and Deltapine 16 with the control, Stoneville 453. All data on nematode population densities were transformed by $\log_{10}(x + 1)$ before analyses.

Results

Field experiments: In 1992, initial population densities (Pi) of *R. reniformis* at planting and final population densities (Pf) at crop maturity in the untreated plots were 110 ± 12 and 789 ± 69 nematodes per 100 cm^3 soil, respectively. The Pi and Pf of *R. reniformis* in fumigated plots were 17 ± 5 and 87 ± 10 nematodes per 100 cm^3 soil, respectively. In 1994, the Pi and Pf of *R. reniformis* in the untreated plots were 189 ± 15 and 1,198 ± 119 nematodes per 100 cm^3 soil, respectively, and the Pi and Pf in fumigated plots were 40 ± 6 and 109 ± 14 nematodes per 100 cm^3 soil, respectively.

Significant treatment × cultivar interactions ($P \leq 0.05$) were observed for first har-

vest yield (data not shown), total yield, and lint percentage (data not shown), indicating that the 12 genotypes differed in their responses to fumigation. Because there were significant treatment × year and cultivar × year interactions for total yield, the results were reported for each year separately. Yields of the 12 genotypes were lower in 1994 compared to 1992.

In 1992, yields ranged from 473 to 1,203 kg/ha in the untreated plots, with seven of the breeding lines producing significantly greater yields than La. RN 1032 and Stoneville 453 (Table 1). In fumigated plots, N220-1-91 and N419-1-91 had ca. 25% greater yields than Stoneville 453 ($P \leq 0.05$). Only C306-91 did not produce significantly greater yields than La. RN 1032, indicating that, compared to La. RN 1032, the genotypes generally had greater yield potential or adaptability to the growing conditions of the LRGV of Texas.

In the 1994 experiment, all of the genotypes had higher yields than Stoneville 453 in untreated plots ($P \leq 0.05$) (Table 1). Breeding lines N320-2-91 and N220-1-91 produced three times as much lint as Stoneville 453 in the untreated plots. Lines N419-1-91, C224-91, C300-91, and N222-1-91 produced more than twice as much lint as Stoneville 453. Lines N320-2-91, N220-1-91, N419-1-91, and C224-91 all produced more than 175% of the lint produced by La. RN 1032. Only C300-91 produced a significantly greater yield than Stoneville 453 in the fumigated plots. However, eight breeding lines produced greater yields than La. RN 1032 ($P \leq 0.05$), indicating a greater yield potential than La. RN 1032 under LRGV growing conditions.

In 1992, lint yields of the 12 entries grown in non-fumigated plots were suppressed 4.1% to 42.8% compared to lint yields in fumigated plots. Lint yields of breeding lines N320-2-91, C306-91, and N220-1-91 were suppressed less than 20%; in comparison, Stoneville 453 and La. RN 1032 had lint yield reductions of 37.5% and 42.8%, respectively. Yield suppression of Stoneville 453 was greater than that of breeding lines C300-91, C224-91, N220-1-91, and N419-1-91 ($P \leq 0.05$). In 1994, yield was reduced 2.8% to 66.8% in the non-fumigated plots compared to the fumigated plots. Seven experimental breeding lines and La. RN 1032 sustained less than one-half of the yield loss observed for Stoneville 453. Averaged across the 2-year study, yield reductions due to *R. reniformis* were less for five of the breeding lines than for Stoneville 453 ($P \leq 0.05$). Two of the breeding lines, N220-1-91 and C224-91, were particularly consistent across both

TABLE 1. Total lint yield of 12 cotton (*Gossypium hirsutum*) genotypes in untreated and in 1,3-dichloropropene-fumigated plots in a field infested with *Rotylenchulus reniformis* in 1992 and 1994.

	Total yield (kg/ha) in 1992		Total yield (kg/ha) in 1994	
Breeding line or cultivar	Untreated	Fumigated	Untreated	Fumigated
N320-2-91	1,203*[a]	1,254*	709*	745
N220-1-91	1,135*	1,373*	669*	688
N419-1-91	953*	1,353*	615*	769
C224-91	924*	1,212	614*	746
C300-91	905*	1,180	549*	810*
N222-1-91	866*	1,243*	497*	640
C306-91	841*	936*	439*	757
C301-91	804	1,173	418*	668
N320-4-91	759	1,157	391*	520*
N226-1-91	607	967	355*	547
Checks				
La. RN 1032 (Resistant)	473*	827*	342*	444*
Stoneville 453 (Susceptible)	673	1,077	221	665
LSD ($P \leq 0.05$)	137	137	119	119

Data for 1992 are means of four replications, and data for 1994 are means of five replications.

[a] Asterisks indicate values that differ from that of the control (Stoneville 453) by one LSD ($P \leq 0.05$).

years for yield response in the fumigated and non-fumigated treatments.

Growth chamber experiments: Six weeks after inoculation with *R. reniformis,* egg production, soil populations of vermiform nematodes, and multiplication factor for N222-1-91 were less than half of those measured for Stoneville 453 ($P \leq 0.05$) (Table 2). Egg production on the related line N320-2-91 was numerically lower than on N222-1-91. Multiplication factors for N222-1-91, N320-2-91, N419-1-91, and La. RN1032 also were lower than on Stoneville 453 at 6 weeks ($P \leq 0.05$). Nematode counts at 10 weeks, however, indicated that all entries were highly suitable hosts for *R. reniformis,* with populations of vermiform nematodes in the soil of 21,000–70,000 nematodes per 100 cm^3 soil. Numbers of *R. reniformis* eggs at 10 weeks after inoculation for N222-1-91 and La. RN 1032 were significantly fewer than for Stoneville 453.

Entries differed markedly in susceptibility to *M. incognita* ($P \leq 0.05$) (Table 3). Cultivar La. RN 1032 and breeding lines N220-1-91, N222-1-91, and N320-2-91 had egg and juvenile densities less than 10% those of Stoneville 453 and 5% those of Deltapine 16. Multiplication factors on these lines were 1.0 or lower, indicating a high level of nematode resistance. Multiplication factors on the cultivar Deltapine 16 and the breeding lines C224-91 and C306-91 were higher than on Stoneville 453. Differences in root galling at both harvest dates paralleled differences in nematode reproduction.

Numbers of vegetative nodes, plant heights, and dry weights of roots and shoots indicated that plants of all cultivars in both experiments 10 weeks after inoculation were comparable in size and at a comparable stage of phenological development (Table 4). The only consistently significant difference between the breeding lines tested and Stoneville 453 was a 40% heavier root system in N320-2-91.

Discussion

Confirming the findings of Jones et al. (1959), results of this study showed that *R. reniformis* can cause significant lint yield reductions in cotton. Micronaire or fiber fineness, the only fiber trait affected significantly, was reduced from 4.0 to 3.9 units. When compared to Stoneville 453, several of the breeding lines produced significantly greater yields in the *R. reniformis*-infested plots and showed less yield reduction due to nematode damage. Most of the breeding lines also produced greater yields than La. RN 1032 in both fumigated and non-fumigated treatments. Results of the field experiments indicate that several of the breeding lines possessed either greater host tolerance or resistance to reniform nema-

TABLE 2. Reproduction of *Rotylenchulus reniformis* on nine cotton (*Gossypium hirsutum*) genotypes 6 and 10 weeks after inoculation with 4,000 *R. reniformis* nematodes (mixed vermiform stages), in a growth chamber.

Breeding line or cultivar	Eggs per gram of dry root		Vermiform nematodes per 100 cm^3 soil		Multiplication factor[a]	
	6 weeks	10 weeks	6 weeks	10 weeks	6 weeks	10 weeks
N220-1-91	6,310	12,460	4,680	28,570	9.9	58.2
N222-1-91	5,170*[b]	4,770*	3,640*	26,900	6.5*	55.4
N320-2-91	3,720*	13,360	5,270	39,340	8.9*	62.1
N419-1-91	5,710	33,190	4,450	21,810	8.3*	53.9
C224-91	10,200	24,340	6,250	55,830	11.2	95.4
C306-91	21,380	57,530	10,410	70,760	19.5	151.8*
La. RN 1032	6,970	8,780*	3,750*	28,570	7.9*	66.8
Deltapine 16	13,100	25,590	8,690	32,530	14.1	60.0
Stoneville 453	12,580	31,890	9,700	33,860	16.7	65.1

Data shown are untransformed means of six replications. Data were transformed by $\log_{10}(x + 1)$ before analysis.

[a] Multiplication factor is defined as the total number of vermiform nematodes and eggs per pot divided by the initial inoculum (4,000).

[b] Asterisks indicate values that differ from that of the control (Stoneville 453) by one LSD ($P \leq 0.05$).

TABLE 3. Reproduction and root galling by *Meloidogyne incognita* on nine cotton (*Gossypium hirsutum* L.) genotypes, 6 and 10 weeks after inoculation with 1,000 *M. incognita* second-stage juveniles (J2), growth-chamber experiment.

Entry	Eggs per gram of dry root		J2 per 100 cm^3 soil		Multiplication factor[a]		Gall rating (0–5)[b]	
	6 weeks	10 weeks	6 weeks	10 weeks	6 weeks	10 weeks	6 weeks	10 weeks
N220-1-91	192*[c]	20*	32*	1*	0.6*	0.1*	1.00*	1.00*
N222-1-91	308*	14*	23*	2*	1.0*	0.3*	2.00*	1.83*
N320-2-91	209*	77*	39*	2*	0.9*	0.3*	1.50*	2.00*
N419-1-91	11,160	8,100	200	30	19.6	19.4*	2.00*	3.33
C224-91	14,760	11,270	770	280*	23.7*	22.4*	4.17*	4.33*
C306-91	22,250	18,220*	1,340	49	25.4*	31.0*	4.00	4.83*
La. RN 1032	130*	20*	56*	2*	0.7*	0.3*	1.33*	2.17*
Deltapine 16	19,150	13,360*	1,040	97	25.8*	26.5*	3.67	4.20*
Stoneville 453	6,000	1,510	700	24	9.1	2.6	3.17	3.17

Data are untransformed means of six replications. All data except gall ratings were transformed by $\log_{10}(x+1)$ before analysis.
[a] Multiplication factor is defined as the total number of juveniles and eggs per pot divided by the initial inoculum (1,000).
[b] Gall rating: 0 = no galls detected, 1 = galls detected on <5% of the root system, 2 = 25 % of the root system galled, 3 = 50% galled, 4 = 75% galled, and 5 = >95% of the root system galled.
[c] Asterisks indicate values that differ from that of the control (Stoneville 453) by one LSD ($P \leq 0.05$).

todes than Stoneville 453 and had better yield potential than La. RN 1032.

In the growth-chamber study, breeding lines N222-1-91, N320-2-91, and N419-1-91 had lower *R. reniformis* multiplication factors than Stoneville 453 at 6 weeks after inoculation, indicating that these lines possessed measurable resistance. However, no differences in multiplication factor of *R. reniformis* were observed at 10 weeks after inoculation. The higher yields and lower yield reductions of the breeding lines in the field may have resulted in part from delayed reniform nematode population development in the early stages of plant development.

Reniform nematode reproduction on the breeding lines was not lower than on La. RN 1032; however, the yield potential of most of the breeding lines was significantly greater. Of particular interest is breeding line C224-91. Although C224-91 appears to be an excellent host for both *R. reniformis* and *M. incognita* race 3, compared to Stoneville 453 it had a consistently lower yield reduction in our study and also has shown good performance in fields infested with *M. incognita*

TABLE 4. Plant-growth parameters of nine cotton (*Gossypium hirsutum*) entries 10 weeks after inoculating 2-week-old seedlings with *Rotylenchulus reniformis (Rr)* or *Meloidogyne incognita (Mi)*.

Entry	Plant height (cm)		Number of nodes		Dry shoot weight (g)		Dry root weight (g)	
	Rr	*Mi*	*Rr*	*Mi*	*Rr*	*Mi*	*Rr*	*Mi*
N220-1-91	34	33	11.5	10.5	6.2	7.8	1.07	1.08
N222-1-91	30	31	11.5	10.2	6.3	7.4	1.21	1.57
N320-2-91	32	29	10.7	10.0	6.7	6.3	1.76*[a]	1.83*
N419-1-91	36	39*	11.2	11.2	5.4	6.7	1.74*	1.45
C224-91	33	33	10.7	11.0	5.8	5.9*	1.26	1.62*
C306-91	27	33	10.8	11.7	4.9	5.4*	1.38	1.57
La. RN 1032	34	35	11.5	11.3	7.0	7.3	1.32	1.49
Deltapine 16	32	33	11.3	10.7	6.6	6.3	1.17	1.27
Stoneville 453	31	31	10.8	10.2	6.1	7.4	1.22	1.24
LSD ($P \leq 0.05$)	NS	5	NS	NS	NS	1.3	0.36	0.36

Data are means of six replications.
[a] Asterisks indicate values that differ from that of the control (Stoneville 453) by one LSD ($P \leq 0.05$).

race 3 (A.W. Scott, pers. comm.). Based on these results and the concepts of Cook and Evans (1987), it appears that C224-91 possesses good tolerance because it is a susceptible host compared to Stoneville 453, but does not sustain an equivalent yield loss.

Results of the growth-chamber experiment indicated also that good resistance to *M. incognita* race 3 was present in three of the six tested breeding lines. Multiplication factors of N220-1-91, N222-1-91, and N320-2-91 were ≤1 at 6 and 10 weeks, indicating a high level of resistance to *M. incognita* race 3. The nematode resistance observed in N220-1-91, N222-1-91, N320-2-91, and N419-91 probably resulted from crossing the root-knot and reniform nematode-resistant germplasm developed by Jones et al. (1988) with high-yielding, locally adapted germplasm. These lines represent new germplasm with the potential to produce high yields on reniform nematode-infested and uninfested soils while limiting population increases by *M. incognita* race 3. This new germplasm, which combines high yield potential and significant resistance to both *R. reniformis* and *M. incognita*, demonstrates the progress of an ongoing effort to develop cotton cultivars capable of minimizing yield losses caused by *R. reniformis* and *M. incognita*.

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