PERFORMANCE EVALUATION OF LONREN AND BARBREN RENIFORM NEMATODE RESISTANT GERMPLASM LINES

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Abstract

In the quest to incorporate host plant resistance to reniform nematode in cotton, two candidate resistant germplasm lines, LONREN 21-4 and BARBREN-713, were the subjects of a uniform field evaluation test. Testing was conducted at three locations: Lubbock, TX, St. Joseph, LA, and Belle Mina, AL. Results suggest that BARBREN-713 combines good reniform nematode resistance with good tolerance against the parasite and promising agronomic potential. Though LONREN 21-4 seems to offer a higher level of nematode resistance, it showed at the same time disturbing signs of lack of tolerance in the form of early season stunting.

Introduction

Reniform nematode (*Rotylenchulus reniformis*) is a sedentary semi-endoparasite, causing considerable yield losses in upland cotton (*Gossypium hirsutum*) (Blasingame *et al*., 2010). Developing host-plant resistance, though a high priority, has proven to be a complex problem. Only weak to moderate resistance to reniform nematode has been reported in upland cotton, but high to very high levels of resistance have been found in other *Gossypium* species (Starr *et al.*, 2007).

At the extreme end of resistance, four accessions of *G. longicalyx* were reported virtually immune to reniform nematode (zero nematode reproduction) (Yik and Birchfield, 1984). This resistance was successfully introgressed into *G. hirsutum* (Robinson *et al.*, 2007), culminating in April 2007 in the release of two reniform nematode resistant germplasm lines named LONREN-1 and LONREN-2 (Starr *et al.*, 2007). During subsequent field testing over a wide range of environments, early season stunting of seedlings of these two lines was observed in areas with high nematode densities (Nichols *et al*., 2010). A hypersensitive reaction in LONREN to reniform nematode has been advanced as a possible explanation for this early season stunting (Sikkens *et al.*, 2011).

As part of the release process of two additional germplasm lines with reniform nematode resistance, it was found judicious to include one season of field testing at various locations with high nematode densities. Candidate germplasm line LONREN 21-4 derives its reniform resistance characteristics from the same *G. longicalyx* source as the earlier released LONREN lines. According to a draft Release Notice, reniform nematode resistance in candidate germplasm line BARBREN-713 was transferred from *G. barbadense* accession 713 (GB713) of the USDA National Germplasm Collection. The draft release note also states that BARBREN-713 incorporates resistance to root-knot

nematode (*Meloidogyne incognita*), originating from the breeding line M-315. This resistance to root-knot nematode was not tested in this evaluation, though one observation on this issue is included at the end of this paper.

Materials and Methods

The protocol for the uniform evaluation of the LONREN 21-4 and BARBREN-713 germplasm lines was formulated towards the end of March 2011. Fields at four locations with high reniform nematode densities in four different states were selected: the Texas AgriLife Research facilities in Lubbock, Texas, the Northeast Louisiana Experiment Station, St. Joseph, Louisiana, the Mississippi Agricultural & Forestry Experiment Station, Mississippi, and the Tennessee Valley Research and Extension Center (TVREC), Belle Mina, Alabama. Soon after the start of the study, the Mississippi location was discontinued for logistical reasons.

Beside the two lines with potential reniform nematode resistance, four commercial cultivars were included in the test as standards: Deltapine (DP) 5415, Stoneville (ST) 5288B2F, Phytogen (PHY) 375WRF and Fibermax (FM) 9160B2F. Seed of LONREN 21-4, BARBREN-713 and the conventional variety DP 5415 received a fungicide treatment (Baytan 30 + Allegiance FL + Vortex FL at 0.5 + 0.75 + 0.08 oz/100 lb seed) in Lubbock prior to distribution to the cooperators. Seed treatment insecticide need was determined by the individual cooperator. In case of the Belle Mina location, Gaucho®600 (Bayer CropScience, 12.78 oz/100 lb seed) was applied to prevent early season thrips damage. At the Lubbock, site, Orthene 90S (3.2 oz/acre) was applied weekly after emergence for 28 days. Initially, the experiment called for a uniform 6 × 6 Latin Square design of 2-row plots of 50 ft in length each. Though the Latin Square design was implemented on all locations, local space considerations altered some plot dimensions, as detailed in Table 1. This table also summarizes the growing dates of each location.

Table 1. Selected characteristics of the 3 experiment locations.

Feature	Lubbock, TX	St. Joseph, LA	Belle Mina, AL
row length	35 ft	50 ft	25 ft
rows per plot	2	2	4
row spacing	40"	40"	40"
seeding rate	4 seed/foot	4 seed/foot	4 seed/foot
irrigation	furrow	none	sprinkler
planting date	May 12	May 16	May 17
harvest date	November 4	October 3	October 18
growing days	176	140	154
harvest method	stripper	picker	50-boll handpicked samples followed by picker
observations	yield reduced due to two weather events: (1) wind, (2) rain	poor stand on all varieties due to drought conditions	± 2 week planting delay due to April 27 tornado damage

Results and Discussion

A number of field observations were made during the growing season. Table 2 presents stand counts made at 30 Days After Planting (DAP) at the Lubbock and Belle Mina locations. At both locations, LONREN 21-4 had the poorest stand. For comparison: at Belle Mina, 30 DAP stand count of LONREN 21-4 on the Latin Square was 51% of planted seeds, compared to 73% on a nearby nematode-free field (500 plant sample, part of a different study). Though the 30 DAP stand of BARBREN-713 at Lubbock represented only 46% of planted seeds, it was not significantly lower than the stands of the four check varieties. There, hot, dry, and windy conditions resulted in rapid drying of the seedbed, so poor emergence was due to seed drying out rather than disease. BARBREN-713 on the Latin Square at Belle Mina had a 30 DAP stand count of 65%, compared to 72% on the nearby nematode-free field.

Stand counts per se are, by various degrees, influenced by the quality of their original seed material. Perhaps a better insight in terms of seedling tolerance towards reniform nematode can be gained by studying the differences in stand count between two early growing season dates. Table 2 also includes a 15 DAP stand count made at the Belle Mina location. Whereas BARBREN-713 and the four checks showed stand count losses in the 4-7% range between June 1 and 16, LONREN 21-4 lost 21% of its germinated seedlings during the same time period.

Table 2. Stand counts at 30 days after planting (DAP). Included are data on stand reduction between 15 and 30 DAP at the Belle Mina location.

Genotype	Lubbock, TX stand count 30 DAP (1)	Belle Mina, AL stand count 15 DAP (1)	Belle Mina, AL stand count 30 DAP (1)	Belle Mina, AL change in count stand between 15 and 30 DAP (2)
	%			
LONREN 21-4	24 c (3)	64	51 c	- 21
BARBREN-713	47 ab	70	65 b	- 7
DP5415	39 b	71	66 b	- 7
ST5288B2F	51 a	82	79 a	- 4
PHY375WR	51 a	83	79 a	- 5
FM9160B2F	57 a	74	69 b	- 7
LSD0.05	14.6		7.8	

(1) Expressed as mean percentages (of 6 replications) of surviving seedlings versus planted seeds.
(2) Expressed as mean percentages (of 6 replications) of seedling loss between 15 and 30 DAP.
(3) Means in columns followed by the same letter do not differ significantly at the 0.05 probability level.

At 30 DAP, 10 plants per plot were excavated on the Lubbock, TX, and St. Joseph, LA locations. The objective of this exercise was to evaluate the condition of the roots and the effects on nematode development. In Texas, there were no differences early in the season among cultivars with respect to the number of reniform nematode feeding in the roots (per g root) (Table 3). However, root growth differences were already apparent, with LONREN 21-4 having less root mass (0.71 g/plant) than PHY 375WRF (0.94 g/plant) and FM 9160B2F (0.89 g/plant) (Table 3). BARBREN-713 also had less root mass (0.76 g/plant) than did PHY 375WRF (Table 3). There was tremendous variability between the number of nematodes/root system at this site, indicating that many more plants would need to be sampled to adequately determine differences among cultivars. At the time of sampling, the weather in Texas was excessively hot, windy, and dry, with soil drying out rapidly between furrow irrigations at this site, so the reniform nematode may have had difficulty moving to roots in areas of the row, increasing the variability.

Table 3. Root and nematode development at 30 days after planting.

Genotype	Lubbock, TX fresh root mass (gr)	RN (1) per plant (-)	eggmass per plant (-)	RN (1) per gram of root (RN/gr)	St. Joseph, LA juveniles per plant (-)	eggs per plant (-)
LONREN 21-4	0.71	58 ab (2)	4.0	82	160 a	528 b
BARBREN-713	0.76	26 b	1.8	34	105 a	305 b
DP5415	0.86	39 ab	2.3	45	204 a	877 ab
ST5288B2F	0.78	72 ab	7.8	93	312 a	1769 a
PHY375WR	0.94	66 ab	4.8	70	147 a	784 b
FM9160B2F	0.89	101 a	4.8	114	189 a	931 ab
LSD0.05		65			181	911

(1) Reniform nematodes that had begun to feed (i.e., some swelling was evident).
(2) Means in columns followed by the same letter do not differ significantly at the 0.05 probability level.

About six weeks after planting, heights of 10 representative plants of each plot were recorded at the Lubbock and Belle Mina locations (Table 4). A node count was also conducted at Belle Mina on the same 10 plants. At this location, two further plant height observations from later in the growing season are available, this time based on estimated row-by-row average plant heights. These observations are also reflected in Table 4. The overall picture emerging from Table 4 is that the development of LONREN 21-4 lagged behind all its peers at both locations, while BARBREN-713 significantly outperformed the growth of all other genotypes at the Belle Mina location.

The protocol for the uniform evaluation test called for two reniform nematode field counts: the first before planting, the second as near as practical to August 15. This requirement was met at the Lubbock location, where a third post-harvest count was added because it finally rained, allowing for deeper sampling than on the August 15 date.

Sampling dates on the two other locations varied considerably, as detailed in Table 5. Though this complicates direct comparison among locations, the obtained results still support some interesting, general conclusions.

Table 4. Plant heights and node counts.

	Lubbock, TX	Belle Mina, AL			
	plant height (1)	plant height (1)	node count (1)	plant height (3)	plant height (3)
date	June 28	June 30	June 30	July 14	Aug. 2
days after planting	47 DAP	44 DAP	44 DAP	58 DAP	77 DAP
genotype	(cm)	(cm)	(-)	(cm)	(cm)
LONREN 21-4	5.5	7.9	5.0	18	43
BARBREN-713	7.5	16.0	7.7	40	91
DP5415	5.7	12.7	7.5	25	57
ST5288B2F	7.7	12.2	6.4	24	52
PHY375WR	8.5	10.2	6.4	23	52
FM9160B2F	7.1	10.9	7.3	22	49

(1) Data are mean heights of 10 representative plants per plot (six replications), in cm.
(2) Data are mean node counts of 10 representative plants per plot (six replications).
(3) Data are means of row-by-row estimated average plant heights (four rows of six replications), in cm.

Table 5. Reniform nematode counts from field samplings taken at various intervals (1).

Location	Genotype	preplant		Aug. 19		Nov. 14	
		-		99 DAP		186 DAP	
		---------- reniform nematode per 100 cm^3 ----------					
Lubbock, TX	LONREN 21-4	173	ab (2)	63		23	d
	BARBREN-713	63	a	223		533	bcd
	DP5415	50	b	293		1893	ab
	ST5288B2F	197	ab	467		767	bcd
	PHY375WR	120	ab	407		2233	a
	FM9160B2F	125	ab	288		1149	abc
	LSD0.05	144				1223	
		May 23		July 5		Aug .16	
		7 DAP		50 DAP		92 DAP	
St. Joseph, LA	LONREN 21-4	3669	a	3157	a	5911	c
	BARBREN-713	1547	c	4325	a	6709	bc
	DP5415	2501	abc	4869	a	11221	abc
	ST5288B2F	2971	ab	4688	a	14432	a
	PHY375WR	3024	ab	5008	a	15957	a
	FM9160B2F	2299	bc	3800	a	14091	ab
	LSD0.05	1256		2373		7597	
		June 22		July 20		Oct. 25	
		36 DAP		64 DAP		161 DAP	
Belle Mina, AL	LONREN 21-4	137	ab	283		601	c
	BARBREN-713	60	b	197		893	bc
	DP5415	112	ab	687		1519	abc
	ST5288B2F	137	ab	352		1983	ab
	PHY375WR	146	ab	403		2086	a
	FM9160B2F	292	a	721		1245	abc
	LSD0.05	187				1196	

(1) All data are means of 6 replications, and reported as reniform nematode counts per 100 cm^3 of soil.
(2) Means in column sections followed by the same letter do not differ significantly at the 0.05 probability level.

At all three locations, three separate nematode counts were conducted. The results, summarized in Table 5, indicate that mean final nematode counts on BARBREN-713 plots were lower than those on plots with susceptible genotypes at all three test locations, though the differences were not always statistically significant. Population reduction was most pronounced on LONREN 21-4 plots. It is, however, open to debate whether this population reduction is only

due to the reniform nematode resistance characteristics of LONREN 21-4. Given the low 30 DAP seedling counts for this entry (Table 2) and its lackluster growth performance (Table 3), an argument can be made that the observed low levels of reniform nematode reproduction could, at least in part, be attributable to the absence of root tissue needed to support optimum reniform nematode reproduction.

At the final sampling on the Lubbock site, a separate set of samples were pulled on the LONREN 21-4 plots, with one set for the "shorter" plants and one set for the "taller" plants. The shorter plants averaged significantly fewer reniform nematodes (23 reniform/100 cm^3 soil), than found in roots and soil from the taller plants in the same plots (620 reniform/100 cm^3 soil). This would suggest that any "tolerance" gains made by LONREN 21-4 over earlier releases are due to a mixture of more susceptible plants combined with the highly resistant plants. However, the population density under the taller plants was not as high as in the susceptible varieties (Table 5), and selfing flowers from the taller plants may result in a less resistant, but a more tolerant alternative.

At the Belle Mina location, 50 boll samples were handpicked prior to plot harvesting, primarily to collect material for lint quality analyses. On the LONREN 21-4 and BARBREN-713 plots, this sampling was done on individual plant basis, that is: 5 bolls were taken from 10 randomly selected plants, separately bagged and separately ginned (60 samples total for each candidate line). All BARBREN-713 seed samples had white fuzz color. Fuzz color of LONREN 21-4 seed varied: 20 plants had predominantly green fuzz seed, while the remaining 40 plants yielded mostly white fuzz seed. The linkage in *G. longicalyx* derived reniform resistant lines, such as LONREN 21-4, between the phenotypic green fuzz trait and resistance to reniform nematode was earlier established (Dighe, 2009). Harvesting results from Belle Mina would lend support to the idea that, when exposed to high reniform nematode infestation conditions, some form of selection occurs within the LONREN 21-4 population.

At Belle Mina, lint yield of BARBREN-713 was a respectable 1015 lbs/acre (Table 6), almost double the yield of the next highest performer (DP 5415). By contrast, LONREN 21-4 was by far the lowest yielding genotype at this

Table 6. Summary of yield data.

Location	Genotype	Seedcotton (lbs/acre)	Lint percentage (%)	Lint yield (lbs/acre)	Observations
Lubbock, TX (*)	LONREN 21-4	163 b (1)	42.0	68 b	(*) Stripper harvested. Seed cotton yields are estimates based on sample seed and lint weights (i.e., after deduction of the contamination component of the harvested samples).
	BARBREN-713	381 a	33.2	125 a	
	DP5415	181 b	33.6	62 b	
	ST5288B2F	184 b	37.0	67 b	
	PHY375WR	161 b	37.3	57 b	
	FM9160B2F	304 a	36.2	112 a	
	LSD0.05	109		39	
St. Joseph, LA (**)	LONREN 21-4	317 c			(**) Seedcotton means are based on yields of four replications only. Stands on the fifth and six replications were too poor to give meaningful yields. Lint turnout data are not available.
	BARBREN-713	513 bc			
	DP5415	350 c			
	ST5288B2F	1232 a			
	PHY375WR	667 bc			
	FM9160B2F	827 b			
	LSD0.05	400			
Belle Mina, AL	LONREN 21-4	776 c	37.8	293 c	
	BARBREN-713	2698 a	37.5	1015 a	
	DP5415	1462 b	36.9	543 b	
	ST5288B2F	1196 bc	40.7	486 bc	
	PHY375WR	1124 bc	42.2	470 bc	
	FM9160B2F	1235 bc	39.9	495 bc	
	LSD0.05	549		224	

(1) Means in column sections followed by the same letter do not differ significantly at the 0.05 probability level.

location; its yield constituted less than 30% of the BARBREN-713 yield. Overall yields at the Lubbock location were only a fraction of the yields obtained at Belle Mina. Here again, though, BARBREN-713 was the best performing entry. The poor stands associated with LONREN 21-4 at this site made it impossible to adequately

evaluate its yield potential. The record setting heat and drought, combined with reniform nematode damage at Lubbock, made it impossible to water these plants enough to obtain reasonable yields for any cultivar. No meaningful yields were obtained from the fifth and sixth replications at the St. Joseph, LA, location due to poor plant stands. The yield picture from the remaining four replications deviates considerably from that presented at the other two locations. At St. Joseph, ST 5288B2F was by far the best performing entry, followed at distance by the two other transgenic varieties. BARBREN-713 was the best performing conventional genotype, with LONREN 21-4 ending again at the low end.

Conclusions

A test conducted in 2010 at Belle Mina found that germplasm lines with *G. longicalyx* based resistance to reniform nematode, such as LONREN-1, LONREN-2 and resistant lines of the cross LONREN-1 × FM966, suffered significant reductions in growth rate, overall plant height, and yield compared to susceptible sister lines of the same cross when cultivated in a field infested with reniform nematode (Weaver *et al*., 2011). As such, it was not entirely surprising that LONREN 21-4, whose resistance to reniform nematode is derived from the same source, performed rather poorly at all three uniform evaluation test locations. Not only did it show signs of early seedling stunting, it's seedling mortality rate at Belle Mina during the first growing month was also considerably higher than that of the other entries.

LONREN 21-4's yields were at or near the bottom at all three locations, affirming its lack of tolerance towards reniform nematode. At the same time, seasonal nematode increases were more limited on LONREN 21-4 plots.

BARBREN-713 performed very well at the Belle Mina location. It did not show any sign of stunting in the uniform evaluation test field, and its lint yield at 1015 lb/acre was quite impressive. Although not reported upon in detail in this paper, it should be mentioned here that both LONREN 21-4 and BARBREN-713 were also included in another yield test at the experiment station in Belle Mina. This test consisted of two fields (of 5 replications each), one with considerable reniform nematode infestation and a second field free from reniform nematode. On the nematode infested field, lint yield for BARBREN-713 was 955 lb/acre (15th out of 46 entries); it yielded 731 lb/acre (43rd out of 46) on the nematode free field.

Agronomic performance of all entries at both the Lubbock and St. Joseph locations suffered from climatic conditions and other constraints. At both sites BARBREN-713 performed better or equal to its peers. At the same time, end of season nematode numbers on BARBREN-713 appear to be lower than that on plots cultivated with non-resistant genotypes.

On the issue of root-knot resistance of BARBREN-713: it was also tested in a variety test at the root-knot nematode nursery in Lamesa, TX and yielded slightly below average, ranking 23rd out of 34 entries.

Bottom line: BARBREN-713 might indeed constitute a promising advancement in the quest for host plant reniform nematode resistance. To rule out any unwanted surprises down the road, more physiological and histological information on BARBREN-713 might be needed to determine the nature of interaction between plant and parasite.

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References

Blasingame, D., M. V. Patel, K. Lawrence, W. Gazaway, M. Olsen, T. Kirkpatrick, S. Monfort, M. Davis, J. Marios, B. Kemerait, P. Colyer, G. Scuimbato, G. Lawrence, A. Wrather, N. Goldberg, S. Koenning, J. T. Pitts, J. Muller, M. Newman, J. Woodard, T. Wheeler and P. Phipps. 2010. 2009 Cotton disease loss estimate committee report. Proceedings of the Beltwide Cotton Conferences 1:237-240.

Dighe, N. D., A. F. Robinson, A. A. Bell, M. A. Menz, R. G. Cantrell, and D. M. Stelly. 2009. Linkage mapping of resistance to reniform nematode in cotton following introgression from Gossypium longicalyx (Hutch and Lee). Crop Science 49:1151-1164.

Nichols, R. L., A. Bell, D. Stelly, N. Dighe, F. Robinson, M. Menz, J. Starr, P. Agudelo, J. Jones, C. Overstreet, E. Burris, C. Cook, R. Lemon, and D. Fang. 2010. Phenotypic and genetic evaluation of LONREN germplasm. Proceedings of the Beltwide Cotton Conferences 1:798-799.

Robinson, A. F., A. A. Bell, N. C. Dighe, M. A. Menz, R. L. Nichols, and D. M. Stelly. 2007. Introgression of resistance to nematode *Rotylenchulus reniformis* into upland cotton (*Gossypium hirsutum*) from *Gossypium longicalyx*. Crop Science 47:1865-1877.

Sikkens, R. B., D. B. Weaver, K. S. Lawrence, S. R. Moore and E. van Santen. 2011. LONREN upland cotton germplasm response to *Rotylenchulus reniformis* inoculum level. Nematropica 41: 68-74.

Starr, J. L., S. R. Koenning, T. L. Kirkpatrick, A. F. Robinson, P. A. Roberts, and R. L. Nichols. 2007. The future of nematode management in cotton. Journal of Nematology 39:283-294.

Weaver, D.B., R.B. Sikkens, S.R. Moore, K.S. Lawrence and R.R. Sharpe. 2011. LONREN × FM966 Progeny Evaluation in a field infested with Reniform Nematode. 2010. *In*: S. Boyd, M. Huffman, B. Robertson (ed.). Proceedings of the Beltwide Cotton Conferences, Atlanta, GA. p. 737-741.

Yik, C. P. and W. Birchfield. 1984. Resistant germplasm in *Gossypium* species and related plants to *Rotylenchulus reniformis*. Journal of Nematology 16:146-153.