LONREN GERMPLASM RESPONSE TO RENIFORM NEMATODE INOCULATION LEVEL

Roelof B. Sikkens
Tingting Wu
David B. Weaver
Department of Agronomy and Soils
Auburn University, AL
Scott R. Moore
Kathy K. Lawrence
Department of Entomology and Plant Pathology
Auburn University, AL

Abstract

Early seedling stunting in LONREN germplasm was studied through a series of experiments. No apparent increased sensitivity to pre-emergence PSII herbicide was found. At high inoculation levels of reniform nematode, root necrosis was observed in LONREN-1, LONREN-2 and a resistant line of the cross LONREN-1 × FM966. No pronounced heightened sensitivity of LONREN germplasm to two common fungi was found.

Background

In March 2007, two germplasm lines of *Gossypium hirsutum* L. (upland cotton) LONREN-1 and LONREN-2 were released, in which resistance to *Rotylenchulus reniformis* (reniform nematode) was introgressed from the diploid *G. longicalyx* (Robinson, 2007). In early 2009, 100 $F_{2:3}$ lines of the cross LONREN-1 × Fibermax966 (FM966) were screened for reniform nematode resistance, with 21 lines identified as being highly resistant and 20 lines as homogeneous susceptible. All lines were advanced to the $F_{2:4}$ generation during the summer of 2009, and a large scale yield and quality trial was planned for the growing season of 2010. Around the same time reports of seedling stunting in LONREN accessions under field conditions started to surface (Bell et al., 2009; Nichols et al., 2010; Jack Jones, personal communication). As such behavior could potentially impact the outcome of the planned field trial, it was decided to initiate a number of greenhouse experiments in order to learn more about the seedling stunting phenomenon. Three possible causes for seedling stunting were hypothesized. Firstly, it was suggested that LONREN genotypes showed a heightened sensitivity to photosynthesis inhibiting pre-emergence (PSII) herbicide (Bell et al., 2009). Secondly, LONREN germplasm itself could have a particular sensitivity to reniform nematode. Such a reaction could, if disrupting reniform nematode reproduction, be observed as a resistance trait at low infestation levels. At higher infestation levels the same reaction could be responsible for growth inhibiting injury. In this regard it is noteworthy that hypersensitivity to reniform nematode was observed in *G. longicalyx* (Agudelo et al., 2005). Thirdly, the possibility of heightened sensitivity of LONREN germplasm to other seedling pathogens was considered. Without a clear indication in which direction to search for such a particular sensitivity, it was decided to start off with an exploratory experiment, encompassing only few common seedling fungi.

Materials and Methods

The experiment on increased sensitivity to pre-emergence (type PSII) herbicide was conducted on 20 resistant lines of the cross LONREN-1 × FM966 and 20 homogeneous susceptible lines of the same cross. Two control groups were added: LONREN-1 and LONREN-2 as resistant controls and Fibermax953 (FM953), FM966 and Paymaster1218 (PM1218) as susceptible cultivars. All entries consisted of 5 individually potted seedlings. Response to Cotoran® 4L (Fluometuron, Makhteshim Agan of North America, inc.) was measured twice, on leaf tissue from 36 and 69-day-old seedlings respectively. On each occasion, the newest fully developed leaf from each seedling was bisected, with one leaf section (of approximately 1 cm^2) immersed in a 1000 ppm Cotoran solution and another similar sized section in de-ionized water. Chlorophyll fluorescence response, expressed as the ratio between variable and maximum fluorescence (Fv/Fm), was measured at the start of immersion and 2 hours thereafter. Chlorophyll fluorescence is a known indicator for photosynthesis (Maxwell and Johnson, 2000).

Six accessions were included in the study into the response of LONREN germplasm to various inoculation levels of reniform nematode. LONREN-1, LONREN-2 and resistant line B104 of the cross LONREN-1 × FM966 formed the group of resistant genotypes, whereas FM966, Deltapine555BR (DP555BR), and susceptible line B108 of the cross LONREN-1 × FM966 constituted the group of susceptible genotypes. Two seeds of each entry, coated with a Bayer

fungicide cocktail (Baytan, Allegiance, Vortex), were planted in 150cc cone-tainers filled with an autoclaved soil mixture of 68% sand, 20% silt and 12% clay. One week after planting, thinning to one plant per cone-tainer preceded inoculation, which took place on February 28, 2010. Inoculation levels were: 0, 500, 1,000, 5,000, 10,000 and 50,000 juvenile and vermiform life stages of *R. reniformis*. There were 10 replications per inoculation level and per accession. Since LONREN stunting is observed as an aboveground expression of an underground event, this experiment focused on documenting the root-shoot relationship. Intermediate data were collected on one plant of each accession and each treatment at 17, 31, and 45 days after inoculation (d.a.i.). Shoot and root development was documented through weighing of fresh and dry tissue and through photographing of entire seedlings. Growth of all remaining plants was similarly documented at the end of the experiment at 62 d.a.i. At that time, nematodes were extracted using a modified Baermann funnel technique (Weaver et al., 2007) and counted. Weekly plant heights and vigor readings were also collected during the experiment.

The setup of the exploratory study into a potential increased sensitivity of LONREN germplasm to other seedling pathogens was quite similar to the one described above for the inoculation density study. The same genotypes were included with the exception of DP555BR which, after showing disappointing growing characteristics in the population study, was replaced by PM1218. Seed were not treated with a fungicide. The experiment included five distinct treatments: (i) no inoculation, (ii) 7,500 reniform nematode only, (iii) 7,500 reniform nematode + *Rhizoctonia solani*, (iv) 7,500 reniform nematode + *Pythium*, and (v) non-sterilized soil from the North-East Louisiana Experiment Station, St. Joseph, Louisiana. An initial nematode count on soil samples from the last entry indicated that it carried a population of approximately 7,500 reniform nematode per 150cc. This population level was matched by inoculation in treatments (ii) to (iv). Inclusion of *Fusarium spp*. as a sixth treatment was prevented due to a lack of inoculant. Planting and data collection in this experiment was identical to the methodology followed in the population study described above, with again 10 replications per genotype and per treatment. Intermediate observations on root and shoot development were made at 17, 32, and 46 d.a.i. (one replication each) and at 62 d.a.i. for the remaining seven replications.

Results and Discussion

The results of the pre-emergence PSII herbicide experiment are summarized in Table 1. In all groups, chlorophyll fluorescence readings after two hours immersion in de-ionized water were virtually unchanged from their pre-immersion values. Leaf sections put in the 1000 ppm Cotoran solution saw their Fv/Fm ratios reduced by 42 to 58%. In order of magnitude, all groups saw comparable reductions. The LONREN control group returned the largest reduction in both data sets, while the larger group of 20 LONREN-1 × FM966 susceptible lines returned the smallest reduction overall.

Table 1. PSII herbicide sensitivity experiment: chlorophyll fluorescence response, expressed as the ratio between variable and maximum fluorescence (Fv/Fm)

		Group means			Fv/Fm reduction	
		Fv/Fm			%	
Group	entries	0 hrs	2 hrs 0 ppm	2 hrs 1000 ppm	no Cotoran	with Cotoran
36 days after planting						
LONREN-1 × FM966 resistant lines	20	0.770	0.776	0.444	-0.8	42.3
LONREN-1 × FM966 susceptible lines	20	0.780	0.794	0.426	-1.8	45.4
LONREN-1 and LONREN-2	2	0.760	0.810	0.370	-6.6	51.3
FM953 / FM966 / PM1218	3	0.786	0.796	0.392	-1.3	50.1
69 days after planting						
LONREN-1 × FM966 resistant lines	20	0.790	0.779	0.361	1.4	54.3
LONREN-1 × FM966 susceptible lines	20	0.803	0.806	0.402	-0.4	49.9
LONREN-1 and LONREN-2	2	0.784	0.782	0.323	0.3	58.8
FM953 / FM966 / PM1218	3	0.781	0.792	0.352	-1.4	54.9

A statistically significant difference in the response of susceptible lines vs. resistant lines was detected in the 69 d.a.i. data set, but such a relationship was absent in the 36 d.a.i. set. We cautiously characterize the overall results of the PSII herbicide sensitivity experiment as non-conclusive, though the scale and consistency of the decrease in readings does tend us to believe that PSII sensitivity might not be the prime cause for stunting of LONREN seedlings.

As stated above, the second experiment concentrated on observing the root-shoot relationship of plants under various reniform nematode inoculation levels. Figure 1 presents a representative array of LONREN-2 plants; the photo is taken 62 days after inoculation. Reduction of root mass at the higher inoculation levels of 10,000 and 50,000 nematode/150cc is clearly visible. The shoots at these inoculation levels are also noticeably shorter than those of seedlings exposed to lower inoculation doses. Root development at lower inoculation levels (500, 1,000 and 5,000 nematode/150cc seems, at least at first sight, not very dissimilar to root growth of the 0 inoculation control.

Figure 1. LONREN-2 plants, 62 days after inoculation. Inoculation levels are, from left to right, 0, 500, 1,000 5,000, 10,000 and 50,000 reniform nematode per 150cc of soil.

Figure 2 presents a representative array of FM966 plants, again taken 62 d.a.i. Here the increase in root mass at the two higher inoculation levels is noticeable. At first sight, root growth at lower inoculation levels seems similar to root growth at the 0 inoculation. None of the seedlings appears stunted, though the shoots at the two highest inoculation levels appear somewhat stressed.

Figure 2. FM966 plants, photographed 62 days after inoculation. Inoculation levels are, from left to right, 0, 500, 1,000, 5,000, 10,000 and 50,000 reniform nematode per 150cc of soil.

Shoot and root dry mass means are presented in Table 2. Shoot dry mass means from all genotypes decreased progressively with increase in nematode numbers. This was not the case with root dry mass means, where the group of resistant genotypes saw decreases with increased inoculation levels, but all three susceptible entries recorded increases in root dry mass means with increased nematode population levels. From the dry mass means, shoot to root dry mass ratios were calculated. The fourth section of Table 2 tries to capture the changes in these ratios relative to the uninoculated controls. The ratios in the resistant group show limited variation in no particular direction.

Table 2. Dry mass means and shoot to root ratios of the reniform nematode various inoculation level study

		Reniform Nematode inoculation density					
Group	Genotype	none	500	1,000	5,000	10,000	50,000
Shoot dry mass means				(g)			
Resistant	LONREN-1	0.48	0.50	0.30	0.37	0.45	0.26
	LONREN-2	0.67	0.60	0.61	0.66	0.73	0.42
	B104	0.69	0.61	0.59	0.54	0.66	0.22
Susceptible	B108	0.47	0.52	0.41	0.44	0.35	0.30
	FM966	0.60	0.46	0.31	0.32	0.41	0.30
	DP555BR	0.36	0.27	0.26	0.36	0.17	0.25
Root dry mass means							
Resistant	LONREN-1	0.42	0.30	0.22	0.32	0.33	0.17
	LONREN-2	0.40	0.28	0.36	0.34	0.36	0.21
	B104	0.36	0.45	0.36	0.30	0.42	0.09
Susceptible	B108	0.20	0.25	0.31	0.53	0.43	0.57
	FM966	0.26	0.34	0.26	0.36	0.57	0.54
	DP555BR	0.25	0.25	0.39	0.44	0.18	0.36
Shoot to root dry mass ratio				(ratio)			
Resistant	LONREN-1	1.3	1.8	1.5	1.4	1.6	1.6
	LONREN-2	2.2	2.3	2.1	2.4	2.2	2.8
	B104	2.1	1.6	1.8	1.9	1.6	2.3
Susceptible	B108	2.5	2.5	1.6	1.8	0.9	0.7
	FM966	2.4	1.5	1.3	1.0	0.8	0.6
	DP555BR	1.6	0.8	0.7	1.0	1.4	1.1
Ratio change versus no inoculation					(%)		
Resistant	LONREN-1		36	15	2	20	23
	LONREN-2		5	-5	8	-2	25
	B104		-23	-17	-12	-25	9
Susceptible	B108		1	-38	-30	-64	-73
	FM966		-40	-46	-60	-69	-76
	DP555BR		-51	-54	-35	-15	-34

The ratios of the group of susceptible genotypes show remarkable decreases, reaching up to 76% in case of higher inoculation densities. The above-ground decrease in shoot mass of seedlings (stunting) of resistant plants appears to be accompanied by decreases in root mass of near comparable magnitude. Shoots of susceptible genotypes also showed signs of stress at higher inoculation levels, either in reduced height or as a less vigorous stand. Those effects, translated in decreases in shoot mass, are marred with significant increases in root mass.

Figure 3 permits a closer examination of the difference in root development of reniform nematode resistant and susceptible genotypes in response to high nematode levels. In case of resistant plants, widespread root necrosis can be seen. Contrary to this, roots of susceptible plants are host to numerous nematode egg masses. Its seems that the presence of reniform nematode leads to root starvation in plants of resistant genotypes, whereas roots of susceptible genotypes attempt to satisfy not only the nutritional needs of the developing shoot, but also those of the female nematode.

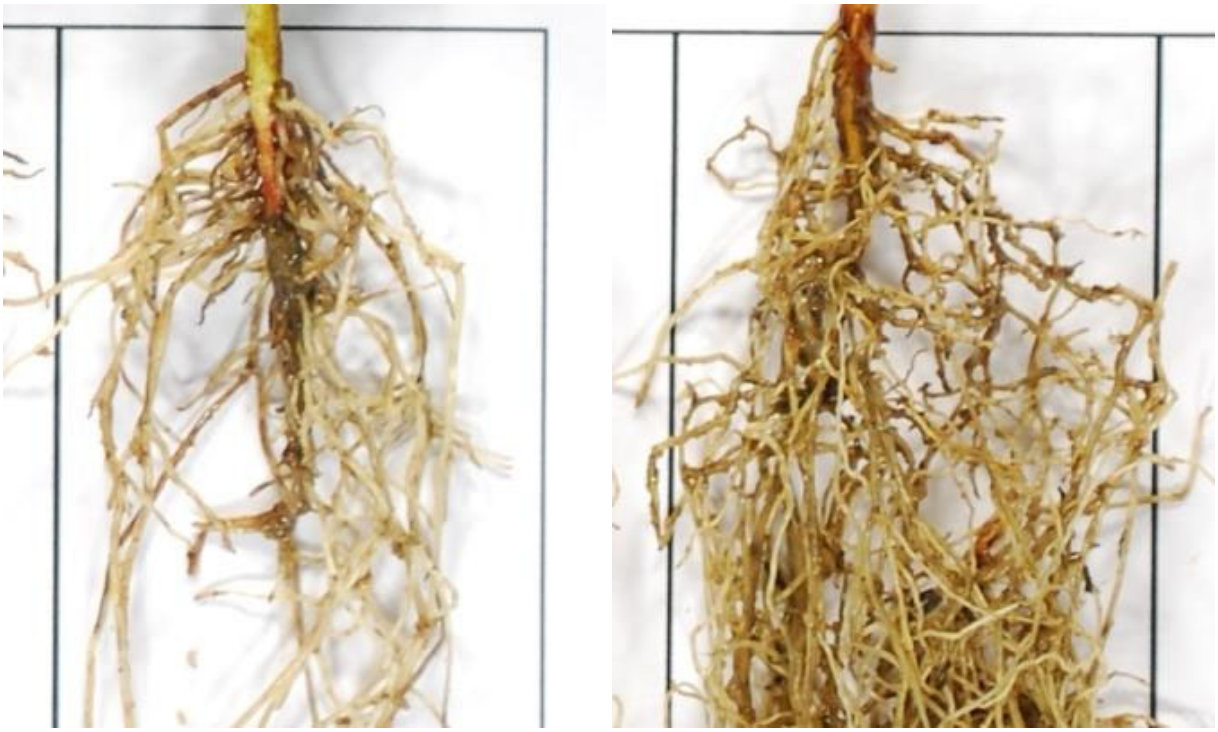

Figure 3. Root details. Left frame shows a detail of figure 1: the LONREN-2 seedling of the 50,000 nematode inoculation level. Widespread root necrosis can be observed. Right frame shows a detail of figure 2: the FM966 seedling of the 10,000 nematode inoculation level. Large numbers of eggmass lodged on lateral roots can be seen.

This above finding is all the more significant when time is taken into account. Figure 4 shows an image of LONREN-1 plants, 31 days after inoculation. At the 50,000 nematode inoculation level, the tap root is heavily damaged and in the process of dying off. At the 10,000 nematode inoculation level, the radicle also shows signs of necrosis. Reniform nematode resistant genotypes, dealing with root damage immediately after germination, face higher odds of survival or healthy development than their susceptible counterparts, where root stress appears to develop more gradually.

Figure 4. Array of LONREN-1 plants, photographed 31 days after inoculation. At the 10,000 and 50,000 reniform nematode inoculation level, damage to the radicle is already very severe at this early stage of seedling development.

Results from the study into potential increased sensitivity of LONREN to other seedling pathogens are summarized in Table 3; for space considerations only shoot to root dry mass ratios and their relative change versus the uninoculated controls are included. The data do not support the notion that LONREN germplasm has an inherited increased sensitivity to the two common pathogens included in the experiment. But the experiment was exploratory in nature, and the results do not preclude the possibility of heightened LONREN sensitivity to seedling diseases not included in this experiment. It is interesting to note that shoot to root dry mass ratios for resistant lines returned, for the various treatments, values similar to or higher than those for the non-inoculated controls. Shoot to root dry mass ratios for susceptible lines, on the other hand, recorded appreciable declines. Here, the results from the pathogen experiment mimic the results of the various inoculation level experiments described above.

Table 3. Shoot to root dry mass ratios from the study into possible LONREN to other cotton seedling pathogens

		Treatment				
Group	Genotype	No Inoculation	7,500 Reniform Nematode	Rhizoctonia solani and 7,500 Reniform Nematode	Pythium and 7,500 Reniform Nematode	St.Joseph (LA) soil (+/- 7,500 Reniform Nematode)
Shoot to root dry mass ratio				(ratio)		
Resistant	LONREN-1	1.0	1.6	0.9	1.8	1.3
	LONREN-2	1.4	1.6	1.8	2.5	2.9
	B104	1.4	1.5	1.5	2.2	1.3
Susceptible	B108	1.7	0.9	1.2	0.9	2.1
	FM966	1.7	0.9	1.4	1.2	1.2
	PM1218	1.7	1.1	1.2	1.3	1.6
Ratio change versus no inoculation				(%)		
Resistant	LONREN-1		52	-14	74	31
	LONREN-2		19	31	81	110
	B104		11	7	63	-5
Susceptible	B108		-50	-27	-48	24
	FM966		-49	-17	-28	-32
	PM1218		-33	-28	-22	-7

Conclusions

Observed root damage of LONREN accessions at high inoculation levels suggests that a hypersensitive reaction to reniform nematode might play an important role in the process of seedling stunting. Though heightened sensitivity of LONREN to other seedling diseases was not demonstrated, it is quite plausible that these do play a critical role in exacerbating the seedling stunting phenomenon, possibly through opportunistic exploitation of root injury remaining after rejecting nourishment to female nematode. Plant hypersensitivity combined with the presence of seedling pathogens might considerably lower the threshold nematode population beyond which consequential seedling stunting in LONREN accessions starts occurring.

Acknowledgement

We would like to thank Dr. Jack Jones of Baton Rouge, LA. for his assistance and, even more specifically, for his insistence that seedling stunting in LONREN germplasm was a serious concern that needed further investigation.

Literature Cited

Bell, A. A., J. L. Starr, J. E Jones, R. Lemon, R. L. Nichols, C. Overstreet and D. M. Stelly. 2009. Nematode resistance and agronomic performance of LONREN and NEMSTACK lines. Proceedings of the 2009 Beltwide Cotton Conferences, pp. 178.

Maxwell, Kate and Giles N. Johnson. 2000. Chlorophyll fluorescence - a practical guide. Journal of Experimental Botany 51: 659-668.

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 2010 Beltwide Cotton Conferences, pp. 798-799.

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

Weaver, D. B., K. S. Lawrence, and E. van Santen. 2007. Reniform nematode resistance in upland cotton germplasm. Crop Sci. 47:19-24.