ADDING ROOT-KNOT NEMATODE RESISTANCE TO GEORGIA-ADAPTED COTTON GERMPLASM, 2009

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Introduction

State surveys of the densities of nematodes reveal that the major cotton-producing counties in Georgia have damaging levels of nematodes (state loss of 137,423 bales, valued at $53,594,970 in 1998) and is increasing from previous years (National Cotton Council, 1998). From 1991 to 1998, almost 98 thousand bales per year valued at a total of $300 million were lost (National Cotton Council, 1998). It is estimated that Georgia producers specifically lose about 77,000 bales of cotton annually from root-knot nematodes (*Meloidogyne incognita,* RKN) damage (Blasingame and Petal, 2001). Crop rotation, while a recommended cultural practice to lessen soil populations of RKN, is not an option for most Georgia growers because of the lack of suitable non-host crops with which to rotate their cotton acreages. Therefore, inherent genetic resistance provides an attractive alternative to pesticides and crop rotation.

Poor profit potential of cotton production from yield stagnation and high pest management costs impels creation of cultivars with inherent genetic resistance to enhance economic returns for cotton producers. Insect, nematode, and weed pest management costs are among the highest expenditures growers face in cotton production (National Cotton Council, 2001), thus their reduction would enhance profitability of cotton production. Since Georgia is the second ranked cotton producing state with 1.4 million acres (NASS, 2006), cotton cultivars adapted for the unique aspects of the environment of Georgia, such as rainfall patterns, soils types and depth, and presence of root-knot nematodes must be developed to give the best available genetics to the GA producer.

Despite the widespread occurrence of RKN in Georgia and most cotton production areas in the Southeast and that genetic resistance to RKN has existed since 1974 (Shepherd, 1974), private cultivar developers have previously exhibited little interest in fulfilling this need. Commonly cited reasons for the slow progress in developing RKN resistant cultivars is that the current screening process is costly, tedious, time consuming and destructive for identifying resistance genotypes. Further, most breeding stations have neither the facilities nor personnel with expertise in nematology to carry out the screening process to identify resistant material. Of those RKN-resistant (CPCSD Acala NemX) or tolerant cultivars (ST LA887 or PM H1560) that have been distributed by commercial cotton seed companies, none are adapted to the Southeast.
Our objective, to develop Georgia-adapted, value-added cotton germplasm with RKN resistance, will benefit the state's producers by providing increased yield and decreased

production costs whereas the increased availability of RKN-resistant germplasm will benefit the cotton industry across the belt.

Materials and Methods

In a previous project, Drs. Chee, May, and Davis developed advanced RKN parents from a backcross breeding population using M120RNR and M155RNR root-knot nematode resistant donor parent with the elite breeding line PD94042 (May, 1999). The best resistant BC3F3 lines were crossed with Georgia adapted, value added lines from our UGA Cotton Breeding program. A ten plant sample of the RKN resistant parental material was challenged twice with a very high rate of RKN in a pot-based greenhouse test following Shen et al. (2006). Further samples were then grown at the Gibbs Farm, University of Georgia-Tifton campus in an RKN infested field following the procedure of Davis and May (2005). The resistant lines were verified in an additional pot-based greenhouse test. Resistant lines 103-7, 201-A, 506-5, and 506-11 were selected as parents to introgress the RKN resistance into the Georgia-adapted germplasm GA 98028 and GA 2001078.

DNA markers developed in a companion project with the preliminary work described by Shen et al. (2006) were to be used to select the resistant offspring using marker-assisted selection (MAS). The chromosomal region bearing the RKN resistance that is indicated by these molecular markers was verified independently (Ynturi et al., 2006), although the work in our lab appears to have markers that were closer to the RKN resistance gene. These markers are polymorphic between the parental line and both original parents of the RKN resistance donors that led to the BC_3F_3 population. Following marker aided selection (MAS), it is expected that selecting for the closely linked markers would also select for the RKN resistance. Three rounds of backcrossing to the agronomic elite parents while ensuring the presence of the markers and the RKN resistance should give Georgia-adapted, value-added cotton germplasm with RKN resistance.

Results & Discussion

The following is our approach after using MAS to maintain the resistance and the marker up to the 2^{nd} backcross. After single plant selections in the BC_2F_1 population of the backcrossing approach along with fiber quality testing, an unreplicated modified augmented design yield test (with every 5th row in the trial assigned to a conventional check cultivar) was to be planted to select for yield and to test/verify the homozygosity of the RKN resistance marker(s). This trial was to be machine harvested and the seed-cotton yield of each F4 progeny row compared with seed-cotton yield of the nearest check row. For the rows that significantly out-yield the nearest check plot, boll samples will be picked for lint %, fiber quality, and for seed in a parallel increase field. Next, the preliminary trial (PT) was to be conducted near Tifton or Plains, GA, depending upon land availability. Advanced generation germplasm lines promoted from the PT were to

be tested in an advanced yield trial (AT) in both Plains and Tifton. Elite germplasm lines from a successful performance in the ATs will be tested in locations throughout the state in both dryland and irrigated fields in the University of Georgia Official Variety Trials. After the preliminary crossing, these most current molecular markers from the companion program mentioned above were to have been used in a three-cycle backcrossing program in the greenhouse to insert the RKN resistance gene during 2007. However, our crossing schedule was disrupted by inviable seed from the second backcross during 2007. F_1 seed were sent to the winter nursery in Mexico to obtain seed for the 2008 growing season to test samples of the F_3 population with the molecular markers for RKN resistance and then to continue with backcrossing with the plants that tested positive with the DNA markers for the RKN resistance.
During 2008, ten F_2 populations of RKN resistance by GA adapted lines from the winter nursery at Tecoman, Mexico were planted at the Gibbs farm, University of Georgia-Tifton campus; one population was lost when no plants emerged. DNA was extracted from ten plants of each F_2 population as well as their RKN-resistant and GA-adapted parents. Three additional advanced lines (155-R2-B1, 120-R1-B3, and 120-R1-B1) that are closely related to the original RKN-resistant parents were also sampled. The DNA markers for the RKN resistance were found to not be polymorphic in any of the populations with any of the RKN-resistant markers in our possession. Furthermore, no polymorphisms showed between the immediate parents of these populations. The linkage between the markers and the RKN-resistance gene must have been broken in the development of the PD 94042 RKN-resistant population. Immediate phenotype testing was not possible due to resource constraints. To continue our aggressive approach, new crosses were made immediately in 2008/2009 utilizing the more advanced RKN-resistant lines (155-R2-B1, 120-R1-B3, and 120-R1-B1) with newer GA-adapted lines to renew the effort to develop better GA-adapted, RKN-resistant cultivars via backcrossing utilizing phenotypic testing if needed.

Early in 2009 year, these crosses were destroyed inadvertently in our greenhouse by technicians from another program spraying 2,4D to control broadleaf weeds in grass plots during the winter of 2008/2009. These grass plots were not next to the greenhouse but the damage showed the classical 2,4D symptoms as compared with photos on the internet (www.weedscience.okstate.edu/cotton.htm [no longer linked, they are redoing their site]). The crosses from available remnant seed along with the backcross parents were planted into the 2009 field crossing block for further crossing/backcrossing. Further crosses with newly released cultivars GA 2004303 and GA 2004230 along with additional elite GA 2007 material were made to develop lines with enhanced GA adaptability along with this superior root-knot resistance. We have also made new crosses to M-120-RNR, the original source of resistance, with GA 2004303 and GA 2004230 to utilize the DNA markers that are presently available and any new ones that may be found. The later generation lines and backcrosses are currently being grown to be phenotypically challenged with RKN in our greenhouse during the winter season of 2009/2010 unless new DNA markers are found that were unaffected by the putative linkage breakage.

The two lines 120-R1-B1 and 120-R1-B3 of the three original lines in 2008 were retested at the UGA-Tifton campus Gibbs Farm in Tifton, GA and the UGA Southwest Georgia Research and Education Center in Plains, GA. Agronomic data was gathered for these improved lines to be used in submitting them for release as germplasm lines. The checks for this test were increased to 5 (GA 2004230, DP 147RF, FM 966, ST 4664RF, and DP 5415) along with PD 94042 which was the original elite breeding line. DP 5415 was eliminated from the lint yield analysis of the Gibbs Farm because it had very poor emergence.

The location by entry interaction term for the lint yield was very highly significant at greater than the 0.001 level. Therefore the lint yield data cannot be averaged across Tifton and Plains in 2009; this was also true in 2008. Plains had a better overall growing condition in 2009 shown by the grand mean of 1460 lbs/acre while Tifton was less at 823 lbs/acre. The line 120-R1-B1 was the best yielder in Plains at 1962 lbs/acre but it was 6th of the 7 lines in Tifton (Table 1 & 2). Line 120-R1-B3 ranked 3rd in Tifton at 865 lbs/acre and 5th in Plains (Tables 1 & 2). Lint % didn't have a location by entry interaction. As it did in 2008, 120-R1-B1 had the highest lint % in 2009 (46.4% across locations); 120-R1-B3 was 2nd in 2009 with 44.6% (Table 3). The fiber, tested by Cotton Incorporated HVI fiber testing, did not show location by entry interaction either. Only GA 2004230 and DP 147RF had better length than these RKN-resistant lines (Table 3). The uniformity index and micronaire were excellent values as good as the recurrent parent, PD 94042, and the checks. Their strength values were significantly less than PD 94042, but they still were as good as all the checks but FM 966.

Line 120-R1-B3 is being released as germplasm under the name "GA 120R1B3" based on its stable, very good resistance to RKN and its very good yield performance and fiber quality.

We are expecting our approach to provide a solid performing release of GA-adapted, RKN resistant germplasms/cultivars. Even though MAS is generally considered a reliable procedure, it is a relatively recent innovation and has not been extensively utilized, and there have been technical problems associated with it. Phenotypic analyses of the new populations are likely to present resource allocation difficulties that will decrease the size of the populations that can be tested.

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Table 1. Lint yield, lint %, and fiber quality comparisons of RKN resistant lines 120-R1-B1 and 120-R1-B3 with the original recurrent parent, PD 94042, and 5 check cultivars at the Southwest Research and Education Center, Plains, GA

ENTRY	Lint Yield[1]	Lint %	UHM[2] in.	UI[3] %	mic[4]	Str[5] g/tex
120-R1-B1	981	47.00	1.24	85.50	4.40	31.87
ST 4664RF	866	43.22	1.20	85.13	4.71	30.30
GA 2004230	863	44.87	1.27	85.47	4.64	30.50
120-R1-B3	849	44.88	1.25	85.60	4.46	31.23
DP 147RF	736	42.16	1.24	85.70	4.31	30.37
DP 5415	703	41.43	1.22	85.17	4.62	31.23
FM 966	656	42.70	1.24	84.27	4.65	38.70
PD 94042	524	43.79	1.19	85.07	4.75	31.17
$LSD_{0.05}$	145[6]	-[7]	-[7]	-[7]	-[7]	-[7]

[1] - lbs/acre
[2] - Length as Upper Half Mean
[3] - Uniformity Index
[4] - Micronaire
[5] - Fiber Strength
[6] - Significant location by entry interaction, no combined analysis
[7] - No location by entry interaction, see Table 3 for the combined analyses.

Table 2. Lint yield, lint %, and fiber quality comparisons of RKN resistant lines 120-R1-B1 and 120-R1-B3 with the original recurrent parent, PD 94042, and 5 check cultivars at the Gibbs Research Farm, UGA-Tifton campus, Tifton, GA

ENTRY	Lint Yield[1]	Lint %	UHM[2] in.	UI[3] %	mic[4]	Str[5] g/tex
FM 966	530	41.2	1.16	85.37	4.51	33.6
DP 147RF	441	42.1	1.25	85.23	4.09	31.9
120-R1-B3	432	44.4	1.22	86.63	4.44	31.9
PD 94042	401	44.3	1.20	85.20	4.54	33.3
GA 2004230	367	43.0	1.27	86.37	4.44	32.5
120-R1-B1	366	45.8	1.21	85.47	4.19	31.1
ST 4664RF	354	41.4	1.15	85.43	4.61	30.0
DP 5415	-	37.5	1.14	84.67	4.58	29.5
$LSD_{0.05}$	98[6]	-[7]	-[7]	-[7]	-[7]	-[7]

[1] - lbs/acre
[2] – Length as Upper Half Mean
[3] - Uniformity Index
[4] - Micronaire
[5] - Fiber Strength
[6] - Significant location by entry interaction, no combined analysis
[7] - No location by entry interaction, see Table 3 for the combined analyses.

Table 3. Lint yield, lint %, and fiber quality comparisons of RKN resistant lines 120-R1-B1 and 120-R1-B3 with the original recurrent parent, PD 94042, and 5 check cultivars
across both GA locations shown in Tables 1 and 2.

ENTRY	Lint Yield[1]	Lint %	UHM[2] in.	UI[3] %	mic[4]	Str[5] g/tex
120-R1-B1	673	46.4	1.23	85.48	4.30	31.47
120-R1-B3	641	44.6	1.23	86.12	4.45	31.55
GA 2044230	615	44.0	1.27	85.92	4.54	31.48
ST 4664RF	606	42.3	1.18	85.28	4.66	30.13
FM 966	593	41.9	1.20	84.82	4.58	36.17
DP 147RF	588	42.2	1.24	85.47	4.20	31.15
PD 94042	455	44.0	1.20	85.13	4.65	32.22
DP 5415	-	39.4	1.18	84.92	4.60	30.37
$LSD_{0.10}$	-[1]	1.3	0.04	NS	0.18	1.62

[1] - lbs/acre
[2] - Length
[3] - Uniformity Index
[4] - Micronaire
[5] - Fiber Strength
[6] - Location by entry interaction, see Tables 1 and 2 for correct, individual analyses