ADDING ROOT-KNOT NEMATODE RESISTANCE TO GEORGIA-ADAPTED COTTON GERMPLASM, 2010

Edward L. Lubbers[1], Peng W. Chee[1], and Richard Davis[2]
[1]Dept of Crop and Soil Science, University of Georgia-Tifton campus, Tifton, GA
[2]Crop Protection and Management Research Unit, USDA-ARS, Tifton, GA

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 in the US (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 Georgia 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

We have developed advanced RKN parents from a backcross breeding population using M120-RNR and M155-RNR root-knot nematode resistant donor parent with the elite breeding line PD 94042. 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. One intention of this project is to assist in further testing of these selections and use any improved material as parents to ultimately develop more elite germplasm.

DNA markers, developed in a companion project with the preliminary work described by Shen et al. (2006 and 2010), were intended 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 on chromosome 11 was verified independently (Ynturi et al., 2006), although the subsequent work in our lab appears to place some markers closer to the RKN resistance gene. The presence of this QTL results in decreased galling compared to susceptible plants when a plant's roots are challenged with RKNs. Another RKN resistance QTL was verified by He et al. (2011) on chromosome 14. It results in decreased numbers of nematode eggs as compared to susceptible plants when the roots are challenged with RKNs. 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. Any additional markers for RKN resistance will be utilized as they become available. Following **m**arker **a**ided **s**election (MAS) expectations, selecting for the closely linked markers will also select for the RKN resistance. Three rounds of crossing/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.

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, a non-replicated 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.

Results & Discussion

Of the three RKN resistant lines 120-R1-B1, 120-R1-B3, and 155-R2-B1 mentioned in the previous section, they were tested in 2008 in our advanced trial AT1 (Lubbers and Chee, 2009). The two better lines 120-R1-B1 and 120-R1-B3 were retested in 2009 at the UGA-Tifton Campus Gibbs Farm in Tifton, GA and the UGA Southwest Georgia Research and Education Center in Plains, GA (Lubbers et al., 2010). The checks for this test were GA 2004230, DP 147RF, FM 966, and ST 4664RF along with PD 94042, the original elite breeding line. Line 120-R1-B3 performed very well and has been released as a germplasm line (Davis et al., 2011). "Registration of GA 120R1B3 Germplasm Line of Cotton" will be published in the Journal of Plant Registrations in early 2011.

The first of the three sets of crosses/backcrosses that we have made since 2007 used RKN-resistant parents derived from a M120-RNR by PD 94042 population that was crossed with transgenic (B2RF) lines derived from GA-adapted lines GA 98028 and GA 2001078. This set was challenged by RKN during the summer of 2010. Out of 633 plants of various crosses, 21 plants (3.3%) were selected as being at least very strongly tolerant with less than 10% root galling (Table 1). Further tests will be done to verify the RKN resistance response of these plants. These will be grown for seed increase in 2011 and then tested agronomically in 2012. The second set that used 155-R2-B1, 120-R1-B3, and 120-R1-B1 as RKN-resistant parents has been crossed over two seasons with a number of more elite lines including the new cultivars GA 2004230 and GA 2004303. Some of these are currently being grown to be phenotypically challenged with RKN in our greenhouse during the off-season of 2010/2011 with the rest being grown to produce BC_1 populations. The third set that are using M120-RNR for the RKN-resistant parents will be backcrossed in the greenhouse during the 2010/2011 off-season and tested using the DNA markers for the chromosome 11 and 14 RKN-resistance QTLs.

Currently, 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. Efforts to develop polymorphic markers are underway with funding from a companion project.

Table 1. Lines with < 10% root galling found in populations of Cotton States (CS) transgenic lines (include Bollgard II and Roundup-Ready flex traits from Monsanto) crossed with select Root-Knot nematode resistance lines developed from M120-RNR by PD 94042 populations.

Populations inoculated with Root-Knot nematodes[1,2]	Number of plants with < 10% root galling
201-A/CS02///CS02	2
506-5/CS11///CS11	4
506-5/CS11///CS12	3
506-5/CS13///CS13	2
506-11/CS02///CS02	2
506-11/CS11///CS11	3
506-11/CS12///CS12	3
506-11/CS13///CS13	2
Total number of plants out of 633 that were classified as at least strongly tolerant to RKN	21 (3.3% of total)

[1]CS02 is derived from GA 98028 and CS11, CS12, and CS13 are derived from GA 2001078
[2]F_4s from the original crosses were crossed with the recurrent parent (except in one of the populations) before being challenged with Root-Knot nematodes in a greenhouse planting.

In summary, we expect our backcrossing 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.

Acknowledgements

We thank the Georgia Cotton Commission (Project No. 06-908GA) for financial support for this project.

References

Blasingame, D., and M.V. Petal. 2001. Cotton disease loss estimate committee report. p. 102-103. *In* Proc. Belt. Cotton Res. Conf. 2001. Anaheim, CA, National Cotton Council, Memphis, TN.

Davis, R.F. and O.L. May. 2005. Relationship between yield potential and percentage yield suppression caused by the Southern Root-Knot Nematode in cotton. Crop Sci 45:2312-2317.

Davis, R.F., P.W. Chee, E.L. Lubbers, and O.L. May. 2011. Registration of GA 120R1B3 germplasm line of cotton. J. Plant Reg. Accepted.

He, Yajun, Khalid Hussain, Xinlian Shen, Richard F. Davis, and Peng W. Chee, Reevaluation of the nematode resistance in the M-120 RNR by Pima S6 population. *In*

Proceeding of the Beltwide Cotton Conferences, January 4-7, 2011. Atlanta GA, National Cotton Council of America, Memphis, TN.

Lubbers, E., S. Walker, L. May, and P. Chee. 2006. Breeding cultivars and germplasm with enhanced yield and quality, 2004. p.136-152. *In* P. Roberts et al. (ed.) 2005 Georgia Cotton Research and Extension Reports. UGA/CPES Research – Extension Publication No. 6, University of Georgia, Athens, GA

Lubbers, E.L. and P.W. Chee. 2009. Breeding cultivars and germplasm with enhanced yield and quality, 2008. p.75-83. *In* M. Toews et al. (ed.) 2009 Georgia Cotton Research and Extension Reports. UGA/CPES Research – Extension Publication No. 6, University of Georgia, Athens, GA

Lubbers, E.L., P.W. Chee and R.F. Davis. 2010. Adding Root-Knot nematode resistance to Georgia-adapted cotton germplasm, 2009 p.101-107. *In* G. Ritchie et al. (ed.) 2010 Georgia Cotton Research and Extension Reports. UGA/CPES Research – Extension Publication No. 7, University of Georgia, Athens, GA

NASS, USDA. 2006. U.S. & All States Data – Cotton. National Agriculture Statistical Service, USDA. http://www.nass.usda.gov/QuickStats/PullData_US.jsp

National Cotton Council. 1998. Nematode survey and education program. http://www.cotton.org/cf/nematodes/survey-6.cfm

National Cotton Council. 2001. Cotton costs and returns – southeast. http://risk.cotton.org:80/CotBudgets/seaboard.htm

Shen, X., G. Van Becelaere, P. Kumar, R.F. Davis, O. L. May, and P. Chee. 2006. QTL mapping for resistance to root-knot nematodes in the M-120 RNR Upland cotton line (*Gossypium hirsutum* L.) of the Auburn 623 RNR source. Theor. Appl. Genet. 113: 1539-1549.

Shepherd, R.L. 1974. Transgressive segregation for root-knot nematode resistance in cotton. Crop Sci. 14:827-875.

Ynturi, P., J.N. Jenkins, J.C. McCarty Jr., O.A. Gutierrez, and S. Saha. 2006. Association of root-knot nematode resistance genes with simple sequence repeat markers on two chromosomes in cotton. Crop Sci. 46:2670-2674.