Report of the Research Coordination Meeting
Genetics of Root-Knot Nematode Resistance in Cotton
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Summary

Our overall objective is to facilitate development of commercial cultivars with high levels of resistance to root-knot nematode (RKN) (*Meloidogyne incognita*). Our consensus is that RKN resistance in its strongest described source, 'Auburn 623,' is conditioned by at least two genes, a major gene derived from the obsolete cultivar 'Clevewilt 6', located on chromosome 11 (A genome), and second gene on chromosome 14 (D genome) derived from the line 'Wild Mexican Jack Jones.' These two genotypes were the parents of the highly-resistant transgressive segregant, Auburn 623, released by Dr. Ray Shepard. Other genes also may be involved. The markers, CIR316 and CIR069, are useful in marker assisted selection for tracking the RKN resistance gene on ch. 11. The marker, BNL 3545, is possibly within ~ 10 cM of the RKN resistance gene on ch. 14. Contributory effects of genes from susceptible lines have been observed in certain populations. USDA-ARS scientists intend to continue with characterization of the RKN resistance gene on ch. 14. The laboratories of Drs. Roberts, Ulloa, and Chee will develop a collaborative strategy to fine map the region of the RKN gene on ch. 11, which appears to be a region where several nematode and other plant disease resistance genes are located.

Presentations

Johnie Jenkins, USDA-ARS, Mississippi State University

Two major genes are involved in RKN resistance lines with the Auburn 623 source (Ynturi 2005 and Ynturi et al. 2006). Current research using RIL and markers confirmed that two genes on two different chromsomes are involved in resistance. One gene is on chromosome 14 and linked to the marker 'BNL 3545.' The marker BNL 3545 is also found in the line Wild Mexican Jack Jones. One gene is on chromosome 11 and linked to the market CIR 316. This gene is also present in Clevewilt and the cultivar, 'NemX.' The lines, 'M240,' 'M315,' Auburn 623, and 'Auburn 634' all have the markers BNL 3545 and CIR 316. Structured crosses intended to determine the quantitative effects of the two genes when they were absent, present separately, or present together were made. Data suggest that in the RIL populations tested, the resistance gene on ch. 11, when present alone, reduces galling and has a major negative effect on RKN reproduction.

The gene on ch. 14 behaves similarly, but has less of an effect on galling, but reduces RKN egg counts (reproduction) more than the ch 11 gene. When both RKN resistance genes are present, both galling and nematode reproduction are reduced more than when either gene is present alone.

Peng Chee, University of Georgia

In the population 'M120' x 'Pima S6,' bulk segregation analysis was used to determine the association between 200 genetically mapped and evenly distributed RFLPs and thereby infer the location of the RKN resistance genes. The RFLPs, pAR111, and PGH243 were associated with a QTL for RKN resistance on linkage group A3 (now ch. 11), and the RFLP G115b with a second QTL on ch. 7. Additional work identified a QLT with dominant effect, *Mi-C11*, between the SSR markers CIR 069 and CIR 316 on chromosome 11 (Shen et al. 2006). Screening of the parents of M120 and other RKN resistant lines showed that CIR316 was present in the RKN resistant parents of M120 and in the resistant cultivars Acala NemX and 'LA 887.' Work is now proceeding on fine mapping the region around the *Mi-C11* locus. About fifteen hundred AFLP primer combinations have been screened. One AFLP falls between CIR 069 and CIR 316 and will likely provide the basis for a more closely linked PCR marker for the RKN resistance gene.

Phil Roberts, University of California at Riverside

Investigation of RKN resistance has been done primarily in recombinant inbred lines (RILs) and other populations derived from the crosses resistant - Acala NemX x susceptible - Acala 'SJ-2' and Acala SJ-2 x susceptible 'Pima S-7,' Certain RILs from Acala NemX x Acala SJ-2 had lower galling indices than did the resistant NemX, thus illustrating transgressive segregation and suggesting that the susceptible SJ-2 carries genes potentially involved in RKN resistance. AFLP work with this population led to development of a CAPs marker, GHACC1, associated with RKN resistance (Wang and Roberts, 2006). Mapping work with several hundred previously mapped SSR markers identified the SSR, CIR 316, tightly linked to the resistance gene designated *rkn1* on chromosome 11 (formerly LG A03). The *rkn1*-mediated resistance is recessively inherited in this population (Wang et al. 2006a,b).

Several lines of *G. hirsutum* and *G. barbadense* that express resistance to RKN were crossed to quantify the relative levels of resistance in the progenies as measured by galling and egg production. In the susceptible Pima S-7 x resistant Acala NemX progenies, galling and egg production are correlated. In addition, extreme phenotypes of highly resistant progenies were observed indicating transgressive segregation, and the F1 of this cross were highly resistant. Results of a test cross of NemX x (Pima S-7 x SJ-2) indicated the presence of a transgressive factor in Pima S-7 contributing to RKN resistance. Screening of previously mapped markers allowed identification of the transgressive segregant, 'TS,' designated an 'RKN2' associated with the SSR marker 'MUCS-088' that mapped to the *rkn1*-CIR 316 region of chromosome 11 and may derive from the diploid A2 genome (Wang et al. 2007). Future work will be saturation mapping of the resistance-gene rich region of ch. 11.

Jinfa Zhang, New Mexico State University

Dr. Zhang has a comprehensive program of population development, marker development, and breeding efforts intended to transfer beneficial traits to Acala cultivars. Work on RKN resistance has focused on the populations '1517-99' x 'Stoneville 5599' and NemX x 'Auburn 634.' Initial work to evaluate methods of phenotyping confirmed that galling index was an effective measure for the populations evaluated (Zhang et al. 2006). Using near-isogenic resistant and susceptible lines, 2 AFLP, 2 RAPD, and 3 resistance gene analog (RGA) markers were found to be polymorphic among the lines. In an F2 population of 'Stoneville 474' x 'Auburn 634,' 2 RAPDs and a sequence tagged site (STS) were mapped to the same chromosome as gene *rkn1* in NemX and associated with a major gene, presumably *Mi2* in Auburn 634 (Nui et al. 2007).

Jim Starr, Texas A&M University

Several sources of RKN resistance have been reported. Using the sources reported in (Robinson and Percival, 1997), a diallele test was done by crossing with the susceptible Delta and Pineland (D&PL) 90 and evaluating the expression of resistance by the progenies. Egg counts from progenies of crosses with Clevewilt, Wild Mexican Jack Jones, TX 1440, and TX 2076 show different patterns of resistance. In these crosses, inheritance of RKN resistance from Clevewilt and Wild Mexican Jack Jones appear recessive; while that from TX 1440 and 2076 appear dominant.

Martin Wubben, USDA-ARS at Mississippi State University

A molecular genetic characterization of root-knot nematode infection was conducted using the differentially resistant lines 'M-8' and 'M-315.' The *MIC-3* gene, (Callahan et al. 1997, Zhang et al. 2002, Callahan et al. 2004) is a novel plant gene that was initially described in the roots of line 81-249 which is a resistant plant with the Auburn 634 source of resistance. cDNA sequencing experiments revealed that at least 14 *MIC-3*-like genes are expressed in cotton roots. Time-course nematode assays showed that *MIC-3* transcript levels reached their maximum in infected resistant plant roots immediately prior to the phenotypic manifestation of resistance. The expression of known cotton defense genes were largely unaffected by the nematode, suggesting that nematode defense mechanisms in roots may differ considerably from those described against foliar pathogens. The exact role of *MIC3* in nematode resistance is not known at this time.

Future Research

The concentration of RKN resistance factors on chromosome 11 indicates that much can be gained by fine mapping this genetic region, including developing tightly linked markers for use in marker assisted breeding and gaining fundamental understanding about the organization of this resistance gene cluster.

In addition, because (1) there are several described sources of genetic resistance to RKN; (2) progenies of several crosses suggest the interaction of at least two genes; (3) several populations exhibit transgressive segregation; and (4) certain RKN resistance

genes show different inheritance patterns in different backgrounds, it is possible that genetic resistance to RKN in cotton involves the interaction of several genes. The combination of two genes confers a high degree of resistance in Auburn 623 and has been documented in at least one segregating population derived from a resistant line of the Auburn 623 source. Recent work by USDA-ARS strongly suggests that the major portion of the resistance in Auburn 623 may be attributed to *rkn1* on chromosome 11 and a recently reported gene, associated with BNL 3545 on chromosome 14 (Ynturi 2005, Ynturi et al. 2006). Additional effort is needed to inventory and characterize the genes conferring RKN resistance, understand their relationships, and their effects in different genetic backgrounds. Our ultimate objective is to accelerate the incorporation of RKN resistance in commercial cultivars by providing the necessary knowledge about the inheritance of RKN genes, developing improved germplasm with the RKN resistance genes and developing selection markers for the resistance genes.

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