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- From: Authors and Editors: Bob Nichols, Peng Chee, Forest Robinson, Phil Roberts, David Stelly, David Weaver, and Roy Cantrell
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- Subject: Report of the June 9, 2005 Meeting in Memphis, Tennessee "Breeding Cotton for Resistance to Nematodes – 2005"
- CC: Al Bell, Charlie Cook, John Creech, Nilesh Dighe, Brian Gardunia, Doug Hinchcliffe, Andrew Kloek, Kathy Lawrence, Mustafa McPherson, Bill Meredith, Edina Moresco, Hal Moser, John Mueller, Melvin Newman, Don Panter, Mike Robinson, Gabe Sciumbato, C. Wayne Smith, Al Wrather, Hongbin Zhang

Summary

Plant parasitic nematodes are common and serious pests of cotton (*Gossypium spp.*). The presently-available options for cultural and chemical control do not prevent economic losses to these pests. In 2002, Cotton Incorporated began to support and coordinate an effort to develop host plant resistance in Upland cotton (*G. hirsutum*) against the number one and two most damaging nematode pests of cotton, the rootknot (*Meloidogyne incognita*) and reniform (*Rotylenchulus reniformis*) nematodes.

Cotton Resistance to Reniform Nematode

The reniform nematode is a serious problem in cotton in the Rio Grande and Tennessee Valleys, Southwest Alabama, and throughout the southern two-thirds of the Mid-South production region. Reniform nematode tolerant lines have been released (Cook et al., 1997; Cook and Robinson, 2005; Jones et al., 1988); however, no commercial cultivars are resistant to reniform nematode. Forest Robinson did a single plant screen of the National Cotton Germplasm Collection (NCGC) followed by replicated testing of accessions that did not support high levels of nematode reproduction in the single plant

screen, and identified six G. hirsutum accessions that were significantly more resistant than the standard, 'DP 16'. He also identified several G. barbadense lines with moderate to good resistance. David Weaver and Kathy Lawrence are presently completing an intensive evaluation of the NCGC accessions. Converted day-neutral and G. barbadense lines and crosses of these resistance sources with G. hirsutum are being evaluated for reniform reaction by USDA-ARS units at Stoneville and at Mississippi State, MS. Several A-genome lines are resistant to reniform nematode. In Mac Stewart's laboratory, Carlos Avila is making G. arboretum x G. trilobum and G. herbaceum x G. trilobum crosses, doubling the chromosome number and then crossing with G. hirsutum. He has also examined a G. arboretum (susceptible) x G. arboretum (resistant) F2 population to determine the number and inheritance of genes conferring reniform nematode resistance, and by using an AFLP approach, to identify molecular markers linked to the resistance. In an integrated approach at College Station, Texas, USDA-ARS and Texas A&M scientists are developing *G. hirsutum* lines with high levels of reniform resistance introgressed from G. longicalyx. Al Bell has made two triple species hybrids, both of which have G. longicalyx as one parent. Back crosses of the hybrids with G. hirsutum are being evaluated for nematode reaction by Forest Robinson and for cytological integrity by David Stelly and Nilesh Dighe. Concurrent progress is being made by Stelly, Dighe, and collaborator Monica Menz in identifying genetic markers linked to the G. longicalyx resistance gene. Also at College Station, F6 generation lines from R. reniformis resistant G. barbadense x G. hirsutum are being evaluated for reniform resistance and productivity by J. L. Starr.

Cotton Resistance to Root-Knot Nematode

The range of the root-knot nematode is coincident with that of cultivated Upland cotton. Whereas, good to excellent genetic sources of resistance have been available in Upland cotton for more than 25 years (Shepherd 1974a, 1974b; Hyer and Jorgenson 1978a, 1978b, 1984, 1985; Hyer et al., 1979; Jones and Birchfield, 1967; Jones et al., 1958, 1988, 1991), few commercial cotton cultivars are resistant to the root-knot nematode of cotton, Meloidogyne incognita. Cultivars sufficiently resistant to suppress nematode populations in the soil are limited to 'Stoneville 5599' of Emergent Genetics, that was derived from 'Stoneville LA887', and its sibling line, 'Paymaster 1560', that was previously 'Hartz 1560'. While these cultivars are only moderately resistant, Acala 'NemX' strongly suppresses some populations of *M. incognita*, but not others; however it is well adapted only to the San Joaquin Valley of California. No commercial cultivars have utilized the Shepherd source lines (Shepherd et al. 1996, Robinson et al., 1999), although Johnnie Jenkins' USDA-ARS laboratory at Mississippi State is advancing and evaluating breeding lines that were initiated by Roy Creech that were developed to express the Shepherd resistance. These backcross populations were derived with 'DES 119', 'Sure Grow 125' and 'Stoneville 213' as the recurrent parents. Three laboratories have developed segregating interspecific populations from G. hirsutum x G. barbadense crosses, and are investigating susceptible vs. resistant polymorphisms using RFLPs or AFLPS and SSRs. In Georgia, Peng Chee's laboratory is working with a G. hirsutum 'M120' x G. barbadense 'Pima S6' population. In Texas, Jim Starr's laboratory is working with a G. hirsutum 'M315' x G. barbadense 'TX-110' population. In California, Phil Robert's laboratory is working with three populations, including populations from Acala NemX x 'Acala SJ2' and 'Acala NemX x Pima S-7' to investigate the genetics of root-knot nematode and Fusarium wilt resistance.

Invited Presentations

Development of Resistance to Reniform Nematode

David Weaver and Kathy Lawrence

"Evaluating Germplasm for Resistance to Reniform Nematode"

The project's intention is to evaluate all unique accessions of *G. hirsutum* for reaction to reniform nematodes. The accessions that are being evaluated are the 'Texas' list, comprised primarily of collected plants, not the collection of obsolete cultivars. Four replications of each accession are being evaluated in the greenhouse. Sets of 50 accessions are challenged as seedlings with a geographically-mixed inoculum of 1500 juveniles. After 60 days of root growth, vermiforms are counted, as an indication of the ability of the nematodes to survive, and eggs are counted, as a measure of nematode reproduction. The cultivar, 'Paymaster 1218', has been used as a control in every set. Some accessions had poor germination. To date, 1603 accessions have been evaluated in the first round. Accessions in the lowest 10th percentile of each parameter were advanced to the next level.

Quantifying the susceptibility of the accessions has been difficult. Eighty-fold differences in vermiform counts and 200-fold differences in eggs on PM 1218 have been observed. Data have been log-transformed and standardized, based on the values found for PM 1218 in each respective set. Histograms of both vermiform and egg data suggest that the logs of counts can be characterized by a mean and variance. In the second round of evaluation, the lowest two entries in each set are being reevaluated. At this point, 134 of 175 entries have been re-tested. Candidate accessions for a third round of evaluation will be selected from those with the lowest counts in the 1st and 2nd rounds. In the third round, the number of replications will be increased and additional standards will be utilized. Final evaluations will be made in the field. Further objectives are to determine the heritability of any resistance that is found, and to incorporate the resistance into adapted lines.

Sally Stetina -- "Reniform Resistance from Texas Day Neutral Lines"

The unit's research program has several objectives, including identifying sources of resistance to reniform nematodes. The project is concurrently developing improved methods for evaluation of nematode resistance and evaluating genotypes. The evaluation of genotypes is concentrated on certain converted, day-neutral lines (McCarty and Percy, 2001). Crosses of three adapted *G. hirsutum* lines, 'DES 119B', 'DES 119H' and '55-3'; and nine primitive *G. hirsutum* lines from the 'T19', 'T1347' and 'T1348' series are being evaluated. Sampling from the field is done by digging up five randomly selected plants in the progeny rows and counting the number of females on the tap and secondary roots. Data are expressed as nematodes/gram of root. The sampling and counting procedure is destructive. In 2003, 889 F2:3 progeny rows and 150 parent rows were screened in the field. Of these, 76 progenies and 31 parental lines are being re-evaluated in the greenhouse in 2005. A review of evaluation methods will be presented at the Society of Nematologists Annual Meeting, in Fort Lauderdale, Florida, July 9-13, 2005.

Jim Starr, C. Wayne Smith, E. Zhou, K. Ripple, and E. Morgan "Reniform Resistance from Gossypium barbadense, 'TX 110""

The original intent was to create an interspecific population to map resistance loci for both root-knot and reniform nematode. The sources of genetic resistance for root-knot and reniform nematode were from 'M315' and 'TX-110', respectively. Estimates of the susceptibilities in the parents and in the F1 families confirmed a high level of root-knot resistance in M315 and the F1s progenies, a high level of reniform resistance in 'TX-110', and moderate to high levels of reniform resistance in the F1s. The crosses were selected for day neutrality, and then selected for the respective nematode resistances through the F5 generation. The plan was to begin selection for agronomic traits at the F5, but the families showed very low fertility. A few F6 lines are being evaluated for productivity, and single plant selections made of the most fertile individuals. A back cross breeding program has been initiated to restore fertility. Plans are to screen the BC1F1 for reniform nematode resistance and produce the BC2F2.

Forest Robinson, Alan Bridges, Ed Percival, Osman Gutierrez, Johnie Jenkins, Jack McCarty, Macon LaFoe, and Clarence Watson

"Use of Resistance Sources Recently Found in the National Collection" Among diploid cottons, immunity to the reniform nematode was previously known in G. longicalyx, and resistance was known in G. arboreum and G. herbaceum. Within tetraploid G. barbadense, there was confirmed resistance in 'TX-110', 'TX-1347', and 'TX-1348'. In addition, eleven breeding lines of *G. hirsutum* are tolerant of reniform nematode (Cook et al. 1997; Cook and Robinson 2005; Jones et al. 1988). In our studies, plants of 2269 G. barbadense and G. hirsutum accessions were screened in the greenhouse, and accessions supporting the lowest levels of nematode reproduction were evaluated in subsequent replicated experiments inside controlled environment chambers. In general, G. barbadense accessions were less susceptible than were G. *hirsutum* accessions. There were six *G. hirsutum* accessions that were significantly less susceptible than 'DP 16'. There were many G. barbadense accessions that were resistant to reniform nematode; the most resistant of these, 'GB 713' supported only 3% of the reniform reproduction of the control, DP 16, in three experiments. A cooperative project was initiated with Osman Gutierrez testing the combining ability of resistance from the six most resistant G. hirsutum accessions identified (TX-25, TX-748, TX-1586, TX-1828, TX-1860, and TX-2469), in hopes of enhancing resistance within G. hirsutum through transgressive segregation, without having to resort to interspecific crosses. The idea is to develop resistance to the reniform nematode using the same approach as was used successfully by Shepherd to develop root-knot nematode resistance in the Auburn lines.

In a different project, also in collaboration with O. Gutierrez and J. Jenkins, the high level of resistance in *G. barbadense* 'GB713' has been recovered in hybrid F1 plants as well as in 24 plants in the first backcross generation. Abundant seed from a second backcross onto agronomic *G. hirsutum* are available from all 24 resistant BC1 parents.

Jack McCarty and Macon Lafoe

<u>"Reniform Resistance Sources from wild Gossypium hirsutums and from G. arboretum"</u> Three day-neutral *G. hirsutum* and seven resistant *G. arboreum* lines were evaluated (Lafoe, 2005). The seven resistant *G. arboretum* lines were crossed with susceptible G. arboretum, 'A2-082'. The reniform inoculum was obtained from Baton Rouge, LA. Infested soil was mixed with sand at a ratio of 3:1 and additional reniform nematodes were pipetted into plots 10 days after planting. At evaluation, the plants were harvested and the nematodes recovered from 1,000 cc of soil using Baerman funnels. The line, 'A2-190' had the lowest mean reniform nematode counts. F2 progeny of A2-190 x A2-082 may fit a 9:7 ratio. In conclusion, the resistance of the parent G. arboretum was confirmed. The F2 populations show skewed distributions towards resistant plants, suggesting that the inheritance may be the result of partially dominant resistance genes.

The day neutral lines, 'MT 1348', 'MT 2468' and 'Mt 2469' were also evaluated in comparison to susceptible 'DPL 61' and 'DPL 5415'. Moderate resistance was confirmed in MT 1348 and the resistance in MT 2468 may be better than moderate.

Carlos Avila, J. Stewart, R. T. Robbins

"Reniform Resistance in the Asiatic Cottons"

About 10% of A-genome (*G. herbaceum* and *G. arboretum*) lines are highly resistant to reniform nematode (Stewart and Robbins, 1995). The first objective was to make fertile hybrids of resistant A-genome and Upland cottons. The approach was to cross diploid A genome with diploid D genome lines, then double the chromosomes using 1% colchicine in lanolin, applied to buds of A2D8 plants to create tetraploid chimeras. Doubling the chromosomes is the bottleneck in the process. For lines in which doubling was achieved, the synthetic allotetraploids were crossed with Upland cotton. Hybridization of reniform resistant *G. arboretum* with cultivated cotton was accomplished by crossing with synthetic allotetraploid $2(A2-194 \times D8)$.

The second objective was to determine the number and inheritance of the genes conferring reniform nematode resistance and to identify molecular markers linked to reniform nematode resistance. The approach was to compare amplified fragment length polymorphisms (AFLPs) of bulk segregants from families of the S x R cross, A2-128 x A2-19. There were 227 F2 plants, and 25 of each parent. F2s were classified for nematode reaction and the AFLPs compared with those of the resistant and susceptible parents. Both the AFLPs and the nematode counts indicated that many of the progeny were heterozygous. A preliminary conclusion is that a single additive A-genome gene confers resistance to the reniform nematode, but molecular markers for the resistance could not be found because the bulked populations were not homozygous. New populations of A2-194 x A2-128 and A1-51 x A2-128 will be made for marker development.

Forest Robinson, David Stelly, Al Bell, Nilesh Dighe, and Monica Menz "Introgression of Resistance from *Gossypium longicalyx*"

The objective of the project is to introgress resistance from *G. longicalyx* into *G. hirsutum* to produce germplasm and markers that will give the planting seed industry the means and incentive to develop agronomically improved cultivars that are resistant to reniform nematode. The project is a cooperative effort between USDA-ARS and Texas A&M University.

Al Bell made two triple species hybrids in the 1980s, (*G. hirsutum* x *G. longicalyx*)² x *G. armourianum* = AD x F to $(FAD)^2 x (FADD) = HLA$; (*G. hirsutum* x *G. herbaceum*)² x *G. longicalyx* = AD x A to $(AAD)^2 x$ F to (AADF) = HHL. Forest Robinson is phenotyping the resistance and identifying nematode-resistant plants. David Stelly, Nilesh Dighe and Monica Menz, are doing cytogenetic evaluations of the crosses, and developing molecular markers. Some families are at BC4, while the most advanced are at BC6.

The objective is to introgress the resistance, maintain normal chromosome pairing and maximize recombination. Chromosome numbers are stabilized at 52 in the BC3. No plants with odd numbers or unpaired chromosomes have been detected in BC3s, BC4s, or BC5s. However, each chromosome may carry a substantial amount of non-hirsutum chromatin. Visualization of the DNA of the resistant progenies, using genomic in-situ hybridization (GISH), suggests that only a small amount of G. longicalyx DNA that has been incorporated into the G. hirsutum BC progenies. BC selfed progeny are being developed for evaluation.

AFLP is the logical technique to use to initiate marker development, as several polymorphisms may be expected in the BC populations that have been derived from the hybrids. The approach will be to test potential AFLP markers in the segregating BC2, then use 4 SSRs per linkage group to attempt to identify the chromosome or linkage group with the reniform resistance gene. Additional work is also planned with cytological analysis, GISH, and field evaluation of advanced generations for agronomic characteristics.

Development of Root-Knot Nematode Resistance

Johnie Jenkins, J. C. McCarty, Roy Creech and John Creech

"Elite Breeding Lines with Resistance to Root-Knot Nematode"

Roy Creech crossed Shepherd-source, root-knot nematode resistant lines with improved cottons including DES 119, Sure Grow 125, and Stoneville 213. The objective was to use the Shepherd M series lines to generate improved agronomic germplasm with high levels of root-knot nematode resistance. Yield tests have been done at two different locations at Mississippi State for two years (total of four site-years). In 2004, twenty-four lines were evaluated for root-knot nematode and Fusarium wilt susceptibility at Tallassee, AL; and 40 lines were evaluated for reaction to root-knot nematode in the greenhouse. Based on the greenhouse evaluation, several lines appear to retain high levels of root-knot nematode resistance, yield well in comparison to 'Stoneville 474', and have good fiber length (1.12-1.14 inch) and strength (28-29 g/tex), but relatively high micronaire (~4.9 units). If a second greenhouse test confirms that the same lines are as resistant as previously determined, the best lines will be publically released as germplasm.

Phil Roberts, Congli Wang

<u>"Root-Knot Nematode Resistance in Acala 'NemX' Crosses"</u> Populations have been developed by crossing G. hirsutum cultivars 'Acala SJ2', 'Acala NemX', and *G. barbadense*, 'Pima S-7' to investigate the genetics of root-knot nematode and Fusarium wilt resistance.

<u>Line</u>	Root-Knot Reaction	Fusarium Wilt Reaction
SJ2	Susceptible (S)	S
NemX	Resistant (R)	S
S-7	S	R

The focus of investigations with the S-7 x NemX population is to determine the inheritance of the NemX root-knot resistance (R) genes, to find markers linked to the resistance, to localize the R-genes, and develop a high-resolution linkage map. The F2 and F2:3 data suggest that one major gene accounts for most of the root-knot nematode resistance expressed in NemX, and that the gene exhibits recessive behavior. Galling indices of F2:7 recombinant inbred lines (RILs) further confirmed a one-gene model, segregating into two distinct groups of resistant and susceptible lines. Transgressive segregation for resistance in the RIL population suggests that susceptible Acala SJ-2 contributes a factor that enhances the NemX R gene, with some lines more resistant than NemX.

Comparisons of AFLPs among the parental lines, SJ2, NemX, and S-7, revealed four polymorphisms. AFLP analysis of the bulked resistant and susceptible RILs confirmed segregation of the previously observed polymorphisms among the susceptible and resistant lines. A dominant cleaved amplified polymorphism (CAP) marker was developed from an AFLP and is closely linked to the NemX resistance gene locus. Further work indicated that the CAP marker was within a few centimorgans (cM) of the simple sequence repeat (SSR) marker 'CIR 316' as located on the linkage map of Nguven et al 2004. Screening of 250-300 SSRs against bulks and individuals showed that the putative resistance gene is on linkage group D02 or a homeologus region on the linkage group A03, between CIR 316 and the SSR 'BNL1231a'. More SSR markers on D02 and A03 are being screened to verify the map position of the resistance. Screening of the CAPs and CIR316 markers across root-knot susceptible and resistant lines suggested a good correlation of these potential markers with root-knot resistance. Although the lines SJ-2 and S-7 are both very root-knot susceptible, when they are crossed with NemX, the galling in the F₁s are different. The crosses, NemX x S-7 and NemX x SJ-2, produce resistant and susceptible F₁s, respectively. The distribution of nematode reaction in the F₁s clearly suggests a two gene model.

Jim Starr, E. Zhou, D. Silvey, E. Moresco, and C. W. Smith

"Evaluating SSRs as Markers for Root-Knot Nematode Resistance in M 315 Genotypes" Root-knot nematode resistance in M315 is derived from two moderately resistant *G. hirsutums*, 'Clevewilt 8-6' x 'Mexico Wild Jack Jones'. A highly resistant selection from the F9 of this cross is 'Auburn 623 RNR' (Shepherd, 1974). Auburn 623 RNR was crossed with Fusarium wilt resistant 'Auburn 56' to produce 'Auburn 634 RNR' (Shepherd, 1982). M315 is a BC2 selection from the cross, Auburn 634 RNR x 'DP 16' (Shepherd et al., 1988). In an attempt to find markers for root-knot nematode resistance, the graduate student, D. Silvey made the cross Upland M315 x Pima TX-100 and screened 192 probes from the map of Reinish et al. 1994. Two RFLP markers were found loosely linked to the probable resistance locus, 'A1214' on linkage group A02 (18.6 cM distant) and 'pAR815' on chromosome 14 (21.6 cM distant). Following the publication of a combined map (Lacape et al. 2003), the effort was shifted to screening the population using SSRs. However, no SSR was found linked to the resistance; therefore, a second population was developed. Using the new mapping population, an SSR, 'CIR 393' has been tentatively linked to resistance on chromosome 7.

Additional Activities:

Six *G. hirsutum* accessions have been reported to have some level of resistance to root-knot nematode (Robinson & Percival, 1997). To establish the number of alleles that may be involved in root-knot resistance, a diallel cross has been made among the 8 parents including, the highly susceptible 'DPL 90', the moderately resistant Clevewilt 8-6, and the six accessions. All crosses have been made and F2s are available except for one combination.

Peng Chee, Lloyd May, and Richard Davis

"Putative Location of Root-Knot Resistance Genes"

A population was made from the cross, resistant G. hirsutum, Shepard source 'M120' x G. barbadense, susceptible 'Pima S-6'. Two F1s were selfed to develop two genetically similar F2 mapping populations. One set of F2s comprised 107 selfed progenies, while the second was 138 progenies. The best indicator for phenotype was eggs/gram of root with correlations of 0.89 and 0.92 in the two F2s, respectively. Approximately 180 RFLPs, covering the entire cotton genome at 20-centimorgans intervals, were screened on 32 individual plants representing 8 extreme susceptible and resistant genotypes from the two populations. Statistical tests identified eight possible markers that were associated significantly with the resistant phenotype, using both gall ratings and eggs/gram of root. These eight markers were further tested for genetic linkage. On linkage group A 03, two linked markers were associated with the resistant phenotype, and another marker on chromosome 7 also was confirmed as significantly associated with resistance by correlation analysis using all individuals from the combined populations. These results strongly suggest that a major resistance gene may be present near these chromosome regions, although the linked markers are not sufficiently close to the gene to explain a large portion of the phenotypic variation. These two chromosome regions are being investigated in more detail by surveying additional markers in order to find markers more closely linked to the resistance genes.

Phil Roberts – Leader – Discussion:

What do We Know about the Genetics of Root-Knot Nematode Resistance in Cotton?

Two sources of root-knot nematode resistance in G. hirsutum are being investigated: 1. The Ray Shepherd source material developed from Auburn 623 that was a selection from the cross, Mexican Wild Jack Jones x Clevewilt 6, (Shepherd et al., 1996), and 2. The resistance in the cultivar Acala NemX. The root-knot resistance in the Shepherd-source line, M315 is the result of backcrossing Auburn 623 through many generations, first onto Auburn 56 to obtain Auburn 634, and subsequently onto Auburn 634 and Deltapine 16, with selection for nematode resistance at each backcross generation. The Shepard source resistance in M315 has been attributed to a two-gene system, $Mi_{1,}$ a dominant gene, and $Mi_{2,}$ an additive gene (McPherson et al. 2004), which are speculated to come from the two parents of the original cross, Mexican Wild Jack Jones and Clevewilt 6.

NemX was developed by Angus Hyer and E. C. Jorgenson of USDA-ARS working at Shafter, California. The information available from their publications (Hyer and Jorgenson, 1978a, 1978b, 1984, 1985) indicates that the source of resistance in NemX is 'N6072', a USDA breeding line developed from Acala 1-2303 and cv. Tanguis. However, other breeding lines and cultivars are known or speculated to be present in the background of Acala NemX, and their respective contributions to root-knot nematode resistance in NemX is not known (Robinson et al., 2001). Roberts and Wang (in preparation) have data indicating that the root-knot resistance in NemX is primarily determined by a single recessive gene, but that the resistance may be augmented by additional genes from certain *G. barbadense* cultivars. Thus a pattern of major and minor genes controlling root-knot nematode resistance is emerging.

Three laboratories, Peng Chee's at the Univ. of Georgia, Jim Starr's at Texas A&M University, and Phil Roberts' at the University of California at Riverside are working to develop markers for resistance from the Shepherd source material with RFLPs and SSRs (Chee & Starr), and from the NemX source with AFLPs and SSRs (Roberts), respectively. Difficulties in identifying and locating the resistance genes include, less than desirable precision in phenotyping the nematode reaction and the lack of SSRs that flank and/or are closely linked to the probable resistance loci. Nonetheless, both the Chee and Roberts laboratories are close to publishing information on SSR markers that co-segregate with the root-knot nematode resistance.

Bob Nichols - Research and Development Needs:

We urge the Chee and Roberts laboratories to publish their respective findings as soon as they are confident in their results. Exchange of information and materials among the Chee, Roberts, and Starr laboratories also is encouraged. All agree that the next step is to confirm the location of the SSRs associated with the resistance and proceed to fine mapping of the hypothesized resistance loci. Commercial utilization of the root-knot nematode resistance sources depends on our collective ability to develop selection markers that may be used by the planting seed industry.

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