New possibilities for some old genes: Improved host plant resistance in cotton
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ABSTRACT

Improved Host Plant Resistance (HPR) has long been a goal of plant breeders. Despite the fact that improvements have been made by a number of researchers over the past 50 years, chemical control of insect pests has remained the preferred method for most production systems. The introduction of Bt cotton and the pressure to decrease insecticide use has renewed interest in increasing the plant’s endogenous defenses against insect pests. A number of simply inherited traits have been identified that can improve the plant’s defenses against various insect pests. Each individual trait is not enough to provide adequate protection, but in combination, should provide a defense network that could further reduce insecticide usage. The traits being combined include semi-smooth leaf (t2t3t3) which deters some insects from feeding and inhibits egg laying, nectarless (ne1, ne2, ne3) which eliminates nectaries that can be an insect attractant, high glancing (Gls1, Gls1) which produces glands on the calyx crown and decreases bud/bollworm feeding and semi-glanded (gl1, gl2, gl3, gl4) which decreases the number of glands in seed, but has near normal glancing on other plant parts. The okra leaf (Lo2Lo2) trait has been suggested to make plants less attractive to tobacco budworm (Heliothis virescens) and bollworms (Helicoverpa zea) and impart better disease tolerance. The parental material also has tolerance/resistance to Verticillium wilt, Fusarium wilt/root knot nematode, and bacterial blight. The parents chosen for combining the traits not only have the trait of interest, but also have good fiber properties and lint yield, thereby eliminating the need for extensive backcrossing to recover a line with acceptable agronomic characteristics. Instead, a forward-crossing scheme is being used where F2 plants from the first cross are crossed onto plants containing the next trait being incorporated. This process is repeated until all the traits have been combined into one line. This genotype building strategy emphasizes combining multiple traits into one line without trying to recover the original phenotype of any of the parental lines. Instead new combinations will be created and selections will be made within the resulting progeny. If the Bt-gene could be incorporated into these new breeding lines, host plant resistance could be further improved.

Introduction

Cotton plants with improved host plant resistance (HPR) mechanisms against a range of insect pests could benefit growers by giving them varieties that maintain stable yields under increased insect pressure. Ideally, the grower could then decrease the amount of pesticide applied, providing significant cost savings and protecting beneficial non-target organisms. Improving a plant’s endogenous defenses against pests is not a new concept, and can be accomplished by avoidance, tolerance or resistance. In the 1890’s, when the boll weevil (Anthonomus grandis Boheman) became a serious pest in the United States Cotton Belt, breeders developed cotton varieties that matured earlier and thus were able to avoid some of the most damaging effects of the boll weevil (Adkisson et al., 1982). In the 1920’s, jassids (leafhoppers, Empoasca spp.) became a problem in Africa and spread to India and Australia. Genes were introduced into cotton that increased the number of plant hairs, and these “hairy” plants were found to be resistant to jassid predation. The mechanism of this resistance has been studied and found to be due to non-preference of the insect for hairy leaves (reviewed by Painter, 1951).

Perhaps the most dramatic example of HPR has been the development of transgenic varieties containing the Bt-gene. These plants produce the delta-endotoxin protein that is toxic to tobacco budworms (Heliothis virescens) and bollworms (Helicoverpa zea) and provides good control of both pests (Jenkins et al., 1997). The original protein has been modified and the toxicity increased to a level where there is concern that populations of budworms and bollworms could quickly develop resistance to the toxin (Sachs et al., 1993; Meredith, 1998). In 2001, over half the hectares in the U.S., were planted to Bt-cotton. As growers have decreased the number of insecticide applications, other insects that are not susceptible to the Bt-toxin have become a problem (reviewed by Meredith, 1998). Incorporation of conventional resistance traits into existing cultivars could slow the build-up of resistance to the Bt toxin and provide suppression of non-susceptible pests such as the tarnished plant bug (Lygus lineolaris). A number of simply inherited morphological traits have been identified that provide some protection from insect damage (reviewed by Jenkins and Wilson, 1996; Jones, 1998). These traits could be combined into one genotype and possibly combined with the Bt-gene, to develop cultivars with a broader range of HPR and provide secondary control mechanisms for budworm and bollworm.

From the group of potential traits that could enhance HPR, five were selected for this project and are summarized in Table 1. The genotypes, from which these simply inherited traits were originally isolated, generally had low yields and poor fiber properties. Extensive breeding efforts over the past forty years have
succeeded in transferring the traits into improved genetic backgrounds. The success of these efforts can be demonstrated by the fact that individual traits are now in elite germplasm and cultivars that are grown commercially and are competitive in various variety trials (www.mississippi.edu/dept/drec/cotton/cotton.htm and http://msa.ars.usda.gov/msa/stoneville/cgrps/ rayburn.html). Jenkins and Wilson (1996) reviewed the literature and found no clear negative “genetic effects” of the HPR traits on agronomic characters. Similar results were reported by Thomson (1987).

How each of the traits reduces insect predation and/or damage varies depending on the trait. Often the trait has multiple effects that interact to produce “resistance” (Figure 1). For example, nectariless cotton not only makes the plants less attractive by eliminating an important food source, it also has a reported antibiosis effect (Benedict et al., 1981). While plants with smooth leaves (glabrous) are more susceptible to some insects (Wilson and George, 1982; Thomson et al., 1987), plants with semi-smooth leaves have sufficient trichomes to physically decrease the ability of insect females to lay eggs (Lee, 1968; Tingey et al., 1975; Jenkins and Wilson, 1996; Meredith, 1998). The okra leaf trait has been identified as a spontaneous mutation in a number of different genotypes. The effect of okra leaf on insect predation is not clear, with some reports attributing earlier maturity (insect avoidance) or decreased attractiveness to bollworms and budworms to this trait (Wilson and George, 1982; Meredith and Wells, 1987; Thomson, 1994).

Cotton has glands distributed over the entire plant. These glands contain a number of toxic substances that can act as feeding deterrents or antibiotic compounds, and provide some protection against a number of pests including bollworm, budworm and plant bugs (reviewed by Jenkins and Wilson, 1996). However, these compounds are toxic not only to insects (Bottger et al., 1964; Lukefahr and Martin, 1966), but also to humans and non-ruminant animals (Aboudinia, 1976). Two genes have been identified (GL2, GL3), that when both are recessive (gl2 gl3 gl3), eliminate glands from the seed and vegetative tissues (McMichael, 1960; Lee, 1962). Using these two genes, breeders made a concerted effort to develop glandless cultivars (Jenkins et al., 1967; Metzer, 1975). Unfortunately, these cultivars were more susceptible to insect predation and generally have not been commercially successful (Metzer, 1975; Jenkins and Wilson, 1996). Genetic studies, by several research groups evaluating gland density and distribution, indicated that glanding on different parts of the plant was differentially influenced by the two genes. The gene GL2 had a greater effect on seed glanding, while GL3 had a greater influence on vegetative parts of the plant (Lee, 1965; Wilson and Shaver, 1973; Wilson and Smith, 1977). Previous researchers have used some effective procedures to identify plants with the genotype (gl2, gl3, GL2, GL3), however, all have limitations (Lee, 1962; Miravell and Hyer, 1962; Rhine and Smith, 1965; Wilson, 1971; Wilson and Smith, 1977). Because leaf glanding varies depending on the developmental stage of the plant, it is essential that the plants be sampled at the same stage and time. This requires collecting large numbers of fresh samples in a short period of time, and being able to make useful comparisons among the samples. New procedures using computer imaging systems and software could help solve these problems. Our group has developed protocols for quickly making digital images of harvested tissue samples (Flashpoint 128, Integral Technologies Inc., www.integraltech.com). These images are saved and later used to make gland density and distribution measurements with the aid of computer imaging software (Image-Pro Plus, Media Cybernetics, www.mediacy.com). Another problem is the difficulty in seeing and counting the small glands. When counts are made visually, human error is another factor. Being able to view the sample on a computer screen, and using automatic counting software, allow the researcher to process a large number of samples quickly and accurately (Scheffler et al., 2001).

The high glanding (HG) trait causes glands to form along the margins of the calyx crown (Calhoun, 1997). This trait is thought to be conditioned by a unique form of the GL3 gene designated G2;GL3. These glands offer protection to the bud and small boll by acting as feeding deterrents and producing compounds with antibiotic effects (Parrot et al., 1989; Hedin et al., 1992).

In seed glands, the compound mainly responsible for the negative effects to mammals and birds is a terpenoid aldehyde called gossypol (Boatner, 1948; Berardi and Goldblatt, 1980). If the amount of gossypol could be lowered to a level that is safe for mammals, but still decrease insect predation, then it would not be necessary to eliminate all the glands in the seed. There are already glanded genotypes available, with seed gossypol content less than 0.60%, (www.cottonseed.com/publications), indicating that developing varieties with reduced gossypol is an achievable goal.

Experimental procedure

Several traits have been shown to improve a plant’s defenses against various insect pests. By using sources of these traits that already have acceptable agronomic characteristics and good fiber properties, the desired resistance traits can be quickly combined into one genotype that is acceptable as a germplasm source for plant breeders.

A sequential crossing scheme is being used to combine simply inherited traits known to decrease damage from and enhance resistance to plant pests. The parents chosen for the crosses not only have the trait of interest, but also have good fiber quality and lint yield, thereby eliminating the need for extensive backcross-
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Feeding tests have shown that this trait makes the plants more resistant to budworms and bollworms (Parrott et al., 1997). Feeding tests have shown that this trait makes the plants more resistant to budworms and bollworms (Parrott et al., 1997).

The first crosses were made in 2001 and F1 seed was advanced at a winter nursery. Approximately 9000 F2 plants were grown at Stoneville in 2002, and nectariless plants with the HG phenotype were then crossed with the semi-glanded genotype A6gGL produced previously by our research group. A6gGL has a decreased number of seed glands while maintaining near normal glancing on the rest of the plant. Genetic analysis indicates that the line has the genotype g|gl, G|GL, for the genes that control glancing in cotton (McMichael, 1960; Lee, 1962). In addition, recent HPLC analysis of the line indicates that A6gGL has less than 0.50 % total seed gossypol. The F1 seed was advanced at the winter nursery and F2 plants will be grown in 2003. Plants with the desired combination of traits will be selected and F3 selfed seed produced. In 2004, F3 progeny rows from approximately 1000 selected plants will be evaluated in the field. Rows containing plants with the desired combination nectariless, semi-smooth leaf, HG and semi-glanded, will be further evaluated for agronomic and fiber quality traits. Preliminary screening for insect resistance/tolerance will be conducted by making insect counts and evaluating anthers for plant bug damage. As closely as possible, insect assessments will be conducted following the guidelines outlined in the MSU Revised Protocol for Scouting Arthropod Pests of Cotton in the Midsouth (Williams et al., 2000). Appropriate checks will be included for comparison. F9a progeny rows will be grown in a replicated single row test and evaluated for the same characters on a row basis. During this season, field screening for plant bugs (Lygus spp.) will be emphasized. Mustard (Brassica juncea L.) will be interplanted within the test to attract and maintain a vigorous plant bug population, then cut at the appropriate stage to force migration of the plant bugs to the adjacent cotton (Laster and Meredith, 1974; Tugwell, 1983; Maredia et al., 1994). F9a lines will be further evaluated in 2006. Future evaluation of selected lines will include laboratory and greenhouse tests for resistance/tolerance to budworm, bollworm and plant bugs using established protocols (George et al., 1983; Hedin et al., 1991, 1992). The development procedure is summarized in Figure 4.

Discussion and Conclusions

This genotype building strategy emphasizes combining multiple simply inherited traits, that have been transferred into agronomically acceptable backgrounds, into one germplasm line. Unlike traditional introgression programs, we will not try to recover the original phenotype of any of the parental lines. Instead, new combinations will be created and selections will be made within the resulting progeny. The best lines will be released as germplasm, which could be combined with the Bt-gene to produce cultivars with a broader range of HPR self defense mechanisms.

References

al of Economic Entomology, **85**: 359-364.


**Table 1.** Summary of the HPR traits used for this project. The germplasm source where the trait was originally identified is given as well as the germplasm source of the trait used for this project.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Genetic Description</th>
<th>Source</th>
<th>Reference</th>
<th>Used for Project</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semi-smooth</td>
<td>Reduced trichomes on leaves</td>
<td>Unknown</td>
<td>Lee, 1968</td>
<td>MD51ne</td>
<td>Meredith, 1993</td>
</tr>
<tr>
<td>Nectarless</td>
<td>ne1, ne2, ne3</td>
<td>G. tomentosum Meyer &amp; Meyer, 1961</td>
<td>MD51ne</td>
<td>Meredith, 1993</td>
<td></td>
</tr>
<tr>
<td>Okra leaf</td>
<td>Lp, Lp</td>
<td>Deeply palmate</td>
<td>LA500</td>
<td>Jack Jones</td>
<td>MD51ne</td>
</tr>
<tr>
<td>High glanding</td>
<td>Gf, Gf</td>
<td>Glands on calyx XG-15 wild G. hirsutum</td>
<td>H1220 (PM)</td>
<td>Calhoun, 1997</td>
<td></td>
</tr>
<tr>
<td>Semi-glanded</td>
<td>glg1, gl2, Gl1, Gl1</td>
<td>Lower seed ganding, near primitive race</td>
<td>Hopi M</td>
<td>McMichael, 1960 A6glGL</td>
<td>Jodi Scheffler</td>
</tr>
</tbody>
</table>

**Figure 1.** Interrelated components of resistance, one or more is frequently present in resistant genotypes, reproduced from Painter (1951).

**NON-PREFERENCE**
For oviposition, food or shelter

**ANTIBIOSIS**
Adverse effect of plant on biology of the insect

**TOLERANCE**
Repair, recovery, or ability to withstand infestation
**Figure 2.**
Upland cotton with
a: normal pubescence on a leaf, b: semi-smooth pubescence on a leaf, c: the “super” pubescent Pilose trait, d: bract nectary, e: bract without nectary, f: leaf nectary, g: leaf without nectary, h: normal leaf glanding (Gl2Gl3), i: normal calyx glanding (Gl2Gl3), j: normal seed glanding (Gl2Gl3), k: glandless leaf genotype (gl2gl3), l: glandless calyx genotype (gl2gl3), m: glandless seed genotype (gl2gl3).

**Figure 3.**
a: Upland cotton with a: normal glanding on calyx (top) and high glanding (bottom), b: close-up of high glanding trait with glands along the calyx margins, c: variation in leaf gland density and distribution for plants with the semi-glanded trait (gl2Gl3), d: variation in calyx gland distribution for plants with the semi-glanded trait (gl2Gl3).
**Figure 4. HPR germplasm development scheme.**

<table>
<thead>
<tr>
<th>Year</th>
<th>Variety Characteristics</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>MD51nc nectarless, semi-smooth okra or normal</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>H1220 (PM1220) high-glanded semi-smooth</td>
<td>F1 Seed</td>
</tr>
<tr>
<td>Winter Nursery 2001/2002</td>
<td></td>
<td>Selfed F2 Seed from Individual Plants</td>
</tr>
<tr>
<td>2002</td>
<td>Grow 9000 plants, select nectarless &amp; high-glanded plants</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>A6g1;Gl, reduced seed glands, high fiber quality, semi-smooth okra or normal, gossypol &lt;0.70</td>
<td>F1 Seed</td>
</tr>
<tr>
<td>Winter Nursery 2002/2003</td>
<td></td>
<td>Selfed F2 Seed from Individual Plants</td>
</tr>
<tr>
<td>2003</td>
<td>Grow 30,000 plants, select those with desired HPR traits and produce F3 seed</td>
<td></td>
</tr>
<tr>
<td>Field 2004</td>
<td>Grow progeny rows from selected plants and evaluate for agronomic and fiber characters, monitor and remove plants without the required HPR traits, produce F4 self seed</td>
<td></td>
</tr>
<tr>
<td>Field 2005</td>
<td>Grow replicated progeny rows of F3:4 plants and evaluate for agronomic and fiber characters, monitor and remove plants without the required HPR traits,</td>
<td></td>
</tr>
<tr>
<td>Field 2006</td>
<td>Grow replicated progeny rows of the F4:5 selected plants and evaluate for agronomic and fiber characters, monitor and remove plants without the required HPR traits, do additional field testing of insect resistance, produce F6 self seed</td>
<td></td>
</tr>
<tr>
<td>Field 2007</td>
<td>Grow replicated progeny rows of the F5:6 selected plants and evaluate for agronomic and fiber characters, monitor and remove plants without the required HPR traits, do additional field and greenhouse testing of insect resistance, produce F7 self seed</td>
<td></td>
</tr>
</tbody>
</table>