Host Response to Reniform Nematode Infection in Resistant and Susceptible *Gossypium arboreum* Accessions

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**RESEARCH PROBLEM**

The reniform nematode (RN), *Rotylenchulus reniformis* Lindford and Oliveira, is a serious threat to cotton production. In fields infested with RN, yield losses are estimated at 340 to 452 kg/ha (Robinson, 2001). Genetic resistance to RN has not been reported for any commercial cultivar of cotton (*Gossypium hirsutum* L.) (Robinson et al., 2004); however, it has been found in *G. arboreum* (Stewart and Robbins, 1994), but little is known about the resistance mechanism. The objectives of this study were 1) describe the response of cotton roots at the transcriptome level in response to RN infection as a tool for the potential development of rational strategies for nematode control and 2) to identify differential genes regulated in resistant and susceptible *G. arboreum* accessions.

**BACKGROUND INFORMATION**

Plants respond to pathogen infection via a complex and integrated set of defenses driven by constitutive and induced responses (Dowd et al., 2004). When a nematode enters the root and initiates feeding, remarkable physiological and morphological changes occur in the cells to accommodate the nematode with a feeding site (Hammond-Khosack and Parker, 2003). Physiological changes in the host can be analyzed through its transcriptome. Since penetration behavior of reniform nematode females in resistant and susceptible *Gossypium* spp. has been reported to be the same, the events that occur in the feeding site (syncytium) establishment may determine the degree of susceptibility of cotton (Carter, 1974; Agudelo, 2004).

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Cotton seeds from *G. arboreum* resistant (A2-194) and susceptible (A2-128) accessions were surface sterilized in a 20% bleach solution, germinated, and transplanted into 500-cc clay pots filled with pasteurized fine sand. Plants were kept in a growth chamber with a 16 h photoperiod at 28°C day/24°C night. Four treatments with 3 reps were applied to 1-month old plants: 1) Resistant-inoculated (RI), 2) Resistant non-inoculated (RNI), 3) Susceptible-inoculated (SI), and 4) Susceptible non-inoculated (SNI). Inoculated treatments received 5,500 vermiform-stage nematodes per pot divided in 5 locations around the plant to cover the entire root system. After 16 days, roots for each treatment were harvested and immediately frozen in liquid nitrogen.

Total root RNA was extracted using a method similar to that reported by Wilkins and Smart (1996). Extracted RNA was cleaned using an RNeasy Plant Mini Kit (Qiagen, Calif.). After quantification, total RNA for each of the 3-reps was bulked to synthesize cDNA from mRNA using the SuperScript III First-Strand Super Mix (Invitrogen, Calif.). Polymerase Chain Reactions with AFLP primers were used to identify polymorphism between treatments. Selected polymorphic bands were cloned using the pGEM T-easy Vector (Promega, Wis.) and sequenced to identify the transcripts.

### RESULTS AND DISCUSSION

After 9 days, roots of monitor plants were stained with Acid Fucsin and observed under a light microscope for RN infection as originally planned. At that point, only a low level of infection was observed. This is thought to be due to the fact that immature vermiform were not infective until they reached maturity. After 16-days plants had a higher infection level and were harvested for RNA extraction.

AFLP analysis was performed using 48 primer combinations on the cDNA of each of the four treatments. Each combination yielded an average of 80 bands. Observed polymorphisms between accessions and between treatments were selected for further analysis.

Expression changes were classified according to their distribution between accession and inoculation. These are summarized in Table 1. Few expression changes were observed between treatments (62 per ~3,840 total bands). Most of the polymorphism observed was due to accession effect (34/62 = 55%), and these may or may not be directly involved in the resistance mechanism.

After cloning and sequencing polymorphic bands, sequences were compared with the NCBI database (www.ncbi.nlm.nih.gov) using the translated query vs. protein database (BLASTx) to obtain a hint of their function. In order to have a better understanding of what was happening between accessions, differentially expressed transcripts were grouped according to their putative biological process involved (Fig. 1). Cellular transport, cell cycle, and DNA processing resulted in more transcripts in the susceptible accession than in the resistant one. It is hypothesized that those processes may be related to syncytia formation. On the other hand, processes that may be involved in resistance mechanism, as cellular rescue, defense, and transcription, had more transcripts in the resistant accession.
Surprisingly, so far no gene-specific expression has been detected in the resistant accession when infected by reniform nematode, suggesting that resistance may be related to a down-regulated gene during nematode feeding, but additional supporting data need to be obtained before the validity of this statement is verified. The only up-regulated transcript observed during nematode infection in A2-194 has similarity to the transcription factor bZIP35, expression of which is related to abiotic stresses in soybean (unpublished data). Similarly, another bZIP transcription family protein was expressed only in the susceptible accession when it was infected by the nematode. The function of transcripts specific to A2-194 expressed in both inoculated and non-inoculated treatments included genes involved in carbohydrate synthesis, protein kinases, carboxyl-terminal peptidases, senescence-associated proteins, transcription factors, mRNA degradation, and a nod-factor-like protein. If these transcripts are involved in resistance, they are expressed without the presence of the nematode (constitutive resistance), but possibly the level of expression could change during infection.

Susceptible inoculated plants showed expression of a transcript similar to Transparent Testa 12 protein (NP_191462) from *A. thaliana*, a multi-drug transporter-like membrane protein, similar to ripening regulated protein DDTFR18 (*Lycopersicon esculentum*). It may be involved in formation of the nematode feeding site (syncytium), which involves the coalition of adjacent cells through cell wall dissolution in response to nematode infection. Likewise, a protein similar to P450 (ABE81447) from *Medicago truncatula*, that is also involved in secondary metabolite biosynthesis, transport, and catabolism, was found in susceptible inoculated plants. Additionally, several transcripts from susceptible inoculated plants had similarity to a protein (Accession NP_566322) from *A. thaliana* with unknown function.

**PRACTICAL APPLICATIONS**

Unveiling the RN resistance mechanism found in *G. arboreum* can be used as a tool for the potential development of rational strategies for nematode control as rotation and pyramiding resistance genes with different mechanisms in order to delay the appearance of nematodes that overcome host resistance. Alternatively, in the absence of markers linked to resistant genes, differentially expressed genes can be used to select for resistance in developing populations. Finally, putative genes involved in syncytia formation can be the targets for gene silencing so as to induce resistance.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


Table 1. Differential gene expression changes in resistant and susceptible genotypes of *G. arboreum*.

<table>
<thead>
<tr>
<th>Gene expression</th>
<th>RI²</th>
<th>RNI</th>
<th>SI</th>
<th>SNI</th>
<th>No. of entries</th>
</tr>
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<tr>
<td>Accession effect</td>
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<td>X</td>
<td>X</td>
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<tr>
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<td>X</td>
<td>X</td>
<td>3</td>
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<td>X</td>
<td>X</td>
<td>4</td>
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<tr>
<td>Up-regulated</td>
<td>X</td>
<td>X</td>
<td>x</td>
<td>X</td>
<td>8</td>
</tr>
</tbody>
</table>

² RI= Resistant inoculated, RNI= Resistant non-inoculated, SI= Susceptible inoculated, SNI= Susceptible non-inoculated, X=band present and x= low intensity band.
Fig. 1. Overview of cell biological processes differentially expressed in resistant and susceptible G. arboreum accessions.