HYBRIDIZATION OF EXOTIC GERMPLASM WITH UPLAND COTTON AS THE FIRST STEP IN TRANSFER OF RENIFORM NEMATODE RESISTANCE

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RESEARCH PROBLEM

Surveys of agricultural areas in several countries have shown that the reniform nematode (*Rotylenchulus reniformis*) is a widespread and persistent pest (Luc et al., 1990). In upland cotton (*Gossypium hirsutum*) the reniform nematode is now considered a serious problem throughout the southern United States (Heald and Robinson, 1990). Cotton yield loss due to this pest may range from 10% to 25% and can be as high as 50% in some situations (Davis and Cummings, 1998). Kirkpatrick and Robbins (1998) reported that, under drought stress, losses caused by reniform nematode in cotton may approach 50%. No commercial cultivar of upland cotton has been reported to have resistance to reniform nematode (Wang, 2001). The objectives of this project are to 1) make hybrids between diploid cotton germplasm resistant to reniform nematode and upland cotton as the first step in trait transfer; and 2) develop molecular markers genetically linked to nematode resistance to aid in following trait introgression.

BACKGROUND INFORMATION

Resistances to reniform nematodes have been found in A-genome diploid cottons (Carter, 1981; Yik and Birchfield, 1984; Robbins and Stewart, 1996). Robbins and Stewart (1996) identified a number of sources of resistance to the reniform nematode in the secondary germplasm pool, especially within *G. arboreum* (A2), *G. herbaceum* (A1), and *G. longicalyx* (F1), the last of which appears to be immune to this nematode. These sources of resistance are diploid species; therefore, the material must be genetically enhanced for use in tetraploid commercial upland cotton. Advances in genetic research methodology have made possible the dissection and analysis of plant genomes at the molecular level. Random Amplified Polymorphic DNA (RAPD) markers

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have been used to rapidly identify loci linked to genes or genomic regions of interest by bulked segregant analysis (Michelmore et al., 1991). Techniques like bulk segregant analysis assist in finding resistant-linked molecular markers that can subsequently be used in progeny selection without specific screening for reniform nematode resistance.

RESEARCH DESCRIPTION

Several strategies are being pursued simultaneously using nematode-resistant diploid cotton. The resistant diploid cotton, including the most resistant plants from an F_2 population of reniform nematode-resistant *G. arboreum* X susceptible *G. arboreum* F_1 hybrid, are being crossed with high-performance upland cottons (which are susceptible to reniform nematodes). In our first approach the resistant diploid is crossed directly with tetraploid upland cotton. The resulting hybrid is expected to be a sterile triploid and requires extensive additional manipulation before introgression into cotton can proceed. In another approach, D-genome wild species are crossed with the A-genome lines that are resistant to the reniform nematodes. A successful hybridization would result in a diploid AD hybrid that, upon having its chromosome number doubled, would be directly compatible with upland cotton. A third approach involves hybridization of a 2(ADD) hexaploid genetic stock with the resistant A-genome species. Success in this approach would yield a hybrid directly compatible with cotton without the need to double the chromosome number. Because most of these crosses will not develop on the mother plant, the pollinated ovules are placed on culture medium for *in ovulo* embryo culture to obtain hybrid embryos (Stewart and Hsu, 1978).

For identifying molecular markers (RAPDs) associated with reniform resistance, bulk segregant analysis was used. DNA was extracted from the ten most resistant and ten most susceptible plants from a segregating F2 population (100 plants) from a cross between a reniform nematode resistant Asiatic line (*G. arboreum*) and a highly susceptible line. DNAs from each group of ten plants were pooled into resistant and susceptible bulks. Random primers were used to perform polymerase chain reactions (PCR) on these two bulked DNA samples. The PCR products from each primer/sample were separated by electrophoresis and examined to detect DNA polymorphism associated with resistance.

RESULTS

Several crosses between resistant A-genome cotton and D-genome *Gossypium aridum* were attempted to create a synthetic AD hybrid. The maternal Asiatic plants have retained a few bolls that were not placed into ovule culture. These bolls have enlarged and are currently in the filling stage of development. A limited number of cross-pollinations have been made between resistant diploid Asiatic cotton and one of three other parental lines including a 2(ADD) hexaploid genetic line and two elite

upland cotton lines. Since these crosses result in empty seeds or will not develop naturally on the plant, the fertilized ovules were placed on a defined culture medium 3 days after pollination. The culture flasks subsequently proved to be contaminated by fungal spores. This approach to obtain hybrids is continuing with improved methods to control microbial contamination.

Identification of molecular markers genetically linked to reniform nematode resistance in Asiatic cotton is in progress. The DNA bulks from the resistant and susceptible plants have been screened with 100 random, 10-nucleotide base primers, thus far. Among these a few RAPD markers have been detected that appear to be associated with the bulked DNA sample from nematode resistant plants. The association of these markers with resistance will be confirmed by testing their presence or absence in individual plants from the F2 segregating population.

PRACTICAL APPLICATION

The need for genetic resistance to the reniform nematode is widely recognized. The first step in transferring resistance from exotic germplasm, such as the Asiatic cottons, into upland cotton is to obtain hybrids between these. The current test for resistance to reniform nematodes requires in excess of two months, is labor intensive, and is subject to wide variation. One or more molecular markers closely associated with resistance could be used in marker-assisted selection to greatly simplify the introgression and breeding of resistance in elite cultivars. This research is continuing.

ACKNOWLEDGMENTS

The financial support of the Delta and Pine Land Company for this research project is gratefully acknowledged.

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