TRANSFER OF RENIFORM NEMATODE RESISTANCE FROM DIPLOID COTTON SPECIES TO TETRAPLOID CULTIVATED COTTON

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RESEARCH PROBLEM

The reniform nematode (Rotylenchulus reniformis) is a semi-endoparasitic organism in which the female penetrates the root cortex establishing a permanent feeding site (Robinson et al., 1997). It has become a serious threat to cotton (Gossypium hirsutum L.) production in Arkansas and the mid-South (Zhao et al., 2000) because yield losses in infested fields may exceed 50% under stress conditions (Smith and Cothren, 1999). The most cost-effective method of pest management is host-plant resistance (Stewart, 1994); however, no commercial cultivars tested to date have shown resistance. Robbins and Stewart (1996) identified a number of sources of resistance within G. arboreum (A2), G. herbaceum (A1), and G. longicalyx (F1). These sources are diploid species; therefore, the material must be genetically enhanced for use in tetraploid cultivated cotton. The objectives of this project are: (1) to develop hybrid materials between resistant reniform nematode diploid cottons and tetraploid cultivated cotton, and (2), to identify molecular markers genetically linked to reniform nematode-resistance gene.

BACKGROUND INFORMATION

Stewart (1994) divided the germplasm resources available for cotton improvement into pools according to genomic affinity. The primary cotton germplasm pool contains the genetic resources that will result in direct genetic recombination between the parental genomes in hybrids. All natural Gossypium allotetraploids fall within this group. The secondary pool includes those genetic resources that require some level of manipulation to obtain fertile hybrids between the source and the cultivated line. However, once fertile hybrids are obtained, recombination potential is high. The A2, A1, and F1 species fall within this group. Molecular markers are very useful for screening and selection of germplasm within breeding programs (Ford-Lloyd and Painting, 1996). Bulked segregant analysis is a method for rapidly identifying markers linked to any specific gene or genomic region. It involves screening for differences between two pooled DNA samples derived from a segregating population that originated from a single cross (Michelmore et al., 1991). Amplified fragment-length polymorphism (AFLP) provides a very powerful DNA fingerprinting technique for DNAs (Vos et al., 1995)
RESEARCH DESCRIPTION

In order to develop hybrid materials between resistant diploid and tetraploid cotton three basic steps were followed: (1) diploid hybridization, (2) chromosome doubling, and (3) introgression into upland cotton. In the first step, several strategies were pursued simultaneously. A genome, reniform nematode-resistant species were crossed both with D-genome species to produce diploid interspecific hybrids, and with a previously produced hexaploid between *G. hirsutum* (AD₁) and *G. armourianum* ([[(AD₁)xD₂₁]6X) to produce compatible tetraploid hybrids. A third strategy consisted in crossing a diploid hybrid between *G. longicalyx* (F₁) X *G. arboreum* (A₂-21) with plants of the D genome and with *G. hirsutum*. The fourth strategy consisted in crossing a previously produced *G. longicalyx* 6X hybrid 2(F₁xAD₁) with D-genome species and upland cotton. The first cross will yield a tetraploid hybrid directly while the second gives a pentaploid that, by recurrent backcrossing with upland cotton, can develop resistance that can be selected through an aneuploid backcross series.

In the second step, colchicine, an anti-mitotic agent, was used to double the number of chromosomes of diploid or triploid hybrids: This was achieved by 1) applying suspension of 1% colchicine in lanolin on the axillary buds of defoliated plant stems; 2) germinating hybrids on 0.8% agar medium containing 10 ppm of colchicine for 12 to 18 d; and 3) placing hybrid cuttings in solutions at two colchicine concentrations: 10 ppm for 2, 4, 6, 8, 10, 15, 20, and 25 d and 20 ppm for 10 and 15 d. Flow cytometry analysis was carried out to determine the ploidy levels of the original and the colchicine-treated hybrids. In the final step, the synthetic tetraploids produced were crossed with upland cotton to transfer the reniform resistance.

To select the bulks for development of molecular markers associated with resistance, 227 plants of an F₂ population of the *G. arboreum* hybrid A2-128 x A2-19, 25 plants of A2-128 susceptible parent, and 25 of A2-19 resistant parent were inoculated with 3,300 nematodes, and after 2 months the nematodes were recovered by the sucrose centrifuge-flotation method (Jenkins, 1964) and counted.

RESULTS AND DISCUSSION

Eleven reniform-resistant diploid AD-hybrids were obtained from approximately seven hundred crosses utilizing the approaches described above. Of these eleven crosses, five were from the cross of *G. herbaceum* accession A1-15 and *G. aridum* (A₁-15 x D₂), but these died by fungus infection eighteen days after germinating them in agar containing 10 ppm colchicine. Six hybrids were obtained from the cross of *G. arboreum* accession A2-194 and *G. trilobum* (A₂-194 x D8); upon germination in agar-colchicine solution, the roots were killed on...
three plants and they died after 10 days. One plant survived the treatment. Of the remaining two hybrids of A₂-190 x D8, one survived after germination in agar-colchicine solution. No embryo was obtained from the remaining crosses. Thus far, the only synthetic tetraploid plant obtained [2(A₂-194 x D₈)] resulted from the treatment of sixty-three plants (680 axillary buds, approximately 6 buds per plant) giving a 0.14% efficiency. Flow cytometry analysis indicated that this was a chimeric plant.

Two triple hybrids have been produced, one from a cross of [2(A₂-194 x D₈)] as the pollen donor with the commercial cultivar DP491 and another with Delta Pearl. These hybrids are 4X and are expected to carry the genetic resistance to reniform nematode of the diploid A-genome species.

After screening the F2 population of the hybrid A2-128 x A2-19 for reniform reproduction, a regression line was traced between plant height (y-axis) and pf (x-axis) (pf = number of nematodes in the final population). The height of the resistant plants was not affected by the nematode but the height of the susceptible ones had an inverse relationship with pf, showing that an increase in pf is related with a decrease of plant height. These results were not statistically significant at \( \alpha = 0.05 \) but showed a clear trend. To remove the effect of outlier observations, student residuals were calculated, and any observation with a residual equal to or more than two was removed. Finally, twenty plants were selected to form DNA bulks of the 10 most resistant and 10 most susceptible plants, to develop a molecular marker linked to reniform resistance. Additional bulks were made of 10 resistant plants with equal pf mean to the resistant control parent and 10 susceptible plants with pf mean equal to the susceptible control parent, A2-19 and A2-128, respectively. Work to identify useful molecular markers is in progress.

**PRACTICAL APPLICATION**

The tri-species hybrids that have been produced will be used as germplasm material to introgress the resistance into upland cotton. The molecular markers closely associated with the resistance gene(s) will greatly accelerate selection and introgression of the resistance into elite cultivars, since MAS can be used in place of the laborious and time-consuming nematode reproduction screens on segregating plant populations that are now required.

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LITERATURE CITED


