DEVELOPMENT OF A TRANSFORMATION CONSTRUCT FOR ENHANCED DISEASE RESISTANCE

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RESEARCH PROBLEM

Insect and microbial pathogens pose an eternal challenge to our crop plants, developing novel ways to tap into food reservoirs. A multitude of management strategies have been tailored to control their proliferation, but the approaches have been successful only at high cost. Pesticide and fungicide usage has been a method of choice, but a newly emerging alternative in disease management is to enhance the resistance of plants by incorporating genes with antibiotic properties from foreign sources. Fungal and bacterial pathogens pose a major challenge to cotton. Fungal pathogens alone contribute to a significant reduction in yield in the United States. This project focuses on incorporating a gene homologue with antibiotic properties into cotton, which will enhance the resistance of cotton to microbial diseases.

BACKGROUND INFORMATION

Several organisms provide us with a source of peptides with potential antibiotic activities. Many of these peptides effectively control various pathogens. One of the recent additions to the antibiotic peptide arsenal is a group called magainins. Magainins are small (approximately 23-30 amino acids) peptides isolated from the skin of the African clawed frog (*Xenopus laevis*). Two isoforms of magainin have been isolated and named magainin 1 and 2. They possess broad-spectrum antiparasitic and antibiotic activities (Zasloff, 1987). The mode of action of magainin is based on its ability to insert into lipid bilayers of membranes, thereby disrupting membrane integrity by forming ion channels. However, membranes of higher plants and animals are relatively insensitive to the peptide (Duclohier *et al.*, 1989; Cruciani *et al.*, 1992). Kristyanne *et al.* (1996) reported the antifungal activity of magainin on several species of fungi pathogenic on cotton such as *Rhizoctonia solani, Fusarium oxysporum, Verticillium dahliae*, *Thielaviopsis basicola,* and *Pythium ultimum*. Magainin 2 at 0.05 µg/µl completely inhibited hyphal growth of all but the last species. Electron microscopy revealed degradation of the mitochondrial and cytoplasmic matrices, a reduction in the number of ribosomes, and vacuolization of the cytoplasm.

RESEARCH DESCRIPTION

Two gene constructs harboring the magainin gene were made. Each of these con-

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structs differed in the signal peptide sequence, which was fused upstream of the magainin gene. The codon usage for the original gene sequence was substituted with the ones most favored by cotton. One of the transit peptides will localize the gene to the endoplasmic reticulum, and the other will transport it into the extracellular space. Oligonucleotides were synthesized for the gene cassettes. The chimeric magainin gene was made utilizing the polymerase chain reaction (PCR). Following synthesis, the PCR products were cloned into pGEM-T vector and sequenced. At this stage, we determined that the gene construct with the extracellular targeting leader peptide did not have the correct sequence. On the other hand, the vector bearing the magainin gene with an endoplasmic reticulum signaling peptide was correct. It was excised from pGEM-T vector and cloned into a pBIN binary vector under the control of the CaMV 35S constitutive promoter. The gene insert was verified by restriction digestion and PCR. Plasmid DNA was isolated and mobilized into *Agrobacterium* super-virulent strain EHA 105. The presence of the gene in *Agrobacterium* was confirmed by PCR analysis.

Tobacco leaf discs were transformed by co-cultivation with *Agrobacterium* harboring the binary vector. Putatively transformed shoots were selected based on resistance to the antibiotic kanamycin. Shoots were transferred to rooting media. After primary roots were formed plants were transferred to pots containing potting soil.

Future research involves the verification of gene integration and expression by Southern and Northern blots. Plants will be tested for resistance against *T. basicola, F. oxysporum*, and *V. dahliae* and selected bacterial pathogens.

PRACTICAL APPLICATIONS

In recent years, many studies have been conducted in the area of exploiting genes with antibiotic activities for disease resistance. It is inevitable that pests will ultimately build resistance and this forces us to look for alternative sources of disease resistance genes. This study will reveal the activity of magainin in plant cells. Transgenic plants bearing the magainin gene will hopefully reduce the production costs by lowering pesticide usage.

LITERATURE CITED

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