VECTORING A BIOACTIVE PEPTIDE FOR COTTON TRANSFORMATION

Satyendra N. Rajguru and James McD. Stewart
Department of Crop, Soil,
and Environmental Sciences
University of Arkansas
Fayetteville, AR

Abstract

Plants are under constant assault by pathogens trying to gain access to the food reservoirs delimited by the cell wall. To efforts to enhance host plant resistance to this attack, several genes encoding antimicrobial peptides have been cloned and engineered into plants by a number of researchers. One of the bioactive peptides that has received such attention is magainin.

Magainins are peptide antibiotics with a broad antimicrobial activities derived from the skin of the African clawed frog (*Xenopus laevis)* (Zasloff, 1987). Magainin 1 and 2, each with 23 amino acid residues, inhibit the growth of fungi and both Gram-positive and Gram-negative bacteria (Zasloff, 1987). These peptides disrupt membrane integrity by forming ion channels across lipid bilayers (Duclohier et al., 1989; Cruciani et al., 1992), and depolarize the membrane (Westerhoff et al., 1989) leading to cell death. Kristyanne et al. (1996) reported the antifungal activity of magainin 2 on several fungal pathogens of cotton including *Thielaviopsis basicola, Rhizoctonia solani, Fusarium oxysporum,* and *Verticillium dahliae*. The peptide completely inhibited hyphal growth at 0.05 µg/µl. We hypothesize that the antimicrobial activity and the selectivity of magainins to pathogenic microbes can be used to our advantage as sources of disease resistance genes for transgenic plants.

Chloroplasts isolated from pea seedlings were exposed for 10 minutes to magainin 2 at concentrations ranging from 0.1 to 0.5µg/µl at 25ºC. The rupture of chloroplasts was measured as a function of loss in chlorophyll concentration determined by spectrophotometry. No decrease in chlorophyll concentration due to magainin was evident.

Polymerase Chain Reaction (PCR) was employed to synthesize a chimeric gene coding for a magainin analog with a transit peptide leader. *BamH I* and *Sac I* sites were incorporated on the 5' and 3' end, respectively, of the chimeric gene fragment to facilitate unidirectional cloning. Following synthesis, the chimeric gene was cloned into pGEM-T Easy vector. White colonies were selected and verified by PCR and restriction digestion for inserts.

Future research involves cloning of the gene under the control of CaMV promoter into the binary vector, pBI 121, without the GUS cassette. Plasmids containing the neomycin phosphotransferase gene without GUS will serve as controls. *Agrobacterium tumefaciens* will be used for plant transformation. Southern, Northern, and Western blots will be performed to obtain molecular confirmation of gene integration and activity in the transgenic plants. Transformed plants will be tested for resistance to various cotton pathogens including *T. basicola, R. solani, F. oxysporum, and V. dahliae.*

References

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