TRANSGENIC EXPRESSION AND EVALUATION OF PLANTS TRANSFORMED WITH A SYNTHETIC ANALOG OF MAGAININ

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Abstract

Magainins are 23-amino acid residue antibiotic peptides isolated from the skin of the African clawed frog (*Xenopus laevis*) (Zasloff, 1987). They inhibit the growth of numerous species of bacteria and fungi by depolarizing cell membranes and making cells leaky to the environment (Matsuzaki et al., 1995), but they are generally benign to cells of higher organisms. The primary objective of this research was to incorporate an analog of the gene encoding a magainin analog into cotton to enhance the resistance of cotton to various disease causing pathogens. Because of the supposed archeomicrobial origin of the chloroplast and mitochondria in eukaryotes, the cytotoxicity of magainin to cell organelles needed to be determined. For this determination isolated intact pea chloroplasts were incubated with 0.1or 0.2μg/ml of magainin 2. No damage to isolated chloroplasts by magainin 2 was detected based on absence of released chlorophyll from treated vs. untreated chloroplasts. Polymerase chain reaction (PCR) was utilized to synthesize a magainin analog gene which was subsequently cloned into pGEM vector. Following confirmation of the correct sequence the DNA fragment was cloned into the binary pBIN vector. The binary vector was mobilized into *Agrobacterium* strain EHA 105 and was used in the transformation of tobacco plants. The integration of the gene was confirmed by PCR and Southern blots. Gene expression was verified by RT-PCR and Northern blots. Bioassays were performed on selected bacterial and fungal pathogens of cotton. Fifty-μl of *Xanthomonas campestris* suspension was diluted either 1.5X or 2X with transgenic plant extracts and colony forming units (cfu) arising on LB plates were counted. The number of cfu were reduced to 30-50% of control dilutions with extracts from plants transformed with selectable marker only. Fungal homogenates of *Verticillium dahliae* or *Rhizoctonia solani* were diluted 3X with transgenic plant extracts. These treatments reduced colony number in the range of 16-50% of controls for both fungal species. Future research includes expressing the transpeptide in cotton and evaluating transgenic plants in greenhouse and field.

References

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