INVESTIGATION OF TRANSFERRING THE BAR GENE INTO COTTON VIA THE POLLEN-TUBE PATHWAY

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Abstract

Foreign genes are often inserted into cotton DNA through the use of a "gene gun" which propels the gene construct into the protoplasm. The technique studied here attempts to transfer genes in solution through pollen tubes. Solutions containing the BAR gene for Glufosinate ('Ignite' or 'Liberty') resistance, coupled to an actin promoter, were applied to severed styles of flowers from cotton in the summer of 1995. Harvested seed were planted in the greenhouse and young plants were sprayed with 'Liberty' (Glufosinate) herbicide. Cotton plants were decisively killed by the application of Liberty herbicide, indicating that the gene was not successfully incorporated into the DNA to provide resistance. The idea of making genes physically present within ovules during fertilization is appealing and perhaps more refinement or attempts are needed to obtain success.

Introduction

Traditional plant breeding has utilized cross pollination procedures to develop genetic diversity from which superior genotypes could hopefully be selected. With the advent of new biotechnology tools, genes from one species are now transferred to others in the laboratory. Frequently, foreign genes are inserted into cotton DNA through the use of a "gene gun" which propels gold particles covered with a gene construct into cotton protoplasm. Tissue culture is required to obtain explants from the treated protoplasm. The technique studied here, first learned of by the principle investigator in China, reportedly transfers genes by direct application of solutions containing DNA or gene constructs through pollen tubes. Successful use of this technique has apparent major advantages, including immediate transfer of foreign genes into established varieties, an end product being a whole seed which may easily be propagated, and elimination of costly time and equipment required for gene guns and subsequent tissue culture. Numerous Chinese scientists have apparently reported success in using this technique, based on variability recorded in progeny. Wu and Luo (1989) reported up to 20% efficiency using this method to transfer DNA into rice in a U.S. laboratory.

Materials and Methods

Solutions containing the BAR gene for Glufosinate ('Ignite' or 'Liberty') resistance, coupled to an actin promoter, were applied to severed styles of flowers from several commercial cotton varieties in the summer of 1995. The time required for the pollen tubes to grow from the stigma to the ovary is generally from 12 to 32 hours. The objective was to apply solutions containing the BAR gene behind gametes going down the pollen tubes, but before fertilization. Styles were severed with a scalpel and usually about 2 microliter of solution was applied with a gas chromatograph needle to the severed surface. Seed were harvested at maturity and later planted in the greenhouse. Plants (226) were sprayed with 'Liberty' (Glufosinate) herbicide on 23 January at a targeted rate of 750 g ai per hectare. Solution was broadcast at 15 GPA using 30 PSI with 8004 tips.

Results

Cotton plants were decisively killed by the application of Liberty herbicide, indicating that the gene was not successfully incorporated into the DNA to provide resistance. In a similar study with soybean, no plants showed resistance to Liberty.

Discussion and Conclusions

Numerous Chinese publications have reported apparent success in transferring DNA into cotton, rice, and soybean using pollen-tube pathways. Papers have reported applying DNA solutions to the stigma or injecting DNA solutions directly into the ovary. Wu and Luo (1989) reported success with this technique in rice. At the present, the authors have not been successful in using this technique. The idea of making genes physically present within ovules during fertilization is appealing and perhaps more refinement of the technique or more attempts are needed to obtain success.

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References

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