NOVEL APPROACHES IN COTTON TRANSFORMATION

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Abstract

Genetic engineering has become a more important strategy for introducing desired genes into cotton germplasms. However, such introduced genes must not only be environmentally friendly but also economical. Means to accomplish these goals include the use of tissue-specific and maternally-inherited promoters for gene expression. A system for evaluating fiber-specific gene introduction and incorporation has been suggested utilizing biolistic bombardment of multi-cellular fibers which develop during ovule culture in media without hormones. In addition, chloroplast promoter-driven gene expression is being investigated with the same system. Multicellular fiber also has been used as a model system to study transgene expression of fiber-specific genes within a short period of time.

Introduction

Cotton fiber is unique as it is a unicellular farm product. Commercial cotton is not a tissue or an organ but a mass of specialized epidermal cells. These cells are characterized by two phases of cell wall deposition, both of which are predominately cellulose.

Appropriate transformation strategies should not only be economical to the farmer (high yield and quality, low costs of production) and plant (efficient metabolically) but also environmentally friendly (low chemicals, no spread to wild relatives, no creation of super-resistant pest/pathogen).

Such schemes could utilize tissue-specific (i.e. fiber-specific) or maternally-inherited gene constructs. Testing of these constructs would require the long-term production of mature, boll-bearing plants in order to assay gene expression.

Because limited information is available on fiber-specific promoters, it would be extremely efficient to have an assay system for rapid screening of expression in fiber cells without having to wait until the plant matures. Ovule culture can be used as a transient assay system for screening of promoter efficiency, copy number and level of expression in the transgenic fiber. Fibers can be grown under culture conditions (Beasley & Ting, 1974) where growth and morphogenesis are very similar to fibers grown *in plant* (Meinert & Delmer, 1977).

The discovery of multicellular fibers during cotton ovule culture (Van't Hof & Saha, 1996) suggests a unique means for evaluating gene expression and incorporation in cotton fiber cells. Multicellular fibers are multiple single cells arranged linearly which have developed from a homogeneous mass of unicellular fiber initials and accordingly, transgenic expression can easily be detected through the different cycles of cell division. Normally, an Upland cotton ovule has about 32,000 fiber cells with about 30% of them dividing within the first 72 hours of culture (Van't Hof & Saha, 1996). Therefore, new cells in a multicellular fiber will express the transgene if transformation is successful.

Materials and Methods

Gossypium hirsutum cultivars MD51 and Coker 312 were used as sources of explants or ovules. Callus was initiated from hypocotyls as per Trolinder and Goodin (1987). Ovules were dissected from bolls collected 1-2 days post-anthesis as per Van't Hof and Saha (1996) and placed on 8% water agar plates for biolistics. After bombardment, the ovules were immediately floated on Beasley's Medium (Beasley & Ting, 1974)) containing no hormones and only floating ovules were assayed subsequently for GUS expression (Jefferson, 1987) and multicellular fiber development (Saha and Van't Hof, 1996).

A universal promoter construct (pAMTGUS25) containing the *UidA* gene linked to the *Chlorella* virus adenine methyl transferase gene promoter was kindly provided by Dr. A. Mitra, University of Nebraska, Lincoln, NE. The chloroplast-specific plasmid (pHD203-GUS) is a dual marker construct consisting of a *psbA* promoter linked to a CAT-coding sequence and a *psbA* promoter linked to a GUS-coding sequence (constructed by H.D.).

Results and Discussion

As a preliminary study, the results with the chloroplast construct have not been as definitive and we are working on improving our assay method. In contrast, we found that individual fibers showed strong, transient expression when transformed with pAMTGUS25, indicating the utility of this construct for further experiments. As the cells divide, only those cells which have incorporated the gene should show expression. Varied expression will not only provide information on the efficiency of transformation but also will elucidate the lineage of the cell divisions in the multicelled fibers.

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