A MERISTEM TRANSFORMATION SYSTEM FOR COTTON

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Abstract

Our present method of inserting a new gene (eg. **Bt**) into cotton consists of infecting cotton hypocotyls with modified strains of Agrobacterium containing the gene of interest, selecting transformed callus from these infected hypocotyls, reducing this selected callus mass into single cells or small cell clumps, further screening of these cells for the presence of the new gene through the use of a linked marker gene, and regenerating new plants from those cells that carry the new gene via somatic embryogenesis. This approach has been highly successful, but several inherent problems are associated with the procedure. The **first** is the time required to complete the process. Under the best of conditions, the process requires nine months to recover a plant plus an additional five months to recover seed. The **second** is that the process is unique to "**Coker 312**" or its sister varieties. A few other varieties can be used, but the time is increased three fold or more. This varietal specificity, due to the backcrossing requirement, greatly increases the breeding effort and adds even more time to the process. The **third** limitation is the induction of somaclonal variations in the regenerated plants. Somaclonal variations result when cells are maintained in culture over an extended period of time. Although the process is not completely understood, it seems that as cells grow in culture they mutate (i.e. change their genetic information) or their chromosomes become broken, rearranged, or damaged. When these cells are regenerated into whole plants, these genetic changes are incorporated into the new plants. Since 99.9% of these changes are deleterious to a plants ability to grow and reproduce, many additional breeding problems result.

Our new approach does not include the use of tissue culture (i.e. development of callus or the regeneration of plants from single cells). The approach includes the use of an Agrobacterium system to insert new genes directly into meristematic cells. Agrobacterium infects plant tissues if they are physically or chemically damaged. Through this process of infection, new genes that have been specifically engineered in the bacterium are transferred to cells of the infected plant. Cotton has a very unique germination process in that the terminal meristem in the ungerminated seed is very immature. This can be seen by the extended period of time required for a cotton seedling to initiate its first true leaves (not the cotyledons). The newly germinated seedling develops its terminal growing point after it imbibes water. In the ungerminated seed, the terminal growing point consist of a few cells that will, upon germination, divide and become the terminal meristem. If the immature meristem is conditioned or treated, so that Agrobacterium successfully infects one or more of these cells, then it is possible to insert a new gene during the early stages of germination.

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