LOCALIZATION OF TRANSGENES INSERTED INTO COTTON, *GOSSYPIUM HIRSUTUM* L., VIA *AGROBACTERIUM TUMEFACIENS* TRANSFORMATION

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Abstract

We found genetic linkage of two independent gene insertions with a single marker locus. The bacterial gene [2,4-D monooxygenase (*tfd*A)] was introduced into cotton to provide resistance to 2,4-dichlorophenoxyacetic acid (2,4-D). The gene was inserted by *Agrobacterium tumefaciens* mediated transformation, and multiple cell lines with the gene insertion were produced. Transformed cell lines were verified first to contain the introduced DNA, and transgenic plants were evaluated for expression of resistance to 2,4-D. Transgenic plants that survived the screening were then progeny tested for inheritance and level of expression of the gene insertion. Separate germlines that exhibited monogenic dominance for resistance to 2,4-D were retained, and we selected two for linkage analysis. Multiple marker lines T582 and T586 were crossed with the two 2,4-D resistant lines. T582 includes the recessive marker loci virescent-1, cup leaf, glandless-1, frego bract, and cluster-1; and T586 includes the dominant marker loci Red plant, Okra leaf, Tomentum, Petal spot, Yellow pollen, Yellow petals, Brown lint, Green lint, and Naked seed-1. F_1, F_2, and backcross/testcross progeny were produced and evaluated for segregation of resistance to 2,4-D and the marker loci. Linkage was found between 2,4-D resistance of both transgenic lines and the Naked seed-1 morphological marker. Only two-point linkage tests were possible, so the orientations on the chromosome with respect to the marker could not be determined. Linkage values from the two transformants were consistently different but not statistically significant, but when intercrossed, there was no recombination between the transformants. The data suggest the same or close locations.

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