PLANTLET REGENERATION COUPLED WITH AGROBACTERIUM-MEDIATED TRANSFORMATION.

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Abstract

Recently, efforts by our laboratory have been focusing on improving the market value of the various cottonseed products. To advance this effort, we initially have focused on the development of a rapid and an efficient plantlet regeneration system and, subsequently, the coupling of this system to Agrobacterium-mediated transformation (Figure 1) (GUS Gene Fusion System Kit). This abstract will focus on the characterization of gene transfer (NPT II & β-glucuronidase/GUS) from Agrobacterium to various tissues of cotton.

The co-cultivation of pre-existing meristems *in vitro* with Agrobacterium resulted in the formation of transgenic plants. This procedure has generated, to date, 70 KAN-selected plantlets and 34 mature viable plants within the efficiency range of 2 - 47 percent. These KAN-selected plants represent the first level of selection for transgenic plants (Figure 1). Next, GUS activity was measured flourometrically (Gallagher, S.R.) in leaf homogenates of some but not all KAN-selected plants: several of these plants (3 of 12) showed a significant level of flourometric GUS activity. The histochemical localization of GUS activity (Stomp, A-H) demonstrated that many of these putative transgenic plants (Paymaster HS26T0s, CA-3076T0s & ST7AT0s) generated the distinguishing blue color reaction in their pollen grains, pollen sacs and pollen tubes. The variation observed in positive GUS pollen grains indicates that these plants, in general, possessed a chimeric growth pattern. Pollen grains of CA-3076T01:T1s (progeny of CA-3076T01) also were GUS positive indicating that the β-glucuronidase gene was inherited by the next generation (Table 1). By Southern blot analyses, the GUS gene was shown to be incorporated into the cotton genome of T0 plants (Figure 2) (Paterson, A.H., *etal*.). The T1 & T2 generations have yet to be analyzed. Using a second KAN-selection procedure for the T1 seedlings, transgenic seeds derived from each T0 boll were tentatively identified. Seeds within each boll and from different bolls of the same T0 plant showed different seed germination patterns which is consistent with the concept that these T0 plants are chimeric. Also, the T1 seeds that germinated developed different growth characteristics. Col-lectively, our results demonstrate the introduc-tion and characterization of foreign genes (NPT II & β-glucuronidase) into several cultivars of cotton.

Reference

GUS Gene Fusion System Kit. CLONTECH Laboratories, Inc., Palo Alto, CA

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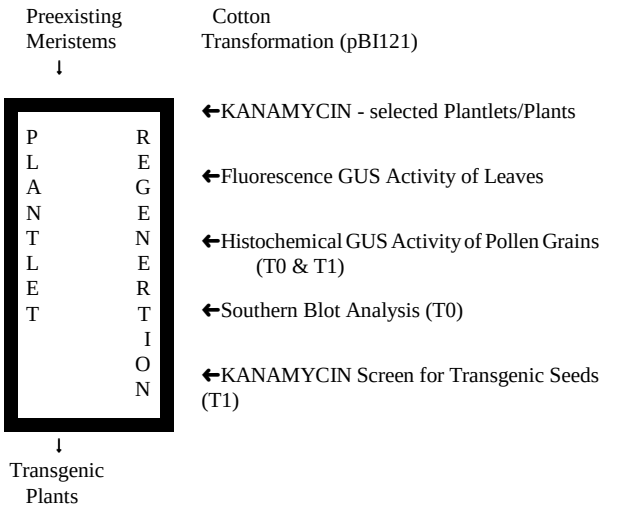

Figure 1. Characterization of GUS trans-formation from Agrobacterium to Cotton Plants.

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Table 1. Histochemical Localization of GUS Activity in Pollen Grains of Putative Transgenic Cotton Plants (CA3076).

Individual Plants	Total # Flowers	Total # Flowers w/+pollen	Pollen in Sac
CA3076T01	9	2	-
CA3076T02	8	2	-
CA3076T03	5	1	2*
CA3076T04	11	2	1
CA3076T11	10	3	-
CA3076T01:T1-00	3	1	-
CA3076T01:T1-01	1	-	-
CA3076T01:T1-02	1	1	-
CA3076T01:T1-03	2	-	-
CA3076T01:T1-04	3	3	-
CA3076T01:T1-05	7	5	-
CA3076T01:T1-06	2	2	-
CA3076T01:T1-07	3	2	-
CA3076T01:T1-08	4	2	-
CA3076T01:T1-09	2	1	-

*Sac stain positive while pollen grains were negative.

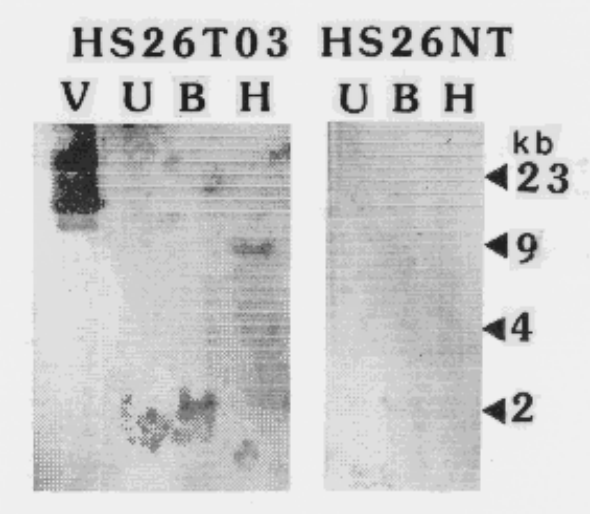

Figure 2. Southern Blot Analyses of HS26T03 and Non-transformed HS26. Cotton genomic DNA was extracted from isolated nuclei from fresh young leaves (Paterson, *etal*.). Purity and yield of DNA preparations were determined spectrophotometrically (Sambrook, J., *etal*.). DNA (15 µg) was digested with 10 units/µl of Bam HI or Hind III and then electrophoresed in 0.8% agarose for 30 minutes at 20 volts and then 2 hours at 60 volts. The genomic DNA was transferred to nylon membrane (Zeta-Probe by BioRad) for hybridization with a random primer-labelled GUS probe (provided by Dr. R. Pirtle's laboratory). The location of the bound GUS probe was identified by an alkaline phosphatase-catalyzed chemi-luminescent reaction (*Gene Images* CDP-*Star* detection module, Amersham LIFE SCIENCE).

When digested with Hind III, cotton genomic DNA hybridized the GUS probe forming a band (9 kb). This band was not evident in the non-transformed genomic DNA. Only one low molecular weight band was seen in each lane containing genomic DNA digested with Bam HI. There were several bands observed in the uncut pBI121 lane because of the different structural states of the vector (supercoiled and relaxed circular forms). Abbrev.: V = vector DNA, 50 ng pBI121; U = undigested genomic DNA; B = genomic DNA digested w/Bam HI; H = genomic DNA digested with Hind III.