Update on Cassava Genetic Transformation at CIAT

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PS7-07

Introduction

In an effort to support breeding of cassava, CIAT generated genetic transformation methodologies via *Agrobacterium tumefaciens* and the particle gun. On this subject attempts have been made to developed varieties with genes for resistance to insects like the cassava hornworm *Erinnyis ello,* and varieties that tolerate herbicides such as Basta®. More recently we have been working on developing varieties with different starch composition. The lines below present the main achievements on the development of tansgenic plants of commercial interest and the optimization of the methodologies to obtain them.

Materials and Methods

Among the plasmid/*Agrobacterium* strain combinations tested there are LBA4404-pGV1040, with the *bar* gene for herbicide tolerance, Agl1-pCAMBIA1305.2, with GBSSI in sense and antisense orientations, and C58C1-pBIGCry, with a *cry*1Ab gene for insect resistance. Gene constructs contain either *npt*II or *hpt* genes for selection with, Kanamycin, Geneticin or Hygromycin, and a *gus* gene for scoring transformation. The use of the *pmi gene* for positive selection with mannose is still under testing.

The starting plant material used in the assays was obtained either from Friable Embryogenic Callus (FEC; Taylor et al 1996) of the varieties 60444 (model variety), Col 2215, CM 3306-4 (Ica Negrita) and the brazilian cultivar Buya Preta 1468. Somatic Embryo Cotyledons (SEC) of variety Per183 (Sarria et al 2000) have also been used in the past for transformation.

The most recent efforts to improve transformation efficiency with FEC, SEC, young leaves and axillary or apical buds, involve vacuum infiltration and cocultivation at lower temperatures. Regeneration of transgenic plants has been usually achieved by embryo germination on solid or liquid medium or by inducing multiple buds (organogenesis).

For insect resistance, bioassays have been run in biosafety greenhouses, where plants have been challenged with the cassava stem borer *Chilomima clarkei* and the hornworm *Erinnyis ello*.

Results and Discussion

Tolerance to the Herbicide BASTA: The first transgenic cassava plants (cultivar Per183) expressing tolerance to BASTA (Sarria et al 2000) don't seem to be protected anymore against herbicide spreads. Evidence from RT-PCR shows that they produce less mRNA compared to transgenic tobacco plants carrying the same gene and transformed at the same time (Figure 1), which suggests that silencing may have occurred since they were produced more than ten years ago. Therefore, the need to produce new transgenic plants, carrying the same gene, to follow closer the expression of this gene in time, led to new transgenic plants of cultivar CM3306-4 (Figure 1 lower panel).

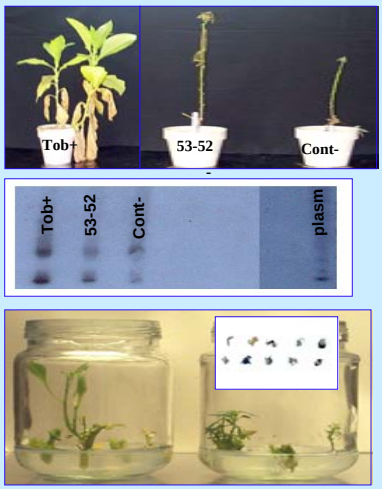

Figure 1 Cassava and tobacco plants after spraying with ppt (200 mg/l for cassava and 1500 mg/l for tobacco). The transgenic cassava plant (53-52), and the non-transgenic control (Cont-), don't show tolerance, which parallels expression of the *bar* gene, in the middle panel, where the probed RT-PCR products detected more mRNA in transgenic tobacco (Tob+). New transgenic plants (lower panel), carrying the *bar* gene, were obtained from gus-expressing embryos (inset) of cultivar CM3306-4, regenerated in 5 mg/l ppt-containing liquid medium.

Figure 2 Plants in the Biosafety greenhouse ready to be transferred to the field. On the right, molecular tests confirm the presence of a *cry*1Ab gene in all transgenic lines.

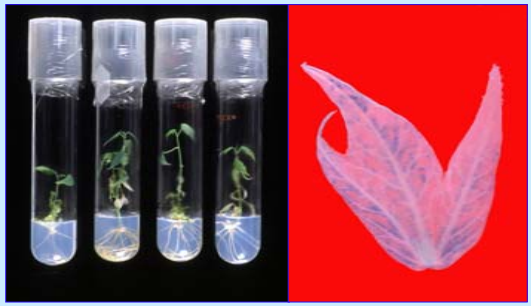

Figure 3. Transgenic plants of cultivar 60444 transformed with a sense copy of cassava's GBSSI gene.

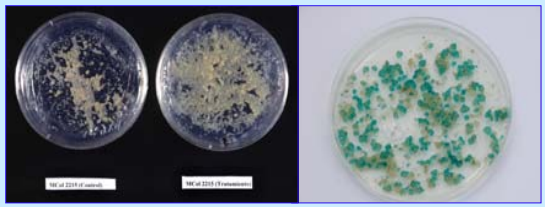

Figure 4. Difference in growth on selective medium (left panel) of FEC lines from cultivar Col2215 after cocultivation with *Agrobacterium* at different temperatures (left plate at 28ºC, right at 21ºC). On the right panel, the intensive gus expression is detected on >50% of the FEC independently transformed lines of cultivar 60444.

Insect Resistance: Transgenic plants of three cultivars, carrying a *cry*1Ab gene, have been produced and are ready to be transferred to the field for increasing planting material and testing (Figure 2). Transgenic lines 55, 80 and 92, from cultivar 60444, were obtained from *Agrobacterium*-mediated transformation of FEC lines. Those from cultivar CM3306-4 and SM1219-9 came from FEC transformed using Bio-ballistics. The level of protection against cassava hornworm (*Erinnyis ello)* is discussed in poster PS4-18.

Starch Modification: Plants from cultivar 60444 were regenerated after being transformed with sense constructs of GBSSI (Figure 3, left panel). They show a patchy pattern of expression of the gus gene (Figure 3, on right). Poster PS4-18 details the achievements on transformation for starch modification.

Improving Transformation Efficiency: Transformation efficiency was recently improved by reducing coculture temperature and by bringing bacteria and FEC in closer contact. The treatment produced roughly 50% transgenic FEC lines with cultivar 60444 (Figure 4). Work is underway to improve the efficiency with more cultivars, including some from Brazil like Buya Preta 1467, which also produces FEC (see Poster PS7-09).

Root-specific Promoters and pmi gene: Recently we started searching for cassava root-specific promoters, in a collaborative work with the University of Freiburg in Germany, to introduce genes that enhance Vitamin-A content in roots. We are also negotiating the Positech system to select transgenic tissues with mannose, for which we have established growth curves on mannose for cultivars 60444 and Col2215.

Field Testing: It will be done for the first time this year. The permit to evaluate transgenics in the field is currently being analyzed by the Colombian Biosafety Technical Committee. An answer is expected in March/2004.

References Taylor et al (1996) Nature Biotechnol 14:726-73; Sarria et al (2000) Plant Cell Rep 19:339-344