DEVELOPMENT OF WAXY STARCH CASSAVA VARIETIES VIA THE ANTI-SENSE MEDIATED SILENCING OF THE GRANULE BOUND STARCH SYNTHASE I (GBSSI)

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INTRODUCTION

Higher incomes from cassava in the developing world where the crop is generally found will require the industrialization of the crop and the development of novel industrial products from cassava. There are several novel products that can be produced from cassava, including modified starches such as 100% amylopectin or 100% amylose starches, from the down regulation of the granule-bound starch synthethase (GBSS) gene or the starch-branching enzyme (SBE) gene. Industrial application of either pure amylopectin or pure amylose starches, or the use of 100% amylopectin in thickeners, pastes and glues, have a market with un limited growth potential. With funds From the Colombian Ministry of Agriculture and Rural Development, a project was been initiated to genetically engineer industrial cassava varieties for the production of waxy starch using an anti-sense and sense construct of the GBSSI gene. Two constructs were made for genetic transformation. They include the full length GBSSI gene in the sense and anti-sense orientation in the binary vector pCAMBIA 1305.2. Friable Embryogenic Callus (FECs) of the cassava genotype TMS 60444, CM 3306-4 and MCol 2215 were transformed via *Agrobacterium tumefaciens* with the two constructs. Results of *GUS* transitory assay revealed a successful incorporation of the gene. One line from transformed FECs of the cassava genotype TMS 60444 were successfully regenerated and four plants transferred to the screen house. Preliminary *gus* assays of leaf revealed successful expression of the construct and a possible endogenous silencing. Transformation events from the other genotypes are being regenerated. Confirmation of the waxy phenotype awaits biochemical tests on roots of the transgenic plants.

MATERIALS AND METHODS

Isolation of a cassava GBSS cDNA clone

More than 87,000 clones of a cassava root and leaf cDNA library cloned in the vector pCMV SPORT (GIBCO BRL, Inc., USA) was gridded onto high-density filters (Mba et al., 2000 unpublished data). The library was screened using a potato GBSS cDNA clones, a gift from Dr. Christine Gerhardt (Max Planck Institute, Cologne, Germany). The potato GBSS gene was labeled with [32P] dATP by random primer labeling and hybridized overnight to the cDNA filters according to standard protocols.

Construction of transformation cassetes

Primers were designer from published sequences of a full-length cassava cDNA of the GBSSI gene (Salehuzzaman et al ., 1993) BamHI and XbaI restriction enzyme recognition sites were incorporated in 5'end or the primers to enable sub-cloning of the cDNA in the sense and antisense orientation into the multiple-cloning (MSC) of the vector pRT101. The primers were used to amplify the cDNA was obtained above, and the PCR product was digested with the appropriate enzyme. A 2.1 Kb BamHI / XbaI fragment was subcloned in the sense and antisense between the 35S promoter and the 35S polyadenylated terminator region of vector pRT101, a gift from Dr. Ryohei Terauchi, Iwate Biotechnology Research Center, Kitakami, Japan. The 35S promoter, GBSSI gene in pRT101 liberated using the restriction enzyme PstI, separated on a agarose gel, eluted and cloned into the PstI site of the binary vector pCAMBIA 1305.2 having the GUSPlus^{R} and HPT reporter genes (Fig.1).

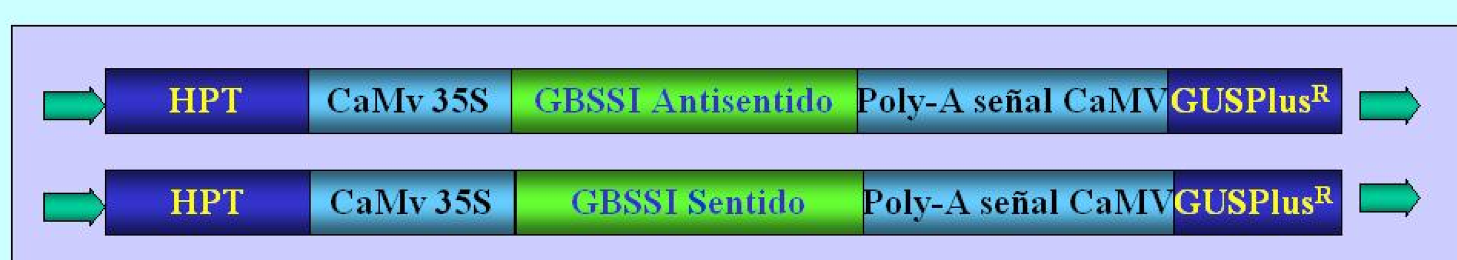

Fig. 1. Constructs in pCAMBIA 1305.2 with the GBSS I gene in anti-sense and sense orientation

Transformation genetic

Friable Embryogenic Callus of the cassava genotype TMS60444, MCol2215 y CM3306-4 was transformed via *Agrobacterium tumefaciens* with the GBSSI gene in antisense- sense orientation (fig. 2).

Fig.2 . Friable Embryogenic Callus of the cassava

RESULTS AND DISCUSSION

Seven GBSSI cDNA clones obtained from screening the cassava library were sequenced, and one was found to be a complete cDNA clone has the ATG start codon 81 bp down stream from the begining of the cDNA sequence and a stop codon about 100 bp from the poly-A tail. PCR amplification with the designed primers yielded a fragment about 2.1 Kb in size that corresponds to the full-length GBSSI cDNA clone (Fig. 3).

The resulting PCR fragment, digested with BamHI and XbaI restriction enzyme, was cloned into the MCS of pRT101. Next, the GBSSI gene, promoter and terminator sequences, excised with PstI and the resulting fragments separated from the vector fragment (sizes 2.8 and 2.7 Kb) by electrophoresis was cloned into the PstI site of pCAMBIA. These are the constructs that were used in the *Agrobacterium*- mediated transformation.

The constructs were transformed into Friable Embryogenic Callus(FEC) of the model transformation genotype TMS 60444 via *Agrobacterium tumefaciens.* Results of *gus* estable assay in callus and leaves revealed a successful incorporation of the gene (Fig.4 and 5).

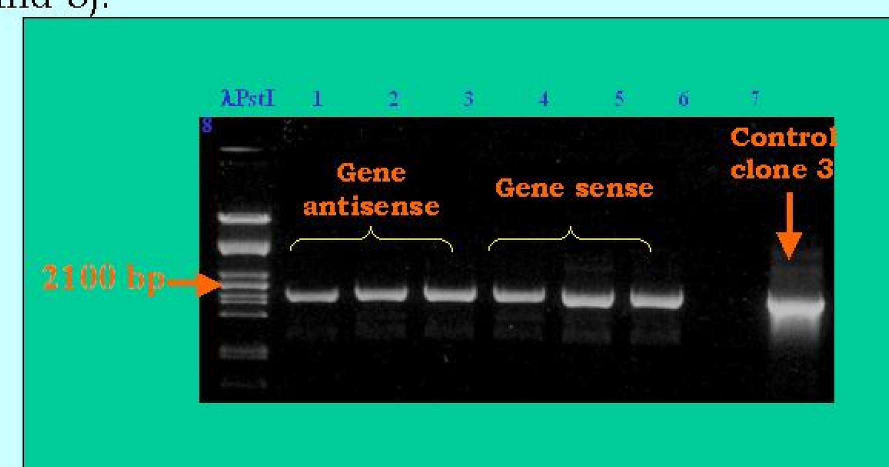

Figure 3. PCR amplification of GBSSI gene

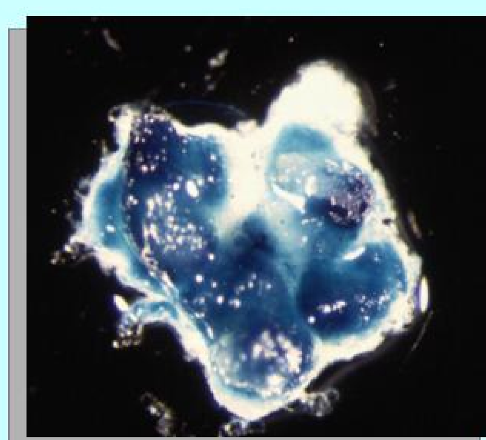

Figure 4. Positive test of *gus* of FEC

Figure 5. Positive test of *gus* of leaf

CONCLUSIONS AND ONGONING WORK

We have successfully transformed full-length sense and anti-sense constructs of the GBSSI gene into the model cassava transformation variety TMS 60444 (Fig. 6).

Transformed calli have been regenerated following which molecular and biochemical tests will be conducted to test stable expression of the gene and the eventually the waxy phenotype. The transgenic plants have been grown in a bio-safety greenhouse at CIAT awaiting molecular and biochemistry tests to determine amylopectin content of the roots.

Fig. 6. Transformed plants of the variety TMS 60444 with GBSS I gene for the production of 100% amylopectin starch

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