

Remy S., Coemans B., **Santos E.**, Buysse S., Möller-Nielsen N., Cannaerts B., Thiry E., Swennen R. and Sági L., 2003. Promoter tagging in banana. 7th International Congress of Plant Molecular Biology. Barcelona, Spain, 23-28 June 2003. Poster abstract.

ABSTRACT

For the identification and isolation of promoters and genes with specific expression patterns a T-DNA trapping technology has been assembled for banana, which allows in planta characterization of candidate promoters without a priori isolation of the corresponding genes. A promoterless luciferase reporter gene linked to the left T-DNA border has been introduced to embryogenic cell suspensions of several banana cultivars. Screening for luciferase activation has been performed without induction as well as via various induction treatments during early (two months) and later (four months) steps of *in vitro* regeneration. Early screening of approximately 35,000 transgenic colonies revealed 26 candidates (0.07%) with constitutive expression. In contrast, late screening of 2,788 proliferating cultures resulted in 12 cultures (0.43%) with constitutive expression comparable to the CaMV35S promoter. Further screening of transgenic colonies, proliferating cultures and *in vitro* plants has been performed under osmotic and aluminum stress conditions as well as after treatment with paraquat. Several positive lines have been regenerated into plants and high expression levels were confirmed in leaves and/or meristems. All candidates have been further propagated for plant regeneration and molecular analysis. Southern hybridization and sequence analysis demonstrated a range of 2-5 insertion sites in the genome with a frequent integration of the vector backbone or complex rearrangements. Therefore, RACE was used to specifically amplify the transcribed copies only and the analysis of 7 flanking regions isolated by TAIL-PCR will be presented. Improved tagging constructs have also been prepared which contain a codon-optimised luciferase gene with or without an intron fused next to the right T-DNA border.