biosafety SOUTH AFRICA

# OUTCOME SUMMARY OF PROJECT BSA 09-010

**BSA 09-010: Development of technology to enable cis-genesis in sugarcane.**

**Project Leader:** Prof Jens Kossmann

**Research Institution:** Institute of Plant Biotechnology, Stellenbosch University

**Duration:** 2 years

## EXECUTIVE SUMMARY

Crop genetic engineering relies on the introduction of foreign DNA into plant genomes. Recently strategies have been developed to genetically engineer plants by transforming them only with their own DNA (cisgenic plants). The aim of this study was to produce cisgenic sugarcane with down-regulated UMP synthase in combination with exclusively sugarcane sequences for promoters, coding sequences, terminators and selection genes.

A SH2 sugarcane promoter sequence from the ADP-glucose pyrophoshorylase subunit SH2 transcriptional regulator was isolated and fused to a GFP reporter gene. Sugarcane callus was bombarded with this construct and transient GFP expression was observed. However, no GFP expression was detected in the mature transgenic sugarcane plants. Numerous other attempts to isolate potential functional sugarcane promoters, based on available sequence data from other plant species and screening of sugarcane BAC libraries, were unsuccessful.

For the cisgenic selection regimes, two plant genes were targeted. The first target gene was acetolactate synthase (ALS). For this, the sensitivity of sugarcane callus towards herbicides from the sulfonylurea and imidazolinone classes was tested. Callus growth was most affected by sulfonylurea herbicides, particularly chlorsulfuron. Herbicide-resistant transgenic sugarcane plants containing mutant forms of a tobacco *als* gene were obtained following biolistic transformation. Post-bombardment, putative transgenic callus was selectively proliferated on nutrient medium and 3.6 µg/l chlorsulfuron. Thirty vigorously growing putative transgenic plants were successfully *ex vitro* acclimatized. Glasshouse spraying of putative transgenic plants with 100 mg/l chlorsulfuron dramatically decreased the amount of non-transgenic plants that had escaped the *in vitro* selection regime. PCR analysis showed that six surviving plants were *als*-positive and that five of these expressed the mutant *als* gene. This research work is the first to describe a selection system for sugarcane transformation that uses a selectable marker gene of plant origin targeted by a sulfonylurea herbicide.

The second plant target gene was protoporphyrinogen IX oxidase (PPO). For this, the sensitivity of sugarcane callus to diphenylether herbicides was tested. Callus growth was mostly affected by fomesafen

and lactofen at 10 mg/l and cell death was also seen in exposed calli. To date a tobacco chloroplastic *ppo* gene was isolated. Two mutations known to induce herbicide resistance in plant cells (Li *et al*. 2003) were introduced at amino acids Y426M and S305L. The mutated *ppo* gene is currently introduced into sugarcane. Attempts are also underway to isolate a native sugarcane PPO gene.

**PROJECT OUTCOMES**

- Isolated and cloned the promoter region of the ADP-glucose pyrophoshorylase subunit SH2 transcriptional regulator from sugarcane. (2011)
- Produced SH2-pGFP containing sugarcane callus and plants. Observed transient reporter gene expression after bombardment but no stable GFP expression was observed. (2011)
- Isolate and cloned a mutated *als* gene from tobacco. (2011)
- This report is the first to describe a selection system for sugarcane transformation that uses a selectable marker gene of plant origin targeted by a sulfonylurea herbicide. The *als* gene was successfully used as selection gene in combination with the herbicide, chlorsulfuron as selection agent in the production of transgenic sugarcane. (2012)
- Isolate and cloned a chloroplastic *ppo* gene from tobacco. (2012)
- Isolate and cloned a partial putative *ppo* sugarcane gene. (2012)
- Mutated a tobacco chloroplastic *ppo* gene at base pair sites known to induce herbicide resistance. (2013)
- In future, the establish ALS selection system can be used in the production of transgenic sugarcane plants eliminating antibiotic genes used in the in vitro selection process. Even though some of these antibiotic selectable markers have been declared safe to use in transgenic crops by the US Environmental Protection Agency (1994), they are still not acceptable to a large portion of consumers. In the long term our project aims to establish technology to genetically transform sugarcane using sugarcane genes only. The first step towards manufacturing cisgenic sugarcane has been taken, as it seems possible to select transformed plants using herbicides targeted to endogenous sugarcane gene products utilizing only point mutations in the corresponding target gene. It seems possible that a cisgenic approach is regarded as self-cloning and falls outside any biosafety regulations.

**PRESENTATIONS**

Van der Vyver C, Stander C, Groenewald H and Kossmann J. Establishment of alternative selection system for transgenic sugarcane callus. *II Genetically Modified Organism in Horticulture Symposium, South Africa,* October 2011.

Van der Vyver C, Stander C, Groenewald H and Kossmann J. Establishment of alternative selection system for transgenic sugarcane callus. *Agriculture: Africa's engine of growth, Harpenden, UK* October 2010.

## PUBLICATIONS

Van der Vyver C., Conradie T., Kossmann J. and Lloyd J. 2013. In vitro selection of transgenic sugarcane callus utilizing a plant gene encoding a mutant form of acetolactate synthase. In Vitro Cellular and Developmental Biology – Plant 49 (2): 198-206. DOI 10.1007/s11627-013-9493-0

Van der Vyver C., Stander C., Kossmann J. and Groenewald H. 2010. Establishment of alternative selection systems for transgenic sugarcane callus. In: Aspects of Applied Biology 96, Agriculture: Africa's "Engine for growth" – Plant science & biotechnology hold the key, pp. 291-295.

## CAPACITY BUILDING

Conradie T.T. 2011. Genetic engineering of sugarcane for increased sucrose and consumer acceptance. MSc thesis, University of Stellenbosch.