SHORT, NON-REFEREED PAPER

IMPROVEMENT OF SUGARCANE IN SOUTH AFRICA USING GENETIC ENGINEERING: REQUIREMENTS FOR POTENTIAL COMMERCIALISATION

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Abstract

Globally, the benefits of growing genetically modified (GM) crops have been widely realised. Several sugarcane industries have declared intent to release GM cane within the next few years, but to date no commercial plantings exist anywhere in the world. For the South African sugar industry to commercialise a GM sugarcane cultivar, participation by all industry sectors is critical. The South African Sugarcane Research Institute's (SASRI) brief in this respect has been to conduct research on improvement of sugarcane by genetic engineering. The purpose of this research has been two-fold, *viz.* to establish proof-of-principle using novel traits and to investigate the genetic basis for sucrose accumulation. To this end, novel traits such as herbicide tolerance, resistance to *Sugarcane Mosaic Virus* and *Eldana saccharina*, as well as metabolic perturbations to increase sucrose content have been introduced to sugarcane. SASRI will continue within its mandate to provide technical advice to the industry on responsible agricultural and environmental stewardship of GM sugarcane, specific legislative requirements for commercial cultivation and potential avenues to overcome intellectual property (IP) ownership barriers that frequently confound commercialisation. Broader issues that require resolution at an industry level include purchase of required IP, developing mechanisms for payment of royalties to IP owners, facilitating positive public and market perceptions, and establishing industry infrastructure and logistics to support processing and marketing of sugar derived from GM cane. While many hurdles remain, the significant progress in GM research that SASRI has made indicates that this technology has potential to significantly increase international competiveness of the South African sugar industry.

Keywords: sugarcane, genetically modified, intellectual property, GM commercialisation, industry approach

Introduction

Biotechnological advances facilitate new avenues to improve crops by introducing exotic genetic material and modifying endogenous gene expression. The first commercial planting worldwide of genetically modified (GM) crops (maize) was in 1996 and it is noteworthy that in 2011 the Republic of South Africa (RSA) grew 2.3 million ha of GM maize, cotton and soybean, making it one of the top 10 countries growing GM crops worldwide (James, 2011).

Although several improvements have been made to commercial sugarcane hybrids through conventional breeding, the opportunity exists with GM technology to modify particular traits, e.g. herbicide tolerance, insect resistance, production of specific alternative products (reviewed by Brumbley *et al*., 2008) and elucidate the effects of up- or down-regulating enzymes involved in sucrose metabolism (Lakshmanan *et al*., 2005). The South African Sugarcane Research Institute (SASRI) has undertaken research on improvement of sugarcane by genetic engineering over the last 18 years (progress reviewed by Watt *et al.*, 2010; Snyman and Meyer, 2011). The purpose of such research has been two-fold, *viz*. to establish proof-of-principle using novel traits and to improve sucrose accumulation.

Proving the concept: Introduction of novel genes to sugarcane

Research and development (R&D) at SASRI began with input traits such as herbicide tolerance (glyphosate and glufosinate ammonium) and assessing resulting plants by herbicide application (Snyman *et al*., 1998; Snyman *et al*., 2001; Leibbrandt and Snyman, 2003). Similarly, insertion of the *cry1A(c)* insecticidal gene conferred improved resistance to *Eldana saccharina* as evidenced by pot trial bioassays (Keeping, 2006; Snyman and Meyer, 2011). Virus resistance was addressed by inserting the coat protein gene from a Midlands-derived *Sugarcane Mosaic Virus* (SCMV), strain D (Sooknandan *et al*., 2003; Snyman and Meyer, 2011). Although field trials demonstrated improved resistance in the transgenic lines compared with wild types, complete SCMV resistance was not observed. In collaboration with the Institute of Plant Biotechnology (IPB, University of Stellenbosch), sucrose metabolism perturbations were performed (Groenewald and Botha, 2007; Roussow *et al.*, 2007; Watt *et al*., 2010). While this work is still underway to confirm increased sucrose content, it has resulted in significant advances in knowledge of sucrose metabolism. SASRI is addressing drought tolerance by inserting genes for dehydration responsive binding elements (DREBs) with a stress inducible promoter. These transcription factors stimulate protective pathways, such as the abscisic acid pathway, when a plant encounters abiotic stress conditions (Golldack *et al*., 2011). To improve nitrogen use efficiency (NUE), the approach demonstrated in rice (Shrawat *et al*., 2008) is being followed in sugarcane with insertion of an alanine aminotransferase gene driven by a root preferential promoter. A GM cultivar containing this gene would require less nitrogen application in the field but still produce expected yields. A summary of characteristics introduced to sugarcane in RSA and the extent of their evaluation is given in Table 1.

Table 1. Summary of genetic modifications carried out on sugarcane at SASRI and IPB (modified from Watt *et al*., 2010).

Characteristic	Gene of interest	Source of gene	Cultivar	Stage of assessment	Freedom to operate status	Reference
Completed projects						
Herbicide resistance: glufosinate ammonium (Basta)	*pat*	*Streptomyces viridochromogenes* (Bayer)	NCo310	Field trials (multiple ratoons)	Research and Development (R&D) only. Proof-of-concept was established in the field. The patent on the gene has recently expired. However, because of stewardship issues, SASRI will have to obtain a licence to use the gene for commercial release.	Snyman *et al*.,1998; Leibbrandt and Snyman, 2003
Herbicide resistance: glyphosate (Roundup)	*cp4 epsps*	*Agrobacterium tumefaciens* (Monsanto)	N12, N19	Field trials (plant cane and first ratoon)	R&D only. Proof-of-concept was established in the field. Monsanto may be willing to issue 3rd party licence to use the gene for commercial release.	Snyman *et al*., 2001
Insect resistance: eldana	*cry1A(c)*	*Bacillus thuringiensis* (Monsanto)	88H0019, N27, 93F0234	Pot trial bioassay	R&D only. Proof-of-concept was established in pot trials. Bayer/Monsanto may be willing to negotiate with SASRI re: access to gene construct and subsequent commercialisation.	Snyman and Meyer, 2011
Virus resistance: mosaic	SCMV strain D coat protein	SCMV coat protein (SASRI)	NCo310, 75E0247	Field trial (plant cane and first ratoon)	Construct was developed at SASRI. No proof-of-concept was established despite conducting field trials. IP restrictions exist for use of viral coat protein technology.	Sooknandan *et al*., 2003; Snyman and Meyer, 2011
Current projects						
Sucrose metabolism perturbations	Several endogenous sugarcane genes	Sugarcane: up or down-regulation of endogenous genes (IPB)	NCo310	Evaluation in glasshouse at IPB, then some lines progressed to field trials at SASRI	Field trials were conducted with PFP, UDP-GH Patents issued to SASRI/University of Stellenbosch for UDP-glucose dehydrogenase (Patent no. 2006/07743) and H+-translocating vacuolar pyrophosphatase (Patent no. 2007/02680).	Groenewald and Botha, 2007; Roussow *et al*., 2007
Drought tolerance	OsDREB1A, DREB 2ACA transcription factors	Rice and *Arabidopsis*, respectively (JIRCAS)	N12, NCo376	Laboratory: molecular analysis	Currently generating transgenic plants for proof-of-concept. R&D agreement signed with JIRCAS.	–
Nitrogen use efficiency	Alanine aminotrans-ferase	Barley (Arcadia Biosciences, USA)	NCo376, N41	Laboratory: molecular analysis	Currently SASRI are generating transgenic plants for proof-of-concept with NCo376. SASRI has signed a commercial licence agreement with Arcadia Biosciences. Once POC has been demonstrated, commercial transformations using another cultivar e.g. N41, will begin.	–

List of abbreviations used in Table 1
IP: intellectual property; IPB: Institute of Plant Biotechnology, Stellenbosch University; JIRCAS: Japan International Research Centre for Agricultural Sciences; PFP: pyrophosphatase:fructose 6-phosphate 1-phosphotransferase; POC: proof-of-concept; R&D: Research and development; SCMV: *Sugarcane Mosaic Virus*; UDP-GD: UDP-glucose dehydrogenase.

Considering the path to potential commercialisation

As a provider of expert agro-technical services to the industry, SASRI's focus on GM sugarcane production has included technology verification, developing an approach to operate within a restrictive intellectual property (IP) environment and compliance with GM legislation.

Overcoming technical constraints
Constraints to commercialisation of GM sugarcane include research limitations such as variations in the response of elite cultivars to the transformation process and sub-optimal gene expression caused by a number of internal cellular processes [e.g. post-translational gene silencing linked to promoter methylation (Brumbley *et al*., 2008)]. In addition, tissue specific expression of the gene of interest may be required and therefore a suitable promoter will have to be identified. Presently there is not a wide range of promoter elements for use in sugarcane (Birch *et al*., 2010; Watt *et al*., 2010).

Addressing intellectual property restrictions
In initial investigations, SASRI produced and tested GM plants under R&D agreements with several multinational agrochemical companies who hold patents for genes conferring novel traits. This arrangement enabled SASRI to (a) develop GM technologies, resident expertise and infrastructure such as the containment glasshouse for testing input traits (e.g. herbicide tolerance and insect resistance); (b) demonstrate that the technology worked in sugarcane under local growing conditions (i.e. proof-of-concept); (c) undertake in-house gene discovery, which in turn could facilitate development of IP. To have freedom-to-operate (i.e. commercialise a GM cultivar) using any IP-protected technology, the sugar industry would have to enter into a licence agreement with the company concerned, coupled with sizeable monetary payments. Should SASRI enter into such negotiations, in-house IP collateral could be used to leverage a more favourable transaction.

Of the two research projects currently underway that have the potential for commercialisation, the NUE project is operating under license from Arcadia Biosciences, USA. The other project is the SASRI-IPB collaboration which has resulted in two patents. A major agrochemical company is interested in licensing this technology from SASRI-IPB and under the tri-partite agreement, SASRI retains the right to use the technology and receives royalty payments. This is a good example of an instance where owning IP enables beneficial engagement with a multinational company.

Fulfilling legislative requirements
Any GM activities in RSA are regulated by the GMO Act (Act 15 of 1997, amended in 2006 to incorporate elements of the Cartegena Protocol on Biosafety). SASRI laboratories and containment glasshouses where GM research is conducted require facility registration with Department of Agriculture, Forestry and Fisheries (DAFF). Permits for field trial evaluation

are compulsory and DAFF representatives regularly inspect these sites. In the event of commercialisation, a General Release Permit (www.daff.gov.za) is mandatory. This comprehensive regulatory dossier encompasses aspects such as food and feed biosafety, environmental risk assessments, molecular and agronomic characterisation and nutritional information. In addition, SASRI scientists have participated in compiling international documents which serve as a baseline for establishing substantive equivalence for all commercial GM sugarcane *viz*. demonstrating that the GM line is comparable to the non-GM wild-type except for the transgene [Organisation for Economic Co-operation and Development (OECD, 2011) www.oecd.org/biotrack].

The RSA sugar industry's role in progressing GM sugarcane

To commercialise GM sugarcane, broader issues that will require consideration and action at an industry-level include:

- managing negative perception of GM-technology amongst sectors of the public and industry customers;
- implementing an industry-wide consultation and decision-making process regarding the GM trait that the industry wishes to commercialise;
- entering into a commercial licencing agreement with the owner of the IP associated with the desired trait;
- allocating significant financial resources associated with payment of royalties for IP-protected technology and the development of a regulatory biosafety dossier to comply with legislation;
- establishing agricultural and environmental stewardship programmes, particularly those associated with traits for insect resistance and herbicide tolerance, should these be the desired traits;
- overcoming logistic and infrastructural challenges for production of sugar and/or alternative products, separation of sugar derived from GM and non-GM sugarcane and mechanism of royalty payment given that sugarcane is a vegetatively propagated crop.

Concluding remarks

The RSA sugar industry has actively supported GM research at SASRI and a progressive approach has been demonstrated by stable gene expression over several ratoons in the field with herbicide-resistant sugarcane (Leibbrandt and Snyman, 2003). The development of related resources e.g. promoter studies, endogenous gene characterisation and sucrose metabolic investigations has contributed to SASRI's progress in the GM arena (Watt *et al*., 2010). In addition, RSA has a robust GM legislative environment that enabled proof-of-concept field trials for over a decade. While continuing with GM research, SASRI has entered into an agreement with a multinational company to progress GM sugarcane with improved nitrogen use efficiency for commercial release. To this end, the sugar industry needs to address issues such as public perceptions, market forces and development of industry infrastructure and logistics to support processing and marketing of sugar derived from GM cane. Despite these constraints, the release of commercial GM sugarcane has potential to benefit all industry stakeholders.

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