

# PROGRAM 1

## Enhanced Sugarcane Farming Systems



This program aims to maximise sugar crop performance and minimise its environmental footprint

## OVERVIEW

The program's research focuses on three important target areas in sugarcane biotechnology. The first is using biotechnology to minimise the industry's environmental footprint. The second is developing new breeding technologies to accelerate delivery of new sugarcane varieties and improve their overall performance. The third is research to pave the way for adopting new GM varieties and integrating GM traits into breeding programs. This includes providing scientifically based assessments of the safety of proposed GM sugarcane releases.

Several projects in this program are coming to fruition in the CRC's sixth year. Highlights this year include identification of novel nitrate-absorbing traits in wild germplasm and completion of the most extensive sugarcane genome map world-wide. The map's application in comparative genomics with other grasses like sorghum is paving the way for sugarcane genome sequencing. Researchers have identified robust markers for key sugar yield traits after a ground-breaking analysis of large sugarcane populations and have developed a new cost-effective strategy for using markers in sugarcane breeding. Scientists in this program continue to publish their outputs in leading sugarcane research journals, maintaining Australia's

reputation for punching well above its weight in sugarcane science and technology development.

### NITRATE RELIEF FOR SUGARCANE

In most sugarcane production soil systems, applied urea fertilisers break down to ammonia which in turn undergoes biological nitrification to eventually form nitrate nitrogen (N). An additional source of nitrogen comes from amino acids released from soil organic matter, especially from the green trash blankets of previously harvested crops. Earlier research in this program has shown that sugarcane varieties strongly prefer amino acids and ammonia over nitrate. This is unfortunate because nitrate leaching into waterways and conversion of nitrate to the potent greenhouse gas nitrous oxide are two of the most potentially undesirable environmental consequences of sugarcane farming. These undesirable effects could be more easily reduced if plants that use nitrate more efficiently were readily available. Encouraging results have been obtained with a pot trial of different varieties, ancestral *Saccharum* species and wild relatives, studying their preference and assimilation rates of various N sources. Indications are that members of the genus *Erianthus* are able to assimilate nitrate

in the presence of competing N sources such as ammonia much more efficiently than other species. Other work with Chinese collaborators has shown that the wild grass *Erianthus rockii* can be crossed with sugarcane. This opens the way to introduce these genes into commercial breeding germplasm. The process may take several years but these preliminary discoveries indicate that development of sugarcane varieties that can reduce damaging nitrate pools in the soil and increase yield returns from costly fertiliser inputs are a real possibility.

### DRILLING DOWN INTO THE GENOME

The program's genome mapping project was completed this year. This has meant that the CRC has now produced the most densely covered map of the sugarcane genome in the world. In practice, this map is important because it also can be matched with the positions of many gene regions, termed quantitative trait loci (QTL), each of which contributes a part of sugar yield traits like content of commercial cane sugar (CCS) and cane yield. Perhaps the most scientifically exciting part of the sugarcane genome mapping has been the ability to start matching the sugarcane map with the known genome sequence of sorghum.

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This is made possible by matching mapped sequenced sugarcane DNA markers to their homologous positions on the sorghum genome sequence. This has revealed that some sugarcane homology groups and sorghum chromosomes have remained relatively intact while others are mosaics with sugarcane chromosomal sequences represented on several sorghum chromosomes. This comparative genomics will greatly help the sequencing of the sugarcane genome and identify candidate genes that can be tested for applications as perfect markers for important sugar yield QTL or as transgenes for effects on sugar yield.

### MOLECULAR SUGARCANE BREEDING PLATFORMS

Molecular breeding in crops is possible either through applying molecular markers in conventional breeding programs or by introducing GM traits. This program has heavily invested in discovering DNA markers to use as surrogates for selecting genotypes that carry important traits. The major strategy for marker discovery has been to use association genetics. This is where specific markers can be identified from large populations that are preferentially associated with genotypes carrying a particular trait. One confounding component of this is that sometimes this association of marker and trait is not derived from genetic linkage, (i.e. that the gene for the trait and the marker exist on

the same chromosome). The association can occur when the trait and marker are derived from recent common parental ancestry in the population and coincidental co-inheritance of traits and markers that has not been broken down by independent chromosomal segregation and frequent recombination events that often occur in multiple generations. This influence of population structure can be analysed and accounted for through careful data analysis; and the CRC's marker team has now identified a suite of markers that account for many important sugar yield traits. The team's modelling of how and when markers could be applied in the breeding process (including cost estimates of marker analysis) has resulted in a strong recommendation that the initial application of markers be made in a rapid parent improvement process. The result will be parents highly enriched in desirable alleles, from which elite progeny can be conventionally selected in stages.

It is also clear that marker systems will be important to rapidly introduce GM traits into multiple lines through a process known as forward GM breeding. In other crops, GM traits are approved for commercial release as events or single insertions of the transgene. Unfortunately at the moment only a



single event is approved and at considerable cost. If this paradigm is maintained for sugarcane, rapid transfer of the GM trait to a wider range of varieties will be required to maximise adoption and maintain the diversity needed for disease protection. The CRC's platform research in marker development and deployment will not only add value to conventional breeding but also to the responsible deployment of GM traits.

## PROGRAM 1 PROJECT SUMMARIES

### MANAGING THE SAFE RELEASE OF GENETICALLY ENHANCED SUGARCANE

The project is greatly progressed and on track to meet its intended outcomes. In the last year, as progress towards understanding if commercial sugarcane can (i) hybridise with other species, (ii) between varieties, and (iii) form viable seed that can establish in the environment, the research team has:

- Confirmed the geographical areas where *S. spontaneum* has synchronous flowering with commercial sugarcane, where potential spontaneous hybridisation may occur, and characterised temporal and regional differences in viable seed production.
- Evaluated the potential of seeds germinating outside cultivation.
- Conducted interspecific crosses to evaluate potential gene flow from the crop to *S. spontaneum* and started evaluating gene flow between commercial canes.
- Modified project plans for 2009>10 following the annual consultative group meeting with regulators.

- Presented results at the 10<sup>th</sup> International Symposium on the Biosafety of Genetically Modified Organisms in New Zealand 2008, and the Tropical Crop Biotechnology Conference in South Africa (2009).

Discussions have continued with regulators, who have sought the CRC's advice on assessing GM field trial applications. Planned discussions with the Gene Technology Group about how best to inform a wider group of end users of the results have taken place ahead of schedule. Project findings and recommendations have also been disseminated to regulators in Malaysia who are implementing a system based on the Australian Office of the Gene Technology Regulator. A research publication schedule has also been drawn up.

IP generated in the project will be published after being collected, collated and analysed. To date, two papers have been accepted for presentation at international conferences. Two journal papers have also been drafted, edited and will be submitted for publication in peer-reviewed journals. A book chapter has been drafted and submitted. Other peer-reviewed publications will follow.

### REDUCING PLANT NITROGEN DEMAND

Acquisition of nitrogen (N) by the plant is a key component of nitrogen use efficiency. Researchers showed that commercial cultivars, Q canes, have a low ability to use nitrate when well supplied with N and when other N forms are present. When screened in controlled glasshouse conditions, *Erianthus spp.* was more able to take up and translocate nitrate than *S. officinarum* and *S. spontaneum*. This is the first step in determining whether nitrate use of commercial varieties could be improved and whether it is therefore a strategy to improve nitrogen use efficiency (NUE). Trials comparing *Erianthus spp.* and commercial cultivars will start in October 2009.

Six genotypes were grown in the field at two N supply rates (0 and 100 kg N/ha urea) to examine biomass production and N uptake over the growth cycle to improve causal understanding of NUE. The final harvest, including excavation of underground stools, finished in October 2008. Excised roots from Q117 sampled 2, 8, 15 and 23 weeks after fertiliser application were incubated with 15N-labelled ammonium, nitrate and glycine to determine N source selectivity in the field.

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Twelve genotypes (*S. spontaneum*, *S. officinarum*, *Erianthus spp.* *Erianthus-Saccharum* hybrids) were screened in controlled glasshouse conditions for their ability to take up, translocate, store and metabolise nitrate. The effect of N form (ammonium nitrate and amino acids) on root growth of commercial cultivar, Q146, has been tested in two experimental systems, sterile plantlets in agar and two-month old plants grown in soil in glasshouse conditions. Plants supplied with organic N produced more root length per gram shoot dry



weight than those supplied with inorganic N. Root morphology analysis is ongoing.

Sugarcane N form preferences have been tested in axenic culture, controlled glasshouse conditions and on excised and intact roots in the field. Further work comparing a broader range of genotypes is planned for October–November 2009. A workshop on fertiliser product developments and sugarcane N source use, involving research staff, extension officers and selected growers is planned for early 2010 in Burdekin or Herbert, Qld. Quantitative data on N uptake over the growth cycle has been collected for six genotypes in the field. Potential exists to evaluate NUE-linked traits and identify useful germplasm from a low N input breeding trial (Mackay 1 rep only) to be harvested in late August.

## MANIPULATION OF SHOOT ARCHITECTURE

This project aims to understand shoot architecture to see if manipulating it can improve yield. The research group seeks to understand the morphological, physiological and molecular relationships governing sugarcane shoot architecture. The project focuses on transgenically modifying genes that control axillary branching, to reduce suckering and lodging and increase crop yield. PhD student Geoff Dunn has shown that modifying the activity of Teosine Branched 1, a

gene controlling tillering in maize, alters branching in sugarcane. Parallel to this, another PhD student has confirmed that expressing a sugarcane homologue of *Arabidopsis thaliana* branching gene More Axillary Growth 4 in rice alters its morphology, including branching. This study signifies the commonality of genetic regulators controlling branching in monocotyledonous and dicotyledonous plants.

## NOVEL APPROACH TO IMPROVE PEST RESISTANCE IN SUGARCANE

This CRC SIIB 1B9 project is a proof-of-concept project to demonstrate a transgenic approach to controlling sugarcane pests. Sugarcane plant lines containing a series of commercial constructs will be selected for bioassay based on the protein expressed from the transgene. Plants showing high transgene expression levels will be screened with appropriate control plants in a bioassay to identify resistant lines to the target pests. This project is expected to identify which transgene or transgene variant in the commercial constructs is most effective in sugarcane for providing resistance to the target pest. This transgenic material will be incorporated into elite sugarcane varieties to enhance existing pest resistance traits.

In 2008–2009, the project's business case was completed. It summarises the project's importance

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to the Australian sugar industry. In the nine months since laboratory work began in the project, researchers have already generated and multiplied transgenic plant lines for five of the 24 constructs, and plant regeneration is under way for the rest. Transgenic plant lines expressing the transgene at high levels are being selected for screening, and two bioassays are already under way with these selected lines for three of the constructs. Identification of the first batch of sugarcane plant lines showing resistance to the target pest is expected in October/November 2009 following harvest of the first bioassay.

Sugarcane transformation experiments have been completed for all 24 constructs (A-X) and plant regeneration is under way. The group has generated and multiplied transgenic plant lines for the first five constructs (A-E). They have assessed for presence of the transgene and are now calculating protein expression levels to select the best sugarcane plant lines for screening. Bioassays will be initiated at three-weekly intervals with selected sugarcane plant lines containing the different constructs.

### DEVELOPMENT OF DArTs

The final sugarcane Diversity Array Technology (DArT) array is complete with 7600 markers. This is now a commercial venture with Australian

germplasm from the Australian Sugarcane Breeding Group being screened across this array. Most of the DArT clones from the array have been sequenced. A paper is being prepared on analysis of these sequences. The sequences will be used to align the genetic maps that contain DArT markers to the sorghum genome. This will allow the targeted mining of the sorghum genome sequence for genes that underlie QTL (quantitative trait loci) identified in sugarcane populations. The final project report has been submitted and accepted.

The CRC has delivered the sugarcane DArT array to the sugarcane industry and it is available for any group to use. The BSES Ltd/CSIRO Australian sugarcane breeding program has already screened several clones from their breeding germplasm across the DArT array.

### COMPLETE GENOME MAP OF SUGARCANE

This project will deliver valuable information on the location of potential genes involved in traits of economic value. The CRC's Q165 genetic map is the most extensive map available of a sugarcane cultivar and will be valuable in the proposed sugarcane genome-sequencing project.

Many project end goals have been successfully completed. The sugarcane genetic map has been greatly improved with increases in marker density.

With DArT and single nucleotide polymorphisms (SNP) markers added, the map has been aligned to the sorghum genome. Researchers now have a greater understanding of the sugarcane genome's structure and how it relates to sorghum. This alignment to the sorghum genome has also identified regions of the sorghum genome that could contain genes underlying agronomically important QTL. Further work is needed to interrogate these regions.

The CRC sugarcane genetic map now has more than 2600 AFLP, DArT, SSR and SNP markers. Using the 919 DArT markers and 128 SNP markers, researchers have started to align the sugarcane genetic map to the sorghum genome. At the macro syntenic level, homology group (HG) 1 and HG 4 align well to a single sorghum chromosome – the other homology groups align to more than one sorghum chromosome. This analysis shows a number of the sorghum chromosomes poorly represented in the Q165 genetic map. In the last part of this project, marker discovery was targeted to these regions using simple sequence repeat (SSR) markers identified in sugarcane EST (expressed sequence tag) sequences.

### HIGH EARLY SUGAR VARIETIES

This project has contributed greatly to understanding the biology of high-early sugar

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(HES) genotypes and has delivered alternatives for early selection of HES clones. Researchers compared natural and chemically induced ripening by exposing a sugarcane variety to a low concentration of the ethylene-forming compound 2-chloroethylphosphonic acid (CEPA) for a lengthy period from shoot emergence. The CEPA-treated plants accumulated significantly higher sucrose in their primary stalks after two and three and a half months post-germination. The team identified three factors that may have contributed to this early higher sucrose accumulation: (i) efficient diversion of photoassimilate to sink tissue (ii) earlier internode formation (iii) faster internode formation. Consequently, more internodes in the treated sugarcane matured earlier and began filling with sucrose sooner. These findings highlight ways in which some sugarcane varieties may naturally accumulate high sucrose early in the season.

## Outcomes:

- Four tools to improve selection of three to four months-old HES clones.
- Detection of highly significant marker-trait associations. However, each marker explained only a small amount of the variation within the trait. These markers can be integrated into a high-throughput marker system when it eventually becomes available to the sugarcane improvement program.



- Results showing that early sucrose accumulation in HES genotypes is under strong genetic control. Application of the Affymetrix genechip platform identified candidate genes that were differentially expressed, highlighting some genes that may be involved in early sucrose accumulation in some varieties.
- Research showing protracted exposure to low ethylene levels from shoot emergence can induce higher sucrose accumulation in sugarcane from an early stage of culm development.
- Knowledge from this project, completed on 30 June 2009, to be captured in several journal publications (one accepted for publication and three in preparation).