

Program Two

**NEW PRODUCT
DEVELOPMENT
FROM
SUGARCANE**



NEW PRODUCT DEVELOPMENT FROM SUGARCANE (BIOPRODUCTS)

Recent research into producing high-value biomaterials from plants has confirmed sugarcane as an excellent plant option. The need to find alternative sources of carbon other than oil to produce bioplastics and biofuels also offers Australia's sugarcane industry new and potentially profitable business opportunities.

These biomaterials can be produced either in sugarcane as alternative products or co-products with sucrose (through R&D from the Biofactory subprogram) or by downstream processing of pre-existing compounds harvested from sugarcane (from the Biorefinery subprogram). To develop these new materials, the industry will rely on this CRC's underlying research knowledge, scientific platforms, enabling technologies and strategic alliances.

Production of High-Value Materials

The aim of the Biofactory subprogram was to establish sugarcane as the preferred host for plant-based production of specific high-value materials and ensure Australia leads the field with associated IP-protected technologies. It brought together different technologies for producing high-value alternative sugars and biopolymers in

sugarcane. Its strategic research helped to inform selection or engineering of high-value sugarcane varieties. High-value varieties potentially produce higher yields or improved compositions of materials that assist downstream biological or chemical conversions into specific value-added products.

Sugarcane's high photosynthetic efficiency and ability to generate high quantities of stored sucrose, cellulosic fibres, lignins and surface waxes make it an ideal vehicle for generating these downstream products. Its capacity to store soluble products and the composition of its lignins and epicuticular waxes also make it an ideal industrial crop for synthesising several types of high-value products. Because it is vegetatively propagated, sugarcane's genetic make-up does not change, making bio-product development extremely reliable. Opportunities also exist for the co-generation of electricity needed to extract and process from bagasse (the fibre or biomass left over after liquid sugar has been extracted from the cane).

There is a growing market for plant-derived food additives and nutraceuticals. Some high-value waxes and flavonoids are already biosynthesised by sugarcane and are likely to be produced by the industry soon. The opportunity exists for the industry to establish as a leader in supplying this growing market if economic co-production levels and separation

processes can be established.

The CRC's approach to this challenge has combined:

- application of gene technologies to understand the biosynthesis of these materials, enhance production levels and shift production to economically preferred forms; and
- development of growing, harvesting and extraction technologies for optimal recovery of these materials, as well as sucrose.

Taking advantage of renewables

While production of high-value materials in plant biofactories is a strategy for the future, production of materials from available renewable resources by chemical and/or biological conversion is well established. Ethanol, organic acids (lactic acid, citric acid), amino acids (lysine, glutamate, phenylalanine), sugar alcohols (e.g. sorbitol) and many fine chemicals (e.g. enzymes, penicillins) are examples of products derived from renewable resources.

The Biorefinery subprogram sought to show the potential of taking greater advantage of sugarcane streams for large-scale production of such materials and identify similar new high-value products for the industry.

Large-scale fermentation facilities are usually sited next to sugarcane mills. This offers

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the advantages of an efficient supply of biomass from the sugar milling stream and electricity co-generated from bagasse, as well as use of dunder - the dregs of cane juice fermentation - to fertilise surrounding cane fields. The chemical and process engineering and molecular biotechnology expertise within this CRC provided substantial scope to generate IP related to increased extraction efficiencies and conversion to high-value downstream products.

In partnership with the Australian sugarcane industry, this CRC helped to develop the base for producing value-added materials from sugarcane-derived feedstocks or harvesting,

milling and refining by-products and wastes. Examples include alcohols, biofuels, fibre products, biopolymers, biosurfactants, industrial enzymes and renewable biomaterials to replace industrial petrochemicals used in plastics manufacture.

PROJECT SUMMARIES

Testing sucrose accumulation

Understanding how sucrose accumulates in sugarcane will underpin future strategies for increasing sucrose or other high-value products in sugarcane. The aim of this research was to

develop improved transformation methods especially for smut-resistant varieties, identify and test key genes in the sucrose accumulation process by manipulation in transgenic sugarcane, test promoters believed to target expression most strongly to the storage parenchyma and complete the PhD training of three PhD students. This project was focused on intellectual property (IP) capture and the provision of a suite of tools to improve the production of transgenic sugarcane plants.

Following are the significant achievements for 2009/10.

1. Further testing a sugarcane promoter in transgenic

2009/10 Highlights

- > The CRC SIIB filled a new patent in its GI Wise™ portfolio. The patent focuses on a glycemic index lowering extract from bagasse. A clinical trial in humans demonstrates that the extract can reduce postprandial glycemia and insulinemia of high GI foods.
- > CRC participants, Metabolix, BSES and The University of Queensland, were successful in an ARC Linkage grant application, which secures a further five years of funding to continue the development of CRC IP on PHA bioplastics production in sugarcane.
- > A new promoter for enriched expression in storage parenchyma was validated.
- > Efficient and sustained down-regulation using of genes involved in sucrose accumulation was demonstrated.
- > Field trials confirmed that three different transgenic technologies can generate commercially useful sugarcane lines.
- > Methods for determining sugar properties were developed and used to produce detailed information on the structure-property relationship of sugars.

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sugarcane which has been demonstrated to drive tissue-enriched transcript expression (exemplified by a reporter gene) in the storage parenchyma of maturing stem in order to enhance its action. One hundred and seventy-three reporter gene-positive plants were confirmed during the year with approximately three hundred more plants to be tested at the very end of the project. This will be followed with the development of assays for reporter gene expression.

2. Sustained down-regulation was demonstrated in transgenic sugarcane of transcripts corresponding to five genes selected for manipulation in transgenic sugarcane to determine their effect on sucrose accumulation.

PHA production in sugarcane

Polyhydroxyalkanoates (PHAs) is a family of natural polymers that can replace plastic produced from non-renewable resources. The commercial partner on this project, Metabolix, is already establishing large-scale microbial production from corn starch. Production of PHAs in sugarcane has the potential to significantly reduce cost of production.

This project focused on determining where and how to best produce PHA in sugarcane.

Steady progress was made in 2009/10 to increase PHB production in sugarcane. Our previous results suggested an opportunity existed to increase PHB production by expressing the plastic in some cellular structures (plastids) in a large area of the leaf (constituting some 50% of the leaf biomass) where expression had previously been absent. Through an understanding of the chemical pathways involved in that area of the leaf, and the controlling influences at each step in the pathway, we have been able to apply treatments which make more of the chemical precursors available in the leaf for PHB production. Applying these treatments to six-month-old glasshouse-grown plants has resulted in the predicted increased levels of PHB, not only in the targeted areas but in other tissues as well.

Additional modifications were made to the lipid synthesis pathway of the plants in an effort to further increase PHB synthesis. As a result PHB has now been expressed in a range of cellular areas not previously used to store the generated PHB.

As a result of our new understanding and experience in the metabolic pathways of sugarcane we have been able to extend a genome scale model of *Arabidopsis thaliana* (AraGEN) to represent C4 plant species by the addition of reactions and transported typically found in C4 plants. This new model

(C4GEN) is now being validated for its ability to synthesize different biomass components and to predict the metabolic pathway activity. C4GEN was also able to predict the classical C4 photosynthesis and its major effect in various plant structures.

Field evaluation of GM sugarcane

This project aims to assess the field performance of GM sugarcane produced by three different transgenic technologies: agrobacterium-mediated transformation, conventional plasmid-based and the new minimal DNA-based biolistic methods.

During 2009/10 accurate yield and CCS data (Stage 3 trial) was obtained for nearly 40 transgenic lines. Analysis of results suggests that growth and cane yield reduction was observed in plants produced by both Agrobacterium-mediated and biolistic transformation methods, relative to wild-type (no transformation or tissue culture) controls. Importantly, growth and yield of 10 to 15 per cent of transgenic lines, depending on the transformation method used, were comparable to wild type suggesting that both techniques could be used to recover agronomically suitable material. Interestingly, a large proportion of the negative impact on growth and cane yield found in transgenic lines was also present in tissue cultured plants.

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Importantly, transgene expression analysis over time and crop class suggests that it remains stable over successive crops and increases with plant age.

Bioactive natural products from sugarcane

This project delivered its objective to identify bioactives from sugarcane waste stream that have commercial potential in terms of pharmaceutical, functional food and nutraceutical applications. During 2009/10, the project focused on refining IP generated by the project and the overall concept was trademarked GIWise™.

To date, four compounds have been identified (two of which are novel) that demonstrate potent inhibitory activity against enzymes involved in the initial digestion of complex carbohydrates. This IP was covered in a provisional patent application and may be of interest to pharmaceutical companies for the treatment of diabetes.

Another IP that was evolved during the year is the development of a process for the large-scale production of an active extract from sugar cane leaf and/or bagasse that shows comparable enzyme inhibitory activity with a commercial drug acarbose. In a clinical trial conducted using a high carbohydrate food i.e. instant mashed potato, it was demonstrated that the extract

is able to reduce glycaemia and insulinemia. Thus, the extract can modify the glycaemic index (GI) of foods, which is important in the management of diabetes. This extract can find applications in the functional food and/or nutraceuticals arena.

Alternative sugars

This project aimed to identify technologies to produce alternative sugars for food applications or as industrial chemicals.

During the past year, researchers cloned and expressed enzymes with the potential to modify sucrose and create new products. The applications of these novel sugars in the food or chemical industries will depend on their physical and sensory properties. Sweeteners ideally need to have similar taste and properties to sucrose but with improved health benefits. CRC researchers have developed laboratory methods to test industry-relevant properties of sugars, including sweetness, crystallisation, digestibility and cariogenicity (ability to form dental caries). The results highlight structure–function relationships among sugars.

Potential IP was identified in two novel sugars which were shown to have equivalent sweetness to glucose. The decision was made not to proceed with protection of this IP because the sweetness was less than that of sucrose and because no economic method of production could be identified.

Other IP delivered is in the form of knowledge of candidate sugars and assay techniques.



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