

SUGAR RESEARCH AND DEVELOPMENT CORPORATION

An Economic Analysis of Investment in Non-Conventional Genetic Modification of Sugarcane Plants for New and Improved Characteristics

Background

SRDC has had a continuing investment for a number of years in genetic modification of the sugarcane plant. Several factors have stimulated a higher level of interest in this area in the past decade including:

- the importance of sugar yield and production cost reductions in a low price environment.
- an increasing likelihood of genetically modified plants being allowed to be produced in Australia.
- the low and fluctuating price for cane having stimulated interest in new uses for the cane plant that may be exploited by some form of genetic manipulation, other than by conventional breeding.
- the sugarcane plant being highly suited to production of industrial products due to its high photosynthetic efficiency and its high capacity for storage of soluble compounds.

The production from sugarcane of precursors to biodegradable plastics was the topic of one of these projects (CRC003). There was a considerable opportunity perceived to produce environmentally friendly natural biopolymers. Currently 350 billion lbs/year of plastics is produced by the global petrochemical industry. According to a new study by BCC Research (2007), this market is growing rapidly. The global market for biodegradable polymers reached 541 million lbs in 2007. This is expected to increase to more than 1.2 billion lbs by 2012. One involved company estimates that bioplastics can capture up to 30% of the total global plastics market. Factors that are growing the market for bioplastics, especially biodegradable plastics, are the environmental impact of petrochemical based plastics, both in production and disposal, the extent of biodegradability of many of the biobased plastics (not all are biodegradable), the rapid increase in the cost of the petroleum feedstock, the instability of the major regions of the world which control the vast majority of the crude oil reserves, global warming and especially the rising demand for plastics worldwide.

One of the current constraints in using polyhydroxyalkanoates (PHAs) to make bioplastics is that they need to be made by bacterial fermentation. This means that they are 3 to 10 times as costly to produce as oil based polymers. The SRDC project CRC003 was designed as a proof of concept to show that a third class of biopolymers with a diverse range of commercially exploitable properties could be produced in the sugarcane plant by genetically engineering the plant using the powerful tools of modern molecular biology.

Another project (CRC004) investigated the potential of genetically engineering sugarcane to produce sorbitol. Sorbitol is an industrially significant compound that can be derived from the same metabolic precursors used to make sucrose. More importantly, sorbitol is one of the twelve building blocks identified by the United States Department of Energy Pacific Northwest National Laboratory and National Renewable Energy Laboratory which can be subsequently converted to a number of high-value bio-based chemicals or materials (Top Value Added Chemicals from Biomass US DOE Report # DOE/GO-102004-1992).

A third project in the cluster (UQ039) isolated promoter gene sequences and tested them to drive the expression of introduced genes specific to the parenchyma cells of mature sugarcane stems. A fourth project addressed the identification of sugarcane proteins involved in gene silencing (ICB007).

A fifth project (BSS237) produced and tested some transgenic lines of sugarcane for their anti-metabolic effect on canegrubs.

The Projects

There were five projects funded in this cluster between June 2002 and June 2008. The project codes, titles, other details and objectives for these projects are shown in Tables 1 and 2.

Table 1: Project Codes, Titles and Other Details

Project Code and Title	Other Details
BSS237: Identification of canegrub-resistant transgenic sugarcane lines for commercial evaluation	Bureau of Sugar Experiment Stations 1999-2003 Peter Allsopp, Jason Geijskes, Kerry Nutt
ICB007: Isolation of sugarcane proteins involved in post – transcriptional gene silencing	Texas A&M University Agricultural Experiment Station 2004-2006 Erik Mirkov
CRC003: Use of sugarcane as a biofactory for production of biopolymers	CRC Sugar Industry Innovation through Biotechnology 2004-2006 Stevens Brumbley, Donald Mackintosh, Michael O’Shea
CRC004: Sucrose derivative production in sugarcane	CRC Sugar Industry Innovation through Biotechnology 2004-2006 Barrie Fong Chong, Stevens Brumbley, Michael O’Shea, Graham Bonnett
UQ039: Gene control sequences for metabolic engineering in sugarcane	University of Queensland 2003-2006 Robert Birch, John Manners, Graham Bonnett, Anne Rae, Rosanne Casu

--	--

Table 2: Project Codes and Objectives

Project Code and Title	Objectives
BSS237: Identification of canegrub-resistant transgenic sugarcane lines for commercial evaluation	<p>The objectives of the project were:</p> <ol style="list-style-type: none"> 1. Negotiate IP and research agreements to allow commercial release of transgenic plants. 2. Determine insecticidal performance of current transgenics expressing snowdrop lectin and protease inhibitors. 3. Test additional products for antimetabolic effects on canegrubs and incorporate active ones into the program. 4. Produce additional transgenic lines using snowdrop lectin and protease inhibitors based on other cultivars.
ICB007: Isolation of sugarcane proteins involved in post – transcriptional gene silencing	<ol style="list-style-type: none"> 1. To isolate host proteins from sugarcane that are involved in post–transcriptional gene silencing.
CRC003: Use of sugarcane as a biofactory for production of biopolymers	<ol style="list-style-type: none"> 1. Investigate the use of the plastid fatty acid biosynthetic pathway for production of PHA copolymers in transgenic sugarcane. 2. Investigate the use of the peroxisomal fatty acid beta-oxidation pathway for production of PHA copolymers in transgenic sugarcane. 3. Investigate the polyketide strategy for the production of polyhydroxyalkanoates (PHAs) in transgenic sugarcane using polyketide synthases (PKS). 4. Protect any intellectual property generated in this area. 5. Appropriately implement the technology to demonstrate commercial use.
CRC004: Sucrose derivative production in sugarcane	<ol style="list-style-type: none"> 1. Demonstrate that sorbitol can be produced in sugarcane in a few simple enzymatic steps and that it can be stored stably in plants. 2. Identify the steps(s) in the conversion process that limit the amount of sorbitol that can be accumulated in sugarcane.
UQ039: Gene control sequences for metabolic engineering in sugarcane	<ol style="list-style-type: none"> 1. Isolate gene control sequences to drive the expression of introduced genes specifically in the sucrose storage tissues of mature sugarcane stems. At the same time the project seeks new insight into unexplained features of gene expression in the polyploid sugarcane genome; with profound implications for all work on promoter isolation, transgene expression and application of molecular markers in sugarcane. <p>Specific objectives were:</p> <ol style="list-style-type: none"> 1. Identify genes expressed preferentially in the sucrose-

	<p>storage parenchyma of mature sugarcane stems, among candidate genes from project UQ22 and the CSIRO EST project.</p> <p>2. For any gene with low copy number and mature-stem parenchyma-specific expression, recover the corresponding upstream genomic sequence from sugarcane and test it for patterns of promoter specificity.</p> <p>3. Isolate a full set of promoter homologues from the polyploid sugarcane genome for one of the mature-stem genes identified above.</p> <p>4. Construct modified versions of the promoter, to test the current hypothetical explanations of mechanisms causing inactivity of most tested promoters in transgenic sugarcane.</p> <p>5. Protect and apply the best mature-stem promoter elements for sugarcane metabolic engineering.</p>
--	---

Project and Cluster Investments

Estimates of SRDC and other funding for the five projects are shown in Tables 3 and 4.

Table 3: SRDC Investment in Cluster for Years ending June (nominal \$)

Year	BSS 237	ICB 007	CRC 003	CRC 004	UQ 039	Total
2000	56,209	0	0	0	0	56,209
2001	67,734	0	0	0	0	67,734
2002	57,767	10,000	0	0	0	67,767
2003	29,662	10,000	0	0	50,000	89,662
2004	0	0	170,000	135,734	120,000	425,734
2005	0	0	184,375	119,783	100,000	404,158
2006	0	0	187,903	126,322	20,000	334,225
Total	211,372	20,000	542,278	381,839	290,000	1,445,489

Source: Project proposals to SRDC

Table 4: Other (non-SRDC) Investment in Cluster for Years Ending June (nominal \$)

Year	BSS 237	CRC 003	CRC 004	UQ 039	Total
2000	118,336	0	0	0	118,336
2001	142,600	0	0	0	142,600
2002	121,617	0	0	0	121,617
2003	62,447	0	0	96,015	158,462
2004	0	38,716	74,957	137,703	251,376
2005	0	41,990	66,148	116,427	224,565
2006	0	42,794	69,760	46,629	159,183
Total	445,000	123,500	210,865	396,774	1,176,139

Source: Project proposal

Table 5 shows the total investment for each year for the cluster.

Table 5: Total Investment for the Cluster for Years Ending June (nominal \$)

Year	SRDC	Other	Total
2000	56,209	118,336	174,545
2001	67,734	142,600	210,334
2002	67,767	121,617	189,384
2003	89,662	158,462	248,124
2004	425,734	251,376	677,110
2005	404,158	224,565	628,723
2006	334,225	159,183	493,408
Total	1,445,489	1,176,140	2,621,629

For the five projects reported above, SRDC has contributed the 55% of resources in nominal terms.

Outputs

A summary of the principal outputs from each of the five projects is reported in Table 6.

Table 6: Summary of Principal Outputs by Project

Project	Principal Outputs
BSS 237	<ol style="list-style-type: none"> 1. The trial design for pot-based screening of transgenic lines has been improved and a method for screening potential insect toxins by microinjection has been developed. Both these improvements can be used in future assays. 2. No transgenic lines developed have been shown to have any significant effect on canegrub weight gain and none can be recommended for commercial release. 3. No new toxins or biocontrol agents were identified. 4. Some lessons were learnt regarding intellectual property negotiations with permission to use some genes only granted towards the end of the project.
ICB 007	<ol style="list-style-type: none"> 1. Identification of sugarcane proteins involved in transcription; included Ring Zinc Finger, LRR Transmembrane Protein Kinase and several unknown proteins. However, none of these proteins activate transcription by themselves nor do they interact non-specifically with a control lamin protein.
CRC 003	<ol style="list-style-type: none"> 1. A number of transgenic lines of sugarcane were produced that both (a) utilised the fatty acid β-oxidation pathway in peroxisomes and b) generated novel fatty acids by introducing genes that tap into fatty acid biosynthesis in the plastids. 2. Detected PHA/PHB copolymer in peroxisomes of transgenic sugarcane plants.

	<p>3. Although the levels of PHA/PHB copolymer are low, they were sufficient to establish proof of concept and for filing of a provisional patent.</p> <p>4. Proof of concept demonstrated for short chain length-medium chain length PHA copolymers in sugarcane plants.</p>
CRC 004	<p>1. A gene for sorbitol synthesis was extracted from apple tissue, cloned into a sugarcane expression vector, the construct authenticated by DNA sequencing and then introduced into sugarcane.</p> <p>2. A large population of transgenic sugarcane plants producing a range of sorbitol levels was obtained. Unexpectedly, it was found that a novel byproduct (a sorbitol-hexose) was also produced as a direct result of accumulating sorbitol.</p> <p>3. It was found that the accumulation of sorbitol led to significant physiological and metabolic changes in the sugarcane plant, with the sorbitol producing lines slightly smaller than non-sorbitol producing lines.</p> <p>4. Some lines accumulated a substantial amount of sorbitol in the leaves. Only low levels of sorbitol were stored in the stems. The levels in the stems would not support a commercially viable operation. However, strategies for increasing sorbitol levels in the stalk are available. Significant new capital investment in sugar mills would be required if sorbitol, or any other value added product is to be produced in sugarcane, including the range of bioplastics or other fine chemicals.</p> <p>5. Market research carried out after the project had commenced, suggested that the market for sorbitol was quite small and mature, that other means of producing sorbitol were competitive, and that producing it in plants was not commercially viable unless there were high concentrations of sorbitol stored in the plant stems. However, this could change if sorbitol becomes one of the 12 feedstock chemicals of the bioeconomy to be used for the production of a wide and diverse range of high value biobased materials and chemicals (Top Value Added Chemicals from Biomass US DOE Report # DOE/GO-102004-1992).</p>
UQ 039	<p>1. Identified promoters (gene control sequences) that drive the expression of introduced genes, particularly in relation to stem tissue specificity.</p> <p>2. Expression was inconsistent and varied between transgenic plants, specifically in sucrose storage tissues in the stems of the sugarcane plant</p> <p>3. Production of insights into constraints to gene expression.</p> <p>4. While some success in promoting gene expression was achieved, most promoters used appeared to have some limitations and inconsistencies. No gene with absolute specificity for expression in the storage parenchyma has yet been detected.</p> <p>5. Exclusion of several possibilities as to why promoters do not work effectively.</p> <p>6. Concluded that gene silencing mechanisms have to be better understood to be able to break through this constraint.</p>

Outcomes

A summary of the principal outcomes from each of the five projects is reported in Table 7.

Table 7: Summary of Principal Outcomes by Project

Project	Principal Outcomes
BSS 237	<ol style="list-style-type: none"> 1. There was no genetic solution identified regarding control of canegrubs in sugar cane from this investment. 2. A continuation of the project with some of the recently acquired genes is occurring in the CRC–SIIB project “Environmentally Sustainable Canegrub Resistance”. 3. The follow on project aimed to select canegrub-resistant transgenic sugarcane lines for field testing and to negotiate freedom to operate for third party IP to allow commercial release of successful transgenic lines. The project included the testing of the insecticidal performance of transgenics produced in BSS237 (expressing snowdrop lectin and protease inhibitors) and searching for additional products with antimetabolic effects on canegrubs for incorporation into the program. Additionally, transgenic lines based on new cultivars were produced. 4. The follow on project in the CRC also tested a different gene (avidin), which was one of the potential genes found in BSS163 (a precursor project to BSS237). 5. However, all transgenic lines produced in the follow on project demonstrated little significant effect on canegrubs.
ICB 007	<ol style="list-style-type: none"> 1. Contributed to an improved understanding of the pathways of post transcriptional gene silencing and plant defense responses. 2. May contribute to the success in expressing trans genes by inhibiting silencing. 3. The work continued in a follow up project to which SRDC did not contribute directly; however, the CRC of which SRDC is a member, did contribute on behalf of the Australian industry.
CRC 003	<ol style="list-style-type: none"> 1. The project has provided proof of concept for production in the sugar cane plant of PHA/PHB copolymers which have a wide range of potential applications in the market place. 2. The research has resulted in a provisional patent. Discussions with Procter and Gamble to continue this line of research are on going. However, in October of 2007 Procter and Gamble Inc. sold PHA IP portfolio to a company called Meredian, a privately held company based in Georgia in the USA. 3. A related CRC project on PHB production on sugarcane, originally funded by an ARC Linkage grant has continued in conjunction with a US company Metabolix as the commercial partner. 3. Metabolix owns over 320 patents on PHAs covering everything from the genes, production in plants and bacteria, extraction, purification and processing into products. It is currently in a commercial venture with

	<p>Archer Daniels Midland to produce 110 million pounds per annum of Nirel (PHA) bioplastics by microbial fermentation.</p> <p>4. Metabolix is working to engineer switchgrass to produce PHAs because they believe this will reduce the overall production costs. The mirror project on sugarcane co-funded by Metabolix in the CRC-SIIB will increase the chances of potential benefits being realised. The US and world market is large for this material (>30 million lbs/year).</p> <p>5. If successful the plants will produce PHAs which can be either used directly for plastic production, can be blended with other products like polylactic acid for different plastics or can be chemically modified for the production of other products.</p>
CRC 004	<p>1. In this project the sugarcane plant's capability to function as a biofactory for the production of alternative sugars was successfully demonstrated. The processes used have provided valuable knowledge on which future initiatives can build to produce other compounds in the sugar cane plant.</p> <p>2. Although concentrations of sorbitol in transgenic plants were not at levels required to be commercially viable, very high levels were achieved in leaves, and strategies were considered to increase both stem and leaf concentrations. One approach was to use tissue specific promoters to increase levels. However, other strategies included sorbitol transporters or engineering enzymes for production of sorbitol in the stem parenchyma vacuoles.</p> <p>3. The CRC concluded that further investment in sorbitol production in plants should not be made due to the market situation and competition from catalytic hydrogenation production technologies. This was largely based on current market conditions and did not consider sorbitol as a major building block chemical of a future biobased economy.</p>
UQ 039	<p>1. Improved understanding of the variable levels of effectiveness of promoters for expression of new genes introduced into sugar cane plants.</p> <p>2. Has emphasised the need for research to overcome the gene silencing constraint in order to obtain reliable expression of introduced genes in desired patterns in sugarcane.</p> <p>3. The details of promoters isolated in this project are maintained in confidence, to retain the opportunity for IP protection while further research is undertaken into the most useful forms of the isolated promoters or other promoter versions from the identified genes, and into approaches to avoid gene silencing. These outcomes need to converge for practical application in sugarcane improvement.</p> <p>4. SRDC has agreed with a proposed mode of commercialisation of the project technology through further development and application in the UQ-CSR SugarBooster program. This follow-on R&D is being supported by UQ, CSR and Australian government agencies including ARC, Ausindustry and SRDC, aimed at enhanced production of both sucrose and high-value sugars in sugarcane.</p> <p>5. For example, the summary of ARC project LP0776937 states: "Sugarcane is one of the world's major crops for food (sugar) and fuel (ethanol, electricity co-generation). It is one of the most appealing target crops for metabolic engineering aimed at</p>

	renewable biomaterials and biofuels. Australia has invested strongly to achieve scientific leadership in gene technologies in our major export crops including sugarcane. Field tests show that development of methods to avoid unstable expression or ‘silencing’ of introduced genes is now a critical requirement for practical application. The current project emerges from industry recognition of the need to understand and avoid transgene silencing. The methods developed using sugarcane are expected to have rapid applicability for wider benefits in agriculture.”
--	---

Benefits

A summary of the principal types of benefits and related costs associated with the outcomes of the projects are shown in Table 8.

Table 8: Principal Prospective Benefits Emerging from the Cluster

Project	Principal Benefits
BSS 237	<ol style="list-style-type: none"> 1. It is possible that there will be some benefits derived in the future in regards to cane grub management for which this project could claim some contribution. 2. Future projects in this area may benefit from the lessons learned regarding intellectual property associated with candidate genes.
ICB 007	<ol style="list-style-type: none"> 1. Gene silencing is a major issue for biotechnology improvements to the sugarcane plant. Potential benefits may be large if and when this constraint can be overcome and this project and its follow on projects could possibly claim some contribution.
CRC 003	<ol style="list-style-type: none"> 1. There is still a number of steps from the proof of concept to the capture of benefits including: <ul style="list-style-type: none"> • Increasing levels of PHA in plant • Extraction of PHA from the harvested plants • Commercialisation of the process • Gaining approval of the Australian gene regulator for field trials and ultimate commercial scale field production 2. Commercial benefits could be very large due to the lowered cost of production of biodegradable plastics.
CRC 004	<ol style="list-style-type: none"> 1. Because this project was terminated by the CRC-SIIB, there will be no foreseeable benefits from this investment at this point in time. However, the investment has built a platform on which future work with different compounds can build. 2. The potential value of the novel sorbitol-hexose compound that was discovered is being assessed by CRC project 2b8. 3. This project has strengthened the awareness of the capability of the sugarcane biofactory in so far as: <ul style="list-style-type: none"> • better understanding the type of chemicals that are suited for plant production • highlighting the importance of product targeting and compartmentalisation in the plant cell

UQ 039	<ol style="list-style-type: none"> 1. Potentially enhanced sucrose concentrations in sugarcane plants 2. Potential conversion of sucrose in the plants to high value added products.
--------	--

Public versus Private Benefits

BSS237: As there were limited benefits derived from this investment, this split is not meaningful. However, if benefits are derived in future, they are likely to be mainly private industry benefits, although grub resistant plants would reduce chemical use and therefore provide some community benefits.

ICB007: Any benefits emerging from this investment would most likely be private and large as silencing of gene introductions to the sugarcane plant is a major barrier to commercial gain at present.

CRC003: Prospective benefits linked to this investment would be a mixture of private and public benefits. Apart from benefits to industry, the lower cost of production of biodegradable plastics would also provide a community benefit (social and environmental) through reducing the cost of disposal and discouragement of use of non-renewable resources with greenhouse and energy implications. An independent life cycle analysis of the Metabolix Mirel was conducted by Dr. Bruce Dale, professor of Chemical Engineering at Michigan State University and will be published later this year. He measured the environmental impact from “cradle to factory gate”. He showed that compared to conventional petrochemical plastics, production of Mirel biobased plastics would require 95% less petroleum and would provide a 200% reduction in greenhouse gases. Mirel has a negative net CO2 footprint.

CRC004: As there were no significant benefits obtained from this investment, either captured or capturable in future, any split may not be meaningful. However, if sorbitol becomes one of the 12 building blocks of future chemical production the above statement may not hold true and sorbitol could become significantly more valuable in a future where the world’s chemicals needs have to be produced from renewable rather than from petroleum based feedstock. Both the US and EU have strategic plans in place to replace their petrochemical industries with biorenewable industries. Also the capacity to investigate other compounds that may be produced by the sugarcane plant has been enhanced.

UQ039: As for ICB007, prospective benefits linked to this investment would most likely be private and large as silencing of gene introductions to the sugarcane plant is a major barrier to commercial gain at present.

Match with National Priorities

The Australian Government’s national and rural R&D priorities are reproduced in Table 9.

Table 9: National and Rural R&D Research Priorities 2007-08

Australian Government	
National Research Priorities	Rural Research Priorities
<ol style="list-style-type: none"> 1. An environmentally sustainable Australia 2. Promoting and maintaining good health 3. Frontier technologies for building and transforming Australian industries 4. Safeguarding Australia 	<ol style="list-style-type: none"> 1. Productivity and adding value 2. Supply chain and markets 3. Natural resource management 4. Climate variability and climate change 5. Biosecurity <p><i>Supporting the priorities:</i></p> <ol style="list-style-type: none"> 1. Innovation skills 2. Technology

Contributions to these priorities for the five projects in the cluster are summarised in Table 10. The assessment of the relative contribution of the cluster to each of the five Rural Research Priorities is:

Rural Research Priority 1 (50%)

Rural Research Priority 2 (50%)

Table 10: Summary of Contribution of Cluster Projects to National and Rural Research Priorities

Project	National Research Priorities	Rural Research Priorities
BSS237	National Research Priorities 1 and 3	Rural Research Priority 1 and Priority Support 1 and 2
ICB007	National Research Priority 3	Rural Research Priority Support 1 and 2
CRC003	National Research Priorities 1 and 3	Rural Research Priority 1 and Priority Support 1 and 2
CRC004	National Research Priority 3	Rural Research Priority Support 1 and 2
UQ039	National Research Priority 3	Rural Research Priority Support 1 and 2

Quantification of Benefits

Introduction

This cluster represents a high risk–high return investment for SRDC. Research in this cluster is highly strategic and long term. To justify the investment, prospective payoffs

need to be very large, unlike the more incremental research also funded by SRDC in the low/medium risk –low/medium return categories.

One feature of this investment cluster is that it is unlikely that one project alone will capture benefits on its own. Hence any quantification of benefits from snapshot investments such as these five projects needs to lean heavily on other projects requiring the use of probabilities and expected values.

Only one of the five projects (CRC003) is seen to have strong prospects for capturing benefits for Australia in the medium term. Two other projects (UQ039 and ICB007) appear to have some prospects in the longer term by building knowledge in the area of understanding gene silencing that may then lead to sugarcane plants more fully expressing new gene introductions or the suppression of undesirable traits.

Assumptions for Estimating Benefits from CRC003

A series of steps is assumed necessary to take the proof of concept demonstrated in CRC003 to the end point of capturing benefits for Australia.

Producing Sufficient Quantities of PHA in the Sugarcane Plant –With and Without CRC003

In an ARC linkage grant between BSES Limited and the UQ Chemical Engineering Department, researchers successfully demonstrated that polyhydroxybutyrate can be produced in sugarcane plants (Petrasovits et al 2007, Purnell et al 2007, Brumbley et al 2002, 2007). At about the same time Dr Brumbley, working with a team at the DuPont Experiment Station, on a project funded by DuPont, successfully produced parahydroxybenzoic acid in sugarcane. These projects resulted in a Patent on plants as Bioreactors (Brumbley, [WO 2004/6657](#)) These two proof of concept projects were the basis of the CRC project on the production of biodegradable plastics in sugarcane.

The CRC research in CRC003 was associated with a specific class of biopolymers polyhydroxyalkanoate/polyhydroxybutyrate (PHA/PHB) copolymers known collectively as Nodax®. This work has resulted in the filing of a provisional patent.

The follow on project being undertaken in the CRC is a continuation of the UQ/BSES Limited ARC Linkage project on production of PHB in sugarcane. Procter and Gamble and Metabolix are competitors in this field and P&G has to date not funded any of the research. They have only contributed background intellectual property including DNA constructs and the time of one of their research scientist Dr Phil Green who helped design the CRC project. However, some of the methods and processes used in CRC003 have been useful in a follow on project. A PhD student project is currently using some of the technologies from CRC003. In this project PHB production is being used to study carbon flow through, and manipulation of that carbon flow, in peroxisomes. It is believed by researchers that the approaches being developed are patentable.

For economic analysis, it is assumed that the quantity of PHA/PHB produced in the transgenic sugarcane plant is 10% of dry matter of which 80% is extractable. Assuming

an average yield of 85 wet tonnes of stalks per ha and 15 wet tonnes of leaves/tops per ha, the total biomass would be about 100 wet tonnes per ha. With a wet to dry matter conversion factor of 30%, this is equivalent to 100 tonnes per ha x 30% (wet to dry) x 80% (extraction efficiency) x 10% content or 2.4 tonnes of PHA per ha of cane harvested.

It is assumed that the ability to achieve this will be attained in mid-2009 at a total cost of \$2 m. It is assumed that the likelihood of achieving this is 75%.

Without the earlier projects (CRC003, ARC Linkage and the DuPont project) it is assumed that the probability of success would have been only 60%. With these three projects it is assumed that the likelihood of achieving success is now 75%. Without CRC003, the likelihood of success is estimated at 70%.

Field Trials and Regulation

To conduct field trials will require the approval of the Office of the Gene Technology Regulator (OGTR). An application is currently being drafted. It is assumed that the likelihood of acquiring this permission is 80%. It is assumed that attaining this permission (submissions etc) will take one year and will be achieved in 2010 at a cost of \$200,000.

Field Trial Success

The maintenance of expression of the PHA in the plants grown in the field is assumed to have a likelihood of 80%. This success is assumed to be achieved in 2012 at a cost of \$300,000.

Economics of Transgenic Sugarcane Production

It is assumed that growing, harvesting and transport costs for cane will be similar to those incurred in cane production and harvesting for sucrose. An additional growing cost will be a royalty paid by cane growers of 50 cents per kg of PHA for the intellectual property owned by Metabolix. The ex-facility price of PHA is assumed to be \$3 per kg. The operating cost for extraction of the PHA is assumed to be \$90 per tonne of PHA, excluding capital costs of the extraction facility. The gross margin from producing PHA is therefore $2.4 \text{ tonnes} \times 1,000 \times (\$3 - \$0.5) - (2.4 \times 90) = \$5,784$ per ha of cane harvested, excluding capital costs for the construction of the extraction facility. This compares with the farm gate net revenue from sucrose production of about \$775 per ha.

Extraction and Pilot Plant Success

It is assumed that the likelihood of successful extraction within a pilot plant will be very high, assumed at 90%, as Metabolix and Procter and Gamble already have patented extraction processes. However, it is believed that new technologies will be developed specifically for plants and especially for sugarcane and that these processes will be patentable. It is assumed that the pilot plant success will be achieved in 2013 at a cost of \$1 m.

Commercial Production and Regulation

Full scale commercialisation will require the approval of the OGTR. It is assumed that the likelihood of acquiring this permission is 80%. It is assumed that attaining this permission (submissions etc) will take one year and will be achieved in 2014 at a further cost of \$200,000. It is assumed that the sucrose production from the transgenic plants will be utilised in industrial products such as ethanol, rather than for human consumption.

Full Scale Commercialisation in Australia

Once these former steps have been achieved, the probability of full scale commercialisation is assumed to be 80%. The size of the extraction plant is assumed to be 50,000 tonnes per annum of PHA and the plant will be located next to an existing sugar cane crushing and sucrose extraction facility. Given the assumed extraction efficiency of 80%, to achieve full utilisation there will be a need to harvest 50,000/2.4 ha of transgenic sugarcane each year (about 20,833 ha of cane). The cost of the PHA extraction facility is assumed to be \$50 m and would be operational in 2016. The area of cane is only a small proportion of the Australian sugar cane crop, which averages over 400,000 ha harvested each year.

Commercialisation Overseas

It is assumed that 100,000 ha of the transgenic cane would be grown in overseas cane growing countries and that there would be a benefit to Australia in the form of a royalty (5c per kg) paid to the CRC for its role in contributing to the technology development. The 100,000 ha would produce 240,000 tonnes of PHA that would provide an annual revenue stream of 240,000 tonnes x 1,000 x \$0.05 = \$12 m. This stream would occur from 2016 onwards.

Other Risk Factors

There are other risk factors involved apart from those listed above. These may include competitors producing PHA at a lower cost, filing a blocking patent etc. The assumption that none of these critical factors will apply is 70%.

Summary of Key Assumptions

A summary of the key assumptions made in the analysis is provided in Tables 11 and 12.

Table 11: Key Assumptions for Economic Evaluation of the Genetic Manipulation Cluster

Step	Status	Probability of Attainment	Year Completed	Cost (m\$)
Commercial quantities of PHA expressed in plants –without CRC003	CRC project with Metabolix	0.70	2009	2.0
Commercial quantities of PHA expressed in plants –with CRC003	CRC project with Metabolix	0.75	2009	2.0
Permission from Australian GM regulator to conduct field trials	Being drafted	0.8	2010	0.2
Field trial success	Not yet attempted	0.8	2012	0.3
Success of pilot scale extraction plant	Not yet attempted	0.9	2013	1.0
Permission from Australian GM regulator for full commercialisation	Not yet attempted	0.8	2014	0.2
Full scale commercialisation (mainly extraction plant)	Not yet attempted	0.8	2016	50
No other critical knockout factors applying	Competitors in US, Brazil, etc	0.7	2016	0

Table 12: Other Assumptions

Factor	Assumption	Source
Cane yield	100 wet tonnes per ha including stems (85t)and leaves/tops (15t)	Agtrans Research after discussions with researchers and others
Wet to dry matter factor	30%	Agtrans Research after discussions with researchers and others

PHA yield	10% dry matter of cane	Agtrans Research after discussions with researchers and others
Potential extraction efficiency	80%	Agtrans Research
Production, harvesting and transport to facility costs for sugarcane	\$20 per tonne of stem produced plus \$2 per tonne of stem, leaves and tops for transport to facility	Agtrans Research after discussions with researchers and others
Price received for PHA	\$3 per kg	Agtrans Research after discussions with researchers and others
Added cost to growers for IP	\$0.50 per kg	Agtrans Research after discussions with researchers and others
Size of extraction facility	50,000 tonnes PHA per annum	Agtrans Research after discussions with researchers and others
Variable extraction costs	\$90 per tonne of PHA	Agtrans Research
Area of transgenic cane harvested in Australia	20,833 ha cane harvested per annum	Area required to service facility capacity
Area of transgenic cane grown overseas	100,000 ha	Agtrans Research
Royalty flow-back to Australia from transgenic cane grown overseas	5 cents per kg PHA	Agtrans Research after discussions with researchers and others
Other risk factors preventing benefits	30%	Agtrans Research

Results

All past costs and benefits were expressed in 2006/07 dollar terms using the CPI. All benefits after 2006/07 were expressed in 2006/07 dollar terms. All costs and benefits were discounted to 2006/07 using a discount rate of 5%.

The base costs for the R&D included the costs for the five cluster projects and analyses were conducted for both total investment in the cluster and for the SRDC contribution to the cluster investment. The expected values of other costs (e.g. follow on R&D, field trials, capital costs of extraction facility) were subtracted from the revenue stream.

The base analysis used the best estimates of each variable, notwithstanding a high level of uncertainty for many of the estimates. All analyses ran for the length of the investment period plus 25 years from the last year of investment (2005/06 to the final year of benefits assumed (2030/31)).

Investment criteria were estimated for both total investment in the cluster and for the SRDC investment alone. Each set of investment criteria were estimated for different time periods of benefits. The investment criteria were positive after a 15 year time period as reported in Tables 13 and 14.

Table 13: Investment Criteria for Total Investment in Cluster
(discount rate 5%)

Criterion	0 years	5 years	10 years	15 years	20 years	25 years
Present value of benefits (\$ m)	0	-0.007	-0.63	2.71	5.33	7.39
Present value of costs (m\$)	3.37	3.37	3.37	3.37	3.37	3.37
Net present value (m\$)	-3.37	-3.38	-4.00	-0.66	1.97	4.02
Benefit cost ratio	0	-0.002	-0.19	0.81	1.58	2.19
Internal rate of return (%)	negative	negative	negative	3.5	7.7	9.2

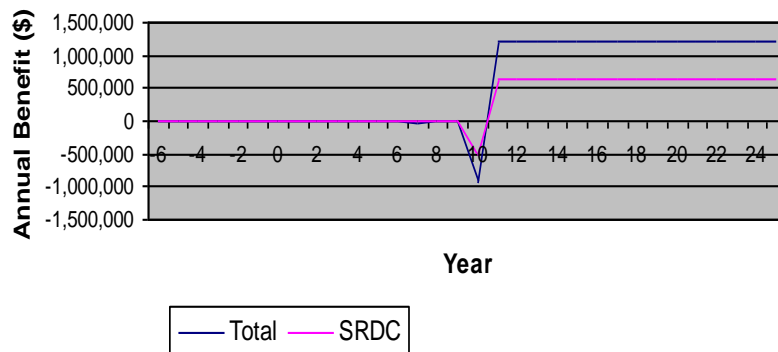
Table 14: Investment Criteria for SRDC Investment in Cluster
(discount rate 5%)

Criterion	0 years	5 years	10 years	15 years	20 years	25 years
Present value of benefits (m\$)	0	-0.004	-0.34	1.48	2.90	4.02
Present value of costs (m\$)	1.79	1.79	1.79	1.79	1.79	1.79
Net present value (m\$)	-1.79	-1.80	-2.14	-0.32	1.11	2.23
Benefit cost ratio	0	-0.002	-0.19	0.82	1.62	2.24
Internal rate of return (%)	negative	negative	negative	3.7	7.9	9.5

In terms of the quantified benefits, it is estimated that all could be attributed to the productivity and adding value component of the rural research priorities. No attempt has been made to value the community benefits involved.

The cash flow of benefits is shown in Figure 1 for both the total investment and for the SRDC investment in the cluster.

Figure 1: Annual Benefit Cash Flow



Sensitivity Analyses

Sensitivity analyses were carried out on a range of variables and results are reported in Tables 15 to 17. All sensitivity analyses were performed using a 5% discount rate with benefits taken over the life of the investment plus 25 years from the year of last investment in the cluster. All other parameters were held at their base values.

The sensitivity of the investment to the capital cost of the extraction facility is shown in Table 15. The break even cost of the extraction facility for the investment in the cluster to still provide a 5% return, given that all other assumptions remained the same, was \$395 million.

Table 15: Sensitivity to Assumption Regarding Capital Cost of Extraction Facility (SRDC investment, 5% discount rate; 25 years)

Criterion	\$25 m	\$50 m (Base)	\$150 m
Present value of benefits (m\$)	4.18	4.02	3.38
Present value of costs (m\$)	1.79	1.79	1.79
Net present value (m\$)	2.39	2.23	1.58
Benefit cost ratio	2.33	2.24	1.88
Internal rate of return (%)	9.8	9.5	8.2

The sensitivity of the investment in CRC003 to the difference made by CRC003 to the probability of success of the Metabolix project is shown in Table 16. The break even increase in probability for the cluster investment to have still returned 5% was 2 percentage points (an increase in probability from 0.70 to 0.72).

Table 16: Sensitivity to Assumption of Increase in Probability of Success due to CRC003 (SRDC investment, 5% discount rate; 25 years)

Criterion	2.5 percentage points	5 percentage points (Base)	10 percentage points
Present value of benefits (m\$)	2.01	4.02	8.05
Present value of costs (m\$)	1.79	1.79	1.79
Net present value (m\$)	0.22	2.23	6.25
Benefit cost ratio	1.12	2.24	4.49
Internal rate of return (%)	5.6	9.5	13.5

Table 17 provides the sensitivities to the yield of PHA. The break even PHA yield was 6.4% DM for the investment to have provided an internal rate of return of 5%.

Table 17: Sensitivity to Assumption of Yield of PHA (SRDC investment, 5% discount rate; 25 years)

Criterion	5%	10% (Base)	15%
Present value of benefits (m\$)	0.91	4.02	7.14
Present value of costs (m\$)	1.79	1.79	1.79
Net present value (m\$)	-0.88	2.23	5.34
Benefit cost ratio	0.51	2.24	3.98
Internal rate of return (%)	1.8	9.5	13.0

No sensitivity to adoption (high, medium, low) has been presented.

Conclusions

Genetic modification of the sugar cane plant for increased sugar yield, suppression of diseases and pests, or its use as a biofactory for industrial materials has not yet materialised despite ongoing investment by SRDC and others. The SRDC investment in this area has been strategic and knowingly made under a high risk–high return part of the Corporation’s investment plan.

Of the five projects funded by SRDC in the past five years in this cluster, only one appears to have sound prospects for delivering specific future benefits. The economic analysis of the prospects for biodegradable plastics made from modified sugarcane plants demonstrates that there are a number of risks involved in taking this investment through to the market place. However, if success is attained, the payoffs will be large.

The analysis is necessarily probabilistic given the uncertainties involved. Given the assumptions made the results are positive with a benefit cost ratio of about 2.2 to 1 and an internal rate of return of just below 10%.

The current analysis places all the costs of the five projects in the cluster against the expected benefits from the bioplastics initiative. However, although not valued in this analysis, there may be other benefits that emerge from the other four projects. The findings from CRC004 and UQ039 in particular have produced knowledge that could form important building blocks for new future innovations. In particular, if the constraint of gene silencing in sugar cane plants is removed, significant future innovations could be unleashed.

Acknowledgments

Peter Allsopp, BSES Ltd

Robert Birch, University of Queensland

Stevens Brumbley, CRC Sugar Industry Innovation through Biotechnology

Barrie Fong Chong, CRC Sugar Industry Innovation through Biotechnology

Peter Twine, CRC Sugar Industry Innovation through Biotechnology

References

BCC Research (2007) 'Biodegradable Polymers', Report ID PLS025C.
(www.bccresearch.com)

Brumbley, S.M., Fong Chong, B., McQualter, R.B., Nielsen, L.K., Petrasovits, L.A., Purnell, M.P. 'Transgenic plants used as a bioreactor' International patent application WO 2004/6657.

Brumbley, S.M., Purnell, M.P. Lars A. Petrasovits, L.A. Nielsen, L.K., and Twine, P.H. (2007) Developing the sugarcane biofactory for high-value biomaterials Internat Sugar J 109:5-14.

Brumbley, S.M., Petrasovits, L., Purnell, M., O'Shea, M.G., Geijskes, J., Lakshmanan, P., Smith G.R and Nielsen, L.K. (2002) Application of biotechnology for future sugar industry diversification. Proceeding of the 24th ASSCT, 29 April – 2 May 2001 Cairns, QLD pp 20-26.

McQualter, Richard B., Chong, Barrie Fong, Meyer, Knut, Van Dyk, Drew E., O'Shea, Michael G., Walton, Nicholas J., Viitanen, Paul V. and Brumbley, Stevens M. (2005) Initial evaluation of sugarcane as a production platform for *p*-hydroxybenzoic acid. Plant Biotechnology Journal 3:29-41.

Petrasovits, L.A., Purnell, M.P., Nielsen, L.K., and Brumbley, S.M. (2007) Polyhydroxybutyrate production in transgenic sugarcane. Plant Biotechnology Journal 5:162-172

Brumbley et al. Polyhydroxyalkanoate production in plants. Provisional patent filed 10 July 2007.

Purnell, M.P., Petrasovits, L.A., Nielsen, L.K., and Brumbley, S.M. (2007) Spatio-temporal characterisation of polyhydroxybutyrate accumulation in sugarcane *Plant Biotechnology Journal*. 5:173-184.

Wondu Holdings (2004) “Bioplastics Supply Chains- Implications and Opportunities for Agriculture”, Report for the Rural Industries Research and Development Corporation, Canberra.