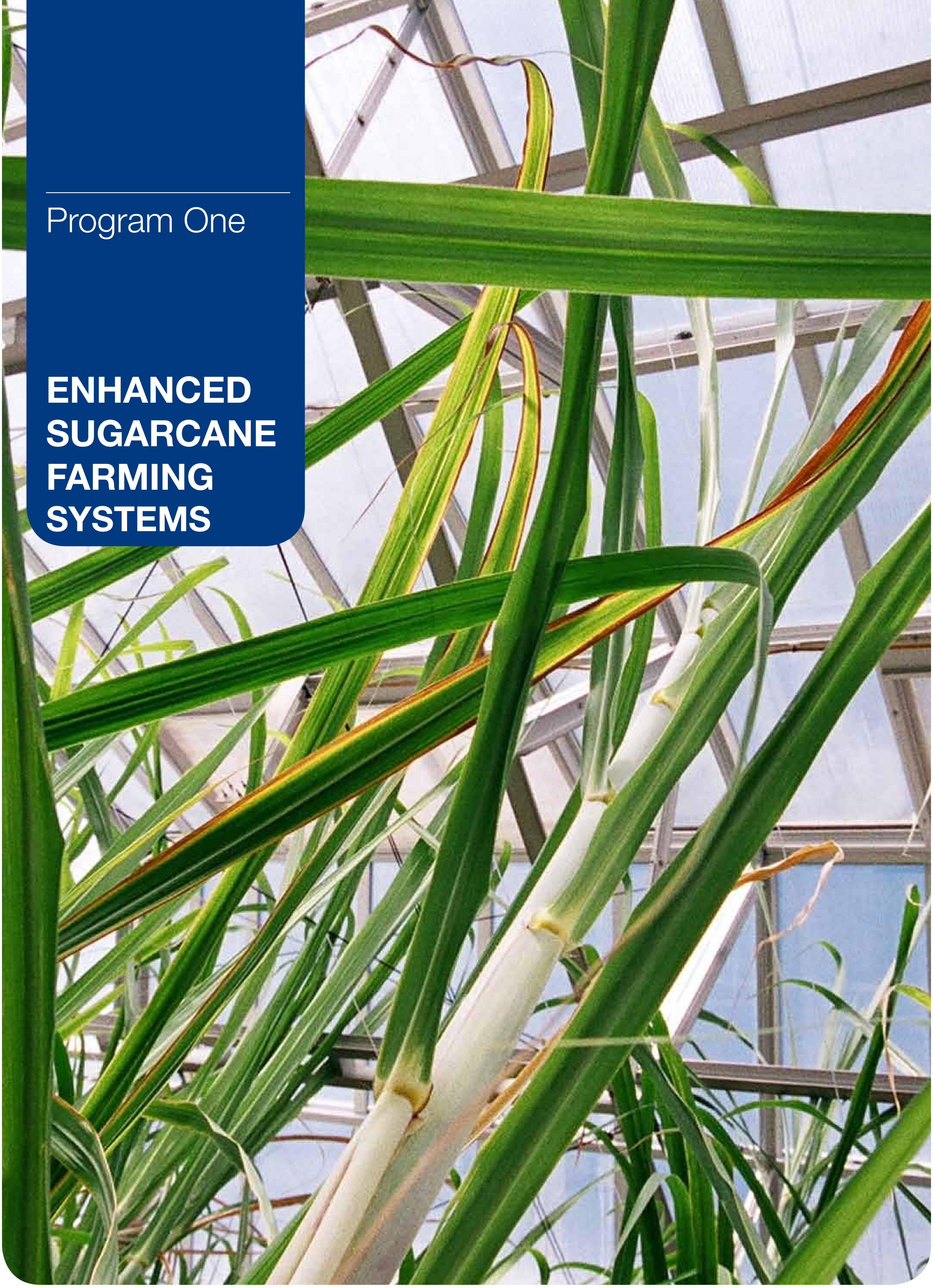
Program One

# ENHANCED SUGARCANE FARMING SYSTEMS



# ENHANCED SUGARCANE FARMING SYSTEMS

Program One focused on three target areas in sugarcane biotechnology research and development. These areas included:

1. The application of biotechnology to minimise the Australian sugarcane industry's environmental footprint.
2. The development of new breeding technologies to accelerate the delivery of new, more profitable sugarcane varieties.
3. Research into the adoption of new GM varieties and the effective integration GM traits into Australian sugarcane breeding programs.

(*The third target area included work by our CRC to provide a basis for the assessment of the environmental safety of future GM sugarcane releases.)*

The CRC SIIB has enabled multiple institutions to work together on these target areas of research. The CRC SIIB team not only made great progress in these areas, several significant milestones were reached. Towards the future, further research, development and commercialisation will be necessary to convert these program outputs into industry outcomes.

To achieve long-term benefits the philosophy of this program has been to support short and long-term research goals. As it takes 10 years to breed new sugarcane varieties, this program did not have the capacity to create technologies for immediate uptake. More importantly, the impact on the Australian sugarcane and supporting industries of the outcomes of Program One will be profound and will stand the test of time.

## Biotechnology to reduce the environmental footprint of sugarcane production

The Australian sugarcane industry is located in a highly environmentally sensitive area adjacent to the Great Barrier Reef and other natural features of significance. Additionally the industry shares its waterways with local communities and other industries.

### 2009/10 Highlights

- Great progress was made towards understanding the biology of sugarcane and its relatives to identify environmental hazards that need investigating to allow science-based evaluation of the release of GM sugarcane.
- Research outcomes showed that improved sugarcane varieties combined with alternative N fertiliser management strategies may increase N acquisition and reduce required N inputs and potential losses from sugarcane farming system.
- Transgenic plant lines with a series of valuable input traits were generated and successfully established in a trail as part of the novel pest resistance work.
- Promising DArT-based markers for high biomass were screened to improve gene map coverage. Individual markers with significant associations to biomass levels in the original population are being scored in a second generation of plants to examine repeatability.
- Great progress was made towards the development of a sugarcane genome map. Sequencing of genomic DNA generated more than 20 billion base pairs of sequence to date.

# Program One (cont'd)

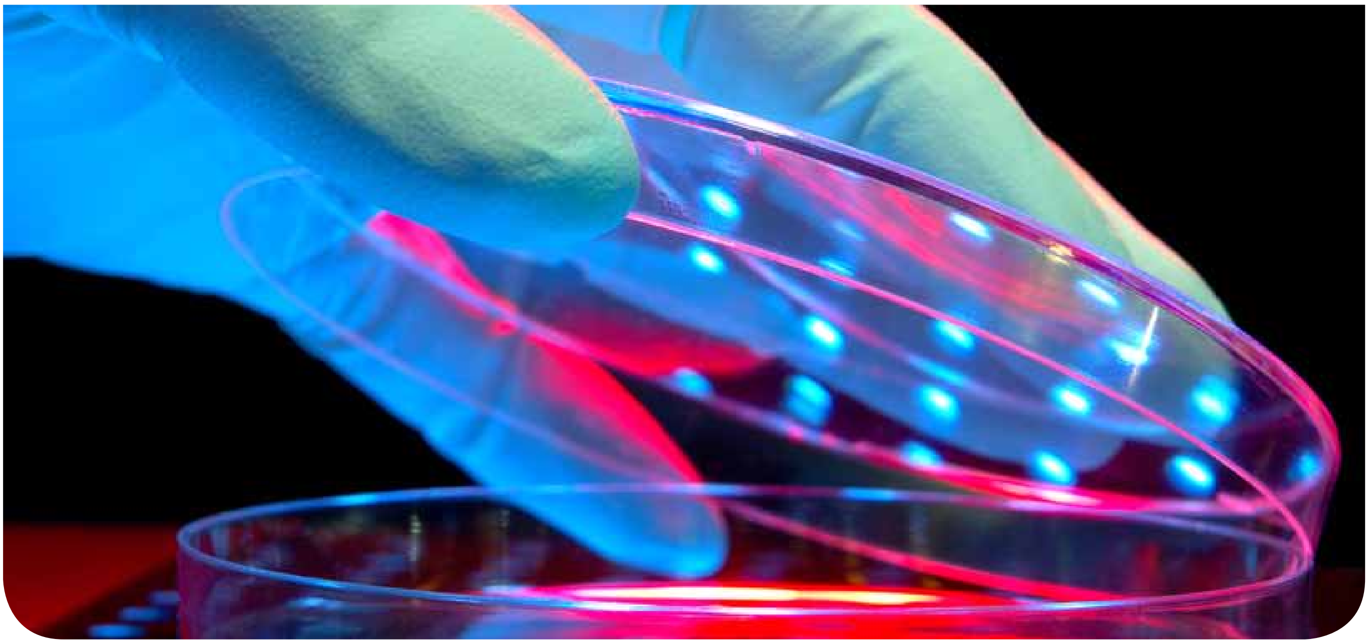
The Queensland State Government enacted the Reef Protection Program to regulate the use of pesticides and fertiliser in sensitive areas. While sugarcane growing targets are currently managed by agronomic practices, in the long-term it is hoped that new sugarcane varieties will help minimise pesticide and fertiliser applications. It is most likely that biotechnology will play an important role in helping to breed these new varieties.

During the past seven years, this program has researched the development of a bioremediation technology with the potential to degrade atrazine residues in the environment. More recently, research involved work with DOW AgroSciences to test gene technologies for the control of nematode pests in transgenic sugarcane. If this work leads to new varieties, it will reduce the current rate of nematicide application by industry.

Both of these technologies will require further development and testing after the CRC. If technically successful, their commercial viability will need to meet the rigours of the marketplace and regulatory system.

A major contribution of this program has been understanding nitrogen (N) utilisation by sugarcane. Our work has rewritten the text book for N assimilation in sugarcane by showing clear physiological differences with other grasses. This new knowledge is important because in most sugarcane production soil systems, applied urea fertilisers break down to ammonia which in turn undergo biological nitrification to eventually form nitrate. An additional source of nitrogen comes from amino acids released from soil organic matter, especially from the green trash blankets of previously harvested crops.

Early research in this program showed 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



Australia has had a dynamic and world-leading sugarcane breeding program.

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.

Most of the CRC's understanding of N assimilation has come from physiological studies with maize, a widely studied crop plant. However, unlike maize that can take up and store nitrate, sugarcane does not accumulate nitrate in its vegetative tissues. Unfortunately it is not possible to cross maize and sugarcane to breed these traits into commercial sugarcane varieties, but encouraging results have been obtained with other wild relatives. Indications are that members of the genus *Erianthus* are also able to assimilate nitrate in the presence of competing N sources, such as ammonia, much more efficiently than other species. Collaboration with Chinese scientists 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 whole process may take several years to complete but these 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.

## New sugarcane breeding biotechnologies

New innovative biotechnologies can be delivered to growers via improved sugarcane varieties. New Varieties represent one of the most readily adopted forms of technology in the Australian sugarcane industry. The maintenance and growth of the industry's production base is reliant on a high-performing delivery pipeline of new varieties with better adaptation, stress tolerance, disease resistance and optimised yield. For a long time, Australia has had a dynamic and world-leading sugarcane breeding program. The adoption of molecular tools represents one of the next technological innovations that will keep this program at the head of the field.

The initial achievement of the CRC in delivering molecular biology to sugarcane breeding was the development of the DArT marker chip and a fee-for-service arrangement for delivery of this genome analysis tool with DArT Pty. Ltd. in Australia. This program has since worked intensively on using DArT and other marker technologies for the discovery of DNA markers that can be used as surrogates for selecting genotypes that carry important traits.

A major strategy in this marker discovery activity has been to use association genetics. This is where specific markers can be identified from large populations associated with genotypes that carry a particular trait. In this approach the program has been able to tap into large industry databases on the field performance of individuals in these populations. Careful statistical analyses have been developed to ensure that the genomic region responsible for the trait and the marker are linked (i.e. exist on the same chromosome)and are not simply derived from coincidental co-inheritance of traits and markers resulting from recent common parental ancestry in the population.

This work has resulted in a suite of markers that explain highly significant proportions of sugar content, yield and diverse disease resistances. 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 DNA code from which elite progeny can be conventionally selected in stages.

The long time frames that are required for sugarcane breeding mean it is important to look over the horizon at traits that may have greater importance in the future and begin to lay a foundation for the introduction of these traits. Renewable energy is seen as an important component in the measures taken to


mitigate against future climate change and the cogeneration of electricity is already an important feature of several sugar mills in Australia. Policies such as the 'Carbon Pollution Reduction Scheme' (now in abeyance) and more recently the Renewable Energy Target Legislation provide a platform to build further cogeneration ventures. Internationally there is increased effort going into the development of technologies for the fermentation on lignocellulose for the production of biofuels. Economically viable processes for this would offer alternative avenues for the commercial exploitation of bagasse.

All of these trends point towards sugarcane fibre having an increased value in the future. Some future sugarcanes may simply be feedstocks for fermentation and energy generation, better termed 'Energy Canes' rather than the current-day sugarcanes dedicated to the crystal sugar business.

The CRC SIIB has been involved in the development of high biomass canes that might fit such future energy production systems. These canes will take full advantage of new gene pools from unexploited *Saccharum spontaneum* accessions and *Erianthus spp*. Currently and into the future, these new energy canes will be tested through the Australian sugarcane industry's Joint Venture for new agronomic characters of value, but early tests indicate that they have the potential to produce extraordinary biomass yields, fermentables and fibre.

While DNA markers have been essential in the characterisation of these potential energy canes a study of the chromosomes of crosses of *Erianthus* into sugarcane backgrounds has indicated that chromosomal recombination is either precluded or is rare, while chromosome addition lines can be generated. It is hoped that arrangements can be established to enable these lines to be tested in the coming years for the presence of useful traits from *Erianthus*, such as water-use and nitrogen-use efficiency, and to tie these traits to specific chromosomes for future breeding.

Finally, a highly pre-competitive area of sugarcane research of international importance is the complete explanation and mapping out of the sugarcane genome sequence. Even though important progress has been made in several other crop genome sequences, including relatives such as sorghum and maize, it is the polyploid nature of the sugarcane genome that makes the goal so unique.

The high polyploidy of sugarcane foils the assembly process used for most genome sequences (i.e. where alike sequences are aligned by overlap). This presents enormous technological challenges. The scientific value in this endeavour may be a greater understanding of how polyploid genomes function and, for sugarcane in particular, how this may be associated with plant vigour and performance.

Polyploid plants are common in nature and this will have impact beyond the *Saccharum* genus. So far the CRC SIIB has allowed Australian researchers to sit at the table of a large international consortium to tackle the sugarcane genome, and some syntony with sorghum has been delineated. The sequencing of the section of the sugarcane genome of specific interest to Australia has been achieved.

If we could take a look over the science horizon into the future, plant, animal, microbe and human genetics will be dominated by genome-based technologies. Access to the first and subsequent sugarcane genomes will enable the discovery of new markers, genes and genetic interactions that will also impact on future breeding and cultivar performance.

## Facilitating science-based regulation of future gm sugarcane

To date, there has been no commercial release of genetically modified (GM) sugarcane in Australia or elsewhere. The rapid growth of the Brazilian sugarcane production system in the past decade and the prospect of another decade of continued growth has now attracted the interest of several of the large multinational companies that commercially exploit GM technology in other crops. It is very likely that the first GM traits that are released in sugarcane will be the same genes and gene combinations that have been successfully developed in crops such as maize.

It is important that Australia maintains at least technological parity internationally in these developments to be competitive in its markets. One attraction of the Australian system is that it has a well defined, robust, transparent and science-based regulatory system for GM products. The CRC SIIB has added to this reputation by funding research aimed at providing data to underpin a regulatory framework that may be used to assess the environmental safety of GM sugarcane releases.

This work has involved the assessment of outcrossing to weedy relatives of sugarcane and the potential for seed set and establishment. Similarly the factors of pollen spread and cross-fertilisation between sugarcane varieties has been established. Interestingly, a series of studies were also undertaken in Panama (as part of a Smithsonian Fellowship) where *S. spontaneum* is an invasive weed. The genotypic/environment interactions that make *S. spontaneum* a successful weed in Panama, but not in northern Australia, were determined.

The outcomes of this work do not preclude the release of GM sugarcane. It will be necessary to assess the risks associated with specific GM traits that are contained in future proposed releases. This work has been the first in the world to address these important questions and has provided scientific evidence for informed regulatory decisions in Australia.

# PROJECT OUTCOMES

## Managing the safe release of genetically enhanced sugarcane

The project made great progress towards understanding the biology of sugarcane and its relatives to identify environmental hazards that need investigating to allow science-based evaluation of the release of GM sugarcane.

During 2009/10 a third season of field observations were collected in northern Australia and data were obtained for more southerly regions. A base line for the frequency of flowering of commercial crops and populations of *S. spontaneum* (a sexually compatible relative of sugarcane) and the timing and levels of viable seed production has been established.

A series of studies were also undertaken in Panama (as part of a Smithsonian Fellowship) where *S. spontaneum* is an invasive weed. The genotypic/environment interactions that make *S. spontaneum* a successful weed in Panama, but not in northern Australia, were determined.

Laboratory analyses to determine the level of crossing in commercial sugarcane fields and how far pollen travels are being completed. Publications for submission to international peer-reviewed journals are being prepared. The projects' findings were also discussed in April 2010 with Government agencies that regulate GM technologies in Australia.

Final outcomes from this work are being generated by providing the information to the designated regulatory authorities in Australia and internationally and also by publication of our results. Ultimately, the information is available for on-going use to inform decisions about the future release of genetically modified sugarcane in Australia.

## Reducing plant nitrogen demand

The acquisition of nitrogen (N) by plants is a key component of nitrogen use efficiency. The CRC SIIB nitrogen use project examined the concept that nitrate is the main N source for sugarcane by comparing use of inorganic (ammonium, nitrate) and organic (amino acids) N forms, all of which are prevalent in Australian sugarcane soils.

Analysis of growth and metabolite profiles has demonstrated that sugarcane uses organic N as a N source in sterile and in glasshouse conditions, and field-grown sugarcane readily takes up organic N. Further, our research trials have shown that organic N modifies root structure which has potential benefits for plant access to water and soil nutrients.

By examining the root structure of many sugarcane genotypes, our research team identified the potential for manipulation of root systems through breeding.

This research has shown that improved sugarcane varieties combined with alternative N fertiliser management strategies may increase N acquisition and reduce required N inputs and potential losses from sugarcane farming system.

## Novel approach to improve pest resistance in sugarcane

The CRC SIIB novel pest resistance project is a proof-of-concept project to demonstrate a transgenic approach to controlling sugarcane pests.

Significant achievements were made during 2009/2010 with the generation and multiplication of transgenic plant lines with a series of input traits. Over three hundred transgenic plant lines expressing the traits at different levels were selected for screening for pest resistance across twelve bioassay experiments. Eight of these bioassay experiments were harvested and analysed.

Pest resistance levels in nineteen promising plant lines identified in the first six bioassay experiments were reassessed in a validation trial that will be harvested in June

2010. Only through validation of the resistance levels in the promising plant lines will we have insight into which of the traits are effective in providing sugarcane plants with resistance to the target pest.

Transgenic material identified through this work may be incorporated in elite future sugarcane varieties to provide specific pest resistant traits.

## High biomass sugarcane

The project has involved following up on CRC SIIB research results that suggest some large quantitative trait loci (QTL) effects for biomass yields in a population of sugarcane recently derived from the wild cane species *S. spontaneum*.

Additional DArT-based markers were screened in this population to improve gene map coverage, and individual markers with significant associations to biomass levels in the original population are being scored in a second generation of plants to examine repeatability. Some of the sugarcane clones in the study have achieved high yields and could have commercial value.

Final recommendation from this work will detail methods for Australian sugarcane breeding programs to cost effectively undertake marker assisted selection for high biomass varieties. This may include specific markers and optimal selection indices. Final recommendations will also apply to other high-value populations identified in future commercial exploitation of potentially high value cross populations in Australian sugarcane breeding programs.

## Marker assisted breeding

The aim of this project is to help facilitate the adoption of marker assisted breeding in the Australian sugarcane breeding program based on CRC SIIB research.

A suite of agronomically important markers have been identified. These markers were selected using several statistical models and represent the current group of markers most likely to produce the best outcomes. These markers will be used in conjunction with a rapid DNA sampling and extraction method that was developed by DArT.

CRC SIIB modelling suggests that at the current estimated operation costs for these markers, application of DNA markers in a 'Marker assisted recurrent selection program' for rapid improvement of parental breeding value, will increase rates of genetic gain in sugarcane breeding programs. Plans to test such a selection program have started as part of the BSES-CSIRO sugarcane breeding program.

Complete genome map of sugarcanThe aim of this project is to deliver valuable information on the location of potential genes involved in traits of economic value.

Sequencing of genomic DNA from sugarcane genotypes has generated more than 20 billion base pairs of sequence to date. An enrichment experiment has been designed using the sorghum genome sequence and selected additional sugarcane specific sequences. This will allow detailed and invaluable sequencing of the gene rich regions of the sugarcane genome. Furthermore, sequencing of the parents of mapping populations will reveal large numbers of polymorphisms and provide markers linked to most genes in the genome.

The CRC work now forms part of an International Sugarcane Genome effort to sequence the complete genome. To this end a workshop was held in Port Douglas in August, San Diego in January and another meeting of the sequencing consortium is planned for August 2010 in France.