FINAL REPORT 2014/053

Phase 1: Advancing yield, disease resistance and ratooning by exploiting new sources of genetic variability from wild relatives of sugarcane

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ABSTRACT

This project was originally planned and designed for six years to allow collection of data over 2 or 3 ratoon crops. In this first phase of the project, all propagations and trials were well established and good quality data collected from the plant crop trial harvests. Analysis and interpretation of results will be an on-going task. Some introgression clones were identified as close to commercial potential. The average performance of introgression clone trials according to their generation was as expected for CCS, Fibre and the selection index rEGV. Continued backcrossing to elite material, after the initial cross to produce the F1, increases CCS and rEGV and decreases Fibre with each backcross generation. The variation observed for each of these traits also decreases with each backcross.

Introgression clones producing high value progeny have been identified and further crossing cycles will focus on using these clones. In general, seedling families derived from *S. spontaneum* appear to ratoon faster than *E. arundinaceus* progeny, regardless of the generation (BC3 or BC4). There was a very high correlation between stalk numbers per plot and stalk numbers of the best 2 stools per plot based on visual assessment, which suggests the latter to be a more efficient and cost effective method of measuring this trait in seedling trials.

High and low nematode treatments were successfully achieved at 2 sites using cover crops and nematicide. No association between nematode numbers and cane yield could be identified at this stage. It is expected that cane yield differences between treatments will be more pronounced in the ratoon crops.

EXECUTIVE SUMMARY

The genetic base of commercial varieties in Australia is narrow, and there is concern that current varieties do not perform or ratoon well under harsh and stressful environments. A large effort in Australia, with Chinese collaboration, over the last 11 years has been addressing the problem of a narrow genetic base: a large number of crosses have been generated using this material, and over 200 clones have been screened for resistance to nematodes, pachymetra, and smut. This material has been tested for yield in a limited number of environments (SRDC/SRA projects 2011/344, 2010/334, 2009/017, 2009/044) but has not been tested extensively in harsh environments or for ratooning ability.

Nematodes are a major component of poor root health in sugarcane soils. There is limited resistance to nematodes in current commercial varieties and the only effective control measures are crop rotation and nematicides. Previous glasshouse research has demonstrated that some basic and introgression clones have high levels of resistance to nematodes and pachymetra root rot. The aim of the nematode trials in this project was to provide field evidence that the nematode resistant clones identified in 2011/344 are resistant under field conditions and to determine the extent of yield losses for different levels of resistance to nematodes.

Breeders and pathologists have been closely involved in the development of this project from the outset, and information and updates have been shared during regular meetings and teleconferences. Productivity Service Companies in the Herbert, Mackay ISIS and NSW were consulted in the requirements and expectations of an introgression program. This has led to a very close and on-going partnership between project staff and local industries.

The aim of the yield trials was to test introgression clones selected from previous research for their performance under harsh conditions. Industry engagement in this process was a critical part of the success of these trials. The sites were chosen after in-depth discussions with industry to describe the requirements and expected outcomes for the trials. Similarly, the sites for the nematode field trials were also discussed in collaboration with industry.

Selected introgression clone types and species crossed with Australian elite parents were chosen for testing in seedling trials. The aim was to determine if different strategies were required for selecting from different species and/or generations.

Nematode trials were designed to test clones under two treatments: high and low nematode pressure. These treatments were applied to the trial sites using cover crops known to suppress or support multiplication of the nematode populations. A nematicide was also applied to the low nematode treatment plots to further reduce the number of nematodes as much as possible.

Trial sites were chosen in regions where nematodes are an issue. Two nematodes, lesion (*Pratylenchus zeae*) and root knot nematode (*Meloidogyne javanica*) were monitored in the field trials.

This project aims to ultimately deliver commercial cultivars containing new sources of genes and germplasm to the Australian Sugar Industry. These cultivars will help provide greater production stability, particularly in areas where harsh or stressful environments exist.

Newly-developed parents will be tested in a pre-breeding program that runs separate, but parallel to the commercial breeding program, with iterative advancements made with each backcross generation.
Introgression clones with high breeding value and/or with specific high value traits will be used by plant breeders in core regional breeding programs as parents in crossing and in selection trials.

Information and details necessary to ensure a targeted approach to utilising introgression material efficiently and successfully is lacking. Introgression material requires different selection strategies to core material. It is imperative that the knowledge and skills required for selection of introgression material (phenotypic and/or markers) is developed and maintained to ensure the delivery of long term successful outcomes. New parents and germplasm with genes for resistance to biotic and abiotic stresses will underpin the success of an introgression program aimed at developing cultivars with improved productivity and yield stability. An enhanced introgression breeding program, working closely and collaboratively with key industry personnel involved in multi-regional trials will lead to greater awareness of the benefits and the long-term nature of introgression breeding. It will also lead to enhanced industry engagement and acceptance of introgression breeding.
Varieties developed from introgression material will be taken up immediately at no extra cost to growers or millers. Improved ratooning and yield under harsh environments will reduce growers' costs and reduce fluctuations in regional productivity. Broadening the genetic base of sugarcane will reduce genetic vulnerability and improve the long-term sustainability of the industry.

All growers and millers will benefit from the release of productive new cultivars with new genes derived from currently untapped sources. Parental clones developed through introgression breeding will be incorporated into the core breeding program immediately at no extra cost to growers or millers. Future use of this parental material will lead to improved ratooning and yield, particularly under harsh environments, will reduce growers' costs, and reduce fluctuations in regional productivity. The project would have significant environmental benefits, reducing the need for chemical control of nematodes and reducing the need for premature plough out and replanting of fields that fail due to Pachymetra root rot, nematodes, or damage caused by frost and drought. Improved control of pachymetra root rot will reduce soil in the cane supply, leading to a reduction in mill mud and ash and wear on equipment in the mills.

Successful introgression programs require long-term investment to produce commercial outcomes. The risks are higher than for conventional breeding programs because unknown and unproven parental germplasm is used. This germplasm contains a number of useful genes breeders will want to exploit, but also contains a large number of genes with negative effects. Production of high breeding value parents combining the positive traits of the commercial type parent with the novel desirable traits of the wild parent can take many crossing cycles. This project aims to deliver new parents with new genes, through a process of "pre-breeding" to the core breeding program.
Once these new parents are developed, the time taken to release varieties from the introgression program will be the same as for the core breeding program material.
There will be no additional cost on top of the current cost associated with development and release of varieties through the conventional program. The clones generated in this project will be used by plant breeders in the core regional breeding programs as parents in crossing and in selection trials.

The outcomes of this work, new commercial cultivars, will be readily taken up by cane farmers, particularly in areas prone to high levels of pachymetra and/or nematodes and/or extreme environments where waterlogging, drought, and frost are prevalent.
This project has also developed close linkages with industry and this has facilitated the flow of results and information to industry through close involvement in the project.

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1. BACKGROUND

The narrow genetic base of commercial varieties is a major concern for the long-term sustainability of the Australian sugarcane industry. Selective breeding across many generations in a limited parental population has slowed down the rate of genetic gain in recent years. Genes for tolerance to biotic and abiotic stresses of serious economic concern (e.g. nematodes, frost, and drought) are limited or unknown in the current parental populations. An introgression program would address these concerns in a more effective manner than using only core breeding material which has a narrow genetic base. Without a follow-up to previous introgression projects, the industry faced the prospect of further loss of genetic diversity, which would ultimately lead to reduced genetic gain, and increased vulnerability to biotic and abiotic stresses.

Introgression of *Saccharum spontaneum* in the early 1900s resulted in improved productivity, adaptation, vigour, ratoonability, and resistance to some major diseases. Further introgression in Australia in the 1960s with the *S. spontaneum* 'Mandalay' resulted in 25 varieties and a new source of resistance to Pachymetra root rot. Louisiana initiated a similar program in the 1960s that was aimed at identifying resistance to sorghum mosaic. This program has been successful, with new resistant varieties now dominating that industry. The success of the original efforts in Louisiana has led to core funding and an expansion in the program's traits of interest to include adaptation to temperate climates. Adapted germplasm is fed into the commercial breeding program and is the basis for the development of stress-tolerant varieties (e.g. cold, drought, and flood tolerance; pest and disease resistance). One of the keys to the success of the Louisiana program is the separation of the pre-breeding and commercial programs. Introgression projects funded in Australia over the past 12 years have been aimed at introducing traits for biotic and abiotic stress resistance from wild species into parental populations. The material generated through these projects has been tested for yield in a limited number of environments (2011/344, 2010/334, 2009/044) but has not been tested extensively in harsh environments or for ratooning ability. Project 2014/053 was initiated to continue the introgression work and capitalise on industry investment in introgression by utilising the knowledge and information already developed in industry-funded projects.

Nematodes are a major component of poor root health in sugarcane soils and have been reported to reduce the yield of plant and ratoon crop by 15 % and 12 % respectively, and cause over $82 M in losses per year (Blair and Stirling, Aust. J. Exp. Ag. 2007, 47: 620-634). There is limited resistance to nematodes in current commercial varieties and the only effective control measures are crop rotation and nematicides, but these are not widely adopted. Pachymetra root rot is widespread throughout Queensland and NSW and can cause losses of 40 % or more in susceptible varieties. Glasshouse research in the current project (2011/344) has demonstrated that some basic and introgression clones have high levels of resistance to nematodes and pachymetra root rot.

Molecular markers have enormous potential to fast-track and improve the efficiency and effectiveness of the development of new introgression material. SNP markers have been developed in other projects, and introgression families were selected for further testing and potential implementation of these markers.

2. PROJECT OBJECTIVES

The objectives of the project were to determine if:

* The germplasm from new introgression crosses made in Australia and overseas improve ratooning, nematode and pachymetra root rot resistance, frost tolerance, and yields in stressful environments
* The nematode resistance ratings from glasshouse studies are reflective of resistance under field conditions
* We can identify and exploit new sources of genes for adaptability, better ratooning, resistance to nematodes and pachymetra root rot and frost tolerance from introgression clones
* We can derive benefit from industry-funded DNA marker projects to "stack" genes in introgression material?

3. OUTPUTS, OUTCOMES AND IMPLICATIONS

3.1. Outputs

This project aims to ultimately deliver commercial cultivars containing new sources of genes and germplasm to the Australian Sugar Industry. Specifically these cultivars will have:

1) Improved root health through novel sources of resistance to nematodes and pachymetra root rot.
2) Increased yield and ratooning ability under harsh conditions.
3) A greater diversity in genetic background to reduce the risks associated with the current narrow genetic base.
4) A strategy for on-going introgression breeding for the Australian sugarcane industry

These cultivars will help provide greater production stability, particularly in areas where harsh or stressful environments exist. Newly-developed parents will be tested in a pre-breeding program that runs separate, but parallel to the commercial breeding program, with iterative advancements made with each backcross generation. Introgression clones with high breeding value and/or with specific high value traits will be used by plant breeders in core regional breeding programs as parents in crossing and in selection trials. Research outcomes were made directly available to the Adoption team through milestone reporting and involvement in annual project meetings. New introgression clones with higher levels of disease resistance are also of great interest to SRA pathologists. Overall, introgression populations could also be useful for molecular geneticists for gene mapping experiments.

Information and details necessary to ensure a targeted approach to utilising introgression material efficiently and successfully is lacking. Introgression material requires different selection strategies to core material. It is imperative that the knowledge and skills required for selection of introgression material (phenotypic and/or markers) is developed and maintained to ensure the delivery of long term successful outcomes. New parents and germplasm with genes for resistance to biotic and abiotic stresses will underpin the success of an introgression program aimed at developing cultivars with improved productivity and yield stability.

An enhanced introgression breeding program, working closely and collaboratively with key industry personnel involved in multi-regional trials will lead to greater awareness of the benefits and the long-term nature of introgression breeding. It will also lead to enhanced industry engagement and acceptance of introgression breeding.

3.2. Outcomes and Implications

All growers and millers will benefit from the release of productive new cultivars with new genes derived from currently untapped sources. Parental clones developed through introgression breeding will be incorporated into the core breeding program immediately at no extra cost to growers or millers. Future use of this parental material will lead to improved ratooning and yield, particularly under harsh environments, will reduce growers' costs, and reduce fluctuations in regional productivity. If the impact of nematodes and Pachymetra was reduced by 30 %, yields could be raised across the industry, lifting profits in excess of $50 M/year. The project would have significant environmental benefits, reducing the need for chemical control of nematodes and reducing the need for premature plough out and replanting of fields that fail due to Pachymetra root rot, nematodes, or damage caused by frost and drought.

4. INDUSTRY COMMUNICATION AND ENGAGEMENT

4.1. Industry engagement during course of project

The key message arising from this project is the very strong interest and willingness to collaborate in introgression/this project from industry groups particularly in the Herbert, Burdekin, Bundaberg, and NSW regions. This interest has developed through regular communication and close collaboration with industry right from the outset of project proposal development. Very close linkages have been developed with HCPSL, Wilmar, ISIS Productivity Services, and NSW Sunshine Sugar. Specific communication activities during the project include:

- 02/04/2014 – project meeting with project staff and Wilmar, Burdekin
- 22 - 23/05/2014 – project meeting with project staff and HCPSL, Ingham
- 10/06/2014 – project meeting with project staff and MAPS, Mackay
- 11 - 12/06/2014 - project meeting with project staff and NSW Sugar
- 18/02/2015 – presentation given at Trial Information Day organised by MAPS
- 14/04/2015 – presentation given at Bundaberg Grower Research Update
- 18/04/2015 – presentation given at Cairns Grower Research Update
- 20/04/2015 – presentation given at Burdekin Grower Research Update
- 22/04/2015 – presentation given at Mackay Grower Research Update
- 23/04/2015 – presentation given at Herbert Cane Industry Walk and Talk Day
- 26/04/2015 – presentation given at SRA Delegates meeting, Mackay
- 10/12/2015 – farm tour highlighting introgression clones in Nematode trial
- 13/05/2016 – project meeting with project staff and ISIS Productivity services staff
- 01 - 05/06/2015 – presentation given at 11th Germplasm & Breeding and 8th Molecular Biology joint ISSCT Workshop

- 30/06/2016 – project meeting with project staff and HCPSL
- 03/08/2016 – presentation given at Ratooning workshop, Brisbane
- 22/08/2016 – Energy can meeting with project staff, NSW Sugar and Cape Byron Power
- 28/09/2016 – project meeting with project staff and HCPSL
- 27/10/2016 – Bus tour funded by Project Catalyst and organised by Reef Catchments and Burdekin Dry Tropics; visit to Macknade introgression seedling trial
- 30/05/2017 – meeting planned between project staff and ISIS productivity services

A successful travel and learning award (2014/318) was also awarded to fund a visit by Dr Anna Hale to Australia to discuss the USA introgression program in-depth and to critically review the current introgression work in Australia (Appendix III). The visit by Dr Hale was a huge success and was highlighted by the three presentations given during her visit:

25/08/2015 – Breeding Sugarcane in Louisiana: Back to the Basics. Presented in Ingham.

27/08/2015 – Sugarcane Breeding in Extreme Conditions: Basic Breeding at the 29th North Parallel. Presented in Broadwater, New South Wales.

28/08/2015 – Extreme Breeding to Beat The Blues: Presented in Brisbane.

4.2. Industry communication messages

Introgression will provide new parents and germplasm with new genes for resistance to biotic and abiotic stresses and a greater diversity in the parent population which will reduce the risks associated with the current narrow genetic base. Successful introgression programs require long-term investment to produce commercial outcomes.

5. METHODOLOGY

5.1. Yield Trials

The requirements for the trials were discussed in detail with local industry personnel (HCPSL, MAPS, and NSW Sugar) and the sites were chosen to represent “harsh” conditions in each of the three regions. The sites are representative of dry conditions on a medium clay soil type (VIC15-62), a clay loam site with high Pachymetra levels (VIC15-61), a poor/marginal soils site (FAR15-61), and a site with poorly drained grey kandosol prone to frost and flooding (BWR15-36).

Most of the introgression clones chosen for the yield trials had previously been tested and evaluated in biomass (2009/017), and water (2010/334) and nitrogen (2009/044) use efficiency projects. We also investigated all other available introgression material (including the Wilmar-derived material) and used all plant and ratoon yield (TCH, CCS, Fibre, TSH, selection index developed in 2009/017) and disease resistance information from previous trials and projects utilising this material to select the best range of material for trialling in the yield and Nematode trials.

Standards Q200^{A}, Q208^{A}, KQ228^{A}, Q232^{A}, and Q240^{A} were included in the yield trials for comparison. During harvest, 6-stalk samples were collected from each plot in yield and nematode trials and processed through SpectraCane for CCS and Fibre calculations. Plot weights were also measured to calculate Tonnes Cane per hectare, Tonnes Sugar per hectare (TSH), and rEGV.

A linear mixed model was applied to the data using a combined analysis of the 3 yield trial sites. The analysed data were uploaded to SPIDNet.

Trial designs and information are described in detail in Table 1, and the full list of introgression clones planted in the yield and nematode trials is provided in Appendix 5.

Table 1: Details of trials planted and harvested in 2014053

Region	Trial	Trial Code	Plant date	Harvest Date	Trial Design	Traits
Herbert	Yield (Pachymetra)	VIC15-61	20-21 May 2015	15 Aug 2016	RCB (2 reps) 4 row x 10 m	TCH, CCS, Fibre, Pachymetra
	Yield	VIC15-62	13-14 Aug 2015	5 Oct 2016	RCB (2 reps) 4 row x 10 m	TCH, CCS, Fibre, Pachymetra
	Root-lesion nematode (RLN)	VIC15-63	11-12 Aug 2015	4 Oct 2016	Split design (5 reps x 2 treatments) 4 row x 10 m	TCH, CCS, Fibre, Nematodes
	Seedlings	VIC15-64	10 Jul 2015	30-31 Aug 2016	RCB (4 reps, 20 seedlings per plot) 139 families	TCH, Brix, Pol, Stalk count, height, diameter
	Slash trial	VIC16-62	27-28 Sep 2016	2017	RCB (2 reps) 4 row x 10 m	TCH, CCS, Fibre
	Seedlings	VIC16-64	11-12 Jul 2016	2017	RCB (3 reps, 20 seedlings per plot) 70 families	TCH, Brix, Pol, Stalk count
Burdekin	Seedlings	KAL15-16	16-22 Jun 2015	1 Jun 2016	Random Family Design	Selection based on appearance only
	Yield	KAL16-26	1 Jun 2016	10 Oct 2016 (Slashed)	RCB (2 reps) 1 row x 5 m	TCH, CCS, Fibre
Mackay	Yield	FAR15-61	13 Aug 2015	29 Aug 2016	RCB (2 reps) 4 row x 10 m	TCH, CCS, Fibre
	RLN	RAC15-61	10 Jun 2015	11 Jul 2016	Split design (5 reps x 2 treatments) 4 row x 10 m	TCH, CCS, Fibre, Nematodes
Bundaberg	Root-knot nematode (RKN)	NEM 15-F1	19-20 Aug 2015	25-26 Jul 2016	Split design (4 reps x 2 treatments) 5 row x 10 m	TCH, CCS, Fibre, Nematodes
NSW	Yield	BWR 15-36	1 Oct 2015	n/a	RCB (2 reps) 4 row x 10 m	TCH, CCS, Fibre

5.2. Seedling Trials

Five clone types x 5 clones x 5 crosses with Australian elite parents x 80 seedlings were targeted for the seedling trials. Five types of families were examined: Ea BC3, Ea BC4, Ss BC1, Ss BC2, and Ss BC3, where Ea = *Erianthus arundinaceus*, Ss = *Saccharum spontaneum*, BC1 = backcross generation 1, etc.

Seed from crosses made at Meringa was germinated at the Herbert SRA station and sent to the Macknade glasshouse for establishment. Seedlings were then potted out to the benches at Macknade and subsequently planted to the field (Table 1) at Macknade (VIC15-64) and Burdekin (KAL15-16; Kalamia, Wilmar). Potting mix and maintenance of seedlings on benches was as per standard SRA procedures.

The Ingham trial (VIC15-64) was planted on a medium clay soil type and the trial design was 1.8 m row spacing with 20 seedlings per plot and 0.7 m spacing between plants. The trial consists of 139 families with a total of 10,800 seedlings, with most of the families (88 %) planted as 4 reps and the rest as 3 reps.

Phenotypic measures taken from trial VIC15-64, 4 - 8 April 2016, included total number of stalks for every seedling stool, and stalk height and diameter from the best two stools in each plot based on a visual assessment.

Trial VIC15-64 also was assessed for germination 27 - 28 September 2016, four weeks after harvest. The percentage of seedlings at four different stages of development was recorded (data not shown), with stage 4 being the most advanced. A speed of ratooning rating (SRR) (or weighted average stage), was calculated for each plot by adding together the product of the percentage of plants at each stage of development. SRR was then calculated as an average for each introgression parent represented in the trial.

During harvest of VIC15-64, 10 stalks were taken at random from each plot and processed through Macknade small mill for collecting brix and pol data on a plot basis. Plot weights were also collected during harvest and converted to tonnes cane per hectare. Family was treated as a fixed effect to determine the effect of 139 families on stalk diameter, stalk count, stalk height, TCH, POL and BRIX. A Box-Cox transformation was done on stalk count, stalk height, TCH, POL and BRIX to improve the normality of residuals. All pairwise mean comparisons were done using Tukey Kramer at 5 % level. All data were analysed using SAS.

The Burdekin seedling trial (KAL15-16) was planted in a random family design, where families were planted in consecutive plots. It was planted to the field at the Kalamia station 22 - 23 June 2015, on a clay loam soil. The trial consists of 177 families with a total of 13,040 seedlings, 1.52 m row spacing, 20 seedlings per plot and 0.75 m spacing between plants. Most of the families planted in VIC15-64 are also represented in KAL15-16. After discussions with Wilmar staff during the project meeting held on 2 April 2014, a different selection strategy was taken with the Kalamia seedling trial. The trial was slashed on 21 October 2015 to enable sampling in 2016 before the crop lodged, and also to potentially allow for ratoonability as an additional selection criterion in the “ratoon” crop. Selections from the Kalamia trial were based on a visual assessment on a clonal basis; this is, the same method employed for the proven crosses in the Burdekin core program. Using this strategy will potentially reduce the selection cycle by one year in the process of identifying and recycling the best selections as parents compared to standard SRA procedures. This will also allow for a comparison between the different selection strategies taken for the Herbert and Kalamia trials.

5.3. Nematode Trials

Glasshouse studies in project 2011/344 identified introgression clones with resistance to nematodes and Pachymetra root rot. The aim of the nematode trials in this project was to provide field evidence that the nematode resistant clones identified in 2011/344 are resistant under field conditions and to determine the extent of yield losses for different levels of resistance to nematodes.

Two nematodes, root-lesion (*Pratylenchus zeae,* RLN) and root-knot nematode (*Meloidogyne javanica,* RKN) were assessed in these field trials. The trials were originally planned for two regions, Herbert and Mackay, however, after industry consultation it was decided to also plant a RKN trial in the southern region. The trial site in Bundaberg (McLennan, Wallaville) has a history of high levels of RKN and appeared to be ideal for this project. Three potential sites for the RLN trial in Mackay and four sites in the Herbert region were visited and soil sampled for nematode and pachymetra spore counts. Based on these counts and the suitability of the sites in terms of location and block size, the sites selected were Muscat (RAC15-61, Oakenden), Russo (VIC15-63, Ingham) and Irlam (VIC15-61, Abergowrie).

5.3.1. Pre-treatments of nematode trials

The nematode trials consist of two treatments: high and low nematode numbers. In order to apply these treatments to the trial sites, cover crops were planted using varieties of soybean and sorghum that either suppress (soybean A6785 suppresses RKN and RLN) or support (soybean Leichardt for RKN; sorghum sweet jumbo for RLN) multiplication of the nematode populations.

In addition to the cover crop pre-treatment for the nematode trials, a nematicide (Nemacur) was applied to the low nematode treatment plots to further reduce the number of nematodes as much as possible. Nemacur application and soil sampling dates are shown in Table 2. Soil samples were taken from the middle rows of every plot of the trials and sent to Woodford for nematode analysis to establish a baseline nematode count for subsequent yield analysis and effects. The nematode trials were analysed for all cane yield components with nematode numbers used as a covariate in the analyses.

Table 2: Nemacur application and soil sampling dates for the three nematode field trials. RLN = Root Lesion Nematode; RKN = Root Knot Nematode

Region	Trial	Trial Code	Nemacur Application date	Soil sampling date
Herbert	RLN	VIC15-63	29/10/15	4/1/2016
Mackay	RLN	RAC15-61	14/7/2015	31/8/2015
Bundaberg	RKN	NEM15-F1	23/10/15	2/12/2015; 11/12/2016; 2/3/2016; 29/3/2016

5.4. DNA Markers

DNA markers developed in CPI025, CPI030, 2013/358 and 2015/025 were used in this project to test their application for selecting introgression clones with desirable characteristics to use as parents. Pachymetra and smut resistance, TCH and CCS were the priority traits for DNA marker selection.

A meeting was held with Drs Aitken and McNeil on 4 August 2016 to discuss options for testing markers in introgression families in this project. Eight families (Table 3) were identified in VIC15-64 that traced back to the *S. spontaneum* ancestor (YN75-1-2) of the QTL mapping population (ROC25 x YN2002-356). This mapping population was used previously to identify markers linked to Pachymetra, smut, and yield traits. Four of the families (1 - 4) are BC2, derived from crosses between a BC1 developed in the mapping population and elite clones. Families 5 to 8 are BC3 derived from crossing BC2 to elite clones. The BC1 clones used to produce these families (QBYN04-26171, QBYN04-26050, and QBYN04-26073) were initially checked to see if they contained the QTL markers for resistance to pachymetra and smut inherited from the *S. spontaneum* ancestor. They were also checked to see if they contained QTL for reduced CCS which is also inherited from the *S. spontaneum* ancestor and the positive effect QTL inherited from the ROC25 ancestor. A further QTL for high TCH was also selected for screening, inherited from the *S. spontaneum* parent. Only QBYN04-26073 inherited this QTL for high TCH. SNP markers were also identified from the association mapping population for high CCS that would be inherited from the commercial parent of the cross.

Leaf samples from the parents of the eight families were sent to CSIRO 11 August 2016 for DNA extraction and preliminary screening of markers to identify which populations are polymorphic for the markers of interest. Populations 1, 2, and 8 were identified from this preliminary screening, and the QTL they carry are shown in Table 4.

Table 3: Eight families identified as candidates for marker testing

Family No.	Family Type	Female	Male	Female Grandparent of Female	Male Grandparent of Female	Female Grandparent of Male	Male Grandparent of Male
1	Ss BC2	QBYN04-26171	Q242[A]	ROC25	YN2002-356	Q170[A]	Q150
2	Ss BC2	QBYN04-26171	QC91-3511	ROC25	YN2002-356	QS79-7202	Q142
3	Ss BC2	QBYN04-26171	QA94-6577	ROC25	YN2002-356	QN85-283	N14
4	Ss BC2	QBYN04-26171	QS87-7140	ROC25	YN2002-356	Q153	H56-752
5	Ss BC3	KQB08-32762	SP86-155	QBYN04-26050	QC91-580	SP78-3081	Unknown
6	Ss BC3	KQB08-32762	SP89-1116	QBYN04-26050	QC91-580	SP71-8210	SP71-1088
7	Ss BC3	KQB08-32762	Q248	QBYN04-26050	QC91-580	QN85-1271	Q170[A]
8	Ss BC3	KQB08-32673	N29	QBYN04-26073	QC91-580	70E457	CP57-614

Table 4: The traits inherited from the parents of the crosses

Trait	QBYN04-26171	QBYN04-26073	ROC25	Q242[A]	QC91-3511
Pachymetra resistance	✓	✓			
Smut resistance	x	✓	✓		
CCS			✓	✓	✓
TCH + stalk No.	x	✓			

Leaf samples from 203 individuals from these three populations were sent to CSIRO 27 September 2016 for DNA extraction and SNP analysis. DNA extraction has been completed, and all the SNP primers have been designed. At the time of writing this report, five SNPType assays had been validated on parents of the selected families. The screening of all the SNPType assays will be completed in 2017.

6. RESULTS AND DISCUSSION

The project was originally planned and designed for 6 years to allow collection of data over two or three ratoon crops. In this Phase one project, all propagations and trials were well established and good quality data collected from the plant crop trial harvests. Analysis and interpretation of results will be an on-going task beyond the life of this project. Results are presented below in sections according to the trial purpose (Yield, Seedling, Nematodes).

6.1. Yield Trials

The rationale for the yield trials was to test introgression clones selected from previous research for their performance under harsh conditions. Industry engagement in this process was a critical part of the success of these trials. The sites were chosen after in-depth discussions with industry to describe the requirements and expected outcomes for the trials.

Trial harvest dates are shown in Table 1. BWR15-36 was planted as a two-year crop, with the plan to collect data during the 2017 harvest season. It has recently been subjected to severe flooding (Figure 1) and clearly there is some important and valuable information that can be collected from this trial to identify introgression clones that can withstand or recover from this stress better than the commercial standards.

Figure 1: Aerial view of flooded NSW yield trial (BWR15-36) taken 06/04/2017. Photo courtesy Michael Grogan, SRA Broadwater.

The quality of the data collected from the trials, as measured by the genetic variance (Vg), heritability (H2) and coefficient of variation (CV) was of a very high standard for the three yield trials (Table 5). For example, H2 for TCH ranged from 0.75 to 0.79, compared to FAT trials that typically achieve 0.50 to 0.70. CCS was also outstanding for H2 (0.94 - 0.96 vs 0.70 - 0.85), as well as Fibre (0.89 - 0.94 vs 0.80 - 0.90). These trait statistics give a high level of confidence in the data generated, and reinforces the validity and importance of collecting ratoon data from these trials in the coming years.

Table 5: Trial data quality estimated by trait statistics for the 3 yield trials[1]

Trial	Trait	Mean	Vg	H2	CV
FAR15-61	TCH	79.66	127.75	0.79	7.15
	CCS	14.49	4.41	0.96	2.59
	Fibre	13.73	4.19	0.93	3.82
VIC15-61	TCH	118.02	368.08	0.75	9.19
	CCS	12.28	4.69	0.94	4.35
	Fibre	15.98	3.74	0.89	4.21
VIC15-62	TCH	94.19	157.57	0.75	7.67
	CCS	14.52	3.57	0.95	2.76
	Fibre	14.08	5.75	0.94	4.15

Four introgression clones were identified from the third ratoon crop of a trial established in a previous project (2009017) as potentially good ratooners (reported in 2014\053 Milestone 3). Two of these clones have performed well in the current yield trials in this project (Table 6; Appendix 3). KQB07-23976, (Ss BC2) was ranked 5th for rEGV while KQB07-24815 (Ss BC1) was ranked 8th for rEGV and 1st for TCH in one trial. KQ08-1040 (Ea BC3) was the top ranked introgression clone for rEGV averaged over two yield trials (VIC15-61 and FAR15-61); it has also performed well in the 2014 Central FAT trials and will continue to be monitored for its performance in ratoon crops and its potential as a commercial variety in the Herbert and Central regions.

KQ08-1347 (Ea BC3) also performed well in the Herbert yield trials with average rEGV of 10.1. Six out of the top ten introgression clones had a much higher (20 %) TCH than the average of the standards.

Table 6: Yield performance of the top ten introgression clones based on rEGV compared to standards.

Clone	Average TCH	Average CCS	Average Fibre	Average rEGV
KQ08-1040	118	14.87	14.39	10.2
KQ08-1347	124	14.39	12.27	10.1
KQ08-1012	106	15.19	13.59	9.87
KQ08-2859	100	15.29	12.97	9.83
KQB07-23976	123	13.49	12.24	9.78
KQB09-23126	123	14.15	14.79	9.75
KQ08-1158	125	13.08	12.91	9.72
KQB07-24815	134	12.43	13.72	9.71
KQ08-1391	95	15.33	13.3	9.7
KQ08-1031	105	14.9	12.3	9.68
KQ228^{A}*	97	16.74	13.24	10.3
Q200^{A}*	105	15.81	15.62	10.05
Q240^{A}*	106	15.47	12.8	10
Q208^{A}*	120	15.58	13.52	10.29
Q232^{A}*	102	15.19	13.67	9.75

*Note: Standards KQ228^{A}, Q200^{A} and Q240^{A} across all three trial sites; Q208^{A} in Herbert trials only; Q232^{A} in VIC15-62 and FAR15-61

The average performance of introgression clones in the yield trials, according to their generation, was as expected for CCS, Fibre and rEGV (Table 7). Continued backcrossing to elite material, after the initial cross to produce the F1, increases CCS and rEGV and decreases Fibre with each backcross generation. The variation observed for each of these traits also decreases with each backcross. This information confirms previous research, and validates the need for backcrossing and will be important in future for developing selection strategies for the introgression program. No similar trend was observed for TCH, perhaps suggesting that less emphasis should be placed on this trait for selection purposes. It will be important information if this trend continues in the ratoon crops.

Table 7: TCH, CCS, Fibre and rEGV averaged by generation with the ranges for each trait shown in brackets

Generation[1]	Average TCH	Average CCS	Average Fibre	Average rEGV
F1	95	7.30	20.79	6.69
BC1	96 (68 – 134)	12.30 (6.79 – 15.0)	15.69 (12.65 – 20.56)	8.25 (6.18 – 9.71)
BC2	103 (84 – 127)	12.82 (8.0 – 15.46)	15.41 (12.08 – 19.73)	8.67 (6.33 – 9.78)
BC3	100 (73 – 125)	14.07 (12.26 – 15.45)	14.29 (12.27 – 16.86)	9.18 (8.15 – 10.20)

[1]A single F1 clone tested in trials, 23 BC1s, 19 BC2s and 34 BC3s.

6.2. Seedling Trials

The objective of this activity was to develop strategies for selecting seedlings from different species and generations of crosses to rapidly identify introgression clones for use as parents in a "pre-breeding" program. Seed of all five cross types were available at Meringa in the seed store from the 2013 crossing season. This seed was developed within project 2011/344 and was built on in this project. Further crossing was conducted at Meringa SRA facilities, including the photoperiod houses, in 2014. To achieve this aim, a list of priority introgression crosses was developed and sent to Meringa prior to the 2014 crossing season. Different approaches were taken between the two seedling trials to gain information and knowledge on the best methods for selection from introgression seedling trials.

The average progeny cane yield for each introgression clone ranged from 42 to 99 TCH (Table 8). This is typical of cane yields achieved in core PATs for most regions. As expected, brix and pol were lower in general than observed in core PATs. KQB07-23976 (Ss BC2) produced the best yielding progeny with an average of 99 TCH. This clone also performed well in the yield trials ranking 5th for rEGV (Table 4). KQB07-23976 will be targeted as a priority for further crossing and used in developing the strategy for future introgression breeding. Other top performing parent clones, including QBYC04-10577, KQB09-20031, KQB07-24619, and QBYN04-10357 will also be targeted for this purpose. KQB07-23227 was the parent of nine families in the trial, six times as a male and three as a female. When used as a male the progeny had a yield almost three times as when used as a female parent (74 vs 27 TCH). Further crossing with this clone will only be considered as a male parent.

Table 8: Introgression parent information and average progeny performance.

Introgression Parent	Progeny Type	Ancestor	Average TCH	Average Brix	Average Pol	Speed of ratooning rating	# of stalks/ stool	# of stalks/ 2 stools	Stalk Diameter (mm)	Stalk Height (cm)
KQB07-23976	Ss BC3	Kepandjen	99	20.69	78.84	2.9	11	17	25.68	244
QBYC04-10577	Ss BC1	Lingshui5	84	19.01	69.99	3	14	20	20.6	248
KQB09-20031	Ss BC2	Vietnam2	75	20.99	79.84	3.04	11	19	24.24	235
KQB07-24619	Ss BC2	SES341 x SES84A	74	21.02	80.53	2.98	13	17	23.07	225
QBYN04-10357	Ss BC1	YN83-157	72	20.1	74.24	2.89	12	18	22.33	237
KQB09-23160	Ep BC3	E. procerus	71	21.56	83.38	2.94	10	15	24.76	221
QBYN04-10356	Ss BC1	YN83-157	71	20.58	76.05	2.98	15	24	19.59	228
QBYN04-26171	Ss BC2	YN75-1-2	68	20.58	77.56	2.76	10	15	24	241
KQ08-2611	Ea BC4	HN92-77	67	22.02	86.47	2.65	10	15	25.03	235
KQ08-1204	Ea BC4	HN92-77	66	20.78	79.47	3.03	11	15	24.5	203
KQ08-1040	Ea BC4	HN92-77	65	22.17	85.89	2.72	9	12	26.11	222
QB00-3014	Ss BC1	IK76-41	63	19.64	73.22	2.84	10	15	24.71	214
QBYN05-20563	Ss BC2	Hainan92-9	62	19.98	74.99	3.05	11	15	21.46	213
KQB08-32762	Ss BC3	YN75-1-2	60	22.18	85.48	2.81	10	13	25.31	218
QBYC06-30296	Ea BC3	HN92-77	60	20.66	79.19	2.75	9	13	26.9	198
KQ08-2664	Ea BC4	HN92-77	59	21.72	83.53	2.89	10	14	24.75	205
KQB07-23227*	Ss BC2	YN83-157	58	20.31	75.72	2.88	10	14	24.84	215
KQB09-23146	Ep BC3	E. procerus	58	21.76	83.57	2.96	10	13	25.83	199
KQ08-2546	Ea BC4	HN92-77	58	20.55	77.89	2.95	9	12	26.14	199
KQB08-32673	Ss BC3	YN75-1-2	58	21.02	79.44	2.85	12	16	22.33	170

Introgression Parent	Progeny Type	Ancestor	Average TCH	Average Brix	Average Pol	Speed of ratooning rating	# of stalks/ stool	# of stalks/ 2 stools	Stalk Diameter (mm)	Stalk Height (cm)
QBYC05-10199	Ss BC1	Hainan92-32	55	20.67	79.35	3	15	22	18.72	215
QBYN04-20250	Ss BC3	YN82-48	54	21.27	81.47	2.94	11	15	22.53	214
QBYC06-30260	Ea BC3	HN92-77	53	21.43	82.06	2.77	8	13	25.02	206
QBYC06-30101	Ea BC3	HN92-77	52	20.36	78.21	2.65	9	13	24.73	190
QBYC06-30381	Ea BC3	HN92-77	52	20.4	76.95	2.67	8	12	27.75	230
KQB07-23989	Ss BC3	51NG2	51	21.45	82.38	2.79	9	14	26.56	221
KQB09-20158	Ss BC2	YN83-157	47	19.9	71.36	N/A	N/A	N/A	N/A	N/A
QBYC06-30376	Ea BC3	HN92-77	42	21.22	80.8	2.76	9	13	23.5	198
KQ08-1046	Ea BC4	HN92-77	42	20.83	79.49	2.71	8	14	22.79	201
		Average	**62**	**20.86**	**79.22**	**2.86**	**10.5**	**15.2**	**24.06**	**216**

Brix and Pol values were highly correlated (Table 9) as expected, and on average (not unexpectedly for introgression material) low. However, there were some introgression parents (BC3, BC4) that produced progeny with relatively high Brix values (e.g. KQ08-1040, KQB08-32762).

A speed of ratooning rating (SRR) was derived from an assessment made on the seedling trial four weeks after harvest. Overall families derived from *S. spontaneum* appear to have higher speed of ratooning rating (SRR) than *E. arundinaceus,* and *E. arundinaceus* progeny were generally slower ratooners regardless of the generation (BC3 or BC4). There was no apparent association between SRR and generation, or SRR and species ancestor.

There was a very high correlation between stalk numbers per plot and stalk numbers of the best two stools per plot based on visual assessment (0.93, Table 9). Stalk Diameter was highly negatively correlated with number of stalks per stool (-0.81), and there was a good correlation between number of stalks and speed of ratooning (0.69, 0.57). Stalk Height and Number of stalks had a positive correlation with cane yield (0.67, 0.51), and there was also a positive correlation between stalk diameter and Brix and Pol.

Based on this information, stalk number of the best 2 stools in a plot can be confidently used to estimate the stalk number of the whole plot. This represents a more efficient and cost effective method for measuring this trait in seedling trials. Stalk number is also moderately correlated with cane yield.

Table 9: Correlation matrix between phenotypic traits measured in VIC15-64 seedling trial.

Trait	*TCH*	*Brix*	*Pol*	*Speed of ratooning rating*	*# of stalks/ stool*	*# of stalks/2st ools*	*Stalk Diameter*
Brix	-0.23						
Pol	-0.24	0.98					
Speed of ratooning rating	0.43	-0.27	-0.30				
#ofstalks/stool	0.51	-0.36	-0.39	0.69			
#of stalks/2stools	0.51	-0.36	-0.40	0.57	0.93		
Stalk Diameter	-0.11	0.37	0.40	-0.50	-0.81	-0.81	
Stalk Height	0.67	-0.19	-0.20	0.14	0.33	0.44	-0.09

6.3. Nematode Trials

Glasshouse studies in project 2011/344 identified introgression clones with resistance to nematodes and pachymetra root rot. The aim of these trials was to provide field evidence that the nematode resistant clones identified in 2011/344 are resistant under field conditions and to determine the level of resistance required to reduce losses from nematodes.

Management of the Herbert RKN site was severely hampered by bad weather. As a result the growth of the cover crop was poor, and weeds and insects could not be controlled due to lack of accessibility at critical times. Nematode numbers in susceptible plots (soybean Leichardt) were very low (<20 nematodes/kg of soil), and this was considered to be unsuitable for the establishment of RKN trial. We decided to exclude this site as a RKN trial. RKN mainly occurs in sandy soil, in contrast, RLN is prevalent in all soil types. Given the numbers of RLN were high in almost all regions, regardless of soil type, another RLN trial was established at Herbert.

Cover crops of soybean and sorghum were successful in suppressing or increasing the number of nematodes within the RLN trial sites, respectively. The resulting nematode counts for the Herbert and Mackay RLN trials (Table 10) showed a 60-fold and 10-fold difference between the treatment plots, respectively. This is exactly the response that we aimed for and demonstrates the effectiveness of the cover crop and nematicide treatments on soil nematode populations.

Table 10: Populations of root-lesion nematodes (per kg of soil) in plots planted with sorghum and soybean in Herbert and Mackay

Clone	Herbert		Mackay	
	Sorghum	Soybean	Sorghum	Soybean
KQ08-1047	8292	148	4652	738
KQ08-1231	-	-	3630	324
KQ08-1347	14926	137	4665	436
KQ08-1348	13561	272	5081	637
KQ08-1359	10783	103	5533	248
KQ08-6014	11782	402	6680	302
KQ228	11452	233	-	-
KQB09-30117	-	-	6292	594

Clone	Herbert		Mackay	
	Sorghum	Soybean	Sorghum	Soybean
KQB09-20048	12823	86	6541	587
MQB88-10825	12078	121	5217	555
Q135	14445	681	5080	502
Q200[A]	12026	214	-	-
Q208[A]	17553	157	5171	355
Q232[A]	18501	237	-	-
QBYC06-30376	9418	148	3985	750
QBYC06-30390	11505	71	-	-
QBYN05-20563	16764	223	5365	321
Mean	**13061**	**216**	**5222**	**488**

We did not see the same treatment response in the Bundaberg Root Knot Nematode (RKN) trial. The initial sampling from every plot (2 December 2015) and nematode counting from this trial indicated there were very few nematodes and no significant difference between the treatments (data not shown). Subsequent sub-sampling and nematode counting of the trial site occurred 11 December 2015 and 2 March 2016 and again failed to show a clear difference in nematode numbers between the treatments (data not shown). Soil samples were again taken from every plot 29 March 2016 and nematode counts from those samples are presented in Table 11. Although some of the plots showed a difference (e.g. Q232[A], Q208[A], and KQB09-30117), in general there was no clear difference in nematode numbers between treatments and the reason for this remains unclear. The site was chosen because of its history of high nematodes but this has not been reflected in our data. A meeting is planned on 30 May 2017 with local industry to discuss the options for this trial and the importance of monitoring nematode numbers in the ratoon crops.

Table 11: Average RKN and RLN numbers per kg dry soil per variety in treated and untreated plots in the Bundaberg trial

Clone	Treated	Untreated
KQ08-1347	169	118
KQ08-1348	20	476
KQ08-1359	94	469
KQ08-6014	119	268
KQ228[A]	500	753
KQB09-20048	198	1337
KQB09-30117	261	3277
MQB88-10825	34	60
Q135	1035	1794
Q200[A]	2652	1144
Q208[A]	761	3427
Q232[A]	246	3689
Q240[A]	99	256
QBYN05-20563	49	5
Mean	446	1219

The nematode trials have been analysed for all cane yield components with nematode numbers used as a covariate in the analyses. However, no association between nematode numbers and yield could be identified at this stage. One reason for this could potentially be that nematode numbers throughout the crop cycle would be a more relevant measure rather than at a single point in time. Nematode numbers at three points in time, from young cane, middle growth and just prior to harvest is expected to give a better indication of variety resistance/ tolerance in the field. We also expect that cane yield differences between treatments would be more pronounced in ratoon crops rather than plant crop.

6.3.1. DNA markers

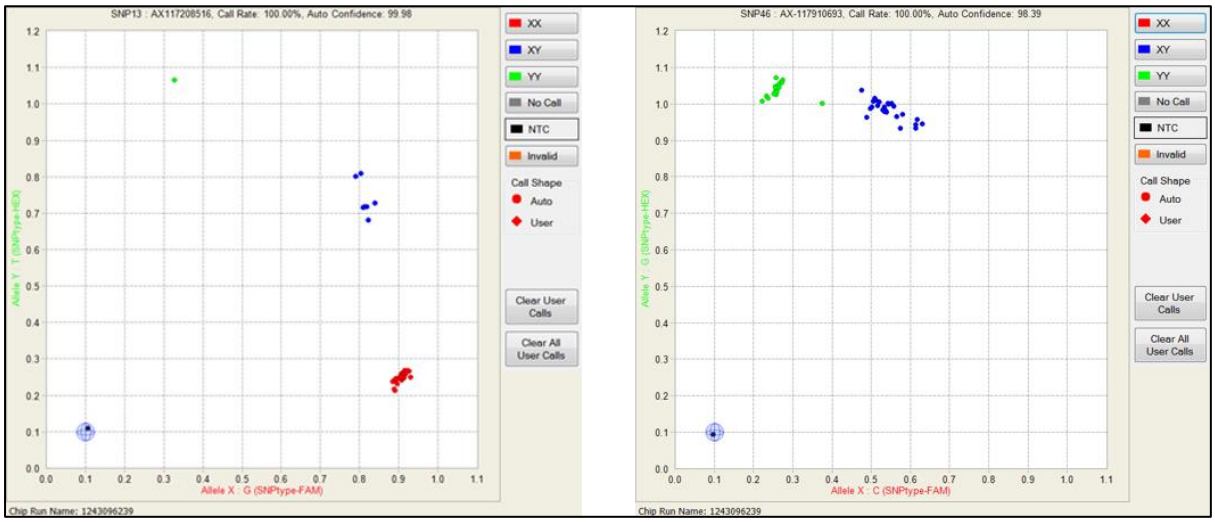
The markers required for this criterion were developed in a CSIRO-led project 2015/025. Eight families were initially identified for preliminary screening and three of these families were selected for further testing (Table 3, Table **4**). SNP markers were identified that were linked to the QTL inherited from both parents for pachymetra and smut resistance and high CCS (Table 12). Once the SNP markers were identified the sequences were sent to Fluidigm to design primers for the SNPType assay.

Table 12: SNP identified for the QTL of interest

Source of QTL marker	Trait	QTL markers	Effect	SNP markers
YN02-356 parent	TCH + stalk number	Acct227s	positive	AX117213140
		418544		AX117196440
		AC9cag109S		AX117298174
	Brix	ACCTC60S	Negative	AX117132968
				AX118125435
				AX117181852
				AX117988103
	Pachymetra	429427	Negative	AX118048308
		426528		AX117210424
		426249		AX118120496
				AX117289784
	Smut		Negative	AX117195428
				AX117290883
				AX117147884
				AX117216833
				AX117966753
				AX117303610
				AX117176181
				AX117180800
ROC25 parent	Pachymetra	QTL1	Negative	AX117891574
				AX118036179
		QTL2	Negative	AX118011967
				AX117266348
				AX118031623
				AX118011974
	Smut		Negative	AX117144387
				AX117318577
				AX117939890
				AX117229541

Source of QTL marker	Trait	QTL markers	Effect	SNP markers
Association Mapping	Pachymetra			AX117910693
				AX117138068
	Smut			AX117208516
				AX117162533
				AX118010398
				AX117983423
	CCS			AX117204528
				AX117201222

The SNPs were then screened across the parents of the selected crosses to verify that the same results was seen in the Affymetrix SNP array as in the Fluidigm SNPType assay. A small number of mismatches is acceptable due to error in either SNP assay (Figure 2).



(a) (b)

Figure 2: SNP Genotyping analysis using Fluidigm SNPType assay. The Fluidigm SNP Genotyping Analysis software automatically analyses the end-point image of a genotyping chip run and generates genotyping calls for each sample. Software generated scatter plot for 48 samples from the association mapping population for SNPs (a) AX117208516: 3 genotypes AA (green dot) AB (blue dot) and BB (red dot) plus negative control (NTC, black dot) and (b) AX117910693: 2 genotypes AA (green dot) and AB (blue dot). There was good correlation of the genotyping results between the Axiom SNP chip and the Fluidigm SNPType assays, there was only one mismatch for AX117208516 and there were four mismatches for AX117910693 for the 48 sugarcane genotypes.

DNA extractions from the 203 individuals of the selected families has been completed, and all the SNP primers have been designed and delivered. At the time of writing this report, five SNPType assays have been validated on parents of the selected families.

The screening of all the SNPType assays will be completed in 2017. A meeting is planned in the coming months to discuss the results when they are available.

7. PUBLICATIONS

No publications have arisen from the project at this stage.

8. REFERENCES

Ponragdee *et al.*, 2013. Proc. Int. Soc. Sugar Cane Technol.

Terajima *et al.*, 2007. Proc. Int. Soc. Sugar Cane Technol.

Blair and Stirling, Aust. J. Exp. Ag. 2007, 47: 620–634). Text

Bakshi Ram; Sreenivasan, T.V.; Sahi, B.K.; Singh, N. (2001). Introgression of low temperature tolerance and red rot resistance from Erianthus in sugarcane. Euphytica 122: 145-153.

Barry Croft; Shamsul Bhuiyan; Robert Magarey; George Piperidis; Eunice Wong; Priyanka Wickramasinghe; Judi Bull; Mike Cox; Graham Stirling; John Foreman; Phillip Jackson (2015). New sources of resistance to major diseases from wild relatives of sugarcane. Proc Aust Soc Sugar Cane Technol 37 pp. 218-226.

Basnayake, J.; Jackson, P.A.; Inman-Bamber, N.G.; Lakshmanan, P. (2012). Sugarcane for water-limited environments. Genetic variation in cane yield and sugar content in response to water stress. Journal of Experimental Botany 63: 6023-6033.

Hale AL, Viator RP, Veremis JC (2014). Identification of freeze tolerant Saccharum spontaneum accessions through a pot-based study for use in sugarcane germplasm enhancement for adaptation to temperate climates. BiomBioenergy 61:53-57.

Kahn, N.A., Bedre, R., Parco, A., Bernola, L., Hale, A.L., Kimbeng, C., POntif, M., Baisakh, N. (2013). Identification of cold-responsive genes in energycane for their use in genetic diversity analysis and future functional marker development. Plant Science. 211:122-131.

Tanksley, S.D. and Nelson J.C. (1996). Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm to elite breeding lines. Theor Appl Genet 92:191-203.

Viator, R.P., White, P.M. Jr., Hale, A.L., Waguespack, H.L. (2012). Screening for tolerance to periodic flooding for cane grown for sucrose and bioenergy. Biomass and Bioenergy. 44:56-63. Online: http://dx.doi.org/10.1016/j.biombioe.2012.04.007.

9. APPENDICES

9.1. Appendix 1 - METADATA DISCLOSURE

Table 13: Metadata disclosure 1

Data	All trial details, planting and harvest data; clone names and pedigrees
Stored Location	SRA, SPIDNet
Access	Available to SRA staff and restricted access to external staff.
Contact	Dr George Piperidis

Table 14 Metadata disclosure 2

Data	SNP data
Stored Location	CSIRO
Access	Unknown/Restricted
Contact	Dr Karen Aitken

9.2. Appendix 2 - Article in the Spring edition of Cane Connection

Project details

Key Focus Area:
Optimally adapted varieties, plant breeding and release

Project name
Advancing yield, disease resistance and ratooning by exploiting new sources of genetic variability from wild relatives of sugarcane

Project number
2014/053

Principal provider
SRA

Project leader
George Piperidis

RESEARCH

Bringing a bit more mongrel into sugarcane varieties

A research project is looking at the role of introgression in improving Australian sugarcane varieties, by bringing in genetics from wild relatives of sugarcane. ***By Rod Fletcher, Adoption Officer, Meringa***

The challenge accepted by Sugar Research Australia (SRA) is to deliver more profit to the Australian sugarcane industry.

One of the approaches SRA leads is exploring ways to manipulate the current commercial sugarcane varieties to improve genetic gain.

SRA currently uses plant breeding for this process, where plant breeders select elite parents to cross together to try and produce new varieties that have the most desirable traits.

This method has been used successfully for several decades by sugarcane breeders and has produced incremental gains in cane yield, CCS, disease resistance, and ratooning.

Over these years, the genetic potential of the breeding program has relied on two main ancestral species from the *Saccharum* (S) family: *S. officinarum*, which is the main source of high sucrose levels; and *S. spontaneum*, which gives it vigour and hardiness.

Dr George Piperidis (Leader Crossing and Selection, SRA) is the Chief Investigator of an SRA funded project that proposes to improve the sugarcane breeding program. The title of the project is *Advancing yield, disease resistance and ratooning by exploiting new sources of genetic variability from wild relatives of sugarcane*.

The project objectives are to:

- Identify and exploit new sources of genes for better ratooning, resistance to nematodes and Pachymetra root rot.
- Examine (ground-truth) identified clones that were resistant to nematodes and Pachymetra root rot in controlled-environment screening tests in field-based trials.
- Select clones with higher yield and ratooning ability under harsh conditions.
- Establish a clear path for future direction and investment in introgression.

This project aims to improve the current sugarcane breeding program by introducing new desirable genes from wild relatives of sugarcane, improving genetic variability, resulting in better yields, disease resistance and ratooning from SRA varieties.

This project and the research findings were communicated at a recent series of Research Forums held throughout the industry. At these forums, Dr Piperidis described the project goals at a grass-roots level. "Think of the current commercial sugarcane varieties as having genetics from a "princess cane" (*Saccharum officinarum*) and a "mongrel" (*S. spontaneum*). What the project hopes to do is to introduce more mongrel from the *Erianthus* (E) and *S. spontaneum* families into the genetic potential," he said.

Above: Lawrence DiBella stands next to sugarcane from the introgression breeding program in Florida, USA (left) and from the SRA introgression breeding trial in the Herbert (right). The question is: whose stalk is bigger?

9.3 Appendix 3 - Full list of analysed yield trial data

Clone	Average TCH	Average CCS	Average Fibre	Average rEGV
KQ228*	97	16.74	13.24	10.3
Q208[A]*	120	15.58	13.52	10.29
KQ08-1040	118	14.87	14.39	10.2
KQ08-1347	124	14.39	12.27	10.1
Q200[A]*	105	15.81	15.62	10.05
Q240[A]*	106	15.47	12.8	10
KQ08-1012	106	15.19	13.59	9.87
KQ08-2859	100	15.29	12.97	9.83
KQB07-23976	123	13.49	12.24	9.78
KQB09-23126	123	14.15	14.79	9.75
Q232[A]*	102	15.19	13.67	9.75
KQ08-1158	125	13.08	12.91	9.72
KQB07-24815	134	12.43	13.72	9.71
KQ08-1391	95	15.33	13.3	9.7
KQ08-1031	105	14.9	12.3	9.68
KQB07-33647	100	15.14	12.08	9.63
KQB09-20048	114	14.6	13.63	9.59
KQB07-23989	107	14.5	12.17	9.57
KQ08-1329	97	15.14	12.88	9.57
QBYC06-30390	115	14.11	17.75	9.56
KQB07-24524	102	14.81	16.18	9.55
KQ09-1736	101	14.71	14.94	9.52
KQ08-1389	92	15.45	12.9	9.5
KQ08-1018	108	14.68	14.88	9.4
KQB07-34350	114	13.55	14.28	9.39
KQ08-2664	91	15.13	15.36	9.36
KQ08-1201	97	14.58	13.76	9.35
KQ08-1028	105	13.95	15.09	9.34
KQB09-20485	102	14.31	13.17	9.33
KQ08-2744	95	14.36	12.68	9.32
KQ08-1231	120	12.51	15.56	9.29
KQB09-30107	89	15.46	12.73	9.26
KQB07-23930	102	13.86	15.65	9.2
KQB08-32673	90	14.88	16.24	9.18
KQ08-1014	109	13.84	15.02	9.14
KQB09-20290	95	14.27	13.63	9.14
KQB09-30110	87	14.92	13.32	9.13
KQ08-2552	115	12.47	14.02	9.13
KQ08-1093	100	13.53	13.42	9.09
KQ08-1073	111	13.22	13.67	9.07
KQ08-1047	105	13.56	16.86	9.04

Clone	Average TCH	Average CCS	Average Fibre	Average rEGV
KQ08-1306	97	14.09	14.1	9.03
KQ08-1287	86	14.88	14.23	9.01
KQ08-1134	109	12.75	16.38	8.99
KQ08-1140	73	15.32	13.34	8.95
KQB07-34851	94	14.17	12.65	8.95
KQ08-1053	94	13.96	15.25	8.93
KQB09-20432	110	12.7	15.57	8.92
KQB09-20481	81	15	13.02	8.85
KQ08-2850	91	13.71	14.71	8.8
KQ08-2915	77	15.27	14.37	8.79
KQB09-20624	97	13.97	13.64	8.74
KQB09-20434	92	14.21	13.86	8.67
KQB09-30117	127	12.56	16.37	8.62
KQB09-20424	90	14.19	14.65	8.59
KQ08-1359	104	12.45	14.43	8.57
KQ08-2546	95	12.44	15.56	8.52
KQ08-1296	76	14.48	16.17	8.5
KQB07-24644	97	12.52	15.62	8.48
KQ08-1348	99	12.26	14.86	8.45
QBYC06-30376	84	13.66	15.47	8.37
KQ08-3482	83	14.13	13.59	8.36
KQB07-23981	102	11.65	17.24	8.29
KQB07-24260	102	11.82	16.57	8.24
KQB07-34872	112	11.15	16.76	8.23
KQB07-24619	91	12.78	15.41	8.19
KQ08-6014	90	12.6	16.24	8.15
KQB09-20328	88	12.73	15.56	8.11
KQB07-33307	101	11.14	19.3	8.1
QBYN04-26166	94	11.83	16.25	8.07
KQB09-20497	98	11.57	15.68	8.06
QBYN04-26127	86	11.94	12.65	7.79
QBYN04-22021	98	11.59	19.73	7.78
KQB07-23980	110	9.7	17.11	7.65
KQB09-30026	95	9.8	16.07	7.17
QBYN05-20563	88	8.42	17.4	6.8
MQB62-10262	95	7.3	20.79	6.69
MQB89-12412	74	10.21	20.56	6.65
MQB89-12420	97	8.31	19.79	6.61
MQB89-12522	91	8	16.6	6.33
MQB88-10825	106	6.79	18.82	6.33
MQB89-12143	68	9.59	19.77	6.18

9.4. Appendix 4- List of introgression clones and number of plots in Yield and Nematode trials

Clone	Clone Type	Female	Male	Yield trials				Nematode trials		
				VIC15-61	VIC15-62	FAR15-61	BWR15-312	VIC15-63	RAC15-61	NEM15-F1
KQ08-1012	Ea BC3	QN80-3425	QBYC06-30376	2	2	2	2			
KQ08-1014	Ea BC3	QN80-3425	QBYC06-30376	2	2					
KQ08-1018	Ea BC3	Q208	QBYC06-30305	2	2					
KQ08-1028	Ea BC3	Q208	QBYC06-30376	2		2	2			
KQ08-1031	Ea BC3	Q208	QBYC06-30376	2	2	2	2			
KQ08-1040	Ea BC3	QN80-3425	QBYC06-30260	2		2	2			
KQ08-1047	Ea BC3	QN80-3425	QBYC06-30138	2	2			10	10	
KQ08-1053	Ea BC3	QN80-3425	QBYC06-30138	2	2	2				
KQ08-1073	Ea BC3	Q208[A]	QBYC06-30296	2	2		2			
KQ08-1093	Ea BC3	Q208[A]	QBYC06-30305	2		2	1			
KQ08-1134	Ea BC3	Q208[A]	QBYC06-30376	2						
KQ08-1140	Ea BC3	Q208[A]	QBYC06-30280	2		2	1			
KQ08-1158	Ea BC3	QN80-3425	QBYC06-30296	2	2	2	2			
KQ08-1201	Ea BC3	QN80-3425	QBYC06-30376	2	2	2				
KQ08-1231	Ea BC3	QN80-3425	QBYC06-30376	2					10	
KQ08-1287	Ea BC3	Q208[A]	QBYC06-30296	2	2	2	2			
KQ08-1296	Ea BC3	MQ239	QBYC06-30413	2	2	2	2			
KQ08-1306	Ea BC3	QN80-3425	QBYC06-30376	2	2	2	2			
KQ08-1329	Ea BC3	Q208[A]	QBYC06-30376	2	2	2	2			
KQ08-1347	Ea BC3	QN80-3425	QBYC06-30415	2	2			10	10	8
KQ08-1348	Ea BC3	QN80-3425	QBYC06-30415	2				10	10	8
KQ08-1359	Ea BC3	QN80-3425	QBYC06-30296	2	2			10	10	8
KQ08-1389	Ea BC3	QN80-3425	QBYC06-30376	2	2	2	2			
KQ08-1391	Ea BC3	QN80-3425	QBYC06-30376	2		2				
KQ08-2546	Ea BC3	QN80-3425	QBYC06-30138	2			2			
KQ08-2552	Ea BC3	Q208[A]	QBYC06-30296	2			2			
KQ08-2664	Ea BC3	Q208[A]	QBYC06-30376	2	2	2				
KQ08-2744	Ea BC3	QN80-3425	QBYC06-30296	2		2	2			
KQ08-2850	Ea BC3	QBYC06-30101	MQ239	2			2			
KQ08-2859	Ea BC3	Q208[A]	QBYC06-30296	2		2				
KQ08-2915	Ea BC3	QBYC06-30101	N29	2	2	2	2			

Clone	Clone Type	Female	Male	Yield trials				Nematode trials		
				VIC15-61	VIC15-62	FAR15-61	BWR15-312	VIC15-63	RAC15-61	NEM15-F1
KQ08-3482	Ss BC3	Q208[A]	MQ239	2	2	2	2			
KQ08-6014	Ea BC3	QN80-3425	QBYC06-30415	2	2			10	10	8
KQ09-1736	Ea BC3	Q208[A]	QBYC06-30376	2	2	2	2			
KQB07-23930	Ss BC1	Q171[A]	QBYN04-10357	2	2	2	2			
KQB07-23976	Ss BC2	Q208[A]	MQB88-10825	2	2	2	2			
KQB07-23980	Ss BC2	Q208[A]	MQB88-10825	2	2	2	2			
KQB07-23981	Ss BC2	Q208[A]	MQB88-10825	2	2	2	2			
KQB07-23989	Ss BC2	Q208[A]	MQB89-12212	2	2	2				
KQB07-24260	Ss BC1	Q208[A]	QBYN04-10357	2	2	2	2			
KQB07-24524	Ss BC1	Q171[A]	QBYN04-10357	2	2	2				
KQB07-24619	Ss BC1	KQ228[A]	MQB88-10850	2	2	2				
KQB07-24644	Sr BC1	KQ228[A]	MQB89-12336	2	2	6	2			
KQB07-24815	Ss BC1	QA89-3305	QBYC05-10199	2			2			
KQB07-33307	Ss BC2	QN80-3425	QBYN04-20250	2		2	2			
KQB07-33647	Ss BC2	QN80-3425	QBYN04-26272	2	2	2				
KQB07-34350	Ss BC2	Q208[A]	QBYN04-26272	2	2	2	2			
KQB07-34851	Ss BC2	QBYN05-20830	KQ228	2	2	2	2			
KQB07-34872	Ss BC2	Q208[A]	QBYC05-20681	2		2	2			
KQB08-32673	Ss BC2	QBYN04-26073	QC91-580	2	2	2	2			
KQB09-20048	Ss BC1	KQ228[A]	QBYC04-10577	2	2			10	10	8
KQB09-20290	Ss BC1	Q208[A]	QBYC04-10865	2	2	2				
KQB09-20328	Ss BC1	KQ228[A]	QBYN04-10472	2	2	2				
KQB09-20424	Ss BC1	KQ228[A]	QBYN05-10390	2	2	2	2			
KQB09-20432	Ss BC1	KQ228[A]	QBYN05-10390	2	2	2	2			
KQB09-20434	Ss BC1	Q208[A]	QBYN05-10420	2	2	2	2			
KQB09-20481	Ss BC1	KQ228[A]	QBYC04-10577	2	2	2	2			
KQB09-20485	Ss BC1	KQ228[A]	QBYC04-10577	2	2	2				
KQB09-20497	Ss BC1	KQ228[A]	QBYC04-10577	2	2	2	2			
KQB09-20624	Ss BC1	KQ228[A]	QBYN05-10420	2	2	2	2			
KQB09-23126	Ep BC2	QC90-353	MQB89-12216	2		2	2			
KQB09-30026	Ss BC2	QBYN05-20643	QBYN05-10383	2	2	2	2			
KQB09-30107	Ss BC2	QBYC05-20721	QC91-580	2	2	2				
KQB09-30110	Ss BC2	QBYC05-20721	QC91-580	2	2	2	2			

Clone	Clone Type	Female	Male	Yield trials				Nematode trials		
				VIC15-61	VIC15-62	FAR15-61	BWR15-312	VIC15-63	RAC15-61	NEM15-F1
KQB09-30117	Ss BC2	C05-20721	QC91-580	2					10	8
MQB62-10262	Ss F1	BADILA	US56-13-7	2			2			
MQB88-10825	Ss BC1	MQB72-12011	Unknown	2	2			10	10	8
MQB89-12143	Ep BC1	MQ81-711	LF65-3660	2	2	2	2			
MQB89-12412	Ep BC1	LF65-3660	QN58-829	2	2	2	2			
MQB89-12420	Ep BC1	MQ84-2B	Q117	2	2	2	2			
MQB89-12522	Ss BC2	MQB88-10800	MQB88-10909	2	2	2	2			
QBYC06-30376	Ea BC2	ROC20	YCE01-102	2	2			10	10	8
QBYC06-30390	Ea BC2	ROC20	YCE01-102	2	2			10	10	
QBYN04-22021	Ss BC2	F172	YN2002-333	2	2					
QBYN04-26127	Ss BC1	ROC25	YN2002-356	2	2	2	2			
QBYN04-26166	Ss BC1	ROC25	YN2002-356	2	2	2	2			
QBYN05-20563	Ss BC1	YN2000-113	YueTang93-159	2				10	10	8