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**FINAL REPORT – SRDC PROJECT BSS250  
IMPROVED SELECTION SYSTEMS AND DATA ANALYSIS  
IN SUGARCANE-BREEDING PROGRAMS**

by

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**SD06003**

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## SUMMARY

Selection represents a costly and critical part of sugarcane-breeding programs. Selection is complicated by the ranking of phenotypes (ie the measured value of a clone in a trial) being often strongly influenced by non-genetic components, such as experimental error effects, effects of competition in small plots (especially for cane yield), and genotype by environment interactions. Maximising the effectiveness and efficiency of selection is an important goal of breeders.

Australian sugarcane breeding programs currently operate using a three-phase selection system. Following a first stage involving selection among families, clones are evaluated in small single-row plots in a single trial. Clones selected from here are then planted in multi-row final assessment trials (FATs) at several sites in the targeted region.

It is well recognised that results from small-plot trials conducted prior to the FAT phase may be unreliable for predicting true genetic value of clones, particularly for cane yield. In a previous SRDC funded project (CS017), it was suggested that a high weighting should be placed on CCS in small plot trials, because of the relatively higher reliability of this measurement in small plots, and because of its high economic value. Other research has also been done recently aiming to account for the effects of competition on cane yield in single row plot trials. Given this background, project BSS250 was developed to help identify optimal selection system designs, data analysis and selection criteria.

Field trials were conducted between 2000 and 2005 in the Burdekin and Central regions. In each region, sub-samples of the same starting populations of clones were entered into two alternative selection-system designs. The first design was similar to that currently used in sugarcane-breeding programs, whilst the second followed a system similar to that suggested in CS017. In the second design, a larger number of clones were screened for CCS and visual grade in replicated smaller (one row by 5 m) plots, prior to selected high-CCS clones being screened in multi-row plot trials, and a small number of selections from this phase being evaluated in FATs. In the field trials, key genetic and statistical parameters that affect gains from selection were estimated and realised gains in both selection system designs were measured by differences between selected and random (unselected clones). Modelling of selection systems was then done to help identify optimised selection systems. A modelling approach to optimisation is necessary because of the impracticality of empirically testing all options in selection system design (eg selection criteria, selection intensity, replicate number, etc, in each stage). The model and predictions were firstly validated by comparing predicted gains with realised gains and then used to predict the consequences of alternative system designs.

Realised gains for economic value were similar in both selection systems. However, it was clear from estimates of genetic and statistical parameters that selection criteria used within both systems in the first clonal selection phase were sub-optimal. Optimal selection indices that maximise gain in economic value from selection in single-row plots in the first phase of clonal selection were determined. While these indices, as expected, weight CCS heavily (eg  $55 \times \text{CCS} + 1 \times \text{TCH}$  for 1 row by 10 m plots in the Burdekin), it is clearly important that cane yield be part of the index in all stages, contrary to a suggestion made from CS017. This was particularly the case in the Burdekin where a consistent strong negative genetic correlation (of around -0.5) between CCS and cane yield was

observed, resulting in a strong negative response in cane yield with very intense selection pressure for CCS alone. Prediction of cane yield in final-stage trials based on single-row-plot data was not improved by a statistical model attempting to account for competition effects.

Predicted gains in economic value from selection based on an optimal index of CCS and visual grade in single row by 5 m plots in the plant crop were similar to those predicted from an optimal index of CCS and cane yield in half the number of single row by 10 m plots across plant and ratoon crops. The latter arrangement is currently used in Australian sugarcane breeding programs. This suggests the use of 5 m plots compared with 10 m plots could save a year in selection process. However, there is a high level of confidence for this prediction only for the Burdekin, with estimates of parameters in the single-row plots in the Central region being compromised because of (unusually) poor germination in some of single-row-plot trials there. In the Burdekin, there is sufficient evidence to support larger-scale implementation, but comparison in further seasonal conditions as part of routine selection operations in core programs is still recommended. It is recommended that gains from 5 m plots be evaluated in regions other than the Burdekin as part of core breeding program operations, and compared with 10 m plots, before large-scale implementation.

Currently in Australian sugarcane breeding programs in each region around 100 clones selected from one-row plot trials are evaluated in a single phase of multi-row trials across several locations, prior to possible release of elite clones. However, it was predicted that a two-phase process of evaluating clones in multi-row plots would provide significantly more gains and should be adopted routinely in sugarcane-breeding programs. This prediction was found to apply for both Burdekin and Central regions, was robust over a wide range of assumptions, and expected to have general application. A two-phase system of evaluation in multi-row plots should first involve testing a larger number of clones than the current system (about 150 instead of approximately 100) across four sites with one replicate per site. The best 25 or so clones from these, mostly identified after the plant-crop results, should be evaluated in a further four trials with two replicates per site. Such a system could be implemented by planting four trials per year with around 200 plots of experimental clones (the same as currently in most regions), but with each containing 150 new clones (one replicate per site) and 25 elite clones (two replicates per site). The additional genetic gains are predicted due to (i) a larger number of clones being evaluated reasonably accurately (in multi-row plots and multi-site trials), and (ii) more accurate comparisons among the best clones and standard cultivars before recommendations on commercial use (8 trials and 3 crop-years in each trial).

## 1.0 BACKGROUND

Selection represents a major part of effort in all breeding programs including sugarcane-breeding programs. Following crossing and production of new genotypes as seedlings, selection among the new recombinants is necessary to identify candidate clones for release as new commercial cultivars or as parents for the next breeding cycle. In the Australian sugarcane-breeding program, the traits primarily targeted in all phases of selection are cane yield and CCS. Disease resistance (to 4-5 diseases depending on region), sugar quality, and fibre content and characteristics are also monitored in the latter phases of these programs to ensure that new cultivars do not have unacceptable performance for these traits.

Selection is complicated by the ranking among phenotypes (ie the observed or measured performance for traits of interest) of a set of genotypes under test being influenced by non-genetic components, with the degree of 'interference' depending on the type of trial involved. These non-genetic components include experimental error effects, effects of competition in small plots (especially for cane yield), genotype by environment interactions, and genetic correlations (favourable or unfavourable) among important traits. The variance of the non-genetic components in many cases is greater than the genetic variance, especially in small-plot trials where both experimental error variance and effects of competition in sugarcane are large. Selection systems aim to select the best genotypes as efficiently as possible while only being able to observe the phenotype in each phase of selection.

Maximising the effectiveness and efficiency of selection is an important goal of breeders because:

- (i) the performance of the breeding program both for short term cultivar release, as well as longer term rates of genetic gains through effective selection of the best parents, is completely dependent on how effective the selection system used is, and
- (ii) a very high proportion of total resources spent in breeding programs is allocated to selection activities.

In all sugarcane-breeding programs multi-stage selection systems operate. The Australian sugarcane-breeding program currently operates on a 3-stage system. This involves firstly screening among a large number (usually greater than 10,000 per region) seedlings generated from annual crossing of parents. Family selection is used in this stage. Seedlings are planted as replicated family plots, and the plots of families (rather than individual seedlings) are measured initially in the plant crop. Elite families are identified, based on a combination of cane yield and CCS measurements. In the ratoon crop, individual seedlings from elite families are selected, based on visual appearance (considering mainly cane yield and plant health). Selected clones are then planted to single-row plots (generally 10 m long) and cane yield and CCS measured in the plant crop. Because cane yield is measured destructively by mechanical harvesting and weighing, good performing clones must be taken from the ratoon crop. Normally a further measurement of cane yield and CCS is done in the ratoon crop for the best (~20-30%) performing clones in the plant crop. The selected clones are propagated for 1 year to produce enough seed cane for establishing in final assessment trials (FATs) the following year. The FATs are normally established at about four sites in each region, and use 4-row by 10-m plots with two replicates per site. Cane yield and CCS are measured in

plant, first-ratoon and second-ratoon crops. Fibre content is measured on a subset of trials, and concurrent screening for disease resistance and sugar quality occurs for promising clones as they advance through the ratoon crops.

It is recognised that a weakness of the current selection system is the unreliability of small-plot trials conducted prior to the FAT phase. This is due to a combination of competition effects, GE interactions and experimental errors. The presence of these effects, which are often large, means that results obtained in a single early stage trial can poorly represent a clone's true performance. Because of this, FATs often include a large proportion of clones that are well below performance levels required for commercial value or as elite parental material, despite favourable comparisons with standard clones in the single-row plot trials. Conversely, it is possible that some elite clones are missed from selection. Inclusion of poor clones in the FAT trials represents a large drain on resources. However, a reduction in the number of clones entering the FATs would mean that accurate data for cane yield would be obtained for an even lower proportion of clones generated in the breeding program, reducing opportunity for identifying rare elite genotypes with high economic value. Overall, reduction in the number of clones evaluated in multi-row plots, therefore, is not seen as a desirable move.

A previous project (CS017) examined limitations to genetic gains in the first clonal stage of selection. Competition effects for cane yield were such that the genetic correlation between cane yield in single-row plots and cane yield in bordered rows in large plots in the same environment were about 0.5, while for CCS the corresponding value was greater than 0.9. This research suggested that a high weighting should be placed on CCS in small-plot trials, because of the relative reliability of this measurement for predicting performance in pure stands, and also because of its relatively high economic value (Jackson and McRae 2001). In fact, selection in early stage trials could focus on CCS alone and this may give maximum genetic gains in economic value. It was further suggested that an optimal selection system could involve evaluating large number of clones in small plots (eg 5 m by 1 row) for CCS, then transfer of a moderate number of high CCS clones (eg 200) into a multi-row plot trial for accurate measurement of cane yield and economic value, prior to a final stage of testing involving fewer clones (eg 25) evaluated in FATs, similar to those currently used.

Other concurrent research aimed to develop statistical models that can better account for the effects of competition in single-row plot trials. It was possible that such analyses could be used to obtain better estimates of cane yield in single-row plots and, therefore, facilitate a greater weighting to be placed on cane yield, and influence decisions regarding optimal selection criteria and system designs.

With this background in mind, BSS250 was developed to explore alternative selection-system designs, with the specific objectives given in Section 2. There were two main aspects of this project and this report. Firstly, field trials were conducted (in the Burdekin and Central regions) to estimate key genetic and statistical parameters that affect gains from selection and alternative selection-system designs. The field trials were conducted between 2000 and 2005. These were based around entering the same populations of clones into two alternative selection-system designs. The first design was similar to that currently used in the Australian sugarcane-breeding program, whilst the second followed a

system similar to that suggested in CS017. Realised gains from selection in practice were also measured.

Secondly, we modelled selection systems to help identify an optimal selection system. A modelling approach to this problem is necessary because:

- (i) the large (infinite) number of possible design options (eg number of stages, selection criteria, selection intensity, replicate number, etc, in each stage);
- (ii) it would be impractical and prohibitively costly to compare a large number of options empirically, and
- (iii) empirical testing may be affected by unusual trials (eg high experimental error variation in any single trial).

Further, we considered a stochastic (rather than analytical) model appropriate because of:

- (i) the complexity in accommodating multi-stages and inter-dependency between stages in analytical models (optimising one stage at a time may not necessarily lead to an optimal overall system), and
- (ii) the mathematical difficulties in determining the effects of selection on variance and population distributions in subsequent stages.

These are both important factors in affecting gains through a multi-stage selection system and to consider in optimising such systems.

At the commencement of this project, we knew that it was unlikely that either of the selection-system treatments imposed in the field trials would represent precisely an optimal system, given the number of parameters that set the design of a selection system (eg selection criteria at each stage, replicate number per trial in each stage, selection intensity at each stage, number of test environments at each stage, etc.). This became even more obvious as the project unfolded, particularly in terms of the selection criteria used. A stochastic selection-simulation model was developed and parameterised using estimates of key genetic and statistical parameters affecting gain from selection (eg starting population genetic variances for cane yield and CCS, error variances for different plot sizes, GE variance, competition variance etc.) obtained in this project as part of objective 1. The model and predictions were validated by comparing predicted gains with realised gains in the field trials, and accounting for any differences. The model was then used to evaluate modifications and refinements in alternative system designs. Because the model predictions are dependent on the assumptions used for the genetic and statistical parameters, the sensitivity of the results (particularly recommendations on best system designs) to changes in these parameters (especially those for which there was most uncertainty) was examined.

Based on the model as well as practical considerations, recommendations are made in relation to implementation of selection-system designs considered to likely maximise gains in the current Australian sugarcane-breeding program, as well as areas where some further inexpensive research, done in association with ongoing core breeding program activity, could be useful to further validate or refine current recommendations.

The relationship between outputs from this project and projects CTA028 (mega GE project) and BSS267 (Maximising whole-of-industry benefits from the Australian sugarcane improvement program through an optimal genetic evaluation system) are

briefly discussed in the recommendations section, since there is some areas of inter-relationship between all three projects.

## **2.0 OBJECTIVES**

The project had three linked objectives, each of which was achieved as summarised below.

***Objective 1 - To compare the selection systems currently used in Australian sugarcane breeding programs with an alternative structure recommended from recent research (primarily SRDC project CS017), in terms of realised selection gains.***

This was achieved through an extensive set of field trials in the Burdekin and Central regions from 2000 to 2005. Key genetic and statistical parameters affecting genetic gains for the key traits cane yield and CCS arising from selection were measured in these trials, in particular using sets of random (unselected clones). Realised genetic gains arising from selection in two different selection systems were measured from differences between selected and random clones.

***Objective 2 - To compare realised selection gains achieved using traditional data analysis procedures with gains achieved using more sophisticated statistical models developed in recent research (primarily SRDC project UQ023).***

This was achieved, but, in place of the UQ023 model, we used the model and analysis procedures developed by Jo Stringer as part of her PhD thesis. This model (like UQ023) was designed to incorporate joint effects of spatial variation within trials and competition effects.

***Objective 3 - From 1 and 2, to determine optimal selection protocols (including specification of selection system structure, data analysis protocols, and selection criteria) for use in core breeding programs that maximise gains per unit resource input.***

This was achieved. A key output developed in the project (in association with the PhD thesis of Hu Fengduo) was a stochastic simulation model designed to simulate the process of selection in a sugarcane-breeding program. A modelling approach is needed to determine optimal selection system designs because of reasons explained in the background section. Recommendations were made (outlined below) on selection system designs considered likely to maximise gains from selection based on the results from the model predictions and on practical considerations.

### **3.0 METHODOLOGY**

#### **3.1 Field trials**

Field trials were conducted in the Burdekin and Central regions. Clones selected from stage 1 trials in the then breeding programs conducted by CSR Limited (Burdekin region) and BSES Limited (Central region) were allocated into two separate selection systems. While there were different clones allocated to each system, these were all drawn at random from the same original starting population in each region. These starting populations are considered representative of those generated annually in core breeding programs targeting these regions.

The clones were established in clonal assessment trials (S2) in both regions in 2000 at the start of BSS250. In each region, two alternative selection-system designs were conducted. The first, SS1 is similar to that conducted presently in the Australian sugarcane-breeding program. This consists of evaluating clones in single-row by 10-m plots for CCS and cane yield. Assessment is generally based on Net Merit Grade (NMG), based on cane yield and CCS measurement in the plant crop. Selections are then propagated for 1 year then planted into FATs at four locations and evaluated in plant, first-ratoon and second-ratoon crops. Plot sizes in the FAT trials are four rows by 10 m, with measurements being made on the middle two rows. The second system, SS2, represented a system following that suggested by Jackson and McRae (2001) arising from research done in CS017. This consisted of evaluating clones in replicated 5-m by 1-row plots, with selection based heavily on CCS and to a lesser degree on visual grade. Because cane is not harvested to measure yield, the selections are transferred to a replicated larger plot (4 rows by 10 m) trial at one site for measurement of cane yield and CCS. A smaller number of selections from this trial (compared with SS1) are then propagated and planted to FAT trials in the same way as described for SS1.

In all trials following S2, a set of random clones was included, with these being taken at random from the clones evaluated in S2. In the Central region, there were 30 random clones, whilst in the Burdekin region there were 48 random clones.

Details of the trials planted and flow of clones in the project for the Central and Burdekin regions are shown in Tables 1 and 2. In the Burdekin, there was a problem with poor establishment of the initial S2 trial planted in 2000. It was decided to abandon this trial and replant the following year. With concurrent propagation and other modifications, the FAT trials were planted without any year's delay in the Burdekin. The additional quantity of planting material that arose from this delay allowed the inclusion of the same set of random clones in S2 trials in both SS1 and SS2 (in the Central region, the random clones were only in the SS2 S2 trials) and allowed all SS1 clones to be replicated in the SS2 trial. This allowed for better estimates of error, heritability and genetic correlations, which allowed for more powerful analysis and interpretation of results for the Burdekin region component.

The field trials were used to:

- (i) Obtain estimates of genetic and statistical parameters that affect gains from selection. These include genetic variance in the starting populations, error variance in different trial designs, GE variance, and genetic correlations between CCS and cane yield; and
- (ii) Obtain estimates of realised gains from selection. Realised gains were measured in stages following S2 from the difference in performance between the group of selected clones (from each of SS1 and SS2) and the group of random clones. These realised gains may be compared against gains predicted by modelling.

**Table 1** Flow chart showing the experimental design used in Central region

Stage	Year	Crop class	Activities
1	1998		Plant 250~300 Families x 2Reps x 20 seedlings/Rep
	1999	P	Harvest families (weight/CCS)
2	2000	1R	Select 'best' clones (using Brix/visual grade) from 'best' families (using NMG) <b>Start of project BSS250</b>
	2000		SS1 Plant trials 1 rep x 1row x 10m plot (1058 clones+standards) (Trial Code: <b>RAC00-32 &amp; RAC00-34</b> )
2	2001	P	Harvest (measure TCH, CCS) all plots Select tentative 400 selections based on NMG
	2001		SS2 Plant trials 2 rep x 1row x 5m plot (1084 clones+standards) (Trial Code: <b>RAC00-33 &amp; RAC00-35</b> ) Harvest (measure CCS and visual grade) 105 clones selected on CCS plus visual grade and 30 clones selected randomly Plant trials (128 clones x 2rep x 4row x 10m) Trial Code <b>RAC01-21 &amp; RAC01-22</b>
3	2002	P (SS2)	Harvest (measure TCH/CCS) and make final selections 132 clones advanced to next stage (66 from RAC00-32 and 46 from RAC00-34)
	2002		Harvest (measure CCS/TCH) and make selection 61 clones advanced to next stage (48 selected by NMG, 30 selected randomly, 7 in common)
3	2003		Propagate 193 selected clones after CS-LHWT (20m/clone) Plant 6 substitution Yield Trials (SYT) Selected clones from each of SS1 (RAC00-32 and RAC00-34) and SS2 (RAC01-21 and RAC01-22) allocated into 2 sub-sets (each subset containing clones from SS1 and SS2) and each sub-set planted in 3 different sites or milling areas. Each clone planted in 2 rep x 4 rows x 10m plot
	2004	P	Harvest plant crop (measure TCH, CCS)
	2005	1R	Harvest 1 <sup>st</sup> ratoon crop (measure TCH, CCS)
	2006		<b>(End of the project BSS0250)</b>

**Table 2** Flow chart showing the experimental design in the Burdekin region

Stage	Year	Crop class	Activities
<b>1</b>	<b>1998</b>		Plant 200 families x 4 replicates x 20 seedlings per replicate
	<b>1999</b>	<b>P</b>	Harvest families (weight/CCS)
	<b>2000</b>	<b>1R</b>	Select 'best' clones (using visual appearance) from 'best' families (using CCS and cane yield)
<b>Start of project BSS250</b>			
<b>2</b>	<b>2000</b>		Plant trials at agricultural college (Clare) 2 rep x 1row x 10m plot Due to the poor germination, trials were not continued, and replanted in 2001 at Kalamia (resulting changes in timetable approved by SRDC)
			Plant trials at agricultural college (Clare) 2 rep x 1row x 5m plot Due to the poor germination, the trials were not continued and replanted in 2001 at Kalamia (resulting changes in timetable approved by SRDC)
	<b>2001</b>		Plant trials at Kalamia 2 rep x 1row x 10m plot (787 clones including 48 random clones same as in SS2)
	<b>2002</b>	<b>P</b>	Harvest (measure TCH, CCS). Select 154 tentative clones and random clones.
<b>Propa- gation (SS1 and SS2) and S3 (just SS2)</b>	<b>2002</b>		154 selected clones hot water treated and propagated into 30 m plots alongside clones propagated from SS2 stage 2 trials.
			Plant trial at Kalamia (114 selected + 48 random clones) x 2 reps x 4 rows x 10m. In addition, 48 random clones also were planted in a single row by 10 meters with 2 replicates.
			Concurrent propagation of 114 selected and 48 random clones into 30 m x 1 row plot for farm trials next year.
	<b>2003</b>		Evaluate 154 tentative selected clones (and random clones) in ratoon crop
<b>Final assess- ment trials</b>	<b>2003</b>		Plant Final assessment trials: consisting of 50 clones selected from SS1, and 25 to 114 clones from SS2 <sup>1</sup>
	<b>2004</b>	<b>P</b>	Harvest plant crop (measure TCH, CCS)
	<b>2005</b>	<b>1R</b>	Harvest 1 <sup>st</sup> ratoon crop (measure TCH, CCS)
	<b>2006</b>		<b>(End of the project BSS0250)</b>

<sup>1</sup> Because selections in the SS2 trial were measured in 2003 in the S3 trial, the first Final assessment trial planted (S4KAL03) included all 114 clones, and the second trial (S4BAN03) had 50 clones and the other two trials (planted after June) had 25 clones.

Cane yield of clones in the S2 trials in the Burdekin comprising one row by 10-m plots was also estimated or predicted using three methods:

- least square means (traditional method);
- BLUPs from analysis using a model that accounts for spatial effects;
- BLUPs from analysis using recently developed (by Jo Stringer and Brian Cullis) methods that model both spatial and competition effects.

CCS in the same plots was estimated or predicted using methods (a) and (b), with method (c) not being used because competition is not important for CCS. Analyses were only done in the Burdekin because the competition effects model requires replication of most clones and this only occurred in the Burdekin. There was considerable overlap in the

groups selected using the different models, but some clones ranked highly by one method but not the others were included in the selections in order to subsequently compare predictions. The results obtained from the different methods were compared for prediction of performance in the FAT trials. This was done initially for the set of random clones and by comparing correlations between predictions (based on S2 data) and actual average performance across the FATs. The best performing clones in the FAT trials based on relative economic value (REV) were also compared for ranking given by the different methods for REV based on S2 data.

### **3.2 Modelling and prediction**

A modelling approach to determining optimal selection systems, as opposed to empirical testing of different systems is necessary because:

- (i) there are a huge (infinite) number of designs of selection systems that can be developed and it is costly and impractical to test through field trials even a moderate number; and
- (ii) empirical testing can be affected by prevailing trial conditions and environments used, which can sometimes be affected by unusual conditions (eg. a poor trial), and, therefore, give results which may not be expected normally.

A selection simulation model was developed to represent the process of selection in a sugarcane-breeding program. The model was parameterised using estimates of parameters obtained during the project, and validated using comparisons of realised and predicted gains. The model was developed mainly by Hu Fengduo under guidance. Details are described in Appendix 4.

The selection simulation model was initially set up to follow the selection regime used in the field trials described above so that we could compare realised and predicted genetic gains. Following this, it was used to explore a range of options for selection system designs and compare these with procedures currently followed in core sugarcane breeding programs in Australia.

## **4.0 RESULTS**

Results are presented in six appendices to this report:

Appendix 1: Field trial results in the Burdekin region.

Appendix 2: Field trial results in the Central region.

Appendix 3: Comparison of methods for analysing S2 data.

Appendix 4: Description of the selection simulation model.

Appendix 5: Comparison of realised and predicted gains.

Appendix 6: Predictions, recommendations and discussion.

Findings of particular interest and practical relevance are:

- For the same level of resource inputs, systems involving three phases of clonal selection (following stage 1) were predicted to provide for greater total gains from selection than the current two-phase selection system following S1. This would

involve one stage of testing clones in single-row plots and two stages of testing clones in multi-row plots. This result applied for both Burdekin and Central regions. It was also robust over a wide range of assumptions on genetic and statistical parameters (eg starting genetic variance, genetic correlation between CCS and cane yield, level of GE interaction), and, therefore, is expected to have general relevance to other regions. Details of such a system are described in our recommendations. The additional genetic gains arising in the recommended system are due to a larger number of clones being evaluated in multi-row, multi-environment trials, and more accurate assessment of the small number of clones being considered for final selection.

- Optimal selection indices that maximise gain in economic value from selection in single-row plots were determined based on estimates obtained in this project on trait heritability, competition effects and genetic correlations between CCS and cane yield. While these indices weight CCS heavily (eg  $55 \cdot \text{CCS} + 1 \cdot \text{TCH}$  for 10-m plots in the Burdekin) as expected, it is clearly important that cane yield be part of selection criteria in all stages. This was particularly the case in the Burdekin where there was a consistent strong negative genetic correlation (-0.5) observed in the Burdekin between CCS and cane yield. This resulted in a negative response in cane yield to intensive selection for CCS.
- Models recently developed to account for competition effects did not provide superior prediction of clone performance in independent multi-row plot trials from performance in single-row plot trials.
- Visual grade based on observations in 5-m plots in the Burdekin had a similar heritability (0.67) as cane yield measured in 10-m plots (0.65). The same result applied in the Central region (visual grade  $H_b = 0.27$ , cane yield  $H_b = 0.24$ ). However, unusually poor germinations and variability of estimates in the S2 trials in the Central region meant that results for these trials were associated with a low level of confidence and the heritabilities for both traits are probably lower than what could be expected in most trials routinely.
- Visual grade in single row by 5-m plots had a genetic correlation with cane yield in multi-row plots in independent trials that was about 25% less on average than cane yield measured in single-row plots.
- As expected, CCS had similar heritabilities in 1 row by 10-m plots (0.75) and 1 row by 5-m plots (0.76) in the Burdekin. However, in the Central region the average heritability in S2 trials involving one row by 5-m plots (0.5 average) was less than in the one row by 10-m plots (0.81 average). However, estimates of error variation were variable in the Central trials, and poor germination could have led to a higher level of experimental error in the 5-m plots.
- An additional experiment not originally planned in the project was conducted in the Burdekin to compare performances of random clones in single-row and four-row plots and compare these with previous research. The results were similar to those reported in CS017 in the Burdekin region. Genetic correlations between cane yield in single-row plots and in the middle two rows of 4-row plots were 0.5 or less, which for CCS genetic correlations were 0.9 or greater. These results showed that in single-row plots, variance due to competition effects are around three times or greater the variance due to genetic variation expected in the absence of competition effects.

- Gains in economic value predicted from selection based on CCS and visual grade in single row by 5-m plots in the plant crop are similar to those from CCS and cane yield measured in single row by 10-m plots in plant and ratoon crops.

## 5.0 OUTPUTS AND RECOMMENDATIONS

The key outputs from this project were:

- estimates of genetic and statistical parameters relevant to modelling selection system designs;
- a selection simulation model that can be used to assess alternative multi-stage selection system designs; and
- results and recommendations on optimising selection system designs.

The outputs lead to the following recommendations:

1. A 2-phase system of multi-row plot testing be adopted in regional breeding programs. This should involve initial testing of about 150 or more clones in singly replicated trials across multiple sites. The top approximately 20-25 clones, based on average performance across trials in the plant-crop sites program, should be then evaluated in a further four trials with two replicates at each site. This would result in little change to the current practice of planting final assessment trials at four sites per year with approximately 100 clones in 2 replicates per site, except with each trial under the new system containing 150 new clones and 25 clones selected in a prior system for elite performance.
2. The use of 5 m plots should be introduced and assessed for practical utility in the core program in the Burdekin. The use of such plots should be evaluated on a smaller scale within core programs in other regions, prior to potential wider scale implementation if similar results to those obtained in this project are repeatable.
3. Appropriate selection indices should be used for all single-row plot trials, which should give appropriate (and heavy) weighting to CCS, but still include cane yield in the index (either measured through mechanical harvesting and weighing or estimated via visual grade). In this project, optimal indices were about 55:1 and 70:1 (CCS:TCH) for 10-m and 5-m plots in the Burdekin, respectively, and about 30:1 for the Central trials. However, these selection indices are sensitive to assumptions on variance components, including competition variance, and the latter was only estimated well in this project in the Burdekin. Some small-scale trials with random clones included in single-row and multi-row trials within the same trial in regions where this has not been done before would be appropriate in developing optimal selection indices for long-term use. In the meantime, best bet assumptions should be used.

Discussion about the results and recommendations from this project has occurred among all sugarcane breeders in the BSES-CSIRO Joint Venture, and who are responsible for all sugarcane breeding activity in Australia. There has been good acceptance of the results and recommendations. Further communication of details will occur through publications

and further discussions at breeder's meetings and conferences - this will be used to help refine and customise recommendations for regions and to investigate key areas requiring further attention through research.

Key statistical and genetic parameters developed in this project should be used as appropriate in project BSS267 (Maximising whole-of-industry benefits from the Australian sugarcane improvement program through an optimal genetic evaluation system). BSS267 is concerned with making optimal use of data in any selection given selection system. BSS250 information will be particularly valuable for determining optimal weighting to give to CCS and cane yield in single-row plots.

Adoption of recommendation 1 may help facilitate adoption of a key recommendation in project CTA028 (mega GxE project). In CTA028, it was recommended that the most elite clones identified in FATs be evaluated directly in FATs in other regions, rather than evaluated in prior single-row plot trials in other regions. However, one concern that some breeders had with this recommendation was that this would use up the limited number of spaces (about 100) available for clones in the annual planting of FATs. The increased number of spaces available for new clones under recommendation 1 would provide for more spaces (about 150) alleviating the numbers pressure. However, the appropriate proportion of spaces that should be allocated to clones selected from FATs in other regions versus clones selected from the local single-row-plot trials is still unclear. This needs to be guided by experience as results from this system is implemented. Initial numbers of about five clones per other region are suggested, and this number could be increased or decreased with time by regional breeders depending on average performance of the different sources of clones.

## **6.0 EXPECTED OUTCOMES**

Adoption of recommendations from this project should result in improved outputs from Australian sugarcane-breeding program in medium (within 5 years) and longer term. The key outputs will be greater genetic gains and better choices on varieties being released.

The increase in gains in the breeding program through alternative selection systems to those currently used are modelled accurately in this project, with these gains being robust to varying a wide range of assumed parameters. However, a complication arises in determining how these predicted increased in relation to initial population means translate to improvements in released varieties and parents, with these improvements likely being greater than improvements in proportion to the initial populations. A 10% improvement from this project over current breeding program in terms of the rate of genetic gains contributed by the Australian sugarcane-breeding program may be a conservative estimate. Assuming an average contribution currently by the breeding program of 1% gain in productivity per year, this would translate to approximately 0.1% *cumulative* improvement in industry revenue with each passing year.

## 7.0 FUTURE RESEARCH NEEDS

Further research is recommended in the following areas:

- (i) Further comparison of gains from use of 1 row by 5-m plots versus 1 row by 10-m plots, particularly in regions apart from the Burdekin. Results from this project suggested the use of 5-m plots with measurements on CCS and visual grade would offer a time saving (1 year) over measuring cane by harvest of 10-m plots (the latter assumed to have half the number of clones and replicates given similar selection system resource inputs). However, reliable parameters to support this prediction were obtained only from the Burdekin region in this project.
- (ii) Use of statistical models attempting to account for competition effects. Results obtained in this project suggested no significant advantage in using such models in terms of realised gains. However, repeated testing of these in other environments, given the efforts devoted to their development to date, would seem justified before drawing final conclusions.

## 8.0 PUBLICATIONS

There have been no papers published to date from this project. A PhD thesis conducted in association with BSS250 (by Hu Fengduo) is expected to be completed within 2 months. We expect one or more scientific papers to be published from work in this thesis and project.

## 9.0 REFERENCES

- Cotterill PP and Dean CA. 1990. Successful Tree Breeding with Index Selection. CSIRO: Melbourne.
- Jackson PA and McRae TA. 2001. Selection of sugarcane clones in small plots: effects of plot size and selection criteria. *Crop Science* 41, 315-322.
- Jackson PA and Morgan TE. 2003. Early stage selection for commercial cane sugar (CCS) in sugarcane clones: effects of time of sampling and irrigation. *Australian Journal of Agricultural Research* 54, 389-396.

## APPENDIX 1 - Field trial results from the Burdekin region

### A1.1 Analyses of variance in stage 2 trials

Results from analysis of variance for nearly all trials indicated that adequate precision was obtained (Table A1.1). Broad-sense heritabilities for most trials were moderate to high (greater than 0.6) for CCS, cane yield and visual ratings.

**Table A1.1 Genetic variance ( $\sigma_g^2$ ) and error variance ( $\sigma_e^2$ ) in each field (sub-trial) for CCS and cane yield (TCH) in the stage 2 trials in selection systems 1 and 2 (SS1 and SS2) for CCS and cane yield (SS1) and visual rating (VR) (SS2). Standard errors for variance components are given in parentheses**

Field	Selection system	CCS				Cane yield (t/ha) (SS1) or visual grade (SS2)			
		Mean	$\sigma_g^2$	$\sigma_e^2$	H	Mean	$\sigma_g^2$	$\sigma_e^2$	H
56A	SS1	12.18	1.55(0.46)	1.27(0.26)	0.71	138.0	736.4(247)	843.4(172)	0.64
	SS2	4.20	1.58(0.28)	1.19(0.14)	0.73	4.32	0.55(0.10)	0.42(0.05)	0.72
60P	SS1	14.10	0.69(0.17)	0.29(0.06)	0.83	152.3	888.6(232)	430.5(93)	0.81
	SS2	2.46	1.54(0.20)	0.81(0.08)	0.79	3.84	1.17(0.17)	0.95(0.11)	0.71
62A	SS1	11.53	1.74(0.25)	1.06(0.11)	0.77	147.5	624.5(137)	1084(117)	0.54
	SS2	2.93	1.63(0.16)	0.99(0.07)	0.77	3.97	0.66(0.07)	0.62(0.04)	0.68
62B	SS1	12.19	0.85(0.20)	0.91(0.14)	0.65	156.2	860.5(207)	987.7(148)	0.64
	SS2	3.68	1.70(0.25)	1.13(0.12)	0.75	3.62	0.52(0.07)	0.47(0.04)	0.69
62C	SS1	12.28	1.35(0.20)	0.80(0.09)	0.77	161.7	835.0(164)	1095(123)	0.60
	SS2	4.09	1.99(0.19)	0.98(0.07)	0.80	3.59	0.55(0.07)	0.70(0.05)	0.61
62D	SS1	12.18	1.10(0.12)	0.54(0.05)	0.80	164.0	1062(153)	1101(102)	0.66
62E	SS2	4.33	1.97(0.17)	1.38(0.09)	0.74	3.47	0.44(0.05)	0.52(0.03)	0.63
Mean	SS1	12.41	1.21	0.81	0.75	153.3	834.5	923.6	0.65
	SS2	3.61	1.73	1.08	0.76	3.80	0.65	0.61	0.67

Among the 200 common clones across the two selection systems, the genetic correlation between CCS in the two trials was 0.62. The average genetic correlation over trials between VR and TCH was -0.29. The negative value is due to VR ranking from the best to the worst (1 to 9). There was a small negative genetic correlation (-0.13) measured in 200 common clones between early CCS measured in the SS2 stage 2 trials and cane yields measured in June in SS1. Genetic correlations between CCS and TCH in each field in the SS1 stage 2 trials, and CCS and visual ratings in the SS2 stage 2 trials were small but mostly slightly negative (Table A1.2).

**Table A1.2 Genetic correlations ( $r_g$ ) in the plant crop between CCS and TCH in the SS1, and between CCS and VR in the SS2**

Field	SS1	SS2
56A	-0.061	0.097
60P	-0.052	-0.215
62A	-0.125	-0.131
62B	0.042	-0.180
62C	0.044	-0.240
62D	-0.273	
62E		-0.083
Mean	-0.071	-0.125

### A1.2 Analysis in stage 3 SS2 trials

These trials included clones selected from the S2 SS2 trial and random clones. Genetic variances for CCS were much less (half or less) in the selected clones compared with the random clones (Table A1.3). This, coupled with the difference in mean in CCS between the random and selected clones clearly indicated a strong effect of selection in S2 for CCS. Heritabilities were high (0.7 or greater) for CCS in the random clones but were lower in the selected clones due to reduced genetic variance.

For cane yield, genetic variance was also reduced in the selected clones compared with the random clones, but cane yield was less in the selected clones than in the random clones. This result was somewhat surprising and suggested a significant negative selection pressure had been imposed on cane yield via the selection for CCS in S2.

Since all clones included in the S3 trial planted in 2002 were also included in the FAT trial planted in 2003, a pooled analysis of variance was done across these two trials. For the random clones the genotype by environment interaction (in this case the confounded effect of different site and year) was about half the size of the genotype variance for both CCS and cane yield. For the selected clones, genetic variance was about half the size of smaller for CCS and cane yield. Variance due to GE interaction was reduced in the selected clones compared with the random clones for CCS, in a proportion roughly in line with the reduction for genetic variance. However, interestingly and unexpectedly, GE variance was much greater in the selected clones than the random clones for cane yield. Reasons for this increase are not clear.

Analyses of variance conducted across the S3 trial and the FAT trial at Kalamia, which included all 114 clones selected from the SS2 S2 trial and the random clones, indicated significant reduction in genetic variance among the selected clones compared with the random clones (Tables A1.4, A1.5). It also indicated significant GE interaction for CCS in the random clones but not for the selected clones. For cane yield, there was an increased genotype x trial interaction for cane yield in the selected clones, but for both classes there was no significant genotype x crop-year interaction for cane yield.

There was some degree of negative genetic correlation observed between CCS and TCH for most situations in the stage 3 trials (Table A1.6). For the random clones, the range for

genetic correlations between CCS and TCH was from -0.19 to -0.24 for both plant crop and first ratoon crop. For selected clones, there was a larger negative genetic correlation between CCS and cane yield observed in March ( $r_g = -0.40$ ). However, this appeared to disappear by June.

**Table A1.3 Genetic variance ( $\sigma_g^2$ ), error variance ( $\sigma_e^2$ ) and heritability for CCS, and cane yield (TCH) measured in the SS2 stage 3 trial in the plant crop (P) and first-ratoon crop (R) for the 114 selected clones and 48 random clones, respectively, in trials at Kalamia planted in 2002 and 2003. Standard errors are given in brackets**

Crop	Trial	Time of measurements	Population	Mean	$\sigma_g^2$	$\sigma_e^2$	H <sub>e</sub>
<b>CCS</b>							
Plant	2002 planted	March	Selected	6.69	1.05 (0.21) <sup>1</sup>	0.91 (0.12)	0.70
			Random	4.55	2.18 (0.50)	0.60 (0.11)	0.88
		June	Selected	12.45	0.53 (0.18)	1.378 (0.18)	0.44
			Random	10.96	2.18 (0.50)	0.54 (0.10)	0.89
	2003 planted	August <sup>2</sup>	Selected	13.33	0.82 (0.24)	1.60 (0.21)	0.51
			Random	12.29	1.60 (0.49)	1.45 (0.30)	0.69
Ratoon	2002 planted	June	Selected	15.33	0.57 (0.12)	0.63 (0.08)	0.65
			Random	14.43	1.39 (0.38)	0.88 (0.16)	0.76
<b>Cane yield (t/ha)</b>							
Plant	2002 planted	March	Selected	122.0	172.7 (44.8)	279.1 (35.6)	0.55
			Random	129.4	278.3 (90.9)	300.1 (55.7)	0.65
		June	Selected	169.5	563.5 (96.2)	302.0 (38.5)	0.79
			Random	192.2	688.2 (179.3)	366.2 (68.0)	0.79
	2003 planted	August	Selected	177.1	230.9 (100.1)	814.6 (108.4)	0.36
			Random	193.4	465.4 (218.5)	984.5 (203.1)	0.49
Ratoon	2002 planted	June	Selected	147.2	233.2 (58.7)	354.9 (45.3)	0.57
			Random	162.1	417.2 (138.7)	469.0 (87.1)	0.64

**Table A1.4 Variance components from analysis of data from the same set of 114 selected clones and 48 random clones evaluated in the stage 3 (2002) trial and in the S4 trial in the following year (2003) in a different field at Kalamia**

Source of variation	Population	d.f.	CCS	TCH
Clones	Random	47	1.295**	389.1**
	Selected	113	0.521**	187.5**
Clones x Environment	Random	47	0.605**	162.7*
	Selected	112	0.181	280.2**
Error	Random	103	0.938	684.0
	Selected	235	1.478	737.5
Mean	Random		11.59	192.9
	Selected		12.89	174.0
C.V.(%)	Random		8.32	13.56
	Selected		9.41	15.69
GE:G ratio	Random		0.47	0.42
	Selected		0.35	1.49

\*\* for  $P \leq 0.01$

**Table A1.5 Variance components from analysis of variance across plant and first-ratoon crops of random and selected clones planted in the S3 trial**

Source of variation	Population	d.f.	CCS	TCH
Clones	48 random	47	1.347**	517.2**
	114 selected	113	0.531**	598.7**
Clones x Crop-year	48 random	47	0.389**	0
	114 selected	113	0.038	0
Error	48 random		0.707	451.4
	114 selected		0.987	582.3
Mean	48 random		12.69	177.14
	114 selected		13.89	159.6
C.V.(%)	48 random		6.61	12.23
	114 selected		7.16	15.71
G x crop-year : G ratio	48 random		0.29	0
	114 selected		0.071	0

\*\* for  $P \leq 0.01$

**Table A1.6 Genetic correlation ( $r_g$ ) between CCS and TCH measured in different time and in different crop classes at Kalamia**

Trial	Time of year and crop	Cane yield			
		March Plant	June Plant	June Ratoon	August Plant
<b>114 selected clones</b>					
S3	CCS March Plant	-0.40	-0.41	-0.32	-0.68
	CCS June Plant	-0.06	0.06	0.02	-0.46
	CCS June Ratoon	-0.09	0.02	0.04	0.04
S4	CCS August Plant	0.10	0.29	0.35	0.05
<b>48 random clones</b>					
S3	CCS March Plant	-0.04	-0.21	-0.33	-0.27
	CCS June Plant	-0.31	-0.24	-0.38	-0.13
	CCS June Ratoon	-0.14	-0.25	-0.19	-0.34
S4	CCS August Plant	-0.08	-0.08	-0.11	-0.18

Genetic correlations between environments (where environments are time of year by trial by crop combinations) for the same trait were high (Table A1.7) particularly for CCS between March and June in plant crop as well as for both CCS and TCH between the plant (measured in June) and first-ratoon crop. The high genetic correlation for CCS between March and June is consistent with results reported by Jackson and Morgan (2003). The genetic correlation for TCH measured between March and June was high (0.73) for the random clones, but was low (0.26) for the selected clones.

**Table A1.7 Genetic correlations ( $r_g$ ) between (i) CCS measurements at different times and crop classes in the S3 and FAT trials planted at Kalamia in 2002 and 2003, respectively, and (ii) cane yield measurements for the same environments**

Year of planting	Time of measurement & crop class	CCS				TCH			
		March Plant	June Plant	June Ratoon	August Plant	March Plant	June Plant	June Ratoon	August Plant
<b>114 selected clones</b>									
2002	March Plant	1				1			
	June Plant	0.86	1			0.26	1		
	June Ratoon	0.24	0.79	1		-0.10	0.74	1	
2003	August Plant	0.22	0.64	0.52	1	0.51	0.49	0.59	1
<b>48 random clones</b>									
2002	March Plant	1				1			
	June Plant	0.77	1			0.73	1		
	June Ratoon	0.63	0.74	1		0.56	0.91	1	
2003	August Plant	0.56	0.70	0.67	1	0.48	0.71	0.41	1

Genetic correlations between stage 2 and stage 3 were determined for the random clones. Correlations for CCS were moderate to high, but lower for cane yield for the 2002 S3 trial (Table A1.8). There were negative genetic correlations between CCS measured in stage 2

and TCH measured in stage 3. Genetic correlations between TCH or visual rating measured in stage 2 and CCS measured in stage 3 were close to zero.

**Table A1.8 Genetic correlations for CCS and TCH measured between the stage 2 and the stage 3 trials for the 48 random clones at Kalamia**

Stage 2 trial	Traits & time of measured in stage 2	Stage 3 trial							
		2002			2003	2002			2003
		CCS March Plant	CCS June Plant	CCS June 1 <sup>st</sup> Ratoon	CCS August Plant	TCH March Plant	TCH June Plant	TCH June 1 <sup>st</sup> Ratoon	TCH August Plant
SS1	CCS June	0.63	0.70	0.71	0.59	-0.13	-0.21	-0.46	-0.54
SS2	CCS March	0.83	0.70	0.51	0.42	0.0	-0.15	-0.25	-0.46
SS1	TCH June	-0.12	0.04	-0.12	0.12	0.16	0.33	-0.02	0.84
SS2	VR February	0.06	0.13	0.08	-0.11	-0.19	-0.20	-0.10	-0.56

***Comparison of performance of 48 random clones in 1-row plots with 4-row plots***

The 48 random clones were planted in single rows as well as four-row plots in the S3 trial planted at Kalamia in 2002. This provided an opportunity to compare genetic variances for 1-row plots and 4-row plots and genetic correlations between these plot designs. For CCS, the 1-row plots gave less genetic variance, but larger error variance than 4-row plots (Table A1.9). For cane yield, genetic variance was larger in 1-row plots compared with 4-row plots. However, error variances in 1-row plots were more than twice as high compared with that in 4-row plots. Similar trends were observed in the study by Jackson and McRae (2001).

**Table A1.9 Genetic variance ( $\sigma_g^2$ ) and error variance ( $\sigma_e^2$ ) obtained from S3 trials in the Burdekin region for 1-row and 4-row plots**

Trials	Plot size	Mean	$\sigma_g^2$	$\sigma_e^2$	$\sigma_g^2/\sigma_e^2$
<b>CCS</b>					
Stage3	10 m by 1 row	10.64	1.17	1.83	0.64
	10 m by 4 rows	10.96	2.18	0.54	4.04
<b>TCH</b>					
Stage 3	10 m by 1 row	159.0	902.9	752.7	1.20
	10 m by 4 rows	192.2	688.2	366.2	1.88

Genetic correlations for CCS between 1-row plots and the middle two rows of 4-row plots were high ( $>0.90$ ) when the measurements were made in the same environment (time by trial by year) (Table A1.10). For cane yield, genetic correlations were 0.47 in the plant crop and 0.04 in the ratoon crop. The plant crop result is similar to results reported by Jackson and McRae (2001), while the ratoon crop result is lower than the levels of 0.74 and 0.17 reported by Jackson and McRae (2001).

**Table A1.10 Genetic correlation ( $r_g$ ) of between measurements made in the 1-row plots with the equivalent traits measured in the middle two rows of 4-row plots, calculated on the set of 48 random clones**

In 4-row	June plant (1-row)	June ratoon (1-row)
<b>CCS</b>		
<b>March Plant</b>	0.82	0.98
<b>June Plant</b>	0.92	1.26
<b>June Ratoon</b>	0.59	1.07
<b>TCH</b>		
<b>March Plant</b>	0.29	0.11
<b>June Plant</b>	0.47	0.45
<b>June Ratoon</b>	-0.05	0.04

### A1.3 Analysis of variance in S4 trials

An analysis of variance of all clones in the S4 trials showed moderate to high heritabilities in most cases for CCS and cane yield, apart from a low genetic variance and heritability in the trial at BSES Burdekin (BRN03) (Table A1.11). The latter trial was not harvested in the ratoon crop due to a mishap with spraying of herbicide in the ratoon crop that affected growth in the trial unevenly.

**Table A1.11 Results from analyses of variance of individual S4 trials, showing genetic and error variances, and heritability, for CCS and cane yield**

Trial	Crop	Trait					
		CCS (%)			Cane yield (t/ha)		
		$\sigma_g^2$	$\sigma_e^2$	H	$\sigma_g^2$	$\sigma_e^2$	H
KAL03	P	1.60	1.45	0.69	465	984	0.49
	1R	5.92	0.39	0.97	161	274	0.53
PIV03	P	0.47	0.60	0.61	143	475	0.38
	1R	2.32	0.82	0.85	138	308	0.47
BAN03	P	0.42	1.33	0.39	255	443	0.54
	1R	0.96	0.27	0.87	187	100	0.78
BRN03	P	1.75	3.23	0.52	142	1536	0.16
Average		1.92	1.15	0.70	213	588	0.48

Pooled analyses of variance were conducted for the different groups of clones (random clones, selections from SS1 S2 trial, selections from SS2 S3 trial). For the random clones, GE interaction for CCS was approximately 0.8 times the size of genetic variance, whilst for cane yield it was much less at about 0.2 times (Table A1.12). Genetic variance was less for selected clones for CCS, with this reduction being particularly marked for the selections from SS2. There was no significant change in ratio of GE/G variances for CCS, so that GE interaction variance was also markedly less for the SS2 selected clones. For cane yield, there was only a small reduction in variance in the selected clones

compared with the random clones. However, interestingly there appeared to be an increase in some GE components due to selection. Reasons for this are not clear and this result was surprising.

**Table A1.12 Variance components from pooled analysis of variance of performance across S4 trials (4 sites, plant and first ratoon crops) for (i) random clones, (ii) clones selected from SS1 S2 trials, and (iii) clones selected from SS2 S3 trial**

Source of variation	CCS	Cane yield
<b>Random clones</b>		
Clones	0.74**	198**
Clones x sites	0.34**	0
Clones x crop-years	0.08	48**
Clones x crop-years x sites	0.24**	0
Error	0.57	319
<b>SS1 selections</b>		
Clones	0.55**	135**
Clones x sites	0.21**	29**
Clones x crop-years	0.09	16.3**
Clones x crop-years x sites	0.36**	85.5**
Error	0.60	191
<b>SS2 selections</b>		
Clones	0.24**	167**
Clones x sites	0.08	0
Clones x crop-years	0.12**	33**
Clones x crop-years x sites	0.13**	41**
Error	0.44	241

\*\* for  $P \leq 0.01$

#### **A1.4 Realised gains from selection**

Selected clones from SS1 had an average across the four final assessment trials of 9.2 t/ha and 0.39 CCS units more than the random clones (Table A1.13).

In SS2, in S3 the selected clones had an average of 1.2 units of CCS more than the random clones. However, most interestingly and unexpectedly, the selected clones had 18 t/ha cane yield less than the random clones. The latter result clearly indicated a strong negative selection pressure on cane yield from the intense selection pressure on CCS in the SS2 stage 2 trials. Selection in the subsequent S3 trial for REV resulted in an improvement in cane yield so that the mean of the selected clones came up to only 8 t/ha behind the random clones when averaged across the final assessment trials, but there was no significant further improvement in CCS.

**Table A1.13 Realised gain measured in the final assessment trials for selections from SS1. Realised gain = mean of selected clones – mean of random clones**

<b>Trial</b>	<b>Crop</b>	<b>Traits</b>	<b>Mean 48 random clones</b>	<b>Mean 50 selected clones</b>	<b>Realized gains</b>	<b>Realized gain as % of mean of randoms</b>
Kalamia FAT	Plant	CCS	12.29	13.18	0.89	7.24
		TCH	193.4	201.8	8.40	4.34
		TSH	23.73	26.63	2.9	12.22
		REV	4196	4873	677	16.13
	Ratoon	CCS	11.5	13.1	1.6	13.8
		TCH	90.0	103.2	13.2	14.6
		TSH	10.4	13.5	3.1	30
		REV	1780	2474	694	38.9
Clare FAT	Plant	CCS	14.85	15.08	0.23	1.55
		TCH	177.1	181.7	4.60	2.60
		TSH	26.18	27.38	1.2	4.58
		REV	5063	5330	267	5.27
	Ratoon	CCS	15.6	15.4	-0.2	-1.2
		TCH	113	121	8	7.0
		TSH	17.6	18.6	1.0	5.6
		REV	3484	3663	179	5.1
BSES FAT	Plant	CCS	11.53	11.60	0.07	0.61
		TCH	188.5	197.4	8.90	4.72
		TSH	21.15	22.99	1.84	8.70
		REV	3588	3940	352	9.81
Giru FAT	Plant	CCS	14.65	14.81	0.16	1.09
		TCH	119.0	130.5	11.50	9.66
		TSH	17.37	19.17	1.80	10.36
		REV	3335	3704	369	11.06
	Ratoon	CCS	15.7	15.7	0	0
		TCH	120	129.9	9.9	8.3
		TSH	18.8	20.2	1.4	7.4
		REV	3730	3995	265	7.1
Average		CCS	13.7	14.1	0.39	2.8
		TCH	142	151	9.2	6.4
		TSH	19.5	21.3	1.8	9.2
		REV	3658	4045	387	10.5

**Table A1.14 Realised gain measured in the final assessment trials for SS2. Realised gain = mean of selected clones – mean of random clones**

Final trials where gain is measured	Crop	Traits	Mean random clones	Mean selected clones	Realized gains	Realized gain as % of mean of random clones
<b>ex-S2 selections</b>						
Kalamia S3	Plant	CCS	10.9	12.5	1.5	13.7
		TCH	192	170	-22	-11.4
		TSH	20.9	21.2	0.3	1.4
		REV	3443	3790	347	10.0
	Ratoon	CCS	14.4	15.3	0.9	6.3
		TCH	162	147	15	9.2
		TSH	23.3	22.5	-0.8	-3.4
		REV	4196	4423	227	5.4
	Average	CCS	12.7	13.9	1.2	9.4
		TCH	177	159	-18	-10.2
		TSH	22.1	21.9	-0.2	1.0
		REV	3820	4106	287	7.5
<b>ex-S3 selections</b>						
Kalamia FAT	Plant	CCS	12.29	13.75	1.46	11.88
		TCH	193.4	177.5	-15.90	-8.22
		TSH	23.73	24.43	0.7	2.95
		REV	4196	4577	381	9.08
	Ratoon	CCS	11.5	13.2	1.70	14.7
		TCH	90.0	81.6	-8.04	-8.9
		TSH	10.4	10.8	0.4	4.0
		REV	1670	1990	320	19.1
Clare FAT1	Plant	CCS	14.85	15.77	0.92	6.20
		TCH	177.1	168.5	-8.60	-4.86
		TSH	26.18	26.54	0.36	1.38
		REV	5063	5264	201	3.97
	Ratoon	CCS	15.6	15.83	0.23	1.4
		TCH	113	107	-6.0	-5.3
		TSH	17.6	16.9	-0.7	-3.9
		REV	3484	3363	-121	-3.4
BSES FAT	Plant	CCS	11.53	12.79	1.26	10.93
		TCH	188.5	192.5	4.0	2.21
		TSH	21.15	24.10	2.95	13.95
		REV	3588	4336	748	20.85
Giru FAT	Plant	CCS	14.65	15.37	0.72	4.91
		TCH	119.0	116.8	-2.20	-1.85
		TSH	17.37	17.96	0.59	3.40
		REV	3335	3523	188	5.64
	Ratoon	CCS	15.7	16.3	0.6	3.8
		TCH	119.8	114.2	-5.6	-4.6
		TSH	18.8	18.6	-0.2	-1.0
		REV	3732	3744	12	0.3
Average	CCS	13.7	14.7	1.1	7.2	
	TCH	142	136	-8	-5.6	
	TSH	19.5	20.0	0.6	3.0	
	REV	3658	3868	210	5.7	

<sup>1</sup> Data presented were for 19 common clones instead of 28 clones

## APPENDIX 2 - Field trial results from the Central region

The general experimental program in the Central region followed that in the Burdekin. However, data analysis from the Central region was limited in three respects. Firstly, clones in the SS1 S2 trials were not replicated. Secondly, the random clones were not included in the SS1 S2 trials and, therefore, genetic correlations between these trials and other trials could not be determined. Thirdly, there was no additional planting of clones in single row plots alongside multi-row plots to directly estimate the size of competition effects and determine the genetic correlation between these two plot types.

### A2.1 Analysis of variance for the S2 trials

Both the SS1 and SS2 clones were planted into two trials in each system, and the results shown in Table A2.1. Heritabilities for both visual grade and cane yield were low, with slightly higher heritabilities observed in RAC00-33 and RAC00-35. The low heritabilities may have been partly due to patchy germinations experienced in some parts of these trials, and this would be considered unusual and not what would be expected in future routine trials. Heritabilities for CCS were higher, but low for the RAC00-35 trial. Again, this may have been due to poor and patchy germination observed, including the possibility of misidentification of the 5-m plots given the poor germination, and this low level of precision may be atypical. A feature of these data, and other trials observed in the Central region in this project were variable sizes of genetic variances in different trials. For example, despite having clones sampled from the same populations, trial RAC00-34 had a genetic variance about half that of RAC00-32, and RAC00-35 had a genetic variance about one-third the size of RAC00-33. Some of this variation in this case could be due to random errors, with high standard errors associated with these data.

**Table A2.1 Summary results from analysis of variance of data from the Central trials, including genetic variance, error variance and broad-sense heritability estimates for each trial in each selection system**

Selection system	Trial	Trait					
		CCS (%)			Cane yield (t/ha) or visual grade		
		$\sigma_g^2$	$\sigma_e^2$	H	$\sigma_g^2$	$\sigma_e^2$	H
SS1	RAC00-32	0.96	0.56	0.77	165	344	0.32
	RAC00-34	0.94	0.33	0.85	80.4	421	0.16
SS2	RAC00-33	1.21	1.51	0.61	0.10	0.24	0.45
	RAC00-35	1.00	3.22	0.38	0.03	0.31	0.15

### A2.2 Analysis of variance for the S3 trials

Results from analysis of variance in the S3 trials in the SS2 system are shown in Table A2.2. Heritabilities were relatively high when all clones were included, but were smaller for the random clones in the case of cane yield in RAC01-21 and CCS in RAC01-22, for

reasons that are unclear. As with the S2 trials, a notable feature was the variable size of genetic variances between the trials in some cases.

**Table A2.2 Results from analyses of variance of the S3 trials in the SS2 selection system**

Population of clones	Trial	Trait					
		CCS (%)			Cane yield (t/ha)		
		$\sigma_g^2$	$\sigma_e^2$	H	$\sigma_g^2$	$\sigma_e^2$	H
All clones	RAC01-21	0.74	0.40	0.78	222	126	0.77
	RAC01-22	0.32	0.33	0.66	204	148	0.73
Random clones	RAC01-21	0.07	1.28	0.10	454	107	0.89
	RAC01-22	0.62	0.59	0.68	64.6	255	0.33

### A2.3 Analysis of variance of the final assessment (FAT) trials

Analysis of variance for individual trials generally showed moderate to high heritabilities in all cases in both plant and first-ratoon crops, indicating likely good quality of data without any individual problem sites. One exception was the low heritability recorded for CCS in the PRO03-31 trial, which was associated with a high error estimate. The low heritability estimate for CCS at RAC03-31 was due to very low genetic variance rather than particularly high error variance.

**Table A2.3 Results from analysis of variance for cane yield and CCS at individual Final assessment trials**

Trial	Crop	Trait					
		Cane yield (t/ha)			CCS		
		$\sigma_g^2$	$\sigma_e^2$	H	$\sigma_g^2$	$\sigma_e^2$	H
FAR03-31	P	161	177	0.64	2.41	0.4	0.92
	1R	77	82	0.65	1.04	0.46	0.81
MAR03-31	P	232	148	0.75	4.38	3.42	0.71
	1R	115	170	0.57	0.63	0.75	0.62
PCK03-31	1R	125	96	0.72	0.27	0.19	0.62
PLY03-31	P	111	104	0.68	0.51	0.62	0.73
	1R	132	65	0.80	0.40	0.04	0.95
PRO03-31	P	96	85	0.69	1.42	0.76	0.78
	1R	219	73	0.85	0.19	1.39	0.21
RAC03-31	P	164	113	0.74	1.36	1.39	0.66
	1R	69	120	0.53	0.13	0.52	0.33
RAC03-32	P	171	47	0.88	0.96	0.37	0.83
	1R	348	100	0.87	0.83	0.24	0.87
Average		155	106	0.72	1.11	0.81	0.69

Pooled analyses of variance across environments were done for the selected and random clones separately. The estimate for variance for clones for CCS in the set of random clones was slightly less than that for the selected clones. This is surprising and different to what would be expected from selection. This may be associated with random error effects, but suggests that the estimate for clone variance for CCS may be a slight underestimate. This possibility is relevant for subsequent modelling predictions (see Appendices 5 and 6). GE interaction components were large relative to clone main effect - in the random clones, the cumulative GE variance was about twice the clone main effect for CCS and 1.8 times the clone main effect for cane yield. Among the selected clones, this ratio was smaller, being less than the clone main effect for both CCS and cane yield. The apparent reduction in GE interaction with selection is the opposite to the effect observed in the Burdekin where the absolute size of GE interaction increased markedly with selection.

**Table A2.4 Results from analyses of variance across sites and crop-years for the random clones and selected clones**

Source of variation	CCS	Cane yield
<b>Random clones</b>		
Clones	0.43 (0.21)	62.4 (30.7)
Clones x sites	0.14 (0.11)	49.5 (12.2)
Clones x crop-years	0.26 (0.13)	42.7 (16.3)
Clones x crop-years x sites	0.49 (0.14)	17.1 (10.1)
Error	0.99 (0.08)	99 (8.8)
<b>Selected clones</b>		
Clones	0.52 (0.08)	35.7 (6.4)
Clones x sites	0.07 (0.04)	19.6 (4.3)
Clones x crop-years	0.15 (0.05)	8.6 (3.4)
Clones x crop-years x sites	0.23 (0.06)	0.12 (6.0)
Error	0.96 (0.04)	109 (4.8)

#### **A2.4 Realised gains from selection**

Realised gains from selection in the S3 trials in SS2 and the final assessment trials were estimated by the difference between the average performance of the selected clones and the random clones in each of SS1 and SS2, and are shown in Tables A2.5 and A2.6. Clearly, gains varied from trial to trial in both SS1 and SS2, but overall gains for both cane yield and CCS were relatively similar in both systems.

One interesting feature in SS2 was the positive gains for cane yield following selection in S2 (realised in the S3 trials) despite selection in S2 being primarily based on CCS. This result is strongly suggestive of a positive genetic correlation between CCS in the S2 and cane yield in the S3 trial, but this relationship was not observed among the random clones between these two environments.

The main value of the measured realised gains in this project was to validate model predictions, rather than to identify the best system, since it was apparent that neither system was optimised particularly for selection criteria. Comparison of the realised gains with predicted gains, and identification of improved selection system designs is discussed in Appendices 5 and 6.

**Table A2.5 Realised gains from clones selected in SS1**

<b>Trial</b>	<b>Crop</b>	<b>Trait</b>	<b>Randoms</b>	<b>Selected clones</b>	<b>Realised gain</b>	<b>% Gain</b>
FAR03-31	P	TCH	53.1	61.7	8.6	16.2
		CCS	17.0	17.9	0.9	5.1
	R	TCH	55.4	61.2	5.8	10.5
		CCS	16.1	17.0	0.9	5.7
MAR03-31	P	TCH	58.7	64.8	6.1	10.4
		CCS	13.2	13.8	0.7	5.0
	R	TCH	44.8	49.9	5.1	11.4
		CCS	15.7	16.6	1.0	6.3
PCK03-310	P	TCH	64.9	73.3	8.4	12.9
		CCS	N/A	N/A	N/A	N/A
	R	TCH	56.5	63.8	7.4	13.1
		CCS	17.8	18.3	0.6	3.1
PLY03-310	P	TCH	58.7	62.2	3.5	5.9
		CCS	17.7	18.2	0.5	2.8
	R	TCH	73.2	77.1	3.9	5.3
		CCS	16.6	17.4	0.8	4.7
PRO03-310	P	TCH	46.3	55.9	9.7	20.9
		CCS	16.1	16.8	0.7	4.5
	R	TCH	76.7	88.2	11.5	14.9
		CCS	16.9	17.4	0.5	3.1
RAC03-310	P	TCH	72.5	80.4	7.9	10.9
		CCS	16.4	17.5	1.0	6.3
	R	TCH	58.2	62.1	3.9	6.7
		CCS	16.8	17.7	1.0	5.7
Average		TCH	59.9	66.7	6.8	11.4
		CCS	16.4	17.1	0.8	4.7

**Table A2.6 Realised gains in selection system 2**

<b>Trial</b>	<b>Crop</b>	<b>Trait</b>	<b>Randoms</b>	<b>Selected clones</b>	<b>Realised gain</b>	<b>% Gain</b>
<b>S3 trials</b>						
RAC01-21	P	TCH	87.6	96.8	9.2	10.5
		CCS	14.9	15.8	1.0	6.7
RAC01-22	P	TCH	83.9	98.4	5.5	6.5
		CCS	15.4	15.4	0	0
Average		TCH	85.8	93.1	7.3	8.5
		CCS	15.1	15.6	0.5	8.5
<b>S4 trials</b>						
FAR03-31	P	TCH	53.1	57.0	3.8	7.1
		CCS	17.0	17.9	0.9	5.2
	R	TCH	55.4	59.9	4.5	8.1
		CCS	16.1	17.2	1.1	6.8
MAR03-31	P	TCH	58.7	63.5	4.8	8.2
		CCS	13.2	14.8	1.6	12.1
	R	TCH	44.8	53.9	9.1	20.3
		CCS	15.7	16.5	0.9	5.7
PCK03-310	P	TCH	64.9	74.0	9.0	13.8
		CCS	N/A	N/A	N/A	N/A
	R	TCH	56.5	63.3	6.8	12.0
		CCS	17.8	18.6	0.8	4.4
PLY03-310	P	TCH	58.7	63.1	4.4	7.5
		CCS	17.7	18.2	0.5	2.8
	R	TCH	73.2	81.8	8.6	11.7
		CCS	16.6	17.2	0.6	3.6
PRO03-310	P	TCH	46.3	53.9	7.7	16.6
		CCS	16.1	17.4	1.4	8.6
	R	TCH	76.7	90.2	13.4	17.4
		CCS	16.9	17.3	0.4	2.3
RAC03-310	P	TCH	72.5	82.0	9.4	12.9
		CCS	16.4	17.5	1.0	6.0
	R	TCH	58.2	62.9	4.7	8.0
		CCS	16.8	18.1	1.3	7.7
Average		TCH	59.9	66.7	7.2	12.0
		CCS	16.4	17.1	1.0	6.1

## APPENDIX 3 - Comparison of methods for analysing S2 data

### A3.1 Comparison of alternative methods of analysis

Cane yield of clones in the S2 trials comprising 1 row by 10-m plots was estimated or predicted using three methods:

- (a) least square means (traditional method);
- (b) BLUPs from analysis using a model that accounts for spatial effects;
- (c) BLUPs from analysis using recently developed methods that model both spatial and competition effects.

CCS in the same plots was estimated or predicted using methods (a) and (b), with method (c) not being used because competition is not important for CCS.

As described in the methods, clones from the original population were allocated at random to each of six trials planted adjacent to each other with 100-300 clones being included in each trial. For methods (b) and (c), the BLUP represents the predicted deviation from the population mean in each trial, with the sum of all BLUPs in each trial being very close to zero. For method (a) the deviation of each clone from the population mean was estimated in a corresponding way by subtracting from the least square mean the grand mean of all plots of the trial in which the clone was.

The different methods produced data that was highly correlated across the clones in most cases (Table A3.1). Correlations between the BLUPs including competition effects were slightly lower (<0.90) and the other two models were slightly lower than correlations between the latter two models (0.94). These results suggest generally similar predictions between the different models.

**Table A3.1 Correlations among clone effects estimated from three methods for CCS and cane yield measured in the SS1 trials (1 row by 10-m plots)**

Parameter	Data	RCB	Spatial	Spatial + competition
CCS	RCB	1.0	0.93	
	Spatial		1.0	
TCH	RCB	1.0	0.94	0.84
	Spatial		1.0	0.88
	Spatial + comp			1.0

The estimates or predictions determined above were then correlated with the average performance of each clone across all of the stage 4 (final assessment) trials across all trials and including both plant and first-ratoon data. The correlations are shown in Table A3.2. Correlations were also done on a per block basis (data not shown) and corresponded very closely on average with the results from all clones combined shown below.

**Table A3.2 Correlations between clone effects estimated from three methods based on S2 results (1 row by 10-m plots) and average clone effects in plant and first ratoon crops in four final assessment trials (4 row by 10-m plots)**

S4 data	S2 results		
	RCB	Spatial	Spatial + competition
CCS	0.58	0.63	
TCH	0.51	0.51	0.46
REV	0.42	0.45	0.41

There was no evidence for an improvement in predictions in the population using the competition model, with correlations between both cane yield and relative economic value in the S2 trial as predicted by the competition model being no greater (and slightly lower but probably not significantly) than the RCB and spatial models. The model accounting for spatial effects realised a small improvement for CCS, but not for cane yield. Therefore, if the more complex models accounting for spatial and competition effects are improving overall predictions, these improvements appear to be small and not detectable in these data.

The alternative models were also investigated by examining the ranking given to the elite clones identified in the S4 trials based on relative economic value. In conducting this analysis, all selected clones and random clones that were evaluated in both the S2 and S4 trials were considered. The average ranking of the top 10 clones based on REV in the S4 trials given by the RCB model in the S2 trial was 33 (out of 98), while that given by the spatial (for CCS) + competition (for TCH) model was 35. Again, these results do not support a hypothesis of improved identification of elite clones by the more sophisticated models.

Overall, there was no evidence for the model incorporating competition effects improving prediction of cane yield or economic value in these experiments.

## APPENDIX 4 Description of the selection-system simulation model

### A4.1 Overview

A stochastic simulation model was developed to represent the process of selection of sugarcane genotypes for CCS and cane yield through a multi-stage selection process. A stochastic (as opposed to analytical) model involves generation of effects that have a random component, eg sampled from a normal distribution. As such, each time the model is run there will be small differences in the result, corresponding to the effects of sampling variation. A stochastic model was considered the best for modelling a multi-stage selection process, since it relies less on assumptions about changes in variance and population distribution with selection, and deals effectively with the multi-stage nature of the problem. Both of these are complex to deal with analytically.

The model was developed as part of a PhD thesis by Hu Fengduo. It was developed within Microsoft Excel and uses Visual Basic code.

The model works by initially generating a population of phenotypes in the first clonal stage of selection (corresponding to stage 2 in the Australian sugarcane-breeding program). Phenotypes for CCS and cane yield (TCH) are generated and then combined as described below to produce a single clone with both CCS and TCH genotype effects and phenotype. The following basic model was assumed for producing phenotypic values for CCS and TCH:

$$Y_{ij} = \mu + g_i + ge_{ij} + c_i + \varepsilon_{ij} \dots \dots \dots (\text{eqn. 1})$$

where

$Y_{ij}$  = observed phenotype;

$\mu$  = mean of all observations;

$g_i$  = genetic effect;

$ge_{ij}$  = genetic x environment interaction effect;

$c_i$  = competition effect among clones;

$\varepsilon_{ij}$  = error effect.

All effects of the linear model (above) are sampled from normally distributed populations (via use of the Excel spreadsheet function Norminv) with variances according to specified assumptions. However, the effects are generated in slightly different ways, reflecting actual selection systems. The genotypic effects ( $g_i$ ) are only generated once in the initial population (stage 2). These then remain unchanged throughout the selection process, since the genetic effect (as opposed to phenotype) does not depend on the trial design or environment in which the genotype is grown. By contrast, all other effects in the linear model change in different trials and environment. The competition effect is defined as the performance of clones measured in small plots minus performance in pure stands. The competition effect is assumed to equal zero when middle rows of multiple rows were measured and is different for CCS and cane yield. The error effect and genotype by environment interaction effect depend on the experimental design used, and therefore are regenerated at each stage of selection following procedures described below.

Assumptions about the variances for generating the population of effects were mostly obtained from experiments in the project, but of course may be varied in the model to

determine how sensitive predictions are to changes in the assumptions. This is particularly important for variance estimates for which there may be some uncertainty or have high standard errors. Competition and error variances differ depending on plot size used in the design.

Following basic statistical theory, error variances and GE variances for each stage are determined by the base value assumed divided by the total number of replicates (replicates per environment times number of environments) and number of environments, respectively, at the stage of selection involved. This provides an appropriate variance for these effects for phenotypes determined by averaging across all replicates and trials.

At each stage of selection, all effects ( $g_i$ ,  $c_i$ ,  $ge_{ij}$  and  $e_{ij}$ ) for each trait are added together to produce a phenotype for each genotype for each of CCS and cane yield. Genotypes are then ranked based on the phenotype of the selection criteria. Depending on selection system design, the selection criteria may consist of CCS, TCH, sugar yield (TSH), net merit grade (NMG) or relative economic value (REV).

The following steps illustrate how the model operates:

***Step 1:***

The population of all effects ( $g_i$ ,  $c_i$ ,  $ge_{ij}$  and  $e_{ij}$ ) for each of CCS and TCH is generated based on the assumed variances specified for each. The number of each effect equals the number of genotypes specified as the starting population size in the selection system design. Genotype effects for CCS are generated using an additional procedure (described below) which allows these effects and genotype effects for cane yield to be generated with a correlation corresponding to the genetic correlation between CCS and cane yield assumed.

***Step 2:***

Competition, GE, and error effects are allocated at random to each of the genotype effects for each of CCS and cane yield and effects for CCS are allocated at random to the cane yield effects.

***Step 3:***

Phenotype effects for cane yield and CCS are calculated by summing up the component effects in equation (1), and criteria for selection (eg. Selection index representing a linear function of CCS and cane yield) is calculated.

***Step 4:***

Genotypes are ranked on the basis of the selection criteria chosen.

***Step 5:***

The top ranked genotypes are transferred to the next stage of selection, with the number selected based on selection intensity specified in the selection system design. These genotypes, consisting of genetic effects for each of CCS and TCH are transferred. In this way, effects of selection on genetic variance are dealt with automatically and appropriately.

**Step 6:**

The effects for  $c_i$ ,  $ge_{ij}$  and  $e_{ij}$  are generated in the same way as stage 2, except using variance specified for the next stage of the selection trial design. As indicated above (stage 2) genotype effects are not generated, but instead comprise effects corresponding to those originally generated.

**Steps 7 and following:**

The same procedures of determining phenotypes, ranking according to selection criteria, selection and transfer to next stage (if there is one) are followed as indicated in the steps for the first stage.

The genetic values of the top ranked six genotypes based on phenotype in the final stage of selection are taken as representing the final performance of the selection system. The choice of six is somewhat arbitrary, representing a compromise between what happens in practice in release of cultivars where one or zero clones are released on average each year, and reducing random variation per simulation.

**A4.2 Generating genetic correlations between CCS and cane yield**

The procedure used to generate genetic effects for TCH and CCS in the model is as follows.

CCS genetic effects for each genotype were generated as two uncorrelated components comprising an effect independent on the genetic effect for cane yield (termed CCS independent genetic effect,  $CCS_{ind}$ ), and an effect dependent on cane yield (CCS dependent genetic effect,  $CCS_{dep}$ ) such that  $CCS_{dep} = b * TCH_g$  where  $TCH_g$  is the genetic effect for cane yield and  $b$  is a constant.

Since  $CCS_{ind}$  and  $CCS_{dep}$  are uncorrelated:

$$\sigma_g^2 (CCS) = \sigma_g^2 (CCS_{ind.}) + \sigma_g^2 (CCS_{dep}) \dots\dots\dots (2)$$

It can be shown that

$$r_g^2 = \sigma_g^2 (CCS_{dep}) / \sigma_g^2 (CCS) \dots\dots\dots (3)$$

where  $r_g$  is the correlation between genetic effects of CCS and TCH.

From (2) and (3):

$$\begin{aligned} \sigma_g^2 (CCS_{ind.}) &= \sigma_g^2 (CCS) - \sigma_g^2 (CCS_{dep}) \\ &= \sigma_g^2 (CCS) - r_g^2 * \sigma_g^2 (CCS) \\ &= (1 - r_g^2) * \sigma_g^2 (CCS) \dots\dots\dots (4) \end{aligned}$$

By definition,

$$\begin{aligned} r_g &= b * \sigma_g (TCH) / \sigma_g (CCS) \\ b &= r_g * \sigma_g (CCS) / \sigma_g (TCH) \dots\dots\dots (5) \end{aligned}$$

From (4) and (5):

$$CCS_{dep} = r_g * \sigma_g (CCS) / \sigma_g (TCH \text{ genetic effect}) * (TCH \text{ genetic effect}) \dots (6)$$

Therefore in the model, the  $CCS_{ind}$  are first generated with variance given by equation (4). The  $CCS_{dep}$  effects are then generated as given by equation (6). This process produces CCS genetic effects with the required variance and the genetic correlation with the TCH genetic effects.

#### A4.3 Accommodating correlated errors in ratoon crops

Error variances specified in the selection-system simulation model are directly applicable to a single crop (eg. plant crop). Evaluation of clones across more than one crop in the same trial should reduce error variance by a factor inversely proportional to the number of crops (eg 0.5 if two crops are involved) if error effects across crop-years are independent. However, error effects are correlated between crop-years in the same trial, because the same plots are measured across crops. This means that error variances will be reduced but not to the full extent predicted if the trials (and error effects) in each crop cycle were independent as initially assumed in the model. Hence, the adjustment of error variance in the model must take account not only of the number of crop-years, but also the covariance of error effects. Details of assumptions and procedures for adjusting the error variance for trials involving multiple crop-years are described below. Specific assumptions used apply to the Burdekin but procedures and final results are applicable generally.

##### *Error variance adjusted in single-row plots (stage2)*

In stage 2 trials, options involving two crop-years (plant and first ratoon) were simulated. Let the error variance in the plant crop =  $\sigma^2_{e1}$  and error variance in the first ratoon crop =  $\sigma^2_{e2}$  and the covariance between error effects in plant and first ratoon crops =  $cov_{e1,e2}$ . The error effect associated with the mean of plant and ratoon crop phenotypes =  $(e1 + e2)/2$ .

$$\text{It is known that } \sigma^2_{(e1+e2)} = \sigma^2_{e1} + \sigma^2_{e2} + 2 cov_{e1,e2} \dots (7);$$

$$\text{and } \sigma^2_{(x*e)} = x^2 * \sigma^2_e; \dots (8)$$

where x is a constant and e is a random variable.

Therefore, from (7) and (8), the error effect of the mean of plant and ratoon crop phenotypes =  $\sigma^2_{(e1+e2)/2} = 1/4 (\sigma^2_{e1} + \sigma^2_{e2} + 2 cov_{e1,e2})$ .

It is assumed (based on published information and analysis of breeding program trial results) that, on average, error variance in plant and ratoon crops are not significantly different. Therefore,

$$\begin{aligned} \sigma^2_{(e1+e2)/2} &= 1/4(\sigma^2_{e1} + \sigma^2_{e2} + 2 cov_{e1,e2}) \\ &= 1/4 (2\sigma^2_{e1} + 2 cov_{e1,e2}) \\ &= 1/2\sigma^2_{e1} + 1/2 cov_{e1,e2} \end{aligned}$$

For cane yield, results from analyses of plant-crop and ratoon-crop data from single-row plots in the SS2 trial (stage 3, planted in 2002) indicated that the covariance of error effects across crop-years equalled  $349 (t/ha)^2$  compared with error variance estimates of  $795 (t/ha)^2$  in plant crop and  $605 (t/ha)^2$  in first-ratoon crop. It was, therefore, assumed for the

purpose of the selection simulation model that error effect covariance across plant and first ratoon crops was equal to 0.5 times the error variance.

$$\begin{aligned} \text{Therefore, from above,} \\ \sigma^2_{(e1+e2)/2} &= 1/2\sigma^2_{e1} + 1/2 \text{cov}_{e1,e2} \\ &= 1/2\sigma^2_{e1} + 1/2 * 1/2\sigma^2_{e1} \\ &= 0.75\sigma^2_{e1} \end{aligned}$$

Therefore, when selection at stage 2 was based on both plant and first-ratoon crop, the variance of error effects for the mean of cane yield across plant and first-ratoon crops was assumed to equal 0.75 times error variance assumed for a single crop-year. For the model, if two crop-years are specified, as a default the model determines error variance based on two environments (ie 2 crop-years) as the error variance divided by 2. Therefore, for options involving two crop-years to adjust for correlated errors regularly observed across crop-years with sites, error variance for cane yield was multiplied by a factor of  $0.75 / 0.5 = 1.5$ .

For CCS, results from analysis of single-row plots across the plant-crop and ratoon-crop data in the SS2 trial planted in 2002 indicated that the covariance of error effects across crop-years was 0.5 compared with 1.79 and 2.33 for error variances in plant and ratoon crops respectively. Based on this, it was assumed that error covariance was equal to 0.25 times the error variance for the simulation model. Then, based on 7. and 8 above and assuming equal error variance in plant and first ratoon crops,

$$\begin{aligned} \sigma^2_{(e1+e2)/2} &= 1/2\sigma^2_{e1} + 1/2 \text{cov}_{e1,e2} = 1/2\sigma^2_{e1} + 1/2 * 1/4\sigma^2_{e1} \\ &= 1/2\sigma^2_{e1} + 1/8\sigma^2_{e1} = 0.625\sigma^2_{e1} \end{aligned}$$

Therefore, when selection at stage 2 was based on both plant and first-ratoon crop, the error variance for error effects for the mean of CCS across plant and ratoon crops was assumed to equal 0.625 times the error variance assumed for a single crop-year, compared with 0.5 times the variance calculated by default in the model if two environments are specified. Therefore, to provide the necessary correction in this situation, the error variance used in the simulation model for this stage of selection was multiplied by 1.25 ( $0.625/0.5$ ).

### ***Error variance adjusted in multi-row plots at final stage***

In final stage of selection for the evaluation of options tested, there were three crop-years. Hence, the error variance applied to the mean across plant, first-ratoon and second-ratoon crop-years was determined for the average of the error effects  $e1$ ,  $e2$ ,  $e3$ , where these are the error effects across plant, first-ratoon and second-ratoon crop-years. Based on published reports and past experience, it was assumed that on average error variances do not differ across crop-years.

$$\begin{aligned} \text{From equation 2. above, } \text{Var}(e1+e2+e3)/3 &= 1/9 (\text{var}((e1+e2)+ e3)) \\ &= 1/9((\text{var}(e1+e2) + \text{var}(e3) + 2 \text{cov}_{(e1+e2),e3}) \end{aligned}$$

It can be shown that  $\text{cov}_{(e1+e2),e3} = \text{cov}_{e1,e3} + \text{cov}_{e2,e3}$

Therefore from 7. it can be shown that

$$\begin{aligned}
 \sigma^2_{(e1+e2+e3)/3} &= \sigma^2_{((e1+e2)+e3)/3} \\
 &= 1/9(\sigma^2_{e1+e2} + \sigma^2_{e3} + 2 \text{cov}_{e1,e3} + 2\text{cov}_{e2,e3}) \\
 &= 1/9(\sigma^2_{e1} + \sigma^2_{e2} + 2 \text{cov}_{e1,e2} + \sigma^2_{e3} + 2 \text{cov}_{e1,e3} + 2\text{cov}_{e2,e3}) \\
 &\text{(Assuming } \sigma^2_{e1} = \sigma^2_{e2} = \sigma^2_{e3}) \\
 &= 1/9(3 \sigma^2_{e1} + 2 (\text{cov}_{e1,e2} + \text{cov}_{e1,e3} + \text{cov}_{e2,e3})) \\
 &= 1/3 \sigma^2_{e1} + 2/9(\text{cov}_{e1,e2} + \text{cov}_{e1,e3} + \text{cov}_{e2,e3})
 \end{aligned}$$

The results from analysis of plant crop and first-ratoon data from the final stage trial (data are not shown) indicated that the genetic correlation between the plant crop and first ratoon was 0.93 for TCH and 0.90 for CCS, respectively.  $\text{Cov}_{e1,e2}$  was approximately 40 % value of the  $\sigma^2_{e1}$  for both of CCS and TCH.

It was assumed that  $\text{cov}_{e1,e2} = \text{cov}_{e1,e3} = \text{cov}_{e2,e3}$

Then,

$$\begin{aligned}
 &1/3 \sigma^2_{e1} + 2/9(\text{cov}_{e1,e2} + \text{cov}_{e1,e3} + \text{cov}_{e2,e3}) \\
 &= 1/3 \sigma^2_{e1} + 2/9(3\text{cov}_{e1,e2}) \\
 &= 1/3 \sigma^2_{e1} + 2/9(3 \times 0.4\sigma^2_{e1}) \\
 &= 0.59 \sigma^2_{e1}
 \end{aligned}$$

Therefore, when selection at the final stage was based on the mean across plant, first-ratoon and second-ratoon crops, the error variances for both CCS and TCH for the model based on error variance estimated in a single crop-year were multiplied by 0.59, accounting for the effects of replication and correlated error effects between crop-years. For the model, if three crop-years are specified, the error variance based on use of three environments will default to the error variance divided by 3. Therefore, to adjust for correlated errors as indicated here, the correlated value is  $0.59 / (1/3) = 1.77$  for multiplying the error variance. The adjustments for  $\sigma^2_e$  estimated are summarised in Table A4.1

**Table A4.1 Factor to multiply  $\sigma^2_e$  in the model (based on number of crop-years)**

Trial design	Selection stage	Factors to apply to the model for CCS	Factors to apply to the model for TCH
2 crop-years	2	1.25	1.50
3 crop-years	Final	1.77	1.77

#### A4.4 Adjustment of genotype x environment interaction variance in the model

The selection-simulation model defaults to determining genotype by environment interaction variance by dividing the basic assumed GE variance specified by the number of environments used for evaluation. Environments consist of combinations of sites by crop-years. However, we know that:

$$\sigma^2_{ge} = \sigma^2_{gs} + \sigma^2_{gc} + \sigma^2_{gcs}$$

where  $\sigma^2_{gs}$ ,  $\sigma^2_{gc}$  and  $\sigma^2_{gcs}$  are variances for effects due to genotype by site, genotype by crop-years and genotype by crop-years by site, respectively. These effects are not equal. Therefore, adjustments were made for genotype by environment interaction variances in some cases to account for differential effects of the different sub-components of total

genotype by environment interaction. Methods and assumptions used in determining these adjustments are described below (specific assumptions shown are for the Burdekin but the same methods apply for the Central).

### ***For CCS***

Based on analysis of past breeding program data and project results it was assumed that

$$\sigma_{gc}^2 = 0 \text{ and } \sigma_{gs}^2 = \sigma_{gcs}^2$$

$$\text{Therefore } \sigma_{gs}^2 = \sigma_{gcs}^2 = 0.5 * \sigma_{ge}^2$$

The following options were specified during testing of different selection system options:

#### *1. Clones tested in two sites and one crop-year:*

$$\sigma_{ge}^2(2 \text{ sites x } 1 \text{ crop-year}) = 1/2\sigma_{gs}^2 + 1/(2 \times 1)\sigma_{gcs}^2$$

$$= 1/4\sigma_{ge}^2 + 1/4\sigma_{ge}^2$$

$$= 1/2\sigma_{ge}^2$$

Since this is the same value as used in the model by default, no further adjustment was necessary.

#### *2. Clones tested at one site and two crop-years:*

$$\sigma_{ge}^2(1 \text{ sites x } 2 \text{ crop-year}) = 1/1\sigma_{gs}^2 + 1/(1 \times 2)\sigma_{gcs}^2$$

$$= 1/2\sigma_{ge}^2 + 1/4\sigma_{ge}^2$$

$$= 3/4\sigma_{ge}^2$$

The model will apply  $0.5 \times \sigma_{ge}^2$  (2 environments) by default, and therefore an upward adjustment of 1.5 times was applied to the size of the GE interaction variance.

#### *3. Clones tested at two sites and two crop-years:*

$$\sigma_{ge}^2(2 \text{ sites x } 2 \text{ crop-year}) = 1/2\sigma_{gs}^2 + 1/(2 \times 2)\sigma_{gcs}^2$$

$$= 1/4\sigma_{ge}^2 + 1/8\sigma_{ge}^2$$

$$= 3/8\sigma_{ge}^2$$

The model will apply a value for  $\sigma_{ge}^2$  of  $\sigma_{ge}^2(2 \text{ sites x } 2 \text{ crop-year}) = 1/4 \sigma_{ge}^2$ , and therefore an adjustment of  $(3/8)/(1/4) = 1.5$  should be applied.

#### *4. Clones tested at four sites and one crop-year:*

$$\sigma_{ge}^2(4 \text{ sites x } 1 \text{ crop-year}) = 1/4\sigma_{gs}^2 + 1/(4 \times 1)\sigma_{gcs}^2$$

$$= 1/8\sigma_{ge}^2 + 1/8\sigma_{ge}^2$$

$$= 1/4\sigma_{ge}^2$$

This is the same value as used in the model by default, no further adjustment was necessary.

#### *5. Clones tested at four sites and 3 crop-years:*

$$\sigma_{ge}^2(4 \text{ sites x } 3 \text{ crop-year}) = 1/4\sigma_{gs}^2 + 1/(4 \times 3)\sigma_{gcs}^2$$

$$= 1/8\sigma_{ge}^2 + 1/24\sigma_{ge}^2$$

$$= 1/6\sigma_{ge}^2$$

In the model,  $\sigma_{ge}^2(4 \text{ sites x } 3 \text{ crop-year}) = 1/12\sigma_{ge}^2$  by default, and therefore an adjustment of 2 times  $((1/6\sigma_{ge}^2)/(1/12\sigma_{ge}^2))$  was applied.

**For TCH**

We know that  $\sigma_{ge}^2 = \sigma_{gs}^2 + \sigma_{gc}^2 + \sigma_{gcs}^2$

Based on personal communication with breeders and analysis of breeding program data, it

was assumed that  $\sigma_{gs}^2 = \sigma_{gc}^2 = \sigma_{gcs}^2$

Therefore  $\sigma_{gs}^2 = \sigma_{gc}^2 = \sigma_{gcs}^2 = 1/3\sigma_{ge}^2$

1. *Clones tested at two sites and one crop-year:*

$$\sigma_{ge}^2(2 \text{ sites} \times 1 \text{ crop-year}) = 1/2\sigma_{gs}^2 + 1/1\sigma_{gc}^2 + 1/(2 \times 1)\sigma_{gcs}^2$$

$$= 1/6\sigma_{ge}^2 + 1/3\sigma_{ge}^2 + 1/6\sigma_{ge}^2$$

$$= 2/3\sigma_{ge}^2$$

In the model,  $\sigma_{ge}^2(2 \text{ sites} \times 1 \text{ crop-year}) = 1/2\sigma_{ge}^2$

Therefore, an adjustment of 1.33 times was applied.

2. *Clones tested at one site and two crop-years:*

$$\sigma_{ge}^2(1 \text{ sites} \times 2 \text{ crop-year}) = 1/1\sigma_{gs}^2 + 1/2\sigma_{gc}^2 + 1/(1 \times 2)\sigma_{gcs}^2$$

$$= 1/3\sigma_{ge}^2 + 1/6\sigma_{ge}^2 + 1/6\sigma_{ge}^2$$

$$= 2/3\sigma_{ge}^2$$

In the model,  $\sigma_{ge}^2(1 \text{ site} \times 2 \text{ crop-year}) = 1/2\sigma_{ge}^2$

Therefore, an adjustment of 1.33 times was applied.

3. *Clones tested at two sites and two crop-years:*

$$\sigma_{ge}^2(2 \text{ sites} \times 2 \text{ crop-year}) = 1/2\sigma_{gs}^2 + 1/2\sigma_{gc}^2 + 1/(2 \times 2)\sigma_{gcs}^2$$

$$= 1/6\sigma_{ge}^2 + 1/6\sigma_{ge}^2 + 1/12\sigma_{ge}^2$$

$$= 5/12\sigma_{ge}^2$$

In the model,  $\sigma_{ge}^2(2 \text{ sites} \times 2 \text{ crop-year}) = 1/4\sigma_{ge}^2$

Therefore, an adjustment of 1.67 times was applied.

4. *Clones tested at four sites and one crop-year:*

$$\sigma_{ge}^2(4 \text{ sites} \times 1 \text{ crop-year}) = 1/4\sigma_{gs}^2 + 1/1\sigma_{gc}^2 + 1/(4 \times 1)\sigma_{gcs}^2$$

$$= 1/12\sigma_{ge}^2 + 1/3\sigma_{ge}^2 + 1/12\sigma_{ge}^2$$

$$= 1/2\sigma_{ge}^2$$

In the model,  $\sigma_{ge}^2(4 \text{ sites} \times 1 \text{ crop-year}) = 1/4\sigma_{ge}^2$

Therefore, an adjustment of 2.0 times was applied.

5. *Clones tested at four sites and three crop-years:*

$$\sigma_{ge}^2(4 \text{ sites} \times 3 \text{ crop-year}) = 1/4\sigma_{gs}^2 + 1/3\sigma_{gc}^2 + 1/(4 \times 3) \times 4\sigma_{gcs}^2$$

$$= 1/12\sigma_{ge}^2 + 1/9\sigma_{ge}^2 + 1/36\sigma_{ge}^2$$

$$= 3/36\sigma_{ge}^2 + 4/36\sigma_{ge}^2 + 1/36\sigma_{ge}^2$$

$$= 2/9\sigma_{ge}^2$$

In the model,  $\sigma_{ge}^2(4 \text{ sites} \times 3 \text{ crop-year}) = 1/12\sigma_{ge}^2$

Therefore, an adjustment of 2.67 times was applied.

The adjustments for  $\sigma_{ge}^2$  estimated above are summarised in Table A4.2

**Table A4.2 Factor to multiply  $\sigma_{ge}^2$  in the model (based on number of sites and crop-years)**

<b>Trial design</b>	<b>Factors to apply to the model for CCS</b>	<b>Factors to apply to the model for TCH</b>
2 sites x 1 crop-year	1	1.33
1 sites x 2 crop-years	1.5	1.33
2 sites x 2 crop-years	1.5	1.67
4 sites x 1 crop-year	1	2
4 sites x 3 crop-years	2	2.67

#### **A4.5 Determining an optimal selection index**

For maximising gain from selection gain in 1-row plots, an optimal selection index for small plots at stage 2 should be applied. Selection based on cane yield in 1 row by 10-m plots or visual grade in 5-m plots were both considered as part of selection system options. These are associated with different statistical parameters and, therefore, will each have different optimal selection indices. Details for determining the optimal index is discussed below. Specific assumptions on values to illustrate the methods are from the Burdekin but the same procedures apply generally.

Consider an optimal selection index combining CCS and TCH. Following terminology of Cotterill and Dean (1990), the index,  $I$ , may be written as:

$$I = b_1 * P_{ccs} + b_2 * P_{tch} \quad (9)$$

where  $P_{ccs}$  and  $P_{tch}$  are an individual clone's phenotypic performance for CCS and TCH, and  $b_1$  and  $b_2$  are the index coefficients for these two traits, respectively. The aim of the index is to maximise the gain in genetic worth for economic value.

The definition of genetic worth is:

$$H = w_{ccs} * G_{ccs} + w_{tch} * G_{tch} \quad (10)$$

where  $H$  is the genetic worth of a sugarcane clone,  $G_{CCS}$  and  $G_{TCH}$  are the true genetic values for CCS and cane yield, respectively, and  $w_{ccs}$  and  $w_{tch}$  are economic weights for each trait.

It is not possible to select directly for a clone having the highest genetic worth  $H$  because only phenotype rather than genotype can be observed. We know the phenotypic values ( $P_{ccs}$  and  $P_{tch}$ ) for each variety in small plots. Therefore, the aim is to select on the index value  $I$  that maximizes gain in genetic worth  $H$  (which integrates unobservable  $g_{ccs}$  and  $g_{tch}$ ). It can be shown that the values of  $b_1$  and  $b_2$  which attain this goal can be determined from the following:

$$[b] = [P]^{-1} * [G] * [w]$$

Where, if there are  $m$  observed traits (in equation 1) and  $q$  genetic values (in equation 10),  $[P]$  is an  $m$  by  $m$  matrix of phenotypic variances and covariances among the observed traits,  $[G]$  is a  $m$  by  $q$  matrix of covariances between the observations on a genotype and the genetic values.

The selection index was calculated using data obtained from the stage 2 and the final trials in the Burdekin. Key steps for determining of selection index for using in small plots (1-row) are outlined below.

#### **A4.6 Determining of economic weights $[W]$ in large plots (pure stands) for the Burdekin region**

It was assumed, based on prior work, that the marginal cost of processing an extra tonne of cane equals \$12, including harvesting, transport and milling costs. The marginal cost of producing an extra tonne of sugar was assumed to equal \$0.5. Sugar price was assumed to equal \$275 per tonne, and mean cane yield and CCS was assumed to equal 150 t/ha and 13%, respectively.

An increase in one unit of CCS is therefore equal to:

$$\text{REV (relative economic value)} = 150 \cdot (275 \cdot 0.13 - 12 - 0.5 \cdot 0.13) - 150 \cdot (275 \cdot 0.12 - 12 - 0.5 \cdot 0.12) = 411.75 (\$)$$

An increase one unit (t/ha) of cane yield is equal to:

$$\text{REV (relative economic value)} = 151 \cdot (275 \cdot 0.12 - 12 - 0.5 \cdot 0.12) - 150 \cdot (275 \cdot 0.12 - 12 - 0.5 \cdot 0.12) = 20.94 (\$)$$

Therefore,

$$\text{Economic weights} = \text{REV}_{(\text{CCS})} : \text{REV}_{(\text{TCH})} = 411.75 : 20.94 \approx 19.7:1$$

$$[w] = \begin{pmatrix} \text{CCS} \\ \text{TCH} \end{pmatrix} = \begin{pmatrix} 19.7 \\ 1 \end{pmatrix}$$

In stage 2 plots, observed traits were cane yield and CCS single row plots, but genetic values are for cane yield and CCS in pure stands. Therefore:

$$[P] = \begin{pmatrix} \sigma^2 p(\text{ccs1row}) & \text{Covp}(\text{ccs1row}, \text{tch1row}) \\ \text{Covp}(\text{ccs1row}, \text{tch1row}) & \sigma^2 p(\text{tch1row}) \end{pmatrix}$$

Where  $\sigma^2 p(\text{ccs1row})$  and  $\sigma^2 p(\text{tch1row})$  are CCS and TCH phenotypic variance in 1-row plots, respectively, and  $\text{Covp}(\text{ccs1row}, \text{tch1row})$  is the phenotypic covariance between CCS and cane yield measured in 1-row plots.

$$[G] = \begin{pmatrix} \text{Covg}(\text{ccs1row}, \text{ccs4row}) & \text{Covg}(\text{ccs1row}, \text{tch4row}) \\ \text{Covg}(\text{tch1row}, \text{ccs4row}) & \text{Covg}(\text{tch1row}, \text{tch4row}) \end{pmatrix}$$

where  $\text{Covg}(\text{ccs1row}, \text{ccs4row})$ ,  $\text{Covg}(\text{ccs1row}, \text{tch4row})$ ,  $\text{Covg}(\text{tch1row}, \text{ccs4row})$  and  $\text{Covg}(\text{tch1row}, \text{tch4row})$  are genetic covariance between CCS 1-row and 4-row, between CCS 1-row and TCH 4-row, between TCH 1-row and CCS 4-row, and between TCH 1-row and 4-row, respectively.

In determining the G matrix, the genetic covariance (common variance due to genotypes) between single-row plots and multi-row plots for the same trait is equal to the genetic variance. For modelling visual grade recorded in 1-row by 5-m plots, this trait was scaled to have the same genetic variance as cane yield, and other parameters (error variance, and a parameter reducing the genetic correlation between visual grade in a single row plot and cane yield in multi-row plots) set in appropriate proportion to this. It was assumed that the genetic variance for CCS = 1.0 and for cane yield = 350. Two options were assumed for determining genetic covariances, namely assuming the genetic correlation between CCS and cane yield equals -0.5 and 0.

By definition,

$$r_{g(\text{CCS}, \text{TCH})} = \text{Cov}_{g(\text{CCS}, \text{TCH})} / (\sigma^2_{(\text{CCS})} * \sigma^2_{(\text{TCH})})^{0.5}$$

Therefore, when the genetic correlation between CCS and cane yield is assumed to equal -0.5,  $\text{covg}(\text{ccs1row}, \text{tch1row}) = -0.50 \times (1 \times 350)^{0.5} = -9.35$ . When the genetic correlation is assumed to equal zero,  $\text{covg} = 0$ .

Therefore, for when the genetic correlation between CCS and cane yield is assumed to equal -0.5,

$$[G] = \begin{pmatrix} 1 & -9.35 \\ -9.35 & 350 \end{pmatrix}$$

and when the genetic correlation between CCS and cane yield is assumed to equal zero,

$$[G] = \begin{pmatrix} 1 & 0 \\ 0 & 350 \end{pmatrix}$$

In determining the P matrix,  $\text{cov}_p = \text{cov}_g + \text{cov}_{gxe} + \text{cov}_{\text{comp}} + \text{cov}_{\text{error}}$ , it was assumed in the model, based on analysis of data, that on average  $\text{cov}_{gxe}$ ,  $\text{cov}_{\text{comp}}$  and  $\text{cov}_{\text{error}}$  between CCS and cane yield equals zero. These values may vary in individual environments, but an analysis of trial data in this project (not shown here) indicated an assumption of zero would be approximately correct overall.

Therefore,

$$\text{Covp}(\text{ccs1row}, \text{tch1row}) = \text{Covg}(\text{ccs1row}, \text{tch1row}) = -9.35$$

Phenotypic variance for CCS and TCH depends on experimental design, and needs to be determined for each specific design. Different design will give different phenotypic variance for CCS and TCH. Three examples are given below for options commonly used in the modelling of selection gains.

(i) 1 row x 10m plots, 1 site x 1 replicate per site x 2 crop-years

$$\text{Phenotypic variance } \sigma_p^2 = \sigma_g^2 + \sigma_c^2 + (\sigma_{ge}^2/n_e)f_1 + (\sigma_e^2/(n_e.n_r))f_2$$

Where  $n_e$  = number of environments,  $n_r$  = number of replicates,  $f_1$  = factor for multiplying GxE interaction variance by when determining GE variance for two crop-years to account for different proportional effects of subcomponents, and  $f_2$  = factor for multiplying error variance by when determining error variance from repeated measures across crop-years.

Based on assumptions used in the model (Table A5.1), for CCS,  $\sigma_g^2 = 1.0$ ,  $\sigma_c^2 = 0$ ,  $\sigma_{ge}^2 = 0.4$ ,  $n_e = 2$ ,  $f_1 = 1.5$ ,  $\sigma_e^2 = 1.0$ ,  $n_r = 1$ , and  $f_2 = 1.25$ . For cane yield,  $\sigma_g^2 = 350$ ,  $\sigma_c^2 = 1050$ ,  $\sigma_{ge}^2 = 52.5$ ,  $n_e = 2$ ,  $f_1 = 1.33$ ,  $\sigma_e^2 = 900$ ,  $n_r = 1$ , and  $f_2 = 1.5$ .

Therefore,

$$\text{CCS } \sigma_{p(\text{ccs1row})}^2 = 1 + 0 + 1/2 \times 0.4 \times 1.5 + 1/2 \times 1 \times 1.25 = 1.93$$

$$\text{TCH } \sigma_{p(\text{tch1row})}^2 = 350 + 1050 + 52.5/2 \times 1.33 + 1/2 \times 900 \times 1.50 = 2110$$

Therefore,

$$[P] = \begin{pmatrix} \sigma_{2p(\text{ccs1row})} & \text{Covp}(\text{ccs1row}, \text{tch1row}) \\ \text{Covp}(\text{ccs1row}, \text{tch1row}) & \sigma_{2p(\text{tch1row})} \end{pmatrix}$$

$$= \begin{pmatrix} 1.93 & -9.35 \\ -9.35 & 2110 \end{pmatrix}$$

$$\text{and } [P]^{-1} = \begin{pmatrix} 0.5295 & 0.000480 \\ 0.002346 & 0.000480 \end{pmatrix}$$

(ii) For 1 row x 10m plots, 1 site x 2 replicates x 2 crop-years.

Assumptions and calculations are the same as for (i) except  $n_r = 2$ . Therefore,

$$\text{CCS } \sigma_{p(\text{ccs1row})}^2 = 1.0 + 0 + 1/2 \times 0.4 \times 1.5 + 1/4 \times 1.0 \times 1.25 = 1.61$$

$$\text{TCH } \sigma_{p(\text{tch1row})}^2 = 350 + 1050 + 52.5/2 \times 1.33 + 1/4 \times 900 \times 1.5 = 1772$$

$$[P] = \begin{pmatrix} 1.61 & -9.35 \\ -9.35 & 1772 \end{pmatrix}$$

$$\text{and } [P]^{-1} = \begin{pmatrix} 0.6408 & 0.003381 \\ 0.003381 & 0.000582 \end{pmatrix}$$

(iii) 1 row x 5m plots, 1 site x 2 replicates per site x 1 crop-year

$$\text{Phenotypic variance } \sigma_p^2 = \sigma_g^2 + \sigma_c^2 + \sigma_{ge}^2/n_e + \sigma_e^2/(n_e.n_r)$$

Based on assumptions used in the model (Table A5.1), for CCS,  $\sigma_g^2 = 1.0$ ,  $\sigma_c^2 = 0$ ,  $\sigma_{ge}^2 = 0.4$ ,  $n_e = 2$ ,  $f_1 = 1.5$ ,  $\sigma_e^2 = 1.0$ ,  $n_r = 1$ , and  $f_2 = 1.25$ . For cane yield (or visual grade),  $\sigma_g^2 = 350$ ,  $\sigma_c^2 = 2100$ ,  $\sigma_{ge}^2 = 52.5$ ,  $n_e = 2$ ,  $\sigma_e^2 = 900$ ,  $n_r = 1$ .

Therefore,

$$\sigma_{p(\text{ccs1row})}^2 = 1 + 0 + 0.4/1 + 1/2 = 1.90$$

$$\sigma_{p(\text{tch1row})}^2 = 350 + 2100 + 52.5/1 + 900/2 = 2953$$

Therefore,

$$[P] = \begin{pmatrix} 1.90 & -9.35 \\ -9.35 & 2953 \end{pmatrix}$$

$$[P]^{-1} = \begin{pmatrix} 0.5346 & 0.001692 \\ 0.001692 & 0.0003439 \end{pmatrix}$$

Based on the above, the selection indices could be determined from  $[b] = [P]^{-1} * [G] * [w]$ . These are shown below, firstly for when the genetic correlation between CCS and cane yield is assumed to equal -0.5, and secondly for when this genetic correlation is assumed to equal 0.

(i) For when genetic correlation between CCS and cane yield equals -0.5

For 1 row x 10m plots, 1 site x 1 replicate per site x 2 crop-years,

$$\begin{pmatrix} b1 \\ b2 \end{pmatrix} = \begin{pmatrix} 0.5295 & 0.000480 \\ 0.002346 & 0.000480 \end{pmatrix} \times \begin{pmatrix} 1.0 & -9.35 \\ -9.35 & 350 \end{pmatrix} \times \begin{pmatrix} 19.7 \\ 1 \end{pmatrix} \approx \begin{pmatrix} 54 \\ 1 \end{pmatrix}$$

For 1 row x 10m plots, 1 site x 2 replicates per site x 2 crop-years,

$$\begin{pmatrix} b1 \\ b2 \end{pmatrix} = \begin{pmatrix} 0.6408 & 0.003381 \\ 0.003381 & 0.000582 \end{pmatrix} \times \begin{pmatrix} 1.0 & -9.35 \\ -9.35 & 350 \end{pmatrix} \times \begin{pmatrix} 19.7 \\ 1 \end{pmatrix} \approx \begin{pmatrix} 55 \\ 1 \end{pmatrix}$$

For 1 row x 5m plots (assuming cane yield estimated via visual grade), 1 site x 2 replicates per site x 1 crop-year,

$$\begin{pmatrix} b1 \\ b2 \end{pmatrix} = \begin{pmatrix} 0.5346 & 0.001692 \\ 0.001692 & 0.0003439 \end{pmatrix} \times \begin{pmatrix} 1.0 & -9.35 \\ -9.35 & 350 \end{pmatrix} \times \begin{pmatrix} 19.7 \\ 1 \end{pmatrix} \approx \begin{pmatrix} 78 \\ 1 \end{pmatrix}$$

(ii) For when genetic correlation between CCS and cane yield equals 0

For 1 row x 10m plots, 1 site x 1 replicate per site x 2 crop-years,

$$\begin{pmatrix} b1 \\ b2 \end{pmatrix} = \begin{pmatrix} 0.5181 & 0.0 \\ 0.0 & 0.0004739 \end{pmatrix} \times \begin{pmatrix} 1.0 & 0.0 \\ 0.0 & 350 \end{pmatrix} \times \begin{pmatrix} 19.7 \\ 1 \end{pmatrix} \approx \begin{pmatrix} 62 \\ 1 \end{pmatrix}$$

For 1 row x 10m plots, 1 site x 2 replicates per site x 2 crop-years,

$$\begin{pmatrix} b1 \\ b2 \end{pmatrix} = \begin{pmatrix} 0.6211 & 0.0 \\ 0.0 & 0.0005643 \end{pmatrix} \times \begin{pmatrix} 1.0 & 0.0 \\ 0.0 & 350 \end{pmatrix} \times \begin{pmatrix} 19.7 \\ 1 \end{pmatrix} \approx \begin{pmatrix} 62 \\ 1 \end{pmatrix}$$

For 1 row x 5m plots (assuming cane yield estimated via visual grade), 1 site x 2 replicates per site x 1 crop-year,

$$\begin{pmatrix} b1 \\ b2 \end{pmatrix} = \begin{pmatrix} 0.5263 & 0.0 \\ 0.0 & 0.0003386 \end{pmatrix} \times \begin{pmatrix} 1.0 & 0.0 \\ 0.0 & 350 \end{pmatrix} \times \begin{pmatrix} 19.7 \\ 1 \end{pmatrix} \approx \begin{pmatrix} 87 \\ 1 \end{pmatrix}$$

Overall, the derivation of an optimal selection index shows that a very strong bias should be placed on CCS during selection, compared with cane yield. This is a reflection of both the greater economic importance of CCS and the reduced effect of competition effect on this trait. As expected, when the genetic correlation between CCS and cane yield reduces, the weighting toward CCS also reduces.

## APPENDIX 5 - Comparison of realised and predicted gains

In this section, genetic gains predicted using the selection simulation model are compared with realised gains observed in the field trials. The model was set with genetic and statistical parameters similar to those estimated in the field trials. Differences between predicted and realised gains are discussed.

### A5.1 Burdekin region

Based on analysis of results from the field experimentation and published data the assumptions in Table A5.1 were used for the selection simulation model. The rationale for these assumptions is described below.

**Table A5.1 Parameters assumed for simulating selection and predicting gains from selection in the field experiments in the Burdekin**

Parameter		CCS	Cane yield
Starting $\sigma_g^2$		1.0	350
$\sigma_c^2$	1-row plots	0	1050
	4-row plots	0	0
$\sigma_e^2$	1-row plots	1.0	900
	4-row plots	1.0	350
$\sigma_{ge}^2 (\sigma_{ge}^2 / \sigma_g^2)$		0.4	0.15 times genetic variance in first clonal stage and 1.5 times in later stages
$r_g$ (CCS versus TCH)			-0.5

Starting genetic variances were assumed from considering results from analyses of variance of the random clones in multi-row plot trials. Since stage 2 trials consisted of single-row plots, estimates of variance due to genotypes in these trials were due to a combination of genetic variance and competition variance. The genetic variances of the random clones differed between trials/environments and were higher in the S3 trials on average than the final assessment trials. However, the values assumed were considered reasonably representative overall.

Estimates of competition variance were obtained from genetic correlations between 1-row plots and 4-row plots as explained below. Results obtained in this project (Table A1.10) were similar to prior results (Jackson and McRae 2001) that suggest a genetic correlation between 1-row plots and multi-row plots of approximately 0.5 for TCH and 1 for CCS. Simulating competition effects is complicated by potential correlations between competition effects and genetic effects, which has been reported as being negative or positive (eg Jackson and McRae 2001). However, for modelling selection, the important issue is the genetic correlation between the apparent genetic effect in small plots (consisting of true genetic effects (g) + competition effect (c)) and true genetic effects. This will be affected by both size of competition variance and genetic correlation between

competition effects and the true genetic effects. Use of a competition effect component in the model that achieves the desired genetic correlation between 1 row plots ( $g + c$ ) and pure stand ( $g$ ) will serve the purpose for a selection model.

Let the genetic correlation between  $g + c$  (ie performance in small plots) and  $g$  (performance in large plots) equal  $r_g$ . By definition  $r_g^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_c^2)$ , assuming no correlation between  $c$  and  $g$ . Therefore,  $\sigma_c^2 = \sigma_g^2 (1 - r_g^2) / r_g^2$ .

If  $r_g = 0.5$  and  $\sigma_g^2 = 1$ , then  $\sigma_c^2 = 3$ . So in 1-row plots, for TCH if  $\sigma_g^2 = 350$ , then  $\sigma_c^2 = 1050$ .

For visual ratings, this trait was assumed to be an estimate of cane yield and treated as cane yield in the model. The heritability for visual rating was found to be similar to cane yield (Table A1.1), but variances were different due to differences in scaling of the units. For the purposes of simulation of selection, all variances associated with visual grade used for modelling (genetic, error, GE) were rescaled to be equivalent to cane yield. However, the genetic correlation of visual ratings in the 5-m plots with cane yield in 4-row plots on average was 25% less than for cane yield measured in 1-row by 10-m plots (-0.5, versus 0.66). This may be due either to increased competition or to other factors such as subjective bias toward factors apart from cane yield in reducing the value of visual ratings in 5-m plots slightly compared with direct measurement of cane yield in 10-m plots. To produce the required genetic correlation observed, the competition effect was set higher for visual rating in the 5-m plots than for cane yield. A variance for competition effects for visual rating equal to 2100 produces a genetic correlation 25% less than that simulated for TCH in 1-row plots and, therefore, this setting was used in the model.

For error variance, estimates varied in different trials. Overall, in both the SS1 and the SS2 trials, variances for CCS were around 1 in both 1-row plots (Table A1.1) and in multi-row plots (Tables A1.3, A1.11). For TCH, error variances in 1-row plots were assumed to be 900 (Table A1.1) and about 350 for multi-row plots (Table A1.9, A1.11).

It was assumed that GE variances for CCS (based on estimates from pooled analysis of variance of the random clones across environments, Table A1.12) were 0.4 times the genetic variance. For cane yield, the ratio of GE:G was assumed to equal 0.15 based on the same results. As mentioned previously (Appendix 1), genotype by environment variance increased following initial selection. Based on analysis of all clones evaluated in SS2 stage 3 across two trials (Table A1.4), the ratio of GE:G for this stage was set at 1.50. However, it should be emphasised that effects of GE variance may vary with individual environments and this could be an important source of differences in realised gains from individual trials.

Genetic correlations between CCS and cane yield varied considerably among different environments (data not shown) but an average reasonable figure for modelling based on overall results was chosen as -0.5. This was also similar to the genetic correlation between CCS in stage 2 and TCH in stage 3 in the SS2 trials. Results achieved in specific environments might vary significantly because of this variation in genetic correlation. Varying genetic correlation should be a focus of sensitivity analysis – both in terms of determining an optimal selection index and in assessing alternative selection systems.

Gains predicted from the model are given below (Table A5.2) and compared with realised gains described above. The model was specified to follow parameters observed for the field trials as closely as possible. Generally, the model predicted gains well, considering the high level of variation that is usually associated with measuring realised gains. The variations between predicted and realised are well within the variation that could be expected due to sampling variation of environments. A possible exception is the larger observed reduction in cane yield from the intensive selection of CCS in S2 than predicted, even though a negative genetic correlation between CCS and cane yield of -0.5 was assumed. This result is suggestive of a non-linear genetic relationship between CCS and cane yield such that extremely high levels of CCS could be associated with a sharper dropping of cane yield than indicated by the relationship among the entire population for these two traits. In practice, this effect could be addressed by some concurrent selection pressure for cane yield in an optimal selection index.

Overall the reasonably tight agreement between the realised and predicted gains provides further confidence that the model is capturing the major sources of variation and may be used for predicting different selection system options.

**Table A5.2 Comparison of realised and predicted gains for CCS and cane yield from selection in the Burdekin trials**

Selection system	Stage	Gain	Trait	
			CCS	Cane yield
SS1	S2	Realised	0.39	9.2
		Predicted	0.29	8.7
SS2	S2	Realised	1.14	-18
		Predicted	1.26	-10.4
	S3	Realised	-0.14	10
		Predicted	0.12	7.8

## A5.2 Central region

Parameters assumed for simulating selection and predicting gains in the Central region experiments are given below (Table A5.3).

**Table A5.3 Parameters assumed for simulating selection and predicting gains from selection in the field experiments in the Central region**

Parameter	CCS	Cane yield
Starting $\sigma_g^2$	0.43, 0.9	60, 120
$\sigma_c^2$	1-row plots	60, 120
	4-row plots	0
$\sigma_e^2$	1-row plots	700
	4-row plots	100
$\sigma_{ge}^2 (\sigma_{ge}^2 / \sigma_g^2)$	2	1.8
$r_g$ (CCS versus TCH)	0.3, -0.3, 0	

Most values initially assumed were based on analysis of the random clones following the rationale described for the Burdekin. Visual grade was assumed to be a representation of cane yield, with heritability as observed in field trials ( $H=0.25$  average), and genetic, error and competition variances scaled to be in line with an assumed genetic variance of 62 as observed in random clones across FATs. Based on the average estimate of genetic variance for cane yield in single row plots (= 120, consisting of the joint action of true genetic variance and competition effects; Appendix 2) in the Central region, a competition variance of 60 was initially assumed. Competition variance for visual grade was determined based on same assumptions for Burdekin trials, so competition variance set at six times genetic variance = 120.

Based on these results, the model was slightly under-estimating gains for SS1 and SS2 (both stages). An interesting feature in the realised gains is the fact that a positive gain for TCH was observed from selection in S2 in SS2, despite strong selection pressure occurring only for CCS. It is extremely unlikely that the very limited pressure for visual grade could have caused this large response. This result is strongly suggestive of a positive genetic correlation between CCS and cane yield in the S2 environment at least. These under-estimates could be attributed to errors in assumptions of the above parameters above. There was some indication that the estimates of genetic variance determined for the random clones may have under-estimated the genetic variance of the starting population especially for CCS, as evidenced by the greater genetic variance that was observed in the whole population in S3. Changing the assumptions on the starting genetic variances to the upper limit of the 95% confidence interval of the original estimates (to 0.9, 120 for CCS and cane yield respectively), and imposing a genetic correlation between CCS and cane yield of 0.3 resulted in gains given in prediction B, which were close to realised gains.

**Table A5.4 Realised and predicted gains for CCS and cane yield in the SS1 (for stage 2) and SS2 (for stage 2 and 3, with stage 3 representing cumulative gains)**

Selection system	Stage	Gain	Trait	
			CCS	Cane yield
SS1	S2	Realised	0.8	6.8
		Prediction A	0.38±0.11	4.0±1.2
		Prediction B	0.77±0.12	8.21±1.1
SS2	S2	Realised	0.5	7.4
		Prediction A	0.5±0.2	0±0.1
		Prediction B	0.8±0.2	5.6±2.5
	S3	Realised	1.0	7.2
		Prediction A	0.66±0.20	2.3±2.1
		Prediction B	1.1±0.21	6.7±3.1

## **APPENDIX 6 - Predictions and discussion**

A wide range of selection systems were modelled. Two broad areas of investigation are presented here:

- (i) comparison of gains predicted from 5-m plots using a combination of CCS measurement and visual selection with gains predicted from using 10-m plots where cane yield is measured destructively and a combination of CCS measurement and cane yield is used for selection; and
- (ii) comparison of selection systems which involved two phases of testing in 4-row plots versus 1 phase (with the latter used at present).

In all cases, it was assumed that the objective is to maximise genetic gain for relative economic value (REV). In all selection system designs an optimal selection index was determined and applied where 1-row plots were used to maximise gains given the trial designs.

### **A6.1 Burdekin region**

Initial assumptions used in the modelling were as given in Table A5.1, with deviations from these parameters described. Because of uncertainty with some assumptions about the parameters, these can be changed and the sensitivity of comparisons among alternative selection systems to the assumptions determined.

#### **A6.1.1 Comparison of 1-row by 10-m plots (current practice) with 1-row by 5-m plots**

With 5-m plots, for the same area of land and planting costs, either twice the number of clones can be evaluated, or alternatively twice the number of replicates (eg two replicates instead of one) can be used. In addition, if visual grade is used instead of cane yield measured via harvesting, selections may be transferred to the next stage of selection in the same year. If cane yield is measured via harvesting, then destruction of cane means that setts must be obtained from these trials in subsequent ratoon crops. Assumptions used for parameters were as described in Table A5.1 above.

Four designs were simulated corresponding to options which are comparable in terms of land used and could be reasonably practically implemented in regional breeding programs. Results are presented in Table A6.1. It was assumed for each design that 100 clones would be selected. In all cases, an optimal selection index designed to maximise gain in each case was used. It should be emphasised that this index is different to that used in the field trials in this project: in the field experiments for the 10-m plots, selections were based on sugar yield (TSH), while for the 5-m plots, selections were based on CCS. Neither of these selection indices was optimal. However, optimal selection indices can be determined (using genetic parameters estimated for the random clones), as described in Appendix 3, and were applied in the simulations below so that maximum gains are compared for each experiment design.

**Table A6.1 Gains from selection (economic value, \$/ha) from selection in different trial configurations in S2**

Assumptions on parameters were as for Table, except for GE interaction variance, where “High GE” corresponds to 0.8 and 1.0 times genetic variance for CCS and cane yield respectively, and “Low GE” corresponds to 0.4 and 0.15 times genetic variance for CCS and cane yield respectively. An optimal selection index was used in all cases to maximise gain in economic value. It was assumed that the top 100 clones were selected in each case. Means and standard errors are based on results from 50 simulations for each design.

Design criteria				Gain in economic value (\$/ha)	
Plot size	Number of crop-years	Number of replicates	Number of clones	High GE	Low GE
1 row x 10 m	1	1	1600	276±8	293±9
		2	800	251±8	281±10
	2	1	1600	297±10	326±11
		2	800	270±10	302±8
1 row x 5 m	1	1	3200	291±11	308±11
		2	1600	276±9	307±10
	2	1	3200	321±10	349±11
		2	1600	305±10	324±11

Overall, these results indicate:

- Gains of about 30% of total system gains are realised in this stage (see next section for total gains).
- Slightly better gains are achieved in all cases by evaluating 2n clones in one replicate rather than n clones in two replicates, but the difference is only small. However, this assessment does not consider the difficult to assess value of a breeder knowing accurately what the error variance is within trials at this stage for CCS and cane yield (or visual grade), and therefore being able to better determine an optimal selection index rather than assume variance parameters for this purpose.
- For the equivalent number of crop-years, the 5-m plots are showing a better gain over the 10-m plots, but the difference is small. Therefore, although visual grade in the 5-m plots for predicting cane yield in a pure stand is not as effective as weighing the same plots via a mechanical harvester, the increased number of clones (and therefore selection intensity) and/or replication with the 5-m plots makes up for this.
- As expected, two crop-years gives a gain over one crop-year, but the difference is only small, especially when GE is assumed to be small. Gains would be limited by the relatively small size of G by crop-year compared with genotypes, and the fact that errors are correlated between crop-years.
- Gains from 5-m plots in one crop-year are very similar to gains from 10-m plots in two crop-years. In practice, the 10-m plot option tested here needs to be conducted over two crop-years, even if measurements are only made in the plant crop, unless there is expensive concurrent propagation of all clones. This is because this assumes cane yield is being measured destructively via harvesting and weighing in

the 10-m plots, and therefore selections can only be cut for planting to the next stage or propagation in the ratoon crop.

- Given the above point, if saving 1 year in the selection system is important then the use of 5-m plots with visual grade estimation for cane yield would provide an option. The cost of this is estimated to be similar to that for measuring plant and ratoon crops with 10-m plots.

### **A6.1.2 Comparison of overall systems in the Burdekin**

The current system widely used in the Australian sugarcane-breeding program involves:

1. Testing clones in 1-row by 10-m plots with one or two replicates in stage 2 in plant and first-ratoon crops,
2. Propagation of selected clones, and planting about 100 clones into final assessment trials (4 rows by 10 m by 2 replicates per site) at four sites, and evaluation in plant, first-ratoon and second-ratoon crops.

This system was used as a benchmark to compare other systems.

Because of the extremely large number of designs possible, an exhaustive evaluation of all options for alternative cannot be done. However, some designs were identified which were predicted as being superior to the current system. Because of uncertainty and potential variability of GE interaction variance and genetic correlations, the superior designs were also compared with the current system under alternative assumptions relating to these parameters. Results are summarised in Table A6.2 for several designs.

Other parameters were also varied (eg genetic correlation between cane yield and CCS set to zero or positive). While absolute gains varied (greatly in the case of changes in genetic correlations for example), in all cases tested the ranking of the three systems was the same – ie options 2 and 3 similar and greater than option 1.

In addition to the above options, a key question was whether more or less effort on stage 2 versus stages 3 and 4 in options 2 and 3 would be best. On the one hand, costs of screening **per genotype** in S2 are clearly much less than in large plots in S3 and S4, and an argument could be made that effort taken from S3 and S4 and put into S2 would lead to much larger populations initially screened, a better chance of generating rare elite clones, and higher selection intensities; all factors that favour greater gains. This line of argument favours systems beginning with very large populations that have higher selection intensities. On the other hand, S2 trials give relatively unreliable data (compared with multi-row plots), effort devoted here may therefore be relatively inefficient, and reduction in numbers of clones being screened in relatively more accurate multi-row trials in S3 could be disadvantageous overall. The latter argument favours systems starting with lower population sizes and with less intensive selection intensities and larger numbers in later phases of selection.

Simulations that represent both sides of the selection intensities in options 2 and 3 were done. In one option (4), the same system design as option 2 above was assumed, except that the starting population was 800 clones, then 200 clones in S3 followed by 25 clones in S4. In another option (5), the same design as option 2 above was assumed, except with a starting population of 2000, then 130 clones in S3 then 25 clones in S4. In both

systems, total land area and costs would be approximately equal. The results indicated that option 4 (lower selection intensity) gave a genetic gain in REV of  $1030 \pm 27$ , slightly lower than the 1079 predicted for option 2 above. Option 5 was predicted to give a gain at  $1099 \pm 28$ , which was slightly more than that predicted for option 2 above but not statistically significant. From these results, it appears that the selection intensity and balance of effort in S2 represents a reasonable balance, although slightly more gains could be achieved through more intense selection pressure in S2. However, the latter program could be associated with greater risk since it is dependent on good results being obtained in the S2 trial and the environment in this trial being well representative of the targeted environments.

**Table A6.2 Predicted genetic gains in relative economic value (REV) for three different selection systems based on parameters assumed in the Burdekin. Two sets of parameters are assumed for the ratio of GE interaction variance to G variance: high assumes ratios of 0.8 and 1.0 for CCS and cane yield, respectively, while low assumes ratios of 0.4 and 0.15 for CCS and cane yield, respectively**

Option	Description of system	Gain (\$/ha)	
		High GE	Low GE
Option 1: Similar to current system	Stage 2: 1600 clones, 1 site, 1 row x 10m plots, 1 replicate per clone, evaluated in plant and first ratoon crops, selection based on optimal selection index	918±30	1011±32
	Stage 3: 100 clones selected from S2, these planted at 4 sites, 4 row x 10m plots, 2 replicates per site, evaluated in plant, first and second ratoon crops.		
Option 2	Stage 2: Same as current system.	1024±28	1079±31
	Stage 3: 150 clones selected from S2, these planted at 4 sites, 4 row x 10m plots, 1 replicate per site, evaluated in plant, first and second ratoon crops; best clones taken based on plant crop data and any superior clones identified in subsequent ratoon crops taken in subsequent years.		
	Stage 4: 25 clones evaluated at 4 sites, 4 row x 10m plots, 2 replicates per site, evaluated in plant, first, and second ratoon crops.		
Option 3	Stage 2: 1600 clones, 1 site, 1 row x 5m plots, 2 replicates per clone, evaluated in plant crop (CCS and visual grade), selection based on optimal selection index	1019±28	1082±26
	Stage 3: As for option 2		
	Stage 4: As for option 2		

## A6.2 Central region

Assumptions used in assessing alternative selection systems for the Central region are given in Table A6.3. Because of variability in observed values, and uncertainty, in some parameters obtained from Central region, it was important to examine how the model predictions were affected by changes in these. Results from changing the following parameters are presented:

- (i) Genetic variance was initially assumed at 0.9 (CCS) and 120 (TCH), and simulations also done with these values doubled.
- (ii) Error variances for the 5-m plots were initially assumed at 0.45 (CCS) and 480 (for TCH, estimated via visual grade). Simulations were also done with these set at the higher of 2.4 and 1440. The initial (low) setting for CCS was lower than observed in the field trials, but was considered representative of those that could be obtained in well-managed trials. The higher values observed in the field trials, as indicated in Appendix 2, were probably unusual and to a large degree associated with poor germination and associated errors. The error variances assumed for cane yield (estimated via visual grade in the 5m plots) corresponded to heritabilities of 0.33 and 0.14 for visual grade.
- (iii) Genetic correlation between CCS and cane yield was initially assumed to equal 0.3, and simulations also done at a setting of -0.3.

**Table A6.3 Sets of parameters used in simulating alternative selection systems in the Central region**

Parameter		CCS	Cane yield
Starting $\sigma_g^2$		0.9, 1.8	120, 240
$\sigma_c^2$	1-row plots	0	60 (10m plots), 120 (5m plots)
	4-row plots	0	0
$\sigma_e^2$	1-row plots	0.4 (10m plots), 0.45 and 2.4 (5m plots)	360 (10m plots), 480 and 1440 (5m plots)
	4-row plots	1.0	100
$\sigma_{ge}^2 (\sigma_{ge}^2 / \sigma_g^2)$		1.7	1.2
$r_g$ (CCS versus TCH)		0.3, -0.3	

The same three selection-system used for the Burdekin were simulated. This again allowed a comparison of two-phase FATs, and a comparison of gains from 10-m S2 plots (with destructive cane yield measurement) with gains from 5-m S2 plots (with cane yield estimated via visual grading).

As indicated previously several parameter sets were used. The first corresponds to values listed first in Table A6.3. Other settings are specified in Table A6.4.

**Table A6.4 Gains predicted from modelling selection systems for the Central region. Parameter assumptions are as described, with the standard setting corresponding to values listed first in Table A6.3. Options 1, 2 and 3 correspond to options described in Table A6.2**

Selection system option	Stage system in	Gain in CCS	Gain in TCH	Gain in relative economic value (\$/ha)
<b>Parameters assumptions – standard parameters assumptions setting</b>				
Option 1	S2	1.11	8.01	653
	FAT	2.15	19.1	1396±29
Option 2	S2	1.00	7.1	583
	S3	1.80	15.5	1141
	FAT	2.3	20.4	1497±33
Option 3	S2	0.95	6.74	554
	S3	1.78	15.3	1126
	FAT	2.3	20.1	1493±32
<b>Parameter assumptions – same as standard parameters except higher error variance assumed for 5m plots (as indicated in table of assumptions above)</b>				
Option 3	S2	0.81	5.11	460
	S3	1.71	14.5	1073
	FAT	2.18	20.3	1445
<b>Parameters assumptions – same as above except that genetic correlation between CCS and cane yield assumed to equal -0.3</b>				
Option 1	S2	1.09	-0.56	428
	FAT	1.8	7.4	944±21
Option 2	S2	0.85	1.1	369
	S3	1.51	6.2	770
	FAT	1.86	10.8	1039±23
Option 3	S2	0.89	-0.93	340
	S3	1.58	4.4	755
	FAT	2.00	7.8	1021±22
<b>Parameters assumptions – same as standard settings except that genetic variance doubled (and GE/G ratio halved to keep same absolute size of GE)</b>				
Option 1	S2	2.01	12.33	1157
	FAT	3.23	29.6	2208±32
Option 2	S2	1.70	12.7	1029
	S3	2.72	24.5	1811
	FAT	3.35	30.7	2302±33
Option 3	S2	1.6	12.0	967
	S3	2.65	23.7	1755
	FAT	3.31	29.4	2242±35

The overall results were found to be similar to those observed in the Burdekin in that:

- (i) A three-stage system is predicted to give greater gains than a two-stage system. Following evaluation of clones in single row plots (either 5 m or 10 m – see point (ii) below), this should involve evaluating around 150 clones in four-row plot trials

at four sites with one replicate per site initially, and then testing around 25 selections from these in four-row plots at four sites with two replicates per site. The gains arise due to (a) testing a larger number of clones in accurate (multi-row) trials at different environments, and (b) more accurate selection of the best final clones (for possible release) due to a greater number of environments and total plot replicates.

- (ii) The use of 5-m plots over 1 year gave a similar gain to use of 10-m plots over two years based on the initial assumptions. However, this comparison was sensitive to assumptions on parameters, particularly error variance, for which there was not a high level of confidence in the Central region. If error variances for 5-m plots similar to the highest observed in the field trials were applied then gains would be lower than those predicted for the 10-m plots.

### **A6.3 Practicalities of a 2-phase final assessment trial system**

As described above for both the Burdekin and Central, following initial selection of clones in single-row plots, it was recommended that around 150 or more clones should be initially evaluated in one replicate per site across four sites in singly replicated trials across multiple sites. The top approximately 20-25 clones based on average performance across trials in the plant crop sites program should be then evaluated in a further four trials with two replicates at each site. This prediction would appear to be robust to changing key assumptions and should have broad application to other regions as well.

From a practical perspective, implementing this system would result in little change to practical operation of planting final assessment trials at four sites per year with approximately 200 plots of experimental clones per site. However, instead of each trial having 100 clones in two replicates, under the recommended system there would be 150 new clones and 25 elite clones selected from a prior series.

Some minor changes to propagation and a 1-year delay to cultivar release would probably be necessary under the recommended system. A recommended arrangement showing timing for propagation for trial planting, planting of the first and second phase of final assessment trials, and propagation for release is shown in Table A6.5.

Clones selected from single-row plot trials in 2006 would be hot-water treated and propagated into 10-m plots. In 2007, (about 150) selected clones would be planted into the initial series of FATs (1 replicate/site by 4 sites). Plant-crop results would be obtained in 2008. In addition, in that year the clones should be propagated into 20-m plots. All clones could be propagated, or alternatively if some plant crop results are obtained prior to the time of propagation, this number could be reduced by discarding the poorest material based on results obtained to that date. Simulation modelling, and practical experience, suggests that the 25 elite clones could usually be mostly selected after the plant-crop results. In 2009, the best clones based on plant crop results would be planted into a further four trials. Not shown is that these trials would also contain another 150 new clones propagated from a subsequent series of seedlings. Also in 2009, first-ratoon results would be obtained from the first phase of FAT trials. Any clones not already selected from the plant crop results and performing outstanding in ratoon crops (this would normally be rare) could be selected and propagated with the latest series of clones

progressing into the propagation phase for the second series of FATs. In 2010, plant-crop results from the second series of FATs and second-ratoon data from the first series would be obtained. Propagation of elite clones (into rows of perhaps 50 m) for distribution agents would be done in 2010. This could comprise all 25 clones included in the second series of FATs, or could be reduced in number if plant-crop results and second-ratoon results indicated some clones were not up to standard at that stage. 2011 and 2012 comprise further propagation and obtaining ratoon data, with only the best clones considered to have release potential making it to the next propagation phase in each case.

**Table A6.5 Recommended timing of propagations for trials, planting of trials, and propagation for release and release for clones undergoing a two-phase FAT system**

<b>Year</b>	<b>Propagation</b>	<b>1st phase FAT</b>	<b>2nd phase FAT</b>	<b>Prop/release</b>	<b>Current</b>
2006	HWT, plant 10m plot				
2007	Plant crop	Plant 4 sites x 1 rep/site x 150 clones			
2008	1R crop, HWT, plant 20m plot	Plant crop results			
2009	Plant crop	1R results	Plant 4 sites x 2 rep/site x 25 clones		HWT Prelim prop for Distribution Agents
2010	1R crop; HWT, plant any ratoon wonders	2R results	Plant-crop results	HWT Prelim prop for Distribution Agents	Plant mother plot
2011			1R results	Plant mother plot	Plant distribution plot
2012			2R results	Plant distribution plot	Release
2013				Release	