FINAL REPORT

SRDC PROJECT CSR017

Optimal plot size and replication for testing clones in early stages of selection

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This project was funded by the Sugar Research and Development Corporation between July 1994 and June 1999. It was conducted jointly by CSR Ltd, BSES and CSIRO Tropical Agriculture. The research organisations are not partners, joint venturers, employees or agents of SRDC and have no authority to legally bind SRDC, in any publication of substantive details or results of this project.

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Summary

The aim of CSR017 was to identify optimal plot design, replication and selection criteria for testing and selecting clones in small plots in early stages of selection in sugarcane breeding programs. Problems associated with the use of small plots are well known in field experimentation. This is particularly so in variety selection trials where measurements in small plots are subject to possible bias due to competition effects when there are significant differences in height between genotypes being compared. In sugarcane breeding programs, small, single row or two row plots are usually used extensively for the first two stages of selection within seedling populations. The reasons for this include the desire to screen large populations of clones within available resource constraints to identify rare, elite recombinants, and the necessity to bulk up planting material from original seedlings before planting to larger plots. Given the level of resources usually devoted to early stage selection trials, it is important that optimal procedures are used so that selection is effective and efficient.

The overall approach used in the project was to obtain estimates of key genetic parameters from field experimentation and then to use these to predict gains from selection among relatively unselected clonal populations in sugarcane breeding programs using different selection options. The clones used in this study were representative of those directly derived from hybridisation in two different sugarcane breeding programs, and were unbiased by any previous selection. As such, the genetic parameters are useful for other studies that may simulate and assess different options for selection from the first stages of selection in sugarcane breeding programs.

The project proceeded following the overall plans outlined in the original project proposal. Briefly, two populations of seedling clones were evaluated at two sites in 1 row x 10m plots, 2 row x 10m plots and 6 row x 20m plots. The middle two rows of the latter was assumed to be an unbiased estimate of performance in pure stand conditions. Cane yield and CCS were determined in each plot in the plant and 1st ratoon crops in both trials and in the second ratoon crop in one of the trials. Sugar yield (kg/ha) on a per plot basis was calculated from the product of cane yield and CCS. Relative economic value (REV, $/ha) on a per plot basis was determined from revenue that would be realised from sugar produced minus variable costs of production.

All planned work in this project was successfully completed and objectives met. The project commenced in July, 1995 and was originally proposed to be completed by June, 1998. Two large field experiments were established in 1995 and excellent data was obtained in the plant crop in 1996. In the first ratoon crop precise data was obtained (1997) at both sites but at one of the sites (the BSES experiment station) cane yields were lower than normal commercial cane crops, believed to be largely because of the short growing period in that trial. An application by BSES was made for further funding (and granted by SRDC) to extend the project for 1 year and obtain data from a second ratoon crop in this trial. This application was made because of concerns the 1st ratoon crop in the BSES trial may have been atypical of normal conditions, and the potential importance of the results in changing current selection

systems was recognised at the time. The single second ratoon crop was harvested (1998) under very wet conditions. Higher error variances than desirable due to suckering and wet conditions were obtained. However, interpretation of results was consistent with that for the other 4 site x year data sets and it was considered that sufficient data was obtained from the project at that stage to more than fully meet the objectives.

The main results and conclusions arising from the project are as follows:

(i) Estimates of cane yield were found to be significantly biased due to competition effects in single row and two-row plots. Estimates of CCS are largely unbiased.

(ii) In single row plots, CCS alone is a near best selection criteria for improving REV ($/ha) in pure stands. An optimal selection index in small plots should involve a very high weighting on CCS; much more than is currently applied using Net Merit Grade or sugar yield.

(iii) The value of mechanically weighing cane in single row plots is questioned and should be the subject of future research. It would appear that visual selection in young cane in single row plots, while not highly effective, is as effective as weighing for improving gains from selection. Visual selection has an advantage over mechanical weighing in that the latter is destructive and in early stage trials (without expensive concurrent propagation of large populations of clones) one needs to wait an additional year to progress selections to the next selection stage.

(iv) An alternative selection system has been proposed based on the findings in CSR17 and other recent research. Such a system involves testing large numbers of clones for CCS in small plots, with discard of particularly poor material based on visual appearance, prior to progressing elite selections to multirow plot trials in the same year. This represents a significant departure from current procedures and further research is recommended to compare realised gains obtained using the current and proposed selection systems prior to widespread adoption of such a system in BSES breeding programs.

Background to research project

Problems associated with the use of small plots are well known in field experimentation. This is particularly so in variety selection trials where measurements in small plots are subject to possible bias due to competition effects when there are significant differences in height between genotypes being compared (eg. Duncan, 1969; Tovey et al, 1973). Despite these potential problems, a large proportion of resources in sugarcane breeding programs is usually devoted to evaluation in small plots in early selection stages, and selection intensities are often high. In sugarcane breeding programs, small, single row or two row plots are usually used extensively for the first two or three stages of selection of seedling clones (Skinner et al, 1987). The reasons for this include the desire to screen large populations of clones within available resource constraints to identify rare, elite recombinants, and the necessity to bulk up planting material from original seedlings before planting to larger plots. Given the level of resources usually devoted to early stage selection trials, it is important that optimal procedures are used so that selection is effective and efficient.

There are few published reports of competition in sugarcane. Skinner (1961) and Skinner and Hogarth (1978) examined competition in Australia. Results from both studies highlighted that variance due to competition was potentially large in sugarcane variety trials, and could seriously bias selection trial results. However, interpretation of results in these reports was limited by the methods used. First, these studies involved evaluating clones derived from previous stages of selection. If competition effects were large among seedling clones, either as individual seedlings in the first selection stage, or in subsequent small plots, prior selection pressure would be expected to discard uncompetitive clones. Such studies may underestimate the importance of competition in original populations, and are of unknown relevance to the earliest stages of selection. Second, these studies only examined trials with plots of a single plot size (eg. 3-row plots) and estimated competition effects using certain assumptions about the relative level of competition expressed in different rows in multirow plots. It was assumed that the outside rows of a three or four row plot would express half the competition effect expressed in a single-row plot, and that the middle row(s) in a three or four row plot would be free of any effects due to competition. However, this may not be the case: if growth in an outside row(s) of a three or four-row plot was strongly affected by inter-plot competition (adversely or favourably), this would result in further inter-row competition effects (in the opposite direction) passed on to the adjacent rows in the same plot.

If the primary objective of variety trials is to select genotypes that maximise economic value in a pure commercial stand, then selection based on any measurement in small plots can be regarded as indirect selection. Using the framework described by Falconer and Mackay (1996), performance in small plots may be regarded as a secondary character to which selection may be applied, with the aim of improving the primary character, performance in pure stand. Accordingly, response to selection in small plots may be predicted based on simple models describing correlated response to selection (Falconer and Mackay, 1996) provided appropriate statistical and genetic parameters are determined from field experimentation for different plot sizes. Using this approach, it is also possible to predict the effectiveness of a wide range of different methods of selection, based on different selection criteria and plot size x replicate configurations.

The study was conducted in the Burdekin region in Australia. Sugarcane crops in this region are fully irrigated, usually have high yields (up to 210 t/ha; average 130 t/ha) and may lodge up to 7 months prior to harvest. It has been suggested that lodging may be detrimental to achieving higher sugar yields under these types of environments (Muchow et al, 1994). If this is the case, resistance to lodging may be one feature of the "ideal" genotype for such environments. Canes which grow tall and thin may be more prone to lodging (Amaya et al, 1996) but are also likely to have a significant competitive advantage in small plots (Tovey et al, 1973). While speculative, it is possible that selection in small plots may bias selection against short, thick stalked genotypes that resist lodging and may have greatest potential in pure stands under high yielding conditions. Therefore, the role of selection in small plots under very high yielding and lodged conditions was also of special interest. Previous reported studies on competition in sugarcane had not focused on such environments.

Objectives

The aim of this project was to identify optimal plot size and replication in early stages of selection in sugarcane breeding programs. During the course of the research and analysis of results it also became apparent that selection criteria in small plots was a key issue in affecting gains from selection, and some key recommendations arising in the project relate to this. The original objectives of the project have been fully addressed. The results from the project have also been considered in relation to other concurrent research conducted by CSR and CSIRO on selection for early CCS in sugarcane and recommendations made based on results from this work as well as CSR017.

Methodology

Overview

The overall approach taken in the project was to obtain estimates of key genetic and statistical parameters from field research on appropriate genetic populations and then to use these to predict effective approaches to selection among relatively unselected clonal populations in sugarcane breeding programs. The clones used in this study were representative of those directly derived from hybridisation in two different sugarcane breeding programs, and were unbiased by any previous selection. As such, the genetic parameters are useful for other studies that may simulate and assess different options for selection from the first stages of selection in sugarcane breeding programs.

Genetic populations and experimental design

Two populations of seedling clones were grown in two experiments. Each population comprised unselected seedling clones derived from sugarcane breeding programs conducted by CSR Ltd. and the Bureau of Sugar Experiment Stations (BSES) in the Burdekin region, Australia. Eleven biparental crosses were selected at random from the first selection stage in each of the breeding programs in 1993 and three clones were taken at random from within each cross. For experiment 1, all thirty-three random clones from the CSR breeding program and fifteen random clones from the BSES breeding program were grown. In addition, two Hawaiian clones (H73-6110 and H78-7234) previously not evaluated locally, and three commercially grown cultivars (Q96, Q117, Eos) were grown. For experiment 2, 31 random seedlings from the BSES program, fifteen random clones from the CSR breeding program, plus three local cultivars (Q96, Q117, Q124) were grown.

Both experiments were established on the alluvial plain of the lower Burdekin River in Australia (lat. 19.0^0 S. long. $147.3\ ^0$ E.). Experiment 1 was planted on a commercial farm on April 7, 1995. Experiment 2 was planted on May 17, 1995 on the BSES experiment station at Brandon. Both experiments were planted and grown according to accepted commercial practices for the Burdekin region, which includes regular furrow irrigation. However, the yield of the first ratoon crop of experiment 2 was significantly lower compared with that normally obtained in the Burdekin region (trial mean yield was 74 t/ha compared with region average of approximately 130 t/ha). The reason for the low yield was at least partly due to the short growing duration between the harvest of the plant crop and the harvest of the first ratoon crop.

In each experiment, each clone was planted to three plot shapes. In the first experiment, plot shapes were 1 row x 10m, 2 rows x 10m and 6 rows x 20m. Each clone x plot shape combination was randomised within each of two blocks, so that the design used was equivalent to a randomised complete block design with two blocks and treatments comprising plot shape x clone combinations. Within each block there were two replicates of each clone in the 1 row x 10m plot shape and one replicate of each clone for the other two plot sizes. Randomisation of clones and plot shapes within each block was done with the constraint that no clone was positioned adjacent to a plot containing the same clone, either laterally or at plot ends.

For the second experiment, plot shapes were 1 row x 10.6m, 2 rows x 10.6m and 6 rows x 21.2m. The design in this experiment was a 7 x 7 simple lattice (Cochran and Cox, 1964) with two complete blocks. Each incomplete block contained all three plot shapes of seven varieties. As with the first experiment, constrained randomisation was used to avoid different plot shapes of the same variety competing as nieghbours.

Measurements

Harvesting of the plant crop occurred on the 15th of July and the 8th of October for experiments 1 and 2, respectively. The first ratoon crops were harvested on the 16th of September and the 16th of June for experiments 1 and 2, respectively. The second ratoon crop for experiment 2 was harvested on the 14 November, 1998. The latter harvest was done later in the season than normal commercial practice and under very wet conditions. Significant suckering was observed in most plots at the time of harvest.

The plant crops of both trials were burnt before harvest in each case, and then harvested using mechanical harvesters. In the ratoon crops, experiment 1 was burnt before harvest, while experiment 2 was harvested without burning. Cane from individual rows of the plots were weighed at harvest using mobile weighing machines. For experiment 1, only the middle two rows of the six row plots were weighed. For experiment 2, a 2m buffer was left at the ends of the 6 row x 21.2m plots, so that only 17.2m was weighed.

At the same time as harvesting, a hand cut sample of four stalks was removed from each individual harvested row for determination of commercially recoverable sugar content (commercial cane sugar, CCS, BSES, 1984).

Sugar yield (kg/ha) on a per plot basis was calculated from the product of cane yield and CCS. Relative economic value (REV, $A/ha) on a per plot basis was determined from revenue that would be realised from sugar produced minus variable costs of production. Because the major costs of harvesting, cane transport and milling are approximately proportional to cane yield, clones that produce high sugar yields via high CCS rather than high cane yield are more profitable. The following formula was used for estimating REV:

REV ($/ha) = [sugar yield (t/ha) x sugar price ($/t)] – [sugar yield (t/ha) x variable costs proportional to sugar production ($/tonne sugar)] – [cane yield (t/ha) x variable costs proportional to cane production ($/tonne cane)],
where sugar price is the long term expected world market sugar price (assumed to equal $US 211/t); variable costs proportional to sugar production (assumed to equal $US 0.40/tonne sugar) include costs of transporting sugar from sugar mills to the ship, variable costs proportional to cane production (assumed to equal $US 12/tonne cane) include harvesting, cane transport and milling costs. For this study, actual prices and costs were based on current estimated values in the study area (Agtrans Research, Ltd, personal communication). While such values fluctuate, both with different regions and with time, they are considered broadly representative of sugar production in an internationally competitive sugar industry. Thus, determination of REV using the process above was considered to provide an adequate indicator of relative economic value of the different clones in this study. Improvement of REV in a pure stand was assumed to represent the objective of selection in sugarcane breeding programs.

While it is suggested that REV ought to be the primary selection criterion, some sugarcane breeding programs also regard improvement of sugar yield in pure stands as the primary selection criterion. Therefore, in this study the effectiveness of selection in small plots was also assessed assuming sugar yield as the primary selection criterion.

The difference between performance in small plots and performance in the bordered rows of the six-row plots for any character was defined as competition for that character. Competition effects were estimated for each clone in each block in each experiment, therefore, could be subjected to the same analyses as any other character.

In experiment 1 some measurements additional to CCS and cane yield in each plot in the plant crop were also taken. Measurements were made in the plant crop on the 28th of September 1995 of the height from the ground to the top of the canopy. The mean of two measurements per plot was recorded, with each measurement being taken at random locations within each plot. Visual ratings were given of overall appearance (1 worst, 9 best), based on apparent cane mass present. Ratings were made by two experienced technical officers independently on December 19, 1995, and averaged for analysis.

Statistical analysis

Results from each experiment were analysed using the SAS statistical package (SAS Institute Inc, NC, USA). For each experiment, analyses of variance were firstly done for each attribute for each plot shape in each crop-year. For analyses of individual attribute x plot shape x crop-year combination, the following model was assumed:

$y_{ij} = \mu + b_j + g_i + e_{ij}$

where μ, b_j, g_i, and e_{ij} are the grand mean, block effect, genotype effect and error effect, respectively. Genotypes were considered to be random effects, generating variance component σ_g^2. Variance components for genotypes and error were estimated from the following expectations of mean squares:

Source of variation	Expected mean square
Genotypes	$\sigma_e^2 + r.\sigma_g^2$
Error	σ_e^2

Analyses of covariance were done for selected pairs of characters within the same experiment. These included characters measured on the same plots or on different plot shapes. This was done in the same manner as the analyses of variance except that sums of cross products and mean cross products were determined, with appropriate covariance components and mean cross products substituted for variance components and mean squares.

Broad sense heritabilities (h^2) for each trait were determined from (Falconer and Mackay, 1996):

$h^2 = \sigma_g^2/\sigma_p^2$,

where σ_g^2 = genetic variance, and σ_p^2 = phenotypic variance. Phenotypic variance was determined from:

$\sigma_p^2 = \sigma_g^2 + \sigma_e^2/r$,

where σ_e^2 = error variance and r = number of replicates.

Genetic correlations between characters X and Y $r_{g(x,y)}$ were determined from:

$r_{g(x,y)} = cov_{g(x,y)}/ (\sigma_{g(x)} . \sigma_{g(y)})$,

where

$cov_{g(x,y)}$ = genetic component of covariance between characters X and Y,

$\sigma_{g(x)}$ and $\sigma_{g(y)}$ = genetic standard deviation for characters X and Y, respectively.

Performance for different characters in single or two-row plots were considered to represent possible secondary indirect selection criteria for predicting performance in the bordered rows in large plots. If performance in the middle two rows of the six row plots is designated character Y, and performance in small plots is designated character

X, then the correlated response in character Y from indirect selection based on character X (CR_y) was determined as follows (Falconer and Mackay, 1996):

$CR_y = i \,.\, h_x \,.\, r_{g\,(x.y)} \,.\, \sigma_{gy}$,

where i is the standardised selection differential; h_x is the square root of the broad sense heritability for trait X; $r_{g(x.y)}$ is the genetic correlation between characters X and Y; and σ_{gy} is the genotypic standard deviation for character Y.

Results and Discussion

Effects of plot shape on variance components

Plot size had a large effect on estimates of genetic variance for cane yield (Table 1). In experiment 1, the genetic variance for cane yield in single-row plots was approximately three times (plant crop) and five times (ratoon crop) that estimated in the well bordered plots. In experiment 2, inflation of genetic variance was even greater, particularly in the plant crop where genetic variance in the single-row plots was about 20 times that estimated in the large plots. This indicates that most genetic variation in cane yield in small plots is associated with competition effects, which is not expressed under pure stand conditions. In both experiments, as expected, genetic variances for cane yield in the two-row plots were intermediate between the single-row and six-row plots.

Table 1. Genetic variance (σ_g^2) and error variance (σ_e^2) in each experiment for different plot sizes. Genetic variances were significant (P<0.01) in all cases except cane yield in the plant crop of experiment 2.

			Plot shape					
			1 row		2 rows		6 rows (middle 2)	
Trait	Crop	Mean	σ_g^2	σ_e^2	σ_g^2	σ_e^2	σ_g^2	σ_e^2
Experiment 1								
CCS[1]	P	11.8	4.02	3.62	4.70	1.18	4.63	0.69
	1R	14.1	2.52	2.59	2.40	1.15	2.00	0.48
Cane yield	P	172	1578	1731	1055	491	536	332
(t/ha)	1R	139	1215	1074	924	250	246	156
Sugar yield	P	20.3	40.9	32.3	34.9	9.77	21.1	5.54
(t/ha)	1R	19.7	30.0	23.1	27.3	5.85	9.19	3.88
REV[2]	P	2.28	0.886	0.704	0.928	0.236	0.721	0.130
($ x 10^{-3})	1R	2.52	0.645	0.481	0.620	0.139	0.265	0.088
Height (cm)	P	85.0	573	136	709	113	777	41.1
Appearance Grade	P	4.68	0.77	0.90	1.12	0.58	1.42	0.40
Experiment 2								
CCS	P	13.9	2.23	3.12	0.83	4.25	3.66	0.97
	1R	13.8	1.94	0.30	1.73	0.28	1.65	0.27
	2R	11.9	0.86	3.26	1.04	2.05	1.92	2.60
Cane yield	P	161	1691	1408	920	911	87.6	326
(t/ha)	1R	73.8	316	368	151	133	78.1	28.3
	2R	132	3250	4764	1060	1360	501	461
Sugar yield	P	22.7	48.7	44.2	23.9	37.8	13.5	7.93
(t/ha)	1R	10.2	8.87	7.13	4.70	3.05	2.50	0.83
	2R	16.1	63.5	35.0	23.6	21.0	12.1	12.5
REV[2]	P	2.90	1.22	0.730	0.468	1.00	0.512	0.185
($ x 10^{-3})	1R	1.33	0.197	0.126	0.110	0.057	0.065	0.018
	2R	1.88	1.03	1.11	0.932	0.831	0.258	0.325

[2`]**Relative economic value**

By contrast, genetic variance for CCS was of similar magnitude in all plot sizes. This suggests that this attribute was less affected by competition in small plots.

The genetic variance of both sugar yield and REV was inflated in the small plots compared with the middle two rows of the six-row plots but this was less than that for cane yield. This result reflects the joint effects of cane yield and CCS in determining these measurements.

As expected, error variances were also inflated in most cases in small plots compared with the large plots. This effect was greatest for cane yield, where error variances were around four to twelve times those in the largest plots (Table 1). Heritability estimated for different plot sizes (data not shown) differed little between plot sizes because both genetic and error variances varied similarly across different plot sizes.

Correlations between performance in different plot sizes

Genetic correlations between characters measured in unbordered and bordered plots in plant and first ratoon crops are given in Table 2. For each character measured in single-row plots and two-row plots in each crop-year, genetic correlations with the same trait measured in large plots in each crop-year are shown. Also shown are genetic correlations between the same characters in unbordered plots and both sugar yield and REV measured in each crop-year in bordered plots. For CCS, genetic correlations between the different plot sizes for the same crop-year were all close to unity. This indicates that measurement of this character in small plots is unbiased. Therefore, providing adequate precision in measuring CCS is obtained, selection in single-row plots would be effective for improving performance of CCS in near pure stands.

For cane yield, the correlations were more variable and in a number of cases, quite low. This indicates the potential for competition effects to affect relative performance of clones under some situations. In experiment 1, the genetic correlation between cane yield in single row plots and cane yield in the bordered plots was 0.48 (±0.13), while the equivalent correlation for experiment 2 was 0.62 (±0.31). Compared with the plant crop, the correlations between ratoon cane yield in single row plots and bordered plots was greater for experiment 1, (0.77 ±0.08) but lower for experiment 2 (0.17±0.20). Correlations for the second ratoon crop of experiment 2 are not shown in Table 2 but were similar or within the range of those in the plant and first ratoon crops in this experiment. In the second ratoon crop, genetic correlations between cane yield, CCS, sugar yield, and REV measured in single-row plots and the same trait measured in the bordered rows of the large plots were 0.47, 0.96, 0.59, and 0.64, respectively. The equivalent correlations for the dual row plots were 0.79, 0.99, 0.74 and 0.71, respectively.

Table 2. Genetic correlations between (i) each trait measured in small plots and the equivalent trait measured in the middle two rows of large plots, and (ii) each trait measured in small plots and REV (relative economic value) in large plots.

			Genetic correlation					
			With equivalent trait		With Sugar yield		With REV	
Trait	Crop	Plot size	P	1R	P	1R	P	1R
Experiment 1								
CCS	P	1 row	0.91±0.04	0.69±0.09	0.69±0.09	0.55±0.12	0.82±0.07	0.63±0.10
		2 row	0.94±0.03	0.73±0.08	0.75±0.07	0.61±0.10	0.87±0.05	0.69±0.09
	1R	1 row	0.88±0.05	0.99±0.04	0.65±0.10	0.63±0.10	0.79±0.07	0.80±0.07
		2 row	0.89±0.05	1.01±0.04	0.70±0.09	0.76±0.09	0.83±0.07	0.89±0.06
Cane yield (t/ha)	P	1 row	0.48±0.13	0.64±0.10	0.48±0.13	0.50±0.13	0.38±0.14	0.35±0.14
		2 row	0.52±0.12	0.68±0.10	0.52±0.12	0.61±0.11	0.41±0.13	0.49±0.13
	1R	1 row	0.26±0.15	0.74±0.08	0.32±0.14	0.57±0.11	0.27±0.15	0.42±0.14
		2 row	0.33±0.14	0.72±0.08	0.37±0.21	0.64±0.10	0.30±0.14	0.52±0.11
Sugar yield (t/ha)	P	1 row	0.65±0.10	0.61±0.11	0.65±0.10	0.61±0.11	0.64±0.10	0.56±0.12
		2 row	0.75±0.08	0.76±0.08	0.75±0.08	0.76±0.08	0.77±0.07	0.74±0.08
	1R	1 row	0.53±0.12	0.77±0.08	0.53±0.12	0.77±0.08	0.54±0.11	0.72±0.09
		2 row	0.57±0.11	0.83±0.07	0.57±0.11	0.83±0.07	0.57±0.11	0.79±0.07
REV ($/ha)	P	1 row	0.72±0.08	0.63±0.10	0.69±0.09	0.64±0.10	0.72±0.08	0.63±0.10
		2 row	0.85±0.06	0.77±0.08	0.79±0.07	0.75±0.08	0.85±0.06	0.77±0.08
	1R	1 row	0.63±0.10	0.81±0.07	0.60±0.10	0.81±0.07	0.63±0.10	0.81±0.07
		2 row	0.66±0.09	0.87±0.05	0.63±0.09	0.88±0.05	0.66±0.09	0.87±0.05
Experiment 2								
CCS	P	1 row	0.74±0.16	0.52±0.14	0.95±0.10	0.42±0.16	0.90±0.10	0.47±0.15
		2 row	1.29±0.26	0.82±0.22	1.46±0.30	0.71±0.22	1.40±0.28	0.82±0.22
	1R	1 row	0.55±0.11	0.94±0.03	0.53±0.12	0.70±0.09	0.54±0.11	0.84±0.05
		2 row	0.59±0.11	0.99±0.02	0.53±0.12	0.70±0.09	0.57±0.11	0.84±0.06
Cane yield (t/ha)	P	1 row	0.62±0.31	-0.15±0.2	0.22±0.17	-0.14±0.2	0.13±0.16	-0.13±0.2
		2 row	0.89±0.34	-0.04±0.2	0.39±0.16	-0.04±0.2	0.29±0.16	-0.02±0.2
	1R	1 row	0.76±0.22	0.17±0.20	0.43±0.16	0.19±0.17	0.34±0.16	0.18±0.18
		2 row	0.87±0.20	0.45±0.16	0.57±0.14	0.43±0.14	0.48±0.14	0.37±0.15
Sugar yield (t/ha)	P	1 row	0.50±0.18	0.01±0.17	0.48±0.15	0.01±0.17	0.58±0.13	0.19±0.16
		2 row	0.74±0.18	0.16±0.17	0.75±0.13	0.16±0.17	0.64±0.14	0.21±0.17
	1R	1 row	0.54±0.14	0.41±0.17	0.54±0.14	0.41±0.17	0.49±0.14	0.46±0.14
		2 row	0.71±0.11	0.65±0.13	0.71±0.11	0.65±0.13	0.66±0.11	0.68±0.10
REV ($/ha)	P	1 row	0.44±0.13	0.11±0.15	0.51±0.12	0.08±0.16	0.44±0.13	0.11±0.15
		2 row	0.83±0.13	0.35±0.17	0.92±0.14	0.28±0.18	0.83±0.13	0.35±0.17
	1R	1 row	0.49±0.13	0.52±0.11	0.54±0.13	0.45±0.13	0.49±0.13	0.52±0.11
		2 row	0.69±0.10	0.78±0.08	0.72±0.10	0.72±0.10	0.69±0.10	0.78±0.08

Visual appearance grades in single-row plots had a genetic correlation with cane yield in the large plots of 0.57±0.14. This was at least as large as the genetic correlation for cane yield measured directly in single-row plots (r_g = 0.48±0.13, Table 2). The advantage of visual appearance grade over cane yield in this respect would appear to be due to competition effects not affecting estimates (at least at the time it was done), as indicated by the high genetic correlation between visual appearance grades in the single-row plots and the large plots (1.07 ± 0.12). Visual appearance grades in small plots also had a similar heritability to cane yield in the same plots (Table 1 – see ratio of genetic variance to error variance). These results suggest that, for single-row plots, visual appearance grade would be at least as effective as cane yield as a selection criterion for improving cane yield in large plots. For the plant crop in experiment 1, which was the only crop where visual grades were recorded, predicted gains in cane yield in large plots from selection (not shown) based on visual grades were greater than using measured cane yield as a selection criterion.

Table 3. Genetic correlations (±standard errors) between competition effects for cane yield (defined as cane yield in single row plots minus cane yield in bordered rows of large plots) and cane yield in bordered rows of large plots in each experiment and crop.

Experiment	Crop	Genetic correlation
1	Plant	-0.04±0.19
	1st ratoon	0.63±0.19
2	Plant	0.41±0.25
	1st ratoon	-0.29±0.15

Competition effects for cane yield showed no consistent correlation with cane yield in the bordered rows of the large plots (Table 3), ranging from –0.29 to 0.57 in the different experiments and crop-years. This is consistent with the findings of Skinner and Hogarth (1978) who showed that among highly selected clones that correlation between competition and "true yield" was highly variable in different environments, with a mean of –0.02 in trials where significant competition effects were apparent.

It was hypothesised that canopy height of a clone at around six months of age might be related with its competitive ability. Modelling studies by Tovey et al (1973) for sugarcane suggested that tall clones would exhibit significant competitive advantage in single row plots compared with short clones. However, there was only a moderate correlation (0.56±0.15) between competition and height. Thus while canopy height was one factor affecting competitive ability, it was only of limited predictive ability, and other factors were also involved.

In all cases for cane yield in Table 2, correlations involving the two row plots were slightly greater than for single row plots (generally by about 0.05 to 0.20), but were still significantly less than 1.0. This indicates that selection for cane yield would be more effective in two row plots, but in some cases would still be seriously limited due to bias associated with competition effects.

Collectively, the genetic correlations obtained for cane yield in different plot sizes suggest that selection for cane yield in unbordered plots would result in improvemed performance in pure stands, due to positive genetic correlations in all cases. However, gains would be limited in most situations, especially in single row plots, due to genetic correlations significantly less than 1.0.

For sugar yield and REV, the genetic correlations between performance in small plots and bordered plots were also variable, but in all cases were significantly less than 1.0. This was particularly the case for sugar yield, reflecting the strong effect of cane yield in determining this attribute. For example, in the plant crop, the genetic correlation between sugar yield in single-row plots and sugar yield in the bordered plots were 0.65 and 0.48 for experiment 1 and experiment 2, respectively.

It may be assumed that the major objective in selection is to improve performance for REV in pure stands. The genetic correlation between a particular character in small plots and REV in large plots is of interest in assessing the potential effectiveness of using that trait in small plots for improving the primary selection criterion. An important result in Table 2 is that the genetic correlation between CCS measured in small plots and REV in large plots was as great or greater than for any other character in small plots, including REV itself. For example, for experiment 1, the genetic correlation between CCS in the single row plots in the plant crop and REV in the 6-row plots was 0.82 (±0.07), while the correlation for REV itself was less at 0.72. The higher genetic correlations associated with CCS compared with other characters in this context was the case for both experiments, although it was most marked for experiment 2.

By contrast, the genetic correlations between cane yield in the small plots and REV in the large plots were quite low (eg. $r_g = 0.38$, 0.13 for single-row plots in the plant crop-year for experiments 1 and 2 respectively). This indicates that gains from selection on the basis of cane yield in small plots for improving REV would be quite limited, even if precise data were obtained. Overall, the high genetic correlations for CCS suggest that effective selection in small plots could be based on selection for CCS alone, which would offer significant practical advantages over selection criteria relying on estimating cane yield, as discussed below.

Practical selection options in breeding programs

As with all selection trials, there is a trade-off between increasing replication, plot size and number of genotypes that can be evaluated, given limited resources (Gauch and Zobel, 1996). Increasing replication will increase precision of estimates of clone means; increasing plot size will increase genetic correlation with yield in pure stand (Table 2) and decrease error variance (Table 1), but decrease genetic variance (Table 1); while increasing number of genotypes being evaluated will allow increased selection intensity. In deciding on an optimal selection system, the trade-offs associated with all factors need to be considered and different options compared for expected gains from selection. Further, in a multi-stage selection system, the optimisation of one stage of selection cannot be done in isolation from other stages. Changes in one stage of selection (eg. number of clones to be evaluated, selection intensity used) will impact on the design and performance of selection stages following or prior to that stage. Proper development of an optimal selection system based on results from this study and other knowledge is a major exercise, and beyond the scope of this paper. However, gains from selection from a limited set of comparisons for selection among sugarcane clones are considered here, as a guide for further investigations. In addition, these comparisons are used to show that more effective approaches than currently used in sugarcane breeding programs can probably be identified.

In practice, the first stage of selection in sugarcane breeding programs involves evaluating clones as single plants grown from true seed, either in family plots, or as individual clones. The second stage involves clonal propagation of cane cut from the individual plants and further testing in plots grown from this cane. The selection options compared here relate to those available immediately following the first seedling stage of selection or propagation, ie. stage 2 in the selection system. Under good growing conditions, individual seedling clones may produce up to about 20m of cane that can be planted to the next selection stage. Therefore, options in the second stage of selection are limited by planting material: total row length in any plot size x replication configuration cannot practically exceed 20m in most situations. The current selection system in breeding programs in Australia generally involves planting single-row plots with either one or two replicates. For a given amount of resources, trials with two replicates of single-row plots, or single replicates of two-row plots would only allow evaluation of half the number of clones than if single-row plots with single replicates were used. Halving the number of clones reduces selection intensity and gains from selection. The results from this study allow different configurations of plot size and replication to be examined, as well as consider impacts of different selection criteria.

Table 4 shows the predicted response from selection based on different traits, based on selection in (i) one-row plots x one replicate per clone, (ii) one-row plots x two replicates per clone, (iii) two-row plots x one replicate per clone. A selection intensity of 5% is assumed for option (i), which corresponds approximately to past practice in Australian sugarcane breeding programs. For the other two options, given equivalent resources, only half the number of clones would be able to be evaluated so that the selection intensity would 10%, assuming an equivalent number of clones were selected for the next stage of selection. For each character considered for selection in Table 4, correlated responses to selection for performance in the large plots are shown for sugar yield, and for REV. It may be assumed that the key objective in selection under most situations would be to maximise gain in REV per unit cost of selection.

Table 4. Correlated response (as a % of the mean) of sugar yield and relative economic value (REV) in the middle two rows of 6 row plots, from indirect selection for different characters in small plots. For the 1 row x 1 rep selection option in each case, selection intensity used for determining gains was doubled since double the number of genotypes could be evaluated using this option compared with the other two options.

			% selection gain in pure stand			
			Plant crop		Ratoon crop	
Trait	Crop	Plot size x rep.no.	Sugar yield	REV	Sugar yield	REV
Experiment 1						
CCS	P	1 row x 1 rep	24	70	12	26
		1 row x 2 reps	23	68	12	25
		2 rows x 1 rep	27	78	14	30
	1R	1 row x 1 rep	21	65	14	32
		1 row x 2 reps	21	64	14	31
		2 rows x 1 rep	23	68	16	35
Cane yield	P	1 row x 1 rep	15	31	11	14
		1 row x 2 reps	15	30	11	13
(t/ha)		2 rows x 1 rep	17	34	13	19
	1R	1 row x 1 rep	11	23	13	17
		1 row x 2 reps	11	22	13	17
		2 rows x 1 rep	13	27	15	22
Sugar yield	P	1 row x 1 rep	23	56	14	24
		1 row x 2 reps	22	54	14	23
(t/ha)		2 rows x 1 rep	26	68	18	31
	1R	1 row x 1 rep	19	48	18	31
		1 row x 2 reps	18	45	17	29
		2 rows x 1 rep	21	52	20	34
REV	P	1 row x 1 rep	25	63	15	27
($/ha)		1 row x 2 reps	24	61	14	25
		2 rows x 1 rep	30	76	18	33
	1R	1 row x 1 rep	22	56	19	35
		1 row x 2 reps	21	54	18	33
		2 rows x 1 rep	24	59	21	38

(Table 4 cont)
Experiment 2

CCS	**P**	**1 row x 1 rep**	**15**	**36**	**12**	**34**
		1 row x 2 reps	**15**	**36**	**12**	**35**
		2 rows x 1 rep	**8.5**	**20**	**7**	**20**
	1R	**1 row x 1 rep**	**20.1**	**50**	**19**	**63**
		1 row x 2 reps	**18**	**44**	**17**	**55**
		2 rows x 1 rep	**18**	**45**	**20**	**59**
Cane yield	**P**	**1 row x 1 rep**	**12**	**19**	**13**	**22**
		1 row x 2 reps	**11**	**19**	**12**	**21**
(t/ha)		**2 rows x 1 rep**	**10**	**17**	**12**	**25**
	1R	**1 row x 1 rep**	**7**	**13**	**13**	**24**
		1 row x 2 reps	**7**	**12**	**13**	**24**
		2 rows x 1 rep	**8**	**13**	**13**	**27**
Sugar yield	**P**	**1 row x 1 rep**	**16**	**32**	**15**	**34**
		1 row x 2 reps	**15**	**31**	**15**	**33**
(t/ha)		**2 rows x 1 rep**	**13**	**27**	**14**	**33**
	1R	**1 row x 1 rep**	**13**	**27**	**19**	**45**
		1 row x 2 reps	**13**	**26**	**19**	**43**
		2 rows x 1 rep	**13**	**25**	**19**	**44**
REV	**P**	**1 row x 1 rep**	**18**	**39**	**17**	**42**
($/ha)		**1 row x 2 reps**	**17**	**37**	**16**	**40**
		2 rows x 1 rep	**13**	**28**	**12**	**31**
	1R	**1 row x 1 rep**	**16**	**34**	**21**	**53**
		1 row x 2 reps	**15**	**32**	**20**	**50**
		2 rows x 1 rep	**14**	**31**	**21**	**50**

As with Table 2, an important result from Table 4 is that gains in REV arising from selection for CCS alone provide similar or greater gains, compared with selection based on either sugar yield, or on REV itself. For example, based on data from experiment 1, it is predicted that selection based on CCS alone would result in a 68% to 78% gain in REV. Selection based on sugar yield or REV itself under the same situation would provide a gain of 54% to 68% and 61% to 76%, respectively. The reason for CCS providing a superior selection criterion compared with other characters is partly due to it being unaffected by competition effects in small plots. Both cane yield and CCS of clones are clearly important in determining their sugar yield and REV. However, measurements of CCS are more reliable in unbordered plots, compared with measurements of cane yield, sugar yield, or REV.

In Australia, selection of clones in trials has frequently been based on sugar yield, or other indices related to sugar yield with additional weighting toward CCS. The results presented here suggest that selection based solely on CCS may be effective, and that very little weighting to cane yield as a character for selection would be appropriate. This result leads to the question of whether measuring cane yield in early stage selection trials represents optimal allocation of resources, or whether reliance on CCS data for selection may be more appropriate in early stages. Measuring cane yield not only is associated with some cost, but it may also add an extra year to selection if harvesting and weighing is practised, as in Australian breeding programs. When a trial is harvested and weighed in the plant crop, the seed source of selected clones in the trial is unavailable until the ratoon crop is grown. Therefore, either a further year is required so that planting material to establish trials for the next stage of selection, or otherwise expensive additional plots of all clones need to be concurrently propagated if this time delay is to be avoided. A high correlation is usually found between performance in plant and ratoon crops in early stage trials (Jackson, 1992; Mirzawan et al 1993) so there is usually little advantage in waiting for ratoon crops in terms of obtaining additional data to use in selection.

If cane yield results from a trial with unbordered plots are obtained, it is likely that an optimal selection index (Smith, 1936, Baker, 1986) involving cane yield and CCS could be identified that would result in greater gains than just using CCS alone. The results obtained in this study indicate that such an index is likely to have a very heavy weighting on CCS and little on cane yield. It is also likely that visual gradings for cane yield could be as effective in a combined selection index with CCS compared with use of cane yield. As indicated previously, visual grades measured in single row plots appear to be as effective as measurements of cane yield for predicting performance in bordered plots. Given such ratings are cheap and do not involve crop destruction, these grades could be used to discard clones which are likely to have extremely low cane yield, without the need to harvest and weigh all plots. However, the use of visual gradings requires further research in order to optimise their application.

Further research is required to examine alternative selection systems and to identify optimal systems. However, based on results reported here it would appear that an optimal system may involve:

(i) Evaluation of a large number of clones in small, single row plots for CCS in the plant crop.

(ii) Selection of clones based largely on CCS, but with discard of material with particularly poor visual grades, and progression of selections to the next stage of selection in the same season as evaluation.

(iii) Evaluation of the high CCS selections in multi-row plots (resembling commercially grown pure stands) for accurate assessment of cane yield and further CCS evaluation.

Impact on sugar industry and future research needs

The research conducted in this project has identified opportunities for improvement in selection systems in breeding programs. The improvements can arise from greater gains from selection per unit resource input, appropriate weighting given to CCS, and faster time to fully evaluate and release varieties. Given the importance of selection in breeding programs it is believed that if adopted, the recommendations could have large, long term favourable impact on performance of breeding programs and therefore on variety development and release.

However, the changes recommended represent significant differences to current practice. It is proposed to test the proposed changes in practice in breeding programs by way of comparing realised gains from the current and proposed selection systems. Such testing would need to be done in a further dedicated research project. Such research would serve as (i) verification of predictions from CSR017 before wider scale adoption, and (ii) an opportunity to refine further the recommendations made to date and allow further customising of recommendations to different situations.

Commercially significant developments, patents etc.

The outputs from this project are primarily information relating to opportunities for improvement in selection systems in sugarcane breeding programs. These outputs are not considered to be readily patentable. Commercial benefits will be achieved after verification and adoption of results and recommendations arising from the project in core commercial breeding programs.

Recommendations on activities to further develop and exploit the project technology

It is recommended that a focused research project be funded and conducted to compare gains from the selection system recommended from this project (and other research) with systems currently used. The changes suggested in this report arising from CSR017, while offering significant improvements, also represent a significant departure to selection systems that have been successfully used in the past. Given the large investment made in selection in sugarcane breeding programs in Australia, and the importance of selection systems in delivering varieties, changes should not be made lightly and without proper verification of benefits. It is envisaged that a joint BSES/CSIRO project will be proposed to examine the recommendations from this project in practice in the Burdekin and Mackay regions. This would measure realised selection gains from the current and proposed selection systems, and may identify further refinements in an optimal selection system beyond those already recommended in CSR017.

Acknowledgements

The competence and dedication of CSR and BSES field staff in conducting the large field experiments associated with this project was central to its success. The efforts of Terry Morgan, Steve Elliott, Geoff Olsen, Trevor Pollard and Steve Attard of CSR Ltd, and Allan Rattey, David Erquiaga, Lionel Jensen and Jamie Summerhayes of BSES are particularly acknowledged. We also acknowledge with thanks the cooperation and help from Jose Ybarlucea for provision of land and crop management expertise for one of the experiments.

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List of publications arising from the project

McRae, T.A. and Jackson, P.A. 1998. Competition effects in selection trials. Proc. ASSCT pp. 148-154.

Matassa, V.J., Basford, K.E., and Jackson, P.A. 1999. Intergenotypic competition in single-row plots of sugarcane variety trials. Proc. ASSCT. pp. 234-241.

Jackson, P.A. and McRae, T.A. Selection of sugarcane clones in small plots: effects of plot size and selection criteria. Submitted to *Crop Science*, August, 1999.

An additional journal paper combining components of work from CSR017 and other research, comparing alternative selection systems, will be prepared for publication for a scientific journal.