BUREAU OF SUGAR EXPERIMENT STATIONS

QUEENSLAND, AUSTRALIA

FINAL REPORT - SRDC PROJECT BSS93
BREEDING CLONES WITH HIGH EARLY SUGAR CONTENT

by

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EXECUTIVE SUMMARY

The project BSS25 'Breeding of clones with high early sugar content' concluded that the potential for increasing CCS through breeding and selection was greatest early in the season. BSS25 commenced a recurrent selection program with short generation interval aimed mainly at population improvement. The aim of BSS93 was to continue a recurrent selection program for early CCS and to assess the realised genetic gain made in the previous project.

At the start of BSS93, BSES had changed its selection program from family assessment in clonal 4-sett plots to family assessment as original seedlings. The recurrent selection program for high early CCS reflected this change. Twenty families with high early CCS parents were selected for planting in New South Wales, southern, central, Burdekin, Herbert and northern regions from 1993 to 1996. At each location, the best 600 out of 1 200 seedlings (based on visual appearance) were sampled in May and June in the following year for CCS. The best 10 clones, based on mean CCS, were selected as parents and sent to Meringa for further crossing. Two hundred clones in total were selected as parents. The 10 parent clones and up to 10 additional clones were selected for testing in Clonal Assessment Trials. A total of 377 clones were selected over the duration of this project. Of these 377 clones, 107 clones were derived from families with at least one recurrent parent from the previous project. Good performing clones from this stage were promoted to advanced selection stages. A number of clones from both the current and previous projects have performed well in advanced trials. To date, two varieties have been released, Q185[A] (central region) and Q205[A] (southern region).

Replicated trials were planted in the southern, central and northern regions to assess the genetic gain realised in the selected clones from the previous project (BSS25). Parents and elite (selected) clones from the families tested were included along with a base population (a group of 29 randomly selected clones from the breeding population) and a core population (a group of 30 clones from core selection programs with known high early CCS). Trials were sampled for CCS in May and June in plant and first-ratoon crops. Mean CCS was calculated and the various populations were compared.

At all locations, the parent population had significantly higher CCS than the base population, and the core and elite populations had significantly higher CCS than the parent population. At Bundaberg, the elite population had significantly higher CCS than the core population, but there were no differences in mean CCS between these two populations at Mackay or Meringa.

In terms of realised genetic gain, at Bundaberg both southern parent and elite populations showed steady gains from 1987 to 1991, averaging about 0.26 unit of CCS per year. There were no indications of a decrease in variability in these populations and it was concluded that it was likely further genetic gains would be sustained in the future.

At Mackay, the central parent populations showed a modest but somewhat inconsistent improvement over the period and this was repeated for these populations tested at Bundaberg and Meringa. The central elite populations showed good improvement for the first 3 years, but this was not sustained over the subsequent 2 years. Extremely difficult selection environments (flooding and extreme moisture stress) impacted on the clones

selected in the final two elite populations and may explain this decline. It was difficult to come to a firm conclusion on continued genetic gain for the central region.

At Meringa, the northern parent populations showed a small, but significant improvement over the 4 years of about 0.13 units of CCS per year. However, the northern elite populations showed no improvement over this period. This was not expected, as the parent populations showed a fairly steady improvement. Interestingly, good improvement was shown by the northern elite populations (first 3 years only) when grown at Bundaberg (0.39 unit CCS per year) and Mackay (0.21 unit of CCS per year). It is difficult to explain these results, but it may indicate that the wet tropics pose some unique difficulties in breeding and selection for high early CCS.

1.0 BACKGROUND

The availability of commercial clones that have higher levels of sucrose early in the season would enable an earlier commencement of crushing. This would increase the potential for mills to handle larger crops and enable more economic utilisation of capital in the harvesting, transport and milling operations of the industry. Such clones would also result in increased sugar production when crushing commences at the currently accepted time. An extra unit of CCS (commercial cane sugar) prior to August throughout Australia was calculated to be worth more than $15 million annually at the time of planning this project.

The potential for increasing sucrose content through breeding and selection has been shown to be greatest early in the season. High early sugar clones selected from the project BSS25 (Breeding of clones with high early sugar content), together with high early sugar selections from routine screening in the core BSES program, have resulted in the formation of an elite breeding population. Utilisation of these clones in a recurrent selection program should result in continued gains in early sugar content. Measures of realised genetic gains to date and the genetic variability in the elite populations are needed to assess the potential for further genetic gain. Benefits to the industry will come from superior early season clones selected either in this sub-program or from crosses involving parents developed in the sub-program. BSES will expand breeding and selection for high early sugar as part of this project to include selection programs in Ingham, Burdekin and New South Wales (funded by BSES) as well as the northern, central and southern programs already catered for.

Breeding and selection for early CCS is likely to be an efficient means of improving productivity in the early part of the season. Recurrent selection of parents with a short generation interval (2-3 years) will result in the most rapid genetic gain, particularly when selection focuses on one specific trait. Assessing the gains already made will indicate the rate of improvement and identify potential impediments to further gains.

2.0 OBJECTIVES

The aim of this project was to determine realised and potential genetic gain for early ccs in a recurrent selection program. Specifically, the objectives were:

- Measure realised genetic gain in early CCS since 1987
- Assess potential limitations to further genetic gain in early CCS
- Continue to assess genetic gain in seedling families through recurrent selection

3.0 METHODOLOGY

3.1 Selection of high early sugar clones

Each year (1993-96), 20 crosses from the stored-seed list were selected for planting in each region on BSES experiment stations (south (S), central (C) and north (N) – SRDC; New South Wales (W), Burdekin (A) and Herbert (H) – BSES). The selected crosses had

high early CCS parents and targeted crossing using these parents was implemented at Meringa during this period. Many 'recurrent' parents (ie those selected from the previous project BSS25 and some from BSS93) were used, continuing the process of population improvement.

Seed was shipped to each region between January and April and germinated in germination cabinets at 36°C for 48 hours. Germination trays of seedlings were then moved to a glasshouse for approximately 6 weeks and then potted into jiffy pots. The potted seedlings were grown on seedling benches outside for approximately 6 weeks and then transplanted to the field. The seedlings were planted in family plots of 20 seedlings per plot and each family plot was replicated three times. Thus families, but not clones, were replicated. Two appropriate standard clones for each region were planted in each replicate, and these were transplanted as for the seedlings but from germinated one-eye cuttings. Rows were 1.5 m apart and seedlings were planted 60 cm apart within the row.
The following year (1994-97) in May and June, a two-stalk sample was taken from the best 10 seedlings in each plot (end seedlings excluded) in each region, based on agronomic appearance. These were crushed through a small mill and CCS was determined using standard procedures (BSES 1984). The mean CCS of these 600 clones was calculated.

The ten highest clones for CCS in each region were selected from the plant crop to be sent to Meringa as parents. Those from NSW, southern and central regions were first sent to quarantine for 2 years, while those from Burdekin and Herbert were sent directly to Meringa.

In the first-ratoon crop, up to 20 clones per region were selected for progression to clonal assessment trials (CAT). These are single row, 10 m plots that are unreplicated. All selected clones were given 6000 serial numbers (eg 93A6001, where 93 = year seedlings went to the field; A = Ayr (region)). Clones that performed well in CATs, based on net merit grade (NMG, an economic index of relative sugar yield) were selected to proceed to the next stage of selection.

Seedling families were planted in the Burdekin in 1993 and 1994, but difficulties in pre-season sampling due to large, lodged crops caused the abandonment of the program in 1995 and 1996.

3.2 Assessment of realised genetic gain

In 1987, a sub-program of population improvement for early CCS was commenced, funded jointly by SRDC and BSES (BSS25). Over a 4-year period, families were selected based on early CCS information on the parents. Bunch seedlings were planted, commencing in 1987 at Bundaberg and Mackay, and 1988 at Meringa. Clonal families were planted as 4-sett plots in the year after bunch seedlings and clones tested in plant and first-ratoon crops for early CCS. The best clones for early CCS with reasonable agronomic type were selected from these families. The parents used during this period and the elite clones selected in each region (southern, central and northern) were propagated at Bundaberg, Mackay and Meringa for a trial to estimate realised genetic gain for early CCS. In addition, a base population of 30 clones was randomly selected from the breeding clones held at Meringa and a group of 30 high early CCS clones were

selected from the core selection program. These were also propagated at each centre. Thus 4 groups of clones were included in trials, designated base, parent, core and elite. Because of difficulties in assembling and propagating all clones at each centre, two years of propagation (1993 and 1994) were needed before the trials could be planted in 1995.

Some parents used in the initial project and some of the elite clones had been discarded by the time this experiment was initiated. One clone from the base population failed to germinate. The number of clones planted in each population group in each trial is given in Table 1.

Table 1
Number of clones in each population group planted into genetic gain trials

Population group	Number of clones		
	Southern	**Central**	**Northern**
Base	29	29	29
Parent	179	179	147
Core	30	30	30
Elite – All	131	121	87
Southern	60[1]	50	24
Central	46	46	32
Northern	25	25	31
TOTAL	**369**	**359**	**293**

[1] Ten clones from BSS93 included in this number

Trials were planted in July-August 1995 on the BSES Experiment Stations at Mackay and Meringa and on a farm (Anderson) in Bundaberg. Plots were 10 m long and a randomised complete-block design was used with two replicates.

Sampling for CCS was done in both May and June in 1996 (plant crop) and 1997 (first-ratoon crop), except at Meringa where the second sampling in 1997 was delayed until September. Two stalks were cut from each plot at each sampling, crushed through a small mill and CCS was determined using standard procedures (BSES 1984). The trials were harvested and weighed as soon after the June sampling as practical. Cane yield and sugar yield (based on mean CCS) was calculated for each clone.

Analyses of variance were conducted at each sampling time in each year and variance components were estimated from expected mean squares. Parents used and elite clones selected were grouped into populations based on the year the original seedlings were planted to the field. Mean CCS over May and June samplings and plant and first-ratoon crops was calculated for individual clones and populations, except at Meringa, where the mean was calculated over May and June in the plant crop and May in the first-ratoon crop. The genotypic variance (σ^2_G) and heritability (H) were calculated as shown below, with standard errors estimated according to Anderson and Bancroft (1952).

σ^2_G = (Mean square clone – Mean square error)/number of replicates
$H = \sigma^2_G / (\sigma^2_G$ + (Mean square error) / number of replicates

The mean CCS of parent populations used to plant seedlings from 1987 to 1991 was calculated along with the mean CCS of elite clone populations derived from these seedlings. This allowed analysis of any improvement in these populations over the period of this work using linear regression.

4.0 RESULTS

4.1 Selection of high early sugar clones

In total, 377 clones were selected from seedling families in all regions. Two hundred of these were sent to Meringa as parents, while all clones were tested in clonal assessment trials (CAT). In terms of parentage of the 377 elite clones selected:

- 131 different parents were involved
- 82 different female parents were involved
- 62 different male parents were involved
- 107 involved at least one recurrent parent from BSS25
- 108 involved at least one foreign parent (mainly CP)

The number of clones selected in each region (1994 to 1997) is shown in Table 2.

Table 2
Number of elite high early CCS clones selected in all regions 1994-97

Region	Number of clones selected in				
	1994	1995	1996	1997	Total
NSW	20	17	21	0	58
Southern	20	22	20	20	82
Central	20	20	20	21	81
Burdekin	20	20	0	0	40
Herbert	20	20	10	15	65
Northern	11	20	20	0	51
All	111	119	91	56	377

Eight families resulted in more than 10 clones selected in total, and these are shown in Table 3. Two of these families involved one recurrent parent (89C6003, 89C6019). Some families resulted in elite clones selected in a specific region (89C6019 * 85C755), while others resulted in elite clones selected in many regions (74S875 * CP57-614 and 83S194 * 89C6003). This indicated that some families were widely adapted for early CCS.

Table 3
Families from which ≥ 10 clones were selected

Family	Number of elite clone selected						
	W	S	C	A	H	N	All
80C203 * 84C497	0	6	6	0	0	3	15
89C6019 * 85C755	0	0	0	0	11	0	11
CP75-1322 * 75S2497	4	7	0	0	0	1	12
Q96 * CP73-341	2	1	0	0	7	0	10
74S875 * CP57-614	0	2	2	2	0	4	10
77S1642 * 79A362	0	0	0	9	0	1	10
83S194 * 89C6003	2	3	2	0	0	4	11
83S2159 * 80C203	9	2	6	0	0	0	17

Sixty-five clones selected in BSS25 and this project, which were tested in CAT, have progressed to advanced stages of selection. To date, two Q varieties have been released (Q185[A] (formerly 89C6004) in the central region and Q205[A] (formerly 88C6002) in the southern region). Fourteen of the 65 clones were still undergoing testing in 2002 and are showing some promise. The remainder have been discarded.

The elite clones selected in both projects are routinely being used as parents and most regional selection programs have now incorporated early CCS as a core activity. In 2002, the stored-seed list contained 5 314 different parental combinations and 440 (8.3%) of these had at least one parent derived from the two projects.

4.2 Assessment of realised genetic gain

The trial mean CCS in May and June, cane yield (TCH) and sugar yield (TSH), together with the ranges and coefficients of variation (CV) for each location and crop class are shown in Table 4.

These data indicate that the trials were conducted with reasonable precision for single-row trials (TCH) and two-stalk samples (CCS). The mean CCS was higher in June than May, as expected, and there was an enormous range in CCS. Estimates of the genotypic variance components from the expected mean squares showed that genetic variability is higher in May than June (Table 5), confirming many previous findings (see Cox *et al.*, 1994). Genetic variability for CCS in September (Meringa second sampling, first ratoon) showed a dramatic reduction compared with that in May (2.49 to 0.40). These results show that the greatest potential to improve CCS occurs early in the season.

Table 4
Mean, range and coefficient of variation (CV) for CCS in May and June, cane yield (TCH) and sugar yield (TSH) at Bundaberg, Mackay and Meringa in plant (P) and first-ratoon (1R) crops.

Trait/Statistic	Bundaberg		Mackay		Meringa	
	P	1R	P	1R	P	1R
CCS May						
Mean	9.2	9.5	10.1	11.3	10.1	11.9
Range	1.9–13.0	1.7–14.7	2.3–13.9	4.0–16.0	3.0–13.5	4.1–15.2
CV	11.6	11.5	12.8	10.3	9.8	11.9
CCS June						
Mean	11.2	11.7	12.8	12.9	12.2	*
Range	4.0–14.2	4.6–14.9	5.2–15.9	4.6–15.9	6.2–15.3	
CV	8.8	8.4	8.3	7.8	7.2	
TCH						
Mean	85.1	118.8	86.5	101.0	72.7	86.6
Range	16–156	9–189	23–127	28–181	29–124	18–170
CV	21.9	17.2	17.6	20.4	17.3	17.9
TSH						
Mean	8.7	12.4	9.8	12.1	8.1	10.4
Range	2.0–16.7	1.2–20.9	2.9–15.6	3.6-22.2	3.2-13.7	2.5–23.9
CV	25.0	17.9	20.5	21.8	17.5	18.7[1]

* June sampling delayed until September
[1] Based on TCH in September and CCS in May

Table 5
Genotypic variance component (σ^2_G) and broad-sense heritability (H), with standard errors for CCS, cane yield (TCH) and sugar yield (TSH).

Trait/Statistic	Bundaberg		Mackay		Meringa	
	P	1R	P	1R	P	1R
CCS May						
σ^2_G	2.27±0.15	3.08±0.19	2.90±0.20	3.33±0.21	2.20±0.16	2.49±0.21
H	0.80±0.01	0.84±0.01	0.78±0.02	0.83±0.01	0.82±0.02	0.71±0.02
CCS June						*
σ^2_G	2.08±0.14	1.97±0.13	1.80±0.13	1.98±0.13	1.92±0.14	0.40±0.05
H	0.81±0.01	0.81±0.01	0.76±0.02	0.79±0.02	0.83±0.01	0.48±0.04
TCH						
σ^2_G	232±23.0	778±52.5	187±17.0	339±31.1	234±18.9	468±35.0
H	0.57±0.03	0.79±0.02	0.62±0.03	0.61±0.03	0.75±0.04	0.80±0.02
TSH						
σ^2_G	2.84±0.30	9.33±0.63	2.21±0.25	5.12±0.49	3.13±0.25	8.73±0.66
H	0.55±0.03	0.79±0.02	0.52±0.04	0.61±0.03	0.76±0.02	0.78±0.02

* Estimates for September

A comparison of the mean CCS of the various population groups (Base, Parent, Core and Elite) is shown in Table 6.

Table 6
Mean CCS and standard deviation (SD) of population groups at Bundaberg, Mackay and Meringa

Population/Statistic	Mean CCS		
	Bundaberg	**Mackay**	**Meringa**
Base			
Mean	**8.9a**[1]	**10.1a**	**9.7a**
No.	29	29	29
SD	1.93	2.10	1.99
Parent			
Mean	**9.8b**	**11.2b**	**11.1b**
% of Base	110.9	111.5	115.3
No.	179	179	147
SD	1.40	1.40	1.37
Core			
Mean	**10.9c**	**12.8c**	**12.2c**
% of Base	123.0	127.6	126.6
No.	30	30	30
SD	1.11	0.95	0.94
Elite			
Mean	**11.4d**	**12.7c**	**12.2c**
% of Base	128.8	126.5	126.4
No.	131	121	87
SD	1.09	1.07	1.10

[1] Means followed by the same letter at each location are not significantly different ($P<0.05$)

At all locations, the mean CCS of the parent, core and elite populations were significantly greater than the base population, a random group of clones from the parent population at the start of this work. At all locations the means of the core and elite populations were significantly greater than the parent population, which included most parents used to produce crosses for project BSS25 during 1987 to 1991, and from which the elite clones were selected. At the start of the project (1987), limited information was available on early CCS and it would be expected that the parents and crosses selected would have improved over the period as more information became available. At Bundaberg, the mean of the elite population was significantly greater than that of the core population (11.4 versus 10.9), but at Mackay and Meringa the means were not significantly different. This may have been because the elite population at Bundaberg included an additional 10 clones selected from the first series of original seedling families in the current project (ie not actually a part of BSS25). These were included to assess genetic gain over a longer period (see below).

One of the key objectives of this project was to assess realised genetic gain from recurrent selection for early CCS. The means of each of the parent and elite populations selected in each region from families that first went to the field during 1987 to 1991 (plus 1993 for the Bundaberg elite population) were estimated and these are plotted over years (Figures 1 to 3).

At Bundaberg (Figure 1), the southern parent and elite populations showed reasonably steady improvement over time. In both cases, linear regression coefficients were significant:

Parents – $Y = 9.50 + 0.27X$, $r^2 = 0.84$, $P < 0.05$;

Elite – $Y = 10.77 + 0.26X$, $r^2 = 0.88$, $P < 0.01$

This indicated that both populations were increasing at slightly more than 0.25 units of CCS per year. Similar estimates of southern population improvement were obtained in Mackay (Figure 2: parent – b=0.27, r^2=0.73, P=0.06; elite – b=0.29, r^2=0.88, P<0.05) and Meringa (Figure 3: parent – b=0.36, r^2=0.89, P<0.05; elite, regression not significant, only three points).

These data indicate that there was little sign of slowing of population improvement in the southern elite clones, with gains continuing in those selected in the current project. Variation within each of these populations over the period was consistent and there were no signs of this diminishing (standard deviations for the six elite populations from 1987 to 1993 were 0.83, 0.90, 0.81, 0.96, 0.93 and 0.89, respectively. It was concluded that continued progress through breeding and selection in the southern region is likely.

At Mackay (Figure 2), the central parent population showed an inconsistent improvement. The regression equation, while not significant (P=0.16, r^2=0.53), indicated modest improvement of 0.14 units of CCS per year. For the same population grown at Bundaberg (Figure 1), the regression approached significance (P=0.07), with an indicative increase of 0.21 units of CCS per year. At Meringa, the regression was positive but not significant.

The central elite population at Mackay (Figure 2) showed good improvement in mean CCS from 1987 to 1989 (12.1 to 13.3), but declined in the 1990 and 1991 populations. This was consistent in these populations tested at Bundaberg (Figure 1) and Meringa (Figure 3). Very difficult selection environments at Mackay from 1991 to 1993 may account for the poorer clones selected from the 1990 and 1991 populations. Severe flooding affected the 1990 population, completely destroying one of the two replicates and badly affecting the other. This dramatically reduced the number of clones available for selection to less than half. Severe drought affected selection in the 1991 population. In the first-ratoon crop, clones were selected for early CCS and agronomic type under extreme drought stress, with cane only waist height. It is difficult to conclude from these data whether genetic gain would have continued if selection had been carried out in the absence of these environmental constraints. As early CCS has high narrow-sense heritability (Cox *et al.,* 1994), it would seem likely that, if the parent populations improved, the clones selected from crosses involving these parents should improve.

At Meringa (Figure 3), the northern parent population showed a small but significant improvement over the 4 years ($Y=11.07+0.13X$, $r^2=0.98$, $P<0.05$). This trend was repeated for these populations grown at Bundaberg (Figure1 – $b=0.24$, $r^2=0.85$, $P=0.08$) and Mackay (Figure 2 – $b=0.14$, $r^2=0.74$, $P=0.14$), although regressions were not significant at $P=0.05$.

The northern elite population showed no improvement over the 4 years. This was unexpected, as the parent populations showed reasonable improvement. However, good improvement was shown by the northern elite populations (1988 to 1990 only) when grown at Bundaberg (Figure 1 – $b=0.39$, $r^2=0.98$, $P=0.08$) and Mackay (Figure 2 – $b=0.21$, $r^2=1.00$, $P<0.05$). It is difficult to explain these results, but it may indicate that it is more difficult to breed and select for early CCS in the wet tropics.

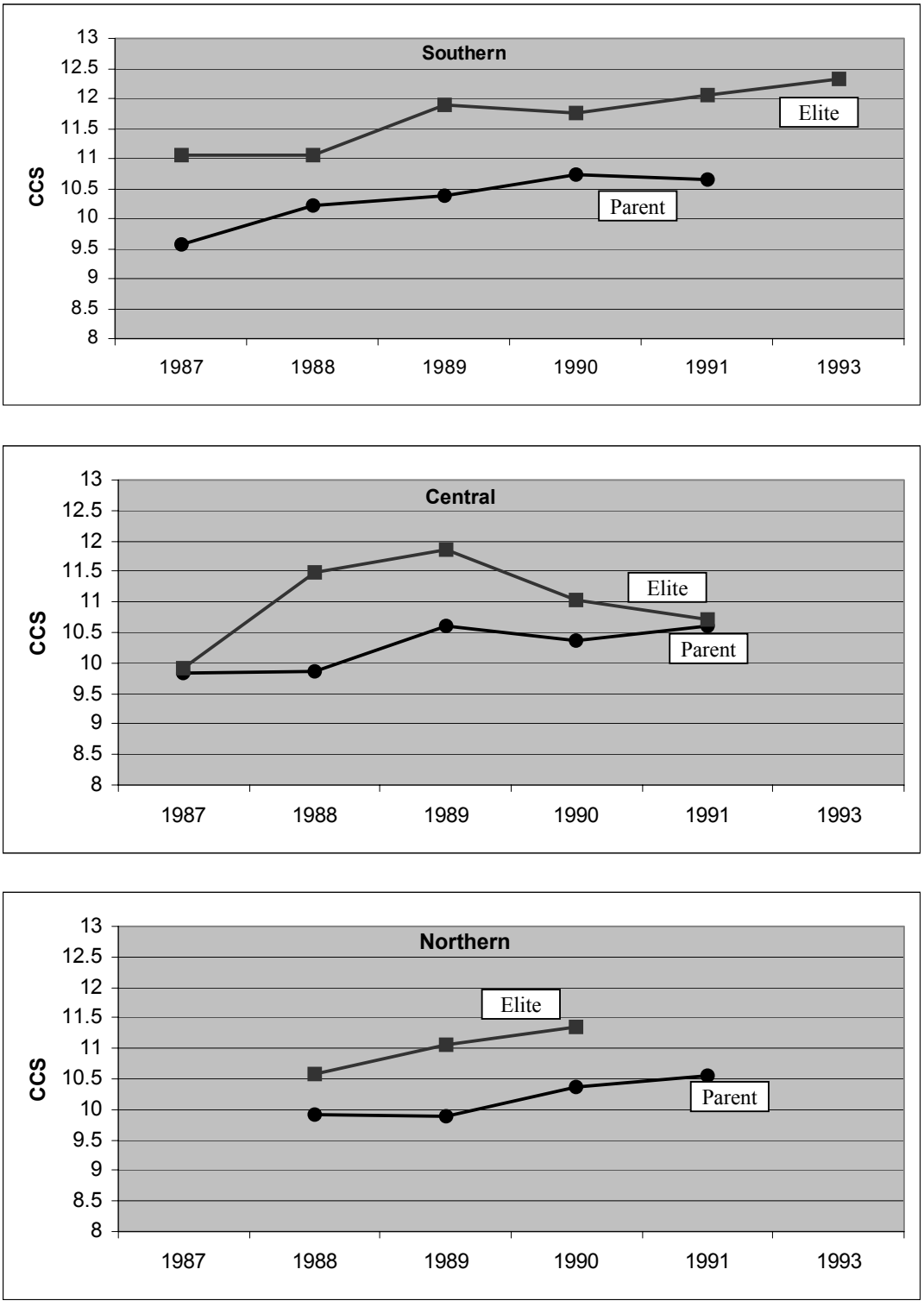

Figure 1 – Southern, central and northern parent and elite populations over the period 1987 to 1993 tested at Bundaberg

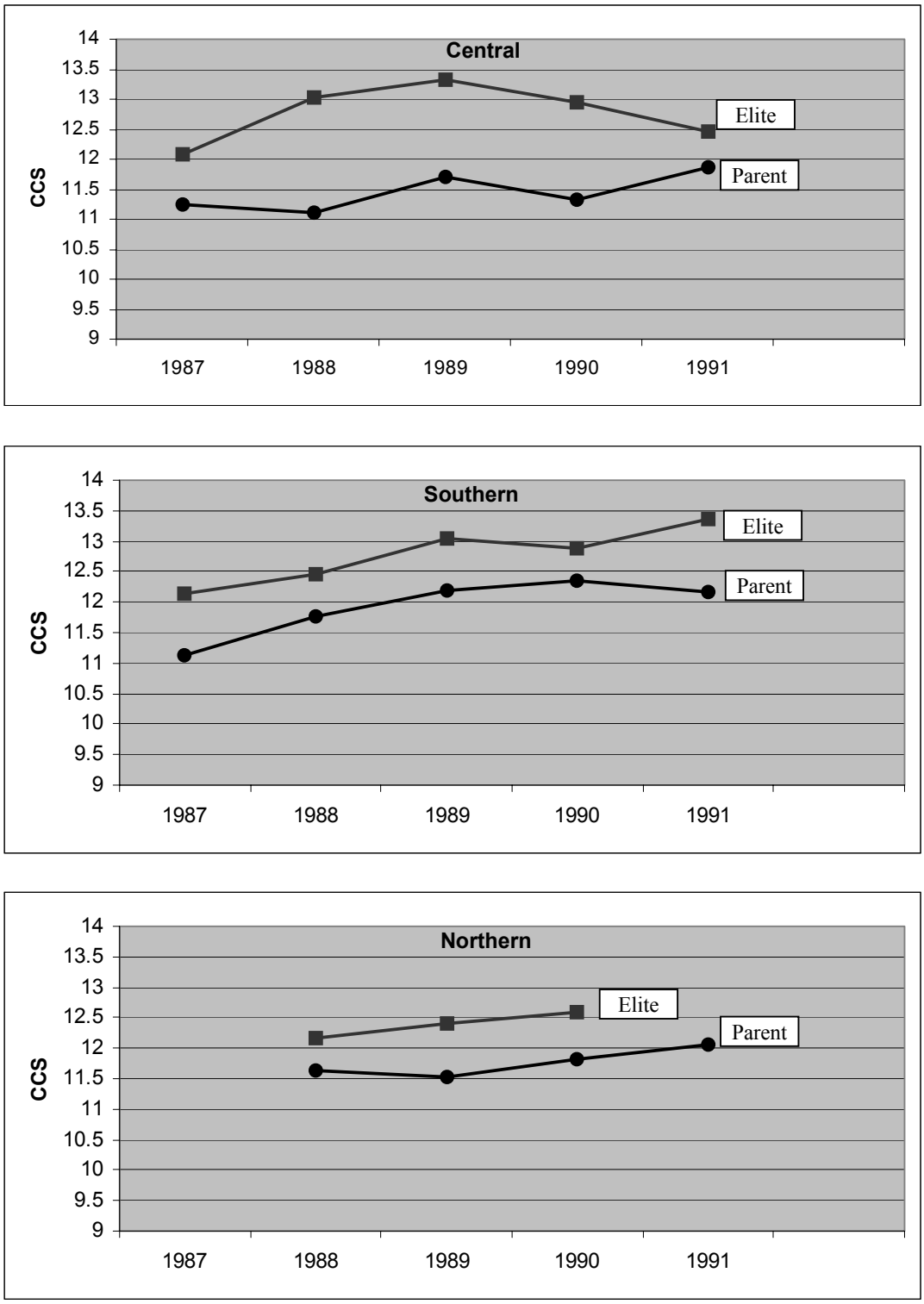

Figure 2 – Central, southern and northern parent and elite populations over the period 1987 to 1991 tested at Mackay

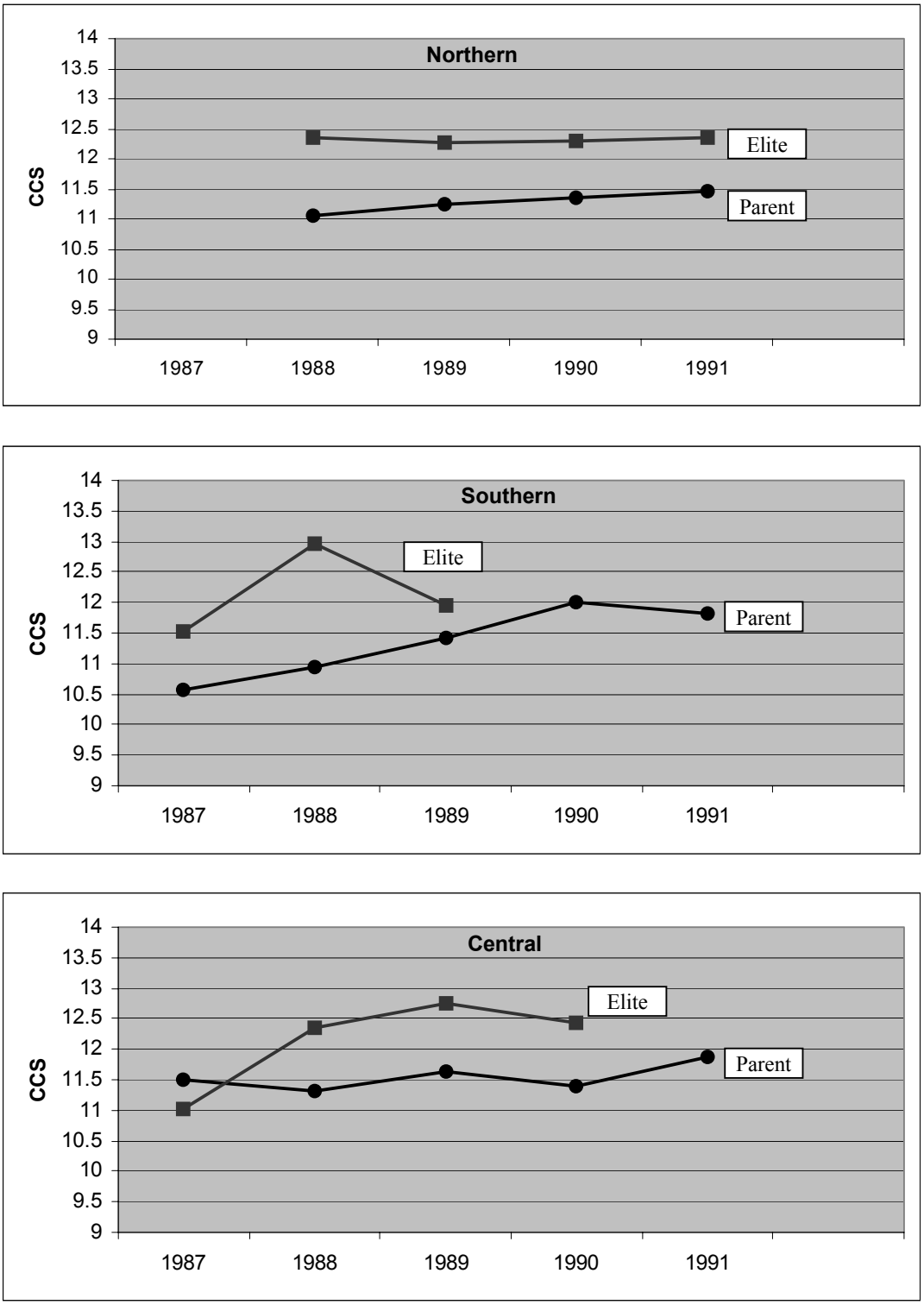

Figure 3 – Northern, southern and central parent and elite populations over the period 1987 to 1991 tested at Meringa

5.0 OUTPUTS

There are two major outputs from this project:

- An elite high early CCS parent population that is being used in further crossing. Apart from the 200 parents from this project, there were 139 clones selected in BSS25 as well as a large number of clones selected from the core selection programs.

- Although BSS25 and BSS93 were basically small recurrent selection subprograms, aimed mainly at population improvement, two varieties have been released as a direct result of these projects (Q185[A] and Q205[A]).

6.0 EXPECTED OUTCOMES

The major outcome of this work has been the incorporation of a routine, high early CCS component to core selection programs in the Herbert, Burdekin, Central and Southern regions. In some cases early CCS or brix is being measured on individual clones in original seedlings. Elite clones selected for high early CCS (they continue to be given 6000 serial numbers to identify them) are sent to Meringa as parents. Thus we would expect continued improvement in early CCS and further varieties released.

7.0 FUTURE RESEARCH NEEDS

No further research needs in the area of early CCS have been identified at this stage. In terms of late CCS, where there may be some increased level of genetic variability due to different clonal responses to summer storm rains, some work in this area may lead to a suite of varieties suitable over the whole period of an extended season.

8.0 RECOMMENDATION

BSES continue to invest resources to develop productive varieties with high early CCS.

9.0 LIST OF PUBLICATIONS

Cox, M C (1999). Improving early season CCS in a sugarcane breeding program. Proceedings of the 11th Australian Plant Breeding Conference, (Vol 2 – Contributed Papers) pp 100-101.

10.0 ACKNOWLEDGMENTS

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11.0 REFERENCES

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