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POTENTIAL GAINS FROM INTROGRESSION BREEDING BASED ON ANALYSIS OF THREE BREEDING POPULATIONS

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Abstract

One of the objectives of South African sugarcane breeders is to balance the ratio of sucrose to fibre in the stalk, since high fibre content causes losses through increased juice extraction costs and loss of sucrose in processing. There are, however, very complex physiological processes controlling this ratio. In addition, these traits are negatively correlated. This poses a challenge for introgression breeding, since most of the wild species of sugarcane contain high fibre levels. The aim of the present study was to determine trends in sucrose and fibre across three generations of progeny resulting from introgression crosses with *Saccharum spontaneum* at the South African Sugarcane Research Institute. Data were collected from single stool trials established from seedlings planted across three regional breeding programmes, (a) irrigated, (b) coastal short cycle high potential and (c) coastal hinterland breeding programmes. Genotypes included *Saccharum* spp. commercial hybrids (commercial crosses), F_1 genotypes (commercial genotype x *S. spontaneum* crosses) and BC_1 genotypes (commercial genotype x F_1 crosses). At harvest age (12 to 18 months, depending on the site), one stalk was sampled randomly from each of the first 20 seedlings in each family plot and analysed in the sucrose laboratory using standard procedures to estimate the quality variables. Results showed that progress with introgression was variable. For the irrigated and coastal short cycle high potential breeding programmes, on average, progeny resulting from commercial crosses had the highest ERC and lowest fibre content, while F_1 progeny had the highest fibre content and lowest ERC, as expected. ERC improved in BC_1 progeny, with a corresponding decrease in fibre content. However, crosses from the coastal hinterland breeding programme displayed a different trend. BC_1 genotypes had the lowest ERC, as well as the lowest fibre content compared to the other crosses, suggesting that the commercial-type parental genotypes used in the BC_1 crosses in the coastal hinterland breeding programme may have been sub-optimal. Future crosses should target high sucrose parents.

Keywords: Introgression, *Saccharum spontaneum*, regional breeding programmes, backcrossing

Introduction

Sugarcane is one of the most important crops globally, providing most of the world's sugar and bio-energy in the form of ethanol and electricity. This contribution has been underpinned by the successful introgression of genes from wild germplasm, particularly from *Saccharum spontaneum*, into the domesticated species, *Saccharum officinarum*, by breeders in the early 1900s. This early introgression resulted in an increase in vigour, ratooning ability and adaptation to adverse environments, compared with the existing varieties at the time.

Following the success of these early interspecific hybridisations, sugarcane breeding programmes around the world have since largely concentrated on utilising clones derived from these early introgressions in further cycles of crossing and selection. This, however, has contributed to most parental clones tracing back to a relatively small number of ancestral clones, thus narrowing the genetic diversity and leading to a renewed interest in introgression breeding.

The complexity and size of the sugarcane genome is a major limitation in genetic improvement. Whilst continued selective breeding for enhanced sucrose accumulation has been able to achieve over half of the yield increase in the past 50 years, it has been reported as having reached a plateau due to limits to the gene pool exploited in traditional breeding programmes (Mariotti, 2002). Based on current reports, introgression with *S. spontaneum* and related species, such as *Erianthus* and *Miscanthus*, is expected to contribute incrementally to genetic gains (Edme *et al*., 2005; Jackson *et al*., 2014).

During sugarcane introgression breeding, the F_1 generation is obtained by crossing commercial-type cultivars (hybrids with high sucrose content) with compatible individuals of different species (e.g. *S. spontaneum*) or genus (e.g. *Erianthus*). The latter usually have little or no sugar but are highly vigorous and resistant to major sugarcane pests and diseases. The F_1 progenies are usually low in sucrose content but acquire most of the desirable features of the 'wild' clones. Introgression for high sucrose is done in successive generations of backcrossing with *S. officinarum* or a commercial-type cultivar with high sugar content. Backcrossing recovers the high sugar phenotype inherited from *S. officinarum* while retaining the disease resistance and good ratooning of the wild *S. spontaneum* parent. Generally, two to three such backcrosses are expected to achieve appreciable sucrose levels and to dilute the undesirable genes of the wild clones (Santchurn, 2010).

Commercial varieties of sugarcane typically contain 30% dry matter and 70% water. Of the dry matter portion, 60% is sucrose and 40% fibre (Moore and Maretzki, 1996). Of these components, sucrose is the most important for sugar production. In the production of raw sugar, high fibre causes losses through increased juice extraction costs and loss of sucrose in processing. Breeders generally aim to increase sucrose content and keep fibre % at levels required for cogeneration to run the mills. There are, however, very complex physiological processes controlling the sucrose:fibre ratio. In addition, these traits are negatively correlated (Botha, 2013). This poses a challenge for introgression breeding, since most of the wild species of sugarcane contain high fibre levels, as seen in Table 1.

Table 1. Mean values for sugar and fibre content of members of the *Saccharum* complex (adapted from Bull and Glasziou 1963).

Species	No. clones	Contents (% fresh wt ± SE)		
		Sucrose	Reducing sugars	Fibre
Erianthus maximus	3	2.24 ± 0.44	0.73 ± 0.23	2.46 ± 0.90
Erianthus arundinaceus	2	0.62 ± 0.16	0.61 ± 0.17	30.30 ± 0.30
Miscanthus floridulus	5	3.03 ± 0.56	0.79 ± 0.24	51.00 ± 2.00
Saccharum spontaneum	10	3.96 ± 0.46	0.44 ± 0.20	33.90 ± 2.10
Saccharum robustum	10	7.73 ± 0.83	0.27 ± 0.02	24.80 ± 1.60
Saccharum sinense	2	13.45 ± 0.02	0.38 ± 0.08	12.80 ± 2.00
Saccharum officinarum	25	17.45 ± 0.35	0.32 ± 0.02	9.80 ± 0.40

At the South African Sugarcane Research Institute (SASRI), the genetic improvement of sugarcane is geared towards the development of new varieties with high cane yield, high sucrose content, resistance to the major pests and diseases, adaptation to various agro-climatic zones, good ratooning ability and suitability for harvesting at different times of the milling season. Imported genotypes and wild germplasm are included as parents to widen the genetic base of the breeding populations, as well as to provide novel sources of genes for disease resistance and other important traits.

The wild species used for introgression breeding in the present study was *S. spontaneum* since it is far more genetically diverse than current commercial cultivars (Alwala *et al.,* 2006; Suman *et al.,* 2010), making it useful for widening the genetic base. The species displays high ratooning ability, is vigorous, has wide adaptation to a range of abiotic stresses (including drought, floods, saline conditions and freezing temperatures), and possesses resistance to most pests and diseases (Santchurn. 2010). Undesirable characteristics include high fibre and low sucrose.

The aim of the present study was to determine trends in sucrose and fibre content across progenies from F_1, BC_1 and commercial crosses, and evaluate implications on introgression breeding. The objective was to summarise results obtained from initial introgression breeding attempts at SASRI in order to inform a more structured way forward. An additional aim was to determine how many backcrosses would be necessary before the resulting progeny could be used as parents in the commercial breeding programme. Results from the present study will be used to inform the SASRI introgression breeding strategy, which in addition to addressing genetic diversity of parental populations, also aims to increase resistance to stem borers, nematodes and drought.

Materials and Methods

Data were collected from seedlings planted as single stools (stage 1 selection trials) for the coastal hinterland breeding programme based at Kearsney Research Station, the coastal short cycle high potential breeding programme based at Empangeni Research Station, and the irrigated breeding programme based at Pongola Research Station (Table 2). The coastal hinterland breeding programme represents crops harvested at 18 months, the ideal harvest age for growing areas along the KZN coastline. The coastal short cycle high potential breeding programme represents areas with good soils and high and well distributed rainfall conducive to high yields, with crops harvested at 12 months. The irrigated breeding programme represents high input sugarcane production in low rainfall areas, with lots of sunshine and water supply using irrigation, and crops harvested at 12 months.

Table 2. South African Sugarcane Research Institute (SASRI) regional breeding programmes included in study

Research station	Altitude (m)	Latitude	Harvest age (months)	Environment
Pongola	308	27°24’	12	Irrigated
Empangeni	102	28°43’	12	Coastal short cycle high potential
Kearsney	241	29°17’	18	Coastal hinterland

The study included progeny from three different generations, viz. commercial-type genotypes (*Saccharum* spp hybrids), F_1 genotypes (hybrids between commercial-type genotypes and *S. spontaneum*), and BC_1 genotypes (first generation backcrosses between F_1 progeny crossed to commercial-type genotypes). Table 3 shows the *S. spontaneum* genotypes used in the study.

A randomised complete block design was used at planting. Different genotypes were planted at each of the locations, since the breeding populations are targeted to address specific agroclimatic conditions. To avoid lodging, stalk sampling was done just before harvest age. One stalk was sampled randomly from each of the first 20 seedlings in each family plot and analysed in the sucrose laboratory using standard procedures to estimate sucrose content. From this data, estimable recoverable crystal (ERC) and fibre content was calculated.

Table 3. Origin of *Saccharum spontaneum* used in study.

Genotype	Origin
HELIUS	Unknown
MANDALAY	Burma
MEX54245	Mexico
NIGERIA1	Nigeria
SES14	India
SES208	India
SES234	India
SES277	India
TAIWAN96	Taiwan

Statistical Analysis

Data were subjected to analysis of variance using the linear mixed model in SAS (SAS Institute, 2013). The linear mixed model used was:

$$Y_{ijk} = \mu + R_i + G_j + RGij + S(RG)_{ijk} \qquad \text{Equation 1}$$

where:

Y_{ijk} is the sucrose or fibre content values measured from the kth seedling (k=1, 2, …, s) in jth generation (j=1, 2, …, g) in the ith replication (i=1, 2, …, r)

μ is the overall mean

R_i is the random effect of the ith replication

G_j is the fixed effect of the jth generation

RG_{ij} is the random interaction effect of the ith replication by the jth generation

$S(RG)_{ijk}$ is the random effect of the kth seedling nested with the interaction of the ith replication by the jth generation and was the residual error.

Mean comparison of the generations was done using the Tukey adjustment (Freund and Wilson, 2003).

Results and Discussion

Conventional breeding at SASRI has contributed to the development of agronomically improved sugarcane cultivars. However, limitations such as complex genome, poor fertility and long breeding cycle are challenges to achieving high genetic gains. In addition, only a small number of original ancestral clones have contributed to the parental material, resulting in a narrow genetic base. The previous strategy of crossing high sucrose parents, followed by selection of high sugar producing progeny in early selection stages has further narrowed the genetic base. Added to this, the long selection cycle of 12-15 years and clonal propagation of sugarcane suggest that the development of cultivars has not involved many meiotic events because of limited opportunities for genetic combination that occurs only at the time of crossing. To address this, introgression breeding is being used to enhance the incorporation of important genetic traits from wild and related germplasm into elite *Saccharum* parents that will in turn be used in commercial breeding. The present study analysed sucrose and fibre content in progeny resulting from introgression crosses derived from three breeding programmes.

There were statistically significant differences ($P<0.001$) in ERC and fibre content among progeny from the irrigated and coastal short cycle high potential breeding programmes. Progeny from both breeding programmes displayed expected trends for sucrose and fibre across commercial, F_1 and BC_1 generations, with fibre increasing and ERC decreasing from the commercial to F_1 generation due to the contribution of the *S. spontaneum* parent. F_1 progeny from the irrigated programme showed approximately 45% less ERC and 19% higher fibre content than that of the commercial progeny (Table 4). In the coastal short cycle high potential programme, F_1 progeny had 19% less ERC and 11% more fibre than the commercial progeny. Fibre then decreased and ERC increased from the F_1 to BC_1 generation due to progeny being backcrossed to high sucrose commercial parents. BC_1 progeny from the irrigated programme showed an approximate 22% increase in ERC and 9% lower fibre content over the F_1 progeny. In the coastal short cycle high potential programme, BC_1 progeny had 11% more ERC and 9% less fibre than F_1 progeny (Table 4). The fact that BC_1 progeny exhibited ERC and fibre contents closer to the commercial progeny suggests that further backcrossing should bring fibre and sucrose content even closer to the level of the commercial genotypes. The authors thus recommend an additional backcross (*Saccharum* spp hybrid x BC_1) to create BC_2 progeny in the irrigated and coastal short cycle high potential breeding programmes.

Table 4: Comparison of ERC and fibre across three SASRI breeding programmes for three generations of crosses. Within each breeding programme, generations represented by the same letter are not significantly different. Numbers in brackets represent the number of genotypes used in the study.

Breeding programme	Generation	ERC	% Commercial	Fibre	% Commercial
Irrigated	F_1 (33)	3.7399^a	55.36	13.4216^a	119.66
	BC_1 (47)	5.2133^b	77.17	12.3546^b	110.14
	Commercial (60)	6.7556^c	100.00	11.2169^c	100.00
Coastal short cycle high potential	F_1 (14)	9.1991^a	81.23	13.5534^a	111.51
	BC_1 (21)	10.4748^b	92.49	12.5118^b	102.94
	Commercial (39)	11.3249^c	100.00	12.1547^b	100.00
Coastal hinterland	F_1 (13)	9.6845^{ab}	96.16	14.5740^a	107.24
	BC_1 (30)	9.3206^a	92.55	13.3785^b	98.44
	Commercial (49)	10.0710^b	100.00	13.5902^b	100.00

In comparison with the previous two breeding programmes, progeny from the coastal hinterland did not follow expected trends. While F_1 progeny had approximately 4% less ERC and 7% higher fibre content than the commercial progeny (Table 4), BC_1 progeny showed an unexpected 4% decrease in ERC and 9% decrease in fibre content compared to the F_1

progeny. These results suggest that low sucrose recurrent parents may possibly have been used in the backcrosses. The recommendation going forward is to recreate BC_1 populations for the coastal hinterland breeding programme using high sucrose-yielding parents from the coastal short cycle high potential and the irrigated breeding programmes. Another option is to use *S. spontaneum* parents with reasonable sucrose levels in the initial F_1 cross. Brown *et al.* (1969) have shown that cycles of breeding and selection within *S. spontaneum* prior to selection of parents should be effective. Roach (1977) also found that selection within *S. spontaneum* for % sucrose prior to introgression was effective in increasing sugar content of the subsequent F_1 hybrids. In the study by Roach (1977), F_1 clones which had been selected for backcrossing on the basis of yield of sucrose per hectare again showed a highly significant advantage over randomly selected clones. Similarly, BC_1 clones produced from these select F_1 clones retained a significant advantage in yield of sucrose per hectare over BC_1 clones derived from randomly selected F_1 clones. For the coastal short cycle high potential programme, an alternative is to use *S. officinarum* parents in place of *Saccharum* spp hybrids in the initial cross with *S. spontaneum*. The F_1 progeny can then be backcrossed to a high-sucrose commercial-type cultivar.

An additional observation is that average sucrose content of progeny resulting from commercial crosses was in the region of 6-11% across the different breeding programmes (Table 4). This is low in comparison to other trial data in the SASRI Plant Breeding Programme, where figures as high as 20% ERC have been obtained (results not shown). The low sucrose content in the present study could possibly be due to the relatively young age of the cane since sampling was done ahead of harvest age in order to avoid lodging, particularly in the irrigated region.

At SASRI, *S. spontaneum* is used as a male parent during crossing because of its heavy pollen production, and more importantly, to avoid self-pollination. The species is known to be able to self-pollinate to a higher degree than commercial-type cultivars (Jackson *et al.* 2014). The commercial-type cultivars are used as female parents because they possess the desirable genetic background. During sexual reproduction in sugarcane, chromosome transmission is usually 2n+n, which means that double the number of chromosomes are donated from the maternal parent and less from the paternal parent. Thus, when *S. spontaneum* is used as a male, a lower proportion of the wild genome is transmitted to the progeny, allowing breeders to perform fewer backcrosses.

The overall major challenge associated with introgression of basic germplasm into highly selected and commercially adapted germplasm in sugarcane is the same as in other crops: that the basic germplasm brings with it many undesirable traits which need to be selected against between cycles of crossing back to the highly bred and commercially superior parental material, while at the same time desirable traits and genes from the wild donor may be diluted or lost with successive generations (Jackson *et al*., 2014). In the case of sugarcane, the major undesirable trait introduced with the use of wild canes is low sucrose content and high fibre. To complicate matters further, these traits are negatively correlated (Botha, 2013), posing a challenge for introgression breeding. Figure 1 shows the relationship between fibre and sucrose content in the present study. Across all three breeding programmes, as fibre content was increased, there was a corresponding reduction in ERC.

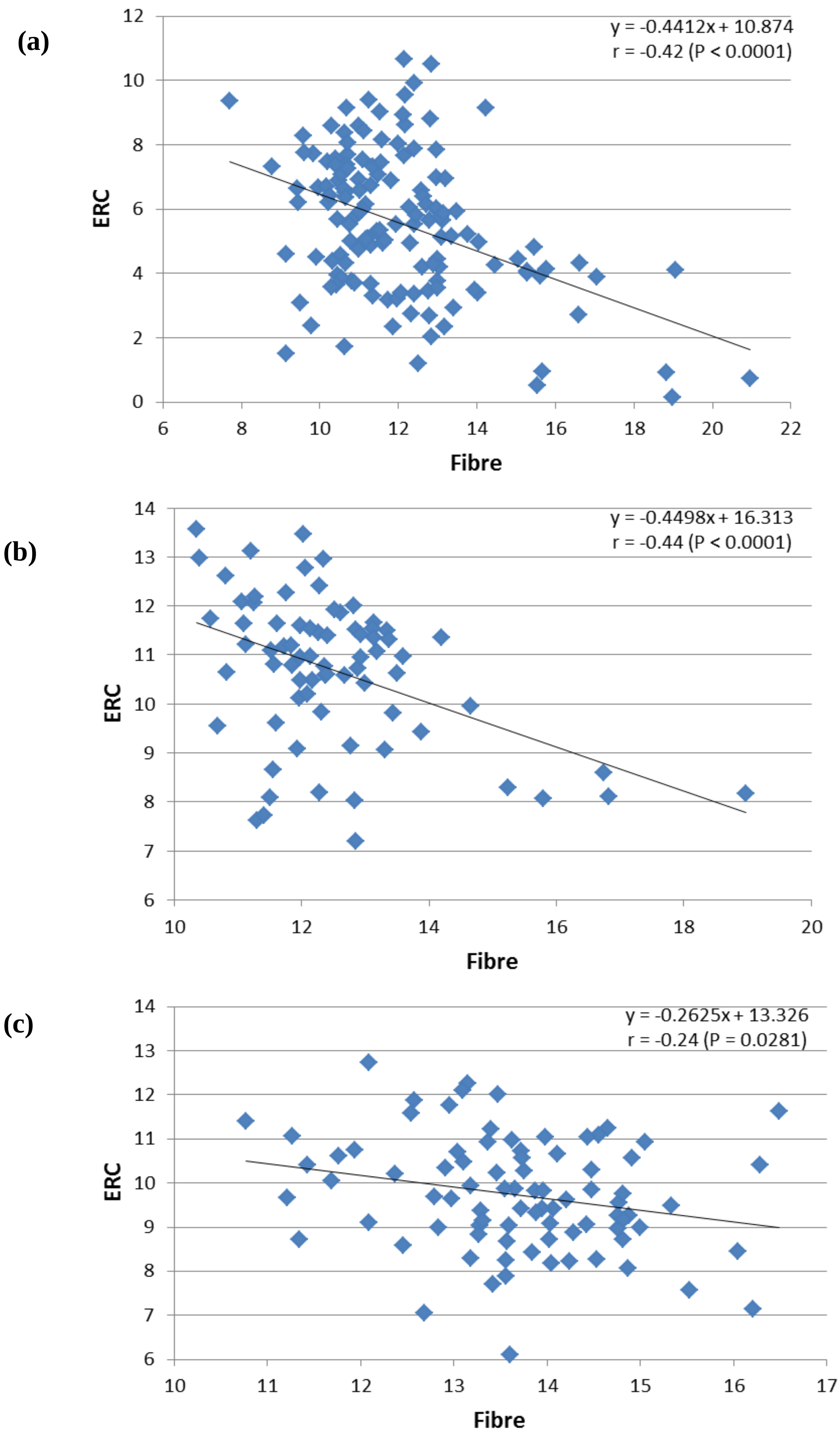

Figure 1. Relationship between fibre and sucrose content in the (a) irrigated, (b) coastal short cycle high potential and (c) coastal hinterland breeding programmes.

Despite the above-mentioned challenges, *S. spontaneum* will continue to be used for germplasm enhancement at SASRI because it possesses desirable traits for hardiness, ratooning ability and high biomass production. It also exhibits high genetic diversity because it is found in almost all continents (Zhou *et al.,* 2013). Studies by Alwala *et al.* (2006) and Suman *et al.* (2010) confirmed the existence of large genetic diversity. One highly successful example of the use of *S. spontaneum* in introgression breeding is the development of numerous important cultivars in Australia within the Bureau of Sugar Experiment Stations (BSES) Limited breeding programme developed from the wild clone 'Mandalay'. In Louisiana, resistance to mosaic virus was successfully transferred to BC_4 progenies in cultivar x *S. spontaneum* crosses culminating in the release of LCP85-384 (Milligan *et al.,* 1994). Although very preliminary, there are some signals that *S. spontaneum* clones sourced from the northern Thailand-Burma region could provide superior breeding material. Commercial successes in Australia and the USA arise from clones selected in this region (Jackson *et al.,* 2014).

Results from the present study will be used to inform the SASRI introgression breeding strategy. The study showed that if parents with high fibre content are used, the progeny produced by introgressions are expected to produce high fibre (as seen in the F_1 generations). By using low-fibre parents, fewer backcrossing cycles would be required to get progeny with the desired levels of sucrose content. Conversely, if it becomes necessary to use a parent known to produce high fibre content because it possesses other desirable traits, then the breeders would be able to plan ahead the possible high number of backcrossing cycles that would be required to lower the fibre content in the resulting progeny populations.

Conclusion

In the present study, trends in ERC and fibre content across the various populations was a reflection of the parental genotypes used to initiate those populations. Early generations of progeny following initial crosses between *S. spontaneum* and *Saccharum* spp hybrids were characterised by levels of sucrose too low for commercial production, together with high fibre levels. Sucrose levels progressively increased in subsequent backcrosses. Choice of recurrent parent is extremely important, as seen in the coastal hinterland breeding programme. The recommendation going forward is to recreate BC_1 populations for the coastal hinterland breeding programme using high sucrose-yielding parents from the coastal short cycle high potential and the irrigated breeding programmes. Another option is to use *S. spontaneum* parents with reasonable sucrose levels in the initial F_1 cross. An alternative is to use *S. officinarum* parents in place of *Saccharum* spp hybrids in the initial cross with *S. spontaneum*. The F_1 progeny can then be backcrossed to a high-sucrose commercial-type cultivar. For the irrigated and coastal short cycle high potential breeding programmes, an additional backcross to create BC_2 progeny should be carried out to bring fibre and sucrose content even closer to the level of the commercial genotypes.

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