A COMPARISON OF SEEDCANE DERIVED FROM TISSUE CULTURE WITH CONVENTIONAL SEEDCANE

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Abstract

Crops grown from seedcane of NCo310, NCo376 and N12 derived from a tissue culture process were compared with crops grown from conventional seedcane in field trials at two sites. The use of seedcane derived from the tissue culture process resulted in the plant crops of all three varieties at both trial sites having increased stalk populations and reduced cane and sucrose yields. The results indicate that the three varieties have retained their yield potential since release, despite several decades of conventional propagation in the case of NCo310 and NCo376, and that the use of tissue culture for the production of seedcane offers no advantages in respect of productivity.

In the first ratoon crop at both sites, plots of all three varieties that had been planted with the tissue culture-derived seedcane contained higher levels of smut than the plots planted with conventional seedcane.

Introduction

One of the characteristic features of sugarcane production is the slow rate of propagation of the crop by traditional means. In South Africa, approximately eight tons of seedcane are planted per hectare and thus a propagation rate of approximately x10 can be achieved from one planting to the next using conventionally produced seedcane.

Two alternative methods permit a more rapid rate of propagation. The use of transplants grown from single-budded seeds in multicelled trays provides a propagation rate of approximately x100. This method is increasingly being used in South Africa for the rapid propagation of new varieties and in seedcane production schemes. An extremely high rate of propagation can be achieved by the production of plantlets **in vitro** from the callus generated by tissue culture. Using appropriate techniques, many thousands of plantlets can be produced from small amounts of ex-plant material with very low rates of somatic variation. Varietal stability is such that collections of sugarcane clones are maintained in tissue culture at a number of institutes and clonal material is exported **in vitro**.

Initially, tissue culture (TC)-derived plantlets are delicate and require special care but they are soon robust enough for planting into the field. Such a process, based on a proprietary tissue culture technique, is being used commercially in the United States. In this use of the technology the plantlets are used to establish propagation plots in which seedcane is produced for commercial plantings. The main advantages claimed are simplification of seedcane procurement for the commercial cane grower and freedom from systemic diseases. The latter could result in substantial gains in yield if the quality of conventional seedcane is poor.

The opportunity to obtain commercially produced, TC-derived plantlets of local varieties for evaluation as a source of seedcane in the South African sugar industry arose in 1985. The main interest was to use this disease-free material as a standard to check the quality of locally produced conventional seedcane, i.e. produced via hot water treatment (HWT). While seedcane produced locally to certified or commercial nursery standards can confidently be claimed to be free or virtually free from the important systemic diseases known to occur in South Africa (viz ratoon stunting disease (RSD), sugarcane mosaic virus (SCMV), smut and leaf scald), there remained the possibility that unknown or unsuspected diseases might have built up in varieties that in some cases were released several decades ago. Two of the varieties used in this study, NCo310 and NCo376, were released in 1945 and 1955 respectively while the third variety, N12, was released in 1980.

Accordingly, several thousand TC-derived plantlets each of the varieties NCo310, NCo376 and N12, produced in the United States and growing in agar medium, were imported in September 1985. The plantlets were used to establish propagation plots from which seedcane was obtained for comparison with locally produced, conventional seedcane in field trials.

Methods

Sources of seedcane

The varieties NCo310, NCo376 and N12 had been exported from South Africa at various times and maintained in a closely supervised quarantine glasshouse of the United States Department of Agriculture. Material obtained from this source was propagated in a glasshouse of quarantine standard by a commercial company and these plants provided the ex-plant material that was subjected to a tissue culture process. Details of this process are proprietary and not available for disclosure, except that young leaf tissue was grown in an agar-based medium under special conditions and the callus tissue generated was induced to form numerous small plantlets. The plantlets were subsequently transferred to a medium in small, transparent containers and, once sufficiently established, were imported into quarantine in South Africa in this form. Each container held approximately 100 plantlets (Figure 1).

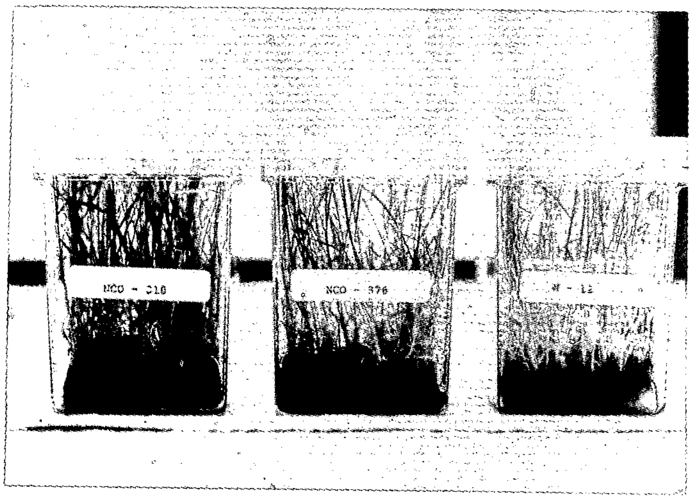


FIGURE 1 Tissue culture-derived plantlets growing on agar medium.

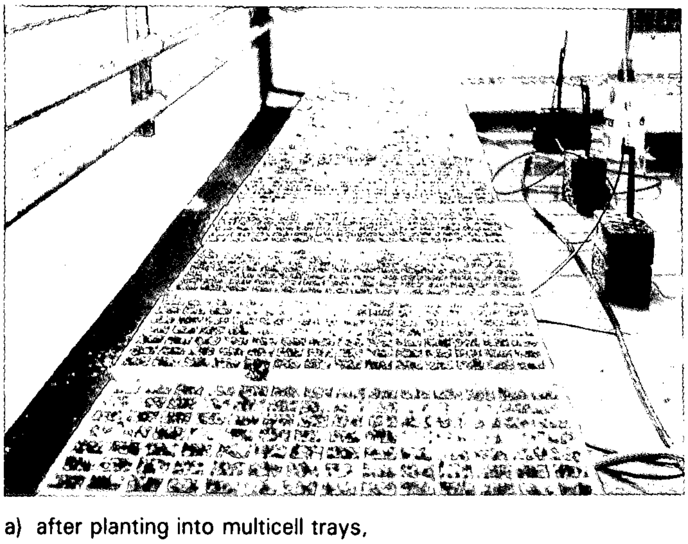
After "hardening" for one week in the containers, one thousand of the TC plantlets of each variety were transplanted into a sterilised vermiculite and compost-based medium in multicelled trays and placed in a temperature controlled compartment of the quarantine glasshouse at the Experiment Station, Mount Edgecombe in late September 1985 (Figure 2a). Temperatures in the compartment were maintained between 24 and 30°C. A mist system was installed to maintain a high humidity to aid in the establishment of the plantlets.

After seven weeks (Figure 2b), 250 plantlets of each variety were trimmed and transferred to a sterilised potting medium in 10 cm pots. After a further five weeks of growth (Figure 2c), in mid-December 1985, these plantlets were sufficiently robust for planting into propagation plots in the field at Mount Edgecombe.

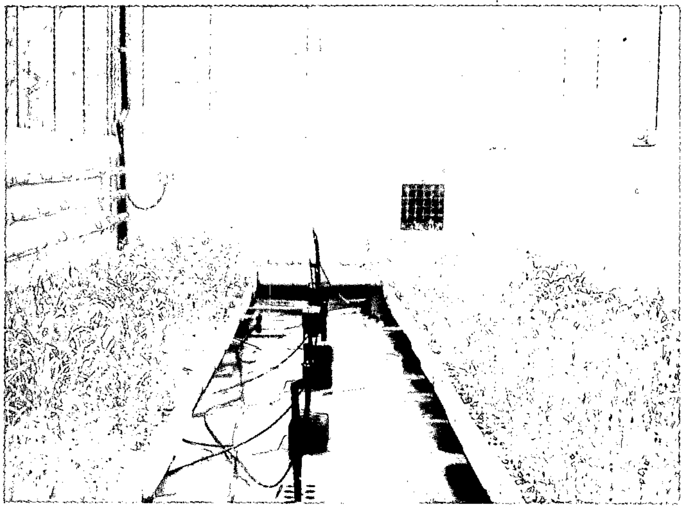
Conditions in the glasshouse were such that almost a 100% survival rate of the transplants was obtained at each of the various stages of production. While in quarantine the plantlets were tested for the presence of SCMV by ELISA with negative results and no signs for other diseases or abnormalities were observed.

The propagation plot of each variety was 140 m^2 in area and the plantlets were spaced 0,5 m apart in furrows at an interrow spacing of 1,4 m. Water (0,5 l per plant) was applied to aid in establishment of the transplants.

Conventional seedcane of the three varieties was, as far as possible, produced under identical conditions for comparison with the TC seedcane. Stalks were taken from the plant crops of nurseries that had been planted with HWT seedcane. Single-eyed setts were pre-germinated and transferred to 10 cm pots at the same time as the TC plantlets, and the resulting plants were transplanted into propagation plots adjacent to the TC plots, with the same interplant and interrow spacing and on the same date.



a) after planting into multicell trays,



b) growth after 7 weeks,

c) ready for planting into the field at 12 weeks.

FIGURE 2 Tissue culture plantlets

The site of the propagation plots was completely free of cane volunteers. The plots were inspected for diseases and off-type plants during growth. Stalks from both "conventional" and TC plants were tested by immunofluorescence microscopy for the presence of RSD, with negative results. Counts of stalk populations and estimates of stalk height were made when seedcane was harvested from the propagation plants in January 1987, 13,3 months after transplanting.

Field Trials

Trials were established in January 1987 at two sites, one under rainfed conditions at La Mercy on the North Coast of Natal and the other under irrigated conditions at Pongola in the eastern Transvaal. The most common disease at both sites is smut but whereas inoculum concentrations are fairly low at La Mercy, they are high at Pongola, where conditions are highly favourable for the development of the disease.

A split-plot design of six replications was used at both sites, with varieties in the main plots and the two sources of seedcane in the sub-plots. Each sub-plot consisted of five rows, 10 m long (nett plots of three rows) at interrow spacings of 1,3 m at La Mercy and 1,4 m at Pongola. Twenty 4-budded setts were planted in each row.

In the plant crop, shoot and stalk populations were counted periodically and the plots were also inspected for diseases. Stalk length and diameter were recorded at harvest. The population of stalks at harvest was recorded at La Mercy but not at Pongola because of severe lodging. Stalk counts and inspections of diseases continued in the first ratoon crops at both sites.

Results

Propagation plots

Both the TC and 'conventional' plots of all three varieties established well, but poor rainfall during the first six months necessitated periodic flood irrigation using a water cart. Conditions for growth were good throughout the latter half of the growing period in these plots.

The most obvious difference between the TC and conventional plots was the greater degree of tillering in the TC plots. This effect persisted until harvest and was most marked in NCo376 and N12 (Table 1). Stalks from the TC plots were also slightly longer, and again this difference was most marked in NCo376 and N12. Stalks from the TC plots were also noticeably thinner, but no measurements were made of stalk thickness.

Table 1

Stalk populations and stalk heights at harvest in propagation plots of tissue culture and conventionally derived plants

Variety	Source	Stalks/ha ($\times 10^{-3}$)	Stalk height (cm)
NCo310	Conventional	170,3	123
	Tissue culture	186,4 (+ 9,5 %)	129 (+ 4,9 %)
NCo376	Conventional	174,6	118
	Tissue culture	231,4 (+ 32,5 %)	127 (+ 7,6 %)
N12	Conventional	209,6	134
	Tissue culture	254,6 (+ 21,5 %)	144 (+ 7,5 %)

Occasional plants (approximately 1%) in the TC plots of all three varieties tillered profusely and remained extremely stunted. These were rogued. Apart from these stools the TC plants exhibited the typical characteristics of the respective varieties. One stool with smut occurred in each of the TC plots of NCo310 and NCo376. Natural infection by mosaic occurred to a greater degree but the extent of this did not differ between the TC and conventional plots. Means of 6,9 4,8 and 1,7% mosaic-infected plants occurred in the plots of NCo376, NCo310 and N12 respectively. All diseased plants found were rogued.

Field trials - growth and yield of plant cane

The condition of the seedcane from the propagation plots was good and uniform stands were obtained at both trial sites. Conditions for growth of the plant crops were generally good at both sites and high yields of cane were obtained. The crops were harvested in May 1988 at Pongola (15,4 months) and in June 1988 at La Mercy (16,6 months). A total of 2 232 mm of rain was recorded at La Mercy, 146 % of the long term mean (LTM), but 1 100 mm of this fell in the three months of September 1987 and February and March 1988. At Pongola, 1 021 mm of rain was recorded (114 % of LTM) and a further 854 mm of water was applied by overhead sprinkler, giving a total of 1 875 mm.

The TC plots of all three varieties had a higher population of stalks at both sites. As in the propagation plots, the difference was greatest in NCo376. At La Mercy, the greater stalk population in the TC plots persisted until harvest but the mean increase among the three varieties of 3,6 % was not significant. At Pongola, the final population count was done eight months after planting and then there was a significantly larger mean stalk population (difference 8,4 %) in the TC plots (Table 2).

In marked contrast to the situation in the propagation plots, stalk length at harvest was generally less in the TC plots. The difference was greatest in NCo376 at both sites. At La Mercy, the decrease in the mean length of stalks of the three varieties in the TC plots was 4,9 % and this was significant (the reduction of 9,8 % in NCo376 was highly significant). At Pongola, stalk length was slightly less in the TC plots of NCo376 and N12, but not in those of NCo310: these differences were not significant (Table 2).

Table 2

Stalk population, length and diameter in tissue culture and conventionally derived plots of NCo310, NCo376 and N12 at La Mercy and Pongola (plant cane. All records at harvest except for stalk population at Pongola at 8 months)

Variety/Treatment		La Mercy			Pongola		
		Stalks/ha ($\times 10^{-3}$)	Length (cm)	Diameter (cm)	Stalks/ha ($\times 10^{-3}$)	Length (cm)	Diameter (cm)
NCo310	TC	153	244	2,1**	155*	278	2,4
	Con	147	254	2,4	146	271	2,3
NCo376	TC	180	220**	1,8**	180*	287	2,0
	Con	169	244	2,1	158	305	2,1
N12	TC	179	231	2,0*	166	280	2,0
	Con	178	234	2,2	159	292	2,2
LSD (0,05)		15	16	0,17	8	30	0,26
(0,01)		—	22	0,23	—	42	—
Means	TC	171	232*	1,98**	167**	282	2,13
	Con	165	244	2,25	154	289	2,20
LSD (0,05)		8	9	0,10	5	18	0,15
(0,01)		12	12	0,13	6	—	—

Similarly, at La Mercy, stalk diameter at harvest of all three varieties was significantly less in the TC plots (Table 2). At Pongola differences in stalk diameter were small.

The mean yield of cane of the three varieties was significantly smaller in the TC plots at both La Mercy (−12,3 %) and Pongola (−7,1 %). At both sites the decrease in yield was greatest in NCo376. With all three varieties decreases were greater at La Mercy than at Pongola (Table 3). It appears that at both sites the slight gains in stalk population in the TC plots were more than offset by the greater stalk length and diameter in the conventional plots.

Cane quality, assessed as estimated recoverable sucrose (ers % cane), was higher in the TC plots of all three varieties at La Mercy; the greatest difference occurred in NCo376 (+13,7 %). Cane quality was poor at Pongola, particularly in NCo310, presumably because of the early harvest date; values of ers % cane were higher in the TC plots of NCo376 and N12 but not significantly (Table 3). Yields of recoverable sucrose were greater in the conventional plots of all three varieties at La Mercy and in the conventional plots of NCo310 and NCo376 at Pongola, though none of these differences was significant. The improved cane quality in TC plots had obviously partly compensated for the poorer yields of cane.

Diseases in plant and ratoon crops

No smut was recorded in the plant crop at La Mercy and there were only low levels at Pongola, indicating that the seedcane planted was free from infection (Table 4). High levels of smut occurred in the first ratoon crops of the susceptible varieties NCo310 and NCo376 at Pongola. At this site there were approximately twice as many smut whips in the TC plots of the three varieties as in the conventional plots. Much lower levels of smut occurred in the first ratoon crop at La Mercy than at Pongola, but there was also substantially more smut in the TC plots of all three varieties at this site.

Table 4

Incidence of smut in tissue culture and conventionally derived plots of NCo310, NCo376 and N12 at La Mercy and Pongola (t=trace).

Varieties and treatments		Smut whips/ha × 10^{-3}			
		La Mercy		Pongola	
		P	1R	P	1R
NCo310	TC	0	2,7	t	44,9
	Con	0	1,6	t	30,4
NCo376	TC	0	1,4	0,6	62,3
	Con	0	0,9	0,1	26,9
N12	TC	0	0,8	t	3,9
	Con	0	0,3	0	1,8
Mean	TC	0	1,6	0,2	37,0
	Con	0	0,9	t	19,7

Discussion

It is accepted that propagation material produced by the tissue culture process is free from systemic diseases. Accordingly the performance of crops grown from seedcane produced from such material should provide a reliable means of assessing the degree to which the vigour of varieties propagated since release by conventional methods has been maintained, and whether unsuspected diseases are present in seedcane stocks of the varieties. The evidence from these trials indicates that the potential productivity of the three varieties under commercial conditions has not diminished since they were released (more than 40 years ago in the case of NCo310). It also appears that no unsuspected diseases are present in commercial seedcane stocks.

Increased tillering of cane plants produced by tissue culture is a commonly reported phenomenon and this, together with apparently increased vigour, was obvious in the propagation plots that were established with the TC plantlets. It

Table 3

Cane and sucrose yields of tissue culture and conventionally derived plots of NCo310, NCo376 and N12 at La Mercy and Pongola

Variety/Treatment		La Mercy (16,6 mth)				Pongola (15,4 mth)			
		Cane t/ha	Ers %	Ers t/ha	Ers t/ha /ann	Cane t/ha	Ers %	Ers t/ha	Ers t/ha /ann
NCo310	TC	144	11,9	17,0	12,2	212	8,1	17,3	13,4
	Con	162	11,7	18,9	13,7	223	8,7	19,5	15,2
NCo376	TC	129*	11,6**	14,9	10,8	229**	9,4	21,3	16,6
	Con	154	10,2	15,6	11,3	263	8,4	22,1	17,3
N12	TC	136	12,2	16,6	12,0	222	9,4	20,9	16,3
	Con	138	11,7	17,3	12,5	228	9,1	20,9	16,3
LSD (0,05)		19	0,7	2,4	—	19	1,1	3,2	—
(0,01)		27	0,9	3,3	—	26	1,5	4,4	—
Means	TC	136**	11,9**	16,2	11,6	221**	9,0	19,8	15,5
	Con	155	11,2	17,3	12,5	238	8,8	20,8	163
LSD (0,05)		11	0,4	1,4	—	11	0,6	1,9	
(0,01)		15	0,5	1,9	—	15	0,9	2,6	—
Means	NCo310	153	11,8	18,0	13,0	218	8,4	18,4	14,3
	NCo376	142	10,9	15,3	11,0	246	8,9	21,7	16,9
	N12	132	11,9	16,9	12,2	225	9,3	20,9	16,3
LSD (0,05)		16	0,7	1,9	—	26	1,0	3,2	—
(0,01)		23	1,0	2,6	—	38	1,4	4,6	—

Table 5

Percentage differences in various attributes of growth and yield between plant crops grown from conventional and tissue culture-derived seedcane

	% differences between conventional & TC crops							
	La Mercy				Pongola*			
	NCo310	NCo376	N12	Mean	NCo310	NCo376	N12	Mean
Stalk population	+ 4,1	+ 6,5	+0,6	+ 3,6	+ 6,2	+13,9	+4,4	+ 8,4
Stalk mass	−15,3	−20,9	−8,4	−15,1	−10,5	−23,5	−6,3	−13,9
Stalk length	− 3,9	− 9,8	−1,3	− 4,9	+ 2,6	− 5,9	−4,1	− 2,4
Stalk diameter	−12,5	−14,3	−9,1	−12,0	+ 4,3	− 4,5	−9,1	− 3,2
Cane yield	−11,1	−16,2	−8,1	−12,3	− 4,9	−12,9	−2,6	− 7,1
Ers % cane	+ 1,7	+13,7	+4,3	+ 6,3	− 6,9	+11,9	+3,3	+ 2,3
Recoverable sucrose	−10,1	− 4,5	−4,0	− 6,4	−11,3	− 3,6	0	− 4,8

* Stalk population and stalk mass at Pongola based on stalk counts at eight months.

is intriguing that increased tillering persisted in the field trials, which represented the next stage of propagation from the original plantlets, and preliminary records indicate that it is still present in the first ratoon crops of both trials.

In contrast to the situation in the propagation plots, in the field trials increased tillering of the TC-derived crops was associated with poorer growth, i.e. shorter and thinner stalks, at both sites. This was unexpected and its cause is uncertain. It is probably not due to increased susceptibility to diseases (despite greater levels of smut occurring in the first ratoon crops derived from TC material) and it is unlikely to be due to the somewhat thinner stalks of the TC seedcane, which germinated vigorously. However, this result is in agreement with poorer yields obtained from TC-derived seedcane of variety CP44-101 in Australia (Anon[1]).

Differences in respect of the various components of growth and yield between the TC and conventional plots demonstrate consistent effects of the TC process in the three varieties at both trial sites (Table 5). The main exceptions to the general trend were stalk length, stalk diameter and ers % cane of NCo310 at Pongola, but these may have been due to the difficulty of representative sampling in the severely lodged cane of this variety. The consistently superior performance of the conventional seedcane shows that the TC-based method of propagation offers no advantages in respect of productivity over good conventional seedcane.

The greater susceptibility of the TC material of all three varieties to smut in this study is an indication of a possibly serious disadvantage of TC-based propagation. The cause of the increased smut is not clear, nor is it known if increased susceptibility to smut is consistently associated with TC methods of propagation. That increased smut was detected in three varieties of widely differing susceptibility suggests that the problem might not be infrequent. Possible causes of greater susceptibility to smut are differences in morphology and histology of the buds or young tillers of the thinner, TC-derived seedcane, or to physiological effects, for example, changes in the levels of protective biochemicals (Lloyd and Pillay[2]). Further studies should show whether the increased susceptibility is only temporary.

Conclusions

A method of propagating sugarcane based on tissue culture resulted in consistent increases in tillering and in reductions in crop growth and yield of varieties NCo310, NCo376 and N12 in the plant crops grown from seedcane which itself was grown from the original TC-derived plantlets. Seedcane originating from tissue culture offered no advantage over conventional seedcane in terms of productivity and in this study it resulted in substantially greater levels of smut in the three varieties used. The project afforded useful experience in the mass handling and establishment of TC-derived plantlets.

Acknowledgements

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