

SHORT COMMUNICATION

## MICROPROPAGATION OF SUGARCANE VIA NOVACANE®: PRELIMINARY STEPS IN COMMERCIAL APPLICATION

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### Abstract

Conventional seedcane schemes are limited by the availability of disease-free material for planting and also by the rate of propagation from transplants. NovaCane® is a micropropagation process whereby sugarcane plants are multiplied *in vitro*, hardened off, field planted and then propagated vegetatively. This approach can contribute to the production of certified disease-free material at improved multiplication rates. Micropropagation of one apical leaf roll can yield up to 700 plants compared to 10 plants per stalk using the transplant route. The project aim was to assess the implementation of NovaCane® in a seedcane scheme with intended future commercial operation. The first step of the project involved indexing sugarcane for the following diseases: ratoon stunt, leaf scald, sugarcane mosaic and sugarcane yellow leaf virus. Subsequently, certified clean material from N19, N23, N25, N32, N40 and N41 was micropropagated and plantlets sent to the Malelane transplant nursery for hardening off. Micropropagation rates of between 30 and 700 plants per stalk were recorded, with efficiency depending on the genotypic response. Plant survival rates of between 87 and 100% were observed at the hardening off stage. High survival rates demonstrated the successful completion of the first stage of the project. The next phase involves field planting and bulking for seedcane generation and will be evaluated by commercial co-operators.

**Keywords:** sugarcane, seedcane, NovaCane®, tissue culture, micropropagation, disease indexing, hardening off

### Rationale

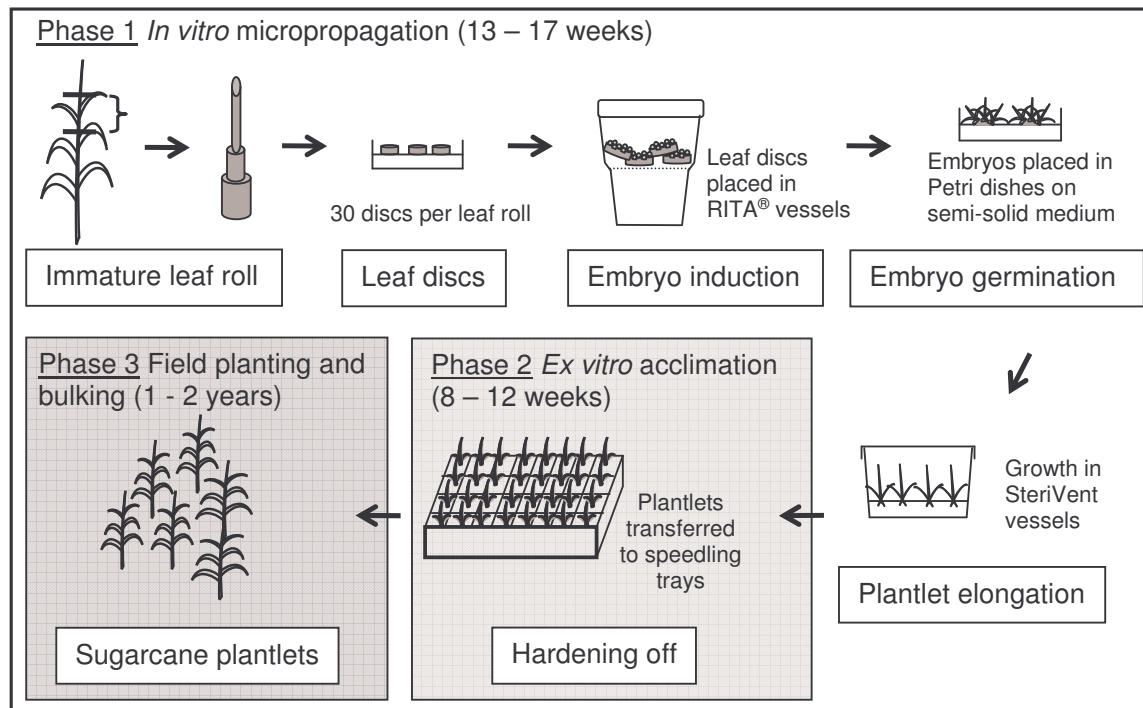
Using the standard protocol for vegetative propagation of sugarcane, the maximum number of propagules (setts) that can be obtained per mature stalk is 10. Large tracts of land and several years are required to bulk up and release a new cultivar to South African growers (Bailey and Bechet, 1989). Tissue culture can increase the propagation potential by 20-35 times (Geijskes *et al.*, 2003; Snyman *et al.*, 2006). In addition, plants can be disease-indexed (Snyman *et al.*, 2007) and healthy material multiplied in half the time compared to the conventional vegetative route.

Research on *in vitro* culture of sugarcane began in the 1960s and several protocols have been documented. These include plantlet regeneration via somatic embryos from callus and immature leaf sections, or directly from apical shoot material (Lee, 1987; Grisham and Bourg, 1989). The South African Sugarcane Research Institute (SASRI) has developed a rapid embryogenic propagation procedure *viz.* NovaCane®. This process involves an *in vitro* phase

of generating plantlets (Phase 1), an *ex vitro* hardening off stage (Phase 2), followed by field planting (Phase 3), as a means of generating large amounts of healthy seedcane (Figure 1). The aim of the study was to evaluate plant production and hardening off for several cultivars.

### Approach

The NovaCane® *in vitro* culture process utilises material from field-grown plants and is summarised in Figure 1. Each stalk was subjected to a disease-indexing procedure for ratoon stunt, leaf scald, sugarcane mosaic and sugarcane yellow leaf virus prior to placement in culture (Snyman *et al.*, 2007). Only certified, pathogen-free material was utilised for culture initiation. The immature leaf roll section from the apical region of cultivars N19, N23, N25, N32, N40 and N41 was cut into 30 transverse sections (each approximately 2 mm thick). Explants were placed on semi-solid MS medium (Murashige and Skoog, 1962; Highveld Biological, South Africa) containing sucrose (20 g/L; commercial grade sugar), casein hydrolysate (0.5 g/L; Sigma), the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D; 3 mg/L; Sigma), and agar-agar (8 g/L; Saarchem, South Africa), pH 5.8, in Petri dishes. Cultures were incubated in the dark at 28°C for two weeks. The leaf discs were quartered and placed in RITA® temporary immersion bioreactors (CIRAD, France) containing agar-free MS medium with 2,4-D (0.6 mg/L), sucrose (20 g/L), casein (0.5 g/L) and an antimicrobial agent, Plant Preservative Mixture (PPM™; 5 ml/L; Plant Cell Technology Inc., USA). Embryo culture proceeded under an immersion interval of 1 min every 12 h and an 8 h dark/16 h light photoperiod. After three weeks, the embryos were placed on semi-solid regeneration medium (MS medium as above, without 2,4-D or PPM™) in Petri dishes (four weeks) for germination to occur. Plant elongation was facilitated on regeneration medium in SteriVent vessels (Duchefa, The Netherlands) for a further four weeks in medium with half-strength MS salts and vitamins, sucrose (5 g/L), casein hydrolysate (0.5 g/L) and agar-agar (8 g/L).



**Figure 1. Diagrammatic representation of micropropagation of sugarcane via the NovaCane® process. The total time required for plant production, ready for field planting, is 21-29 weeks.**

Plantlets were removed from SteriVent vessels and placed in sealed plastic bags containing moist tissue paper, for transport to the Malelane transplant nursery site. Plantlets approximately 10 cm in size with a well-developed root system were hardened off by planting in a potting mix of peat moss:vermiculite (1:1; Grovida) in 98-well polystyrene speedling trays and placed in an enclosed tunnel, covered with 60% shade-cloth. The irrigation regime consisted of 6.5 L water per tray per day, applied for five minutes every three hours via four-way fogger nozzles. Plants were fertilised with 3:1:5 (Wonder) granules (two granules per well every two weeks).

The hardening off efficiency was determined one month after planting on the basis of plantlet survival.

### Results and Discussion

Cultivars placed in culture displayed a range of regeneration rates in this study (Table 1). This genotype variability has been reported in sugarcane previously (Taylor *et al.*, 1992; Fitch and Moore, 1993; Snyman *et al.*, 2006, 2007). Two cultivars that responded favourably to the standard culturing regime were N32 and N41, with 735 and 600 plantlets respectively, being produced per leaf roll placed in to culture (Table 1). Total plant numbers of 5 880 and 13 599 were generated for N32 and N41 respectively.

**Table 1. A comparison of regeneration and hardening off efficiencies for a range of cultivars micropropagated *in vitro*.**

Cultivar	Numbers of plants regenerated <i>in vitro</i> per leaf roll initiated (n=5-22)*	Hardening off efficiency (%)
N19	114	96.5
N23	32	90.1
N25	184	100
N32	735	88
N40	43	87
N41	600	90

\*data collection precluded statistical analysis

Using standard culturing conditions, plants were regenerated from N19 and N25, but cultivars N23 and N40 did not respond well, as indicated by their low regeneration efficiencies (Table 1). Possible reasons for this may be: (i) Seasonal variation – these cultivars were placed in to culture during winter and although conditions are controlled in laboratory growth rooms, the field-derived parent plants may not have been ideal starting material. This may account for the poor response of N40 in this study because, in a previous study, regeneration efficiency for this variety was extremely high (7 000 plants per leaf roll; Meyer *et al.*, 2007), and (ii) genotype variation – previous authors have observed differing responses from cultivars (Taylor *et al.*, 1992; Fitch and Moore, 1993). Cultivar N23 appeared to be unresponsive in culture and produced phenolic compounds that adversely affected embryo production.

Hardening off efficiencies ranged from 87 to 100% at the Malelane transplant nursery (Table 1). Previously, Meyer *et al.* (2007) reported an efficiency range between 35 and 52%, depending on the hardening off site. Hardening off in that study was negatively affected by hyperhydrated plant production, a phenomenon associated with liquid temporary immersion systems.

## Conclusions

Despite cultivar differences in terms of regeneration, all six cultivars were hardened off with high efficiency. The third and final phase of the propagation procedure, to be conducted in the near future, is to assess clonal fidelity and plant performance in the field. The preliminary results obtained from this study indicate that the *in vitro* propagation protocol, NovaCane®, successfully produces an abundant source of pathogen-free plants that can be efficiently hardened off. This has application for enabling rapid release of new cultivars from SASRI.

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