SHORT COMMUNICATION

NOVACANE® AS A TOOL FOR RAPID PROPAGATION OF MATERIAL FOR THE SASRI PLANT BREEDING PROGRAMME

MEYER G M[1], BANASIAK M[1], KEEPING N[1], PILLAY N[1],
PARFITT R[1] AND SNYMAN S J[1,2]

[1]*South African Sugarcane Research Institute, P/Bag X02, Mount Edgecombe, 4300, South Africa*
[2]*School of Biological and Conservation Sciences, University of KwaZulu-Natal, P/Bag X54001, Durban, South Africa*
gwethlyn.meyer@sugar.org.za

Abstract

The South African Sugarcane Research Institute (SASRI) Plant Breeding Programme evaluates new sugarcane germplasm for pest and disease resistance and performance in a range of environmental conditions. The time taken for selection and release of a commercial cultivar is 11-16 years. One of the bottlenecks in the breeding process is the length of time required to produce sufficient material for both evaluation (selection) and final-stage propagation (bulking) as a result of the propagation ratio (eight plants from a single sugarcane stalk). NovaCane® is a technique for the rapid multiplication of sugarcane via *in vitro* culture. In this study, plantlet production after five months varied between 800 and 2,400 per apical meristem depending on the genotype, representing an average propagation ratio of 1:1,600. NovaCane® is currently being used to eliminate Sugarcane Yellow Leaf Virus enabling transfer of high potential genotypes from northern to southern regions of the South African sugar industry. This technique also has the potential to (i) reduce the propagation stages of the plant breeding selection programme, (ii) reduce time-frames for generation of regional information on pre-release genotypes, (iii) eliminate disease and simultaneously multiply imported sugarcane in the quarantine facility and (iv) supply larger volumes of material for either mill area bulking plots or for export to countries with which SASRI has cultivar agreements.

Keywords: sugarcane, propagation, plant breeding, NovaCane®, *in vitro* culture

Introduction

Evaluation of progeny from the SASRI Plant Breeding Programme requires 10-15 years. An initial 250,000 genotypes are taken through five stages of assessment and propagation resulting in the release of one to three cultivars per agroclimatic region of the sugar industry per annum (Anonymous, 2003). Seedlings progress through Single Stool (Stage 1), Single Line (Stage 2), Observation Trial (Stage 3), Primary Variety Trial (VT1) (Stage 4) and Secondary Variety Trial (VT2) (Stage 5) before being multiplied for release (Parfitt, 2005). Each of these evaluation stages occur in five major regions (Irrigated North, Midlands, Hinterland, Coastal high potential and Coastal average potential), and a genotype with potential selected at Stage 5 in one region will be transferred to other regions for evaluation (Anonymous, 2003).

There are a number of bottlenecks in the current selection programme. These include a constraint to transfer outstanding genotypes selected in the north to the southern regions. This restriction has been instituted to reduce transfer of Sugarcane Yellow Leaf Virus (ScYLV) between regions. Secondly, genotypes can be in the field for up to 16 years before entering the final propagation stage. One of the reasons why this process is lengthy can be attributed to the low profileration rates of conventional vegetative propagation, where one stalk produces only eight plants. Furthermore, large tracts of land and concomitant labour are required to accommodate the entire evaluation process (Bailey and Bechet, 1989).

NovaCane®, a tissue culture propagation technique (Snyman *et al*., 2008), is able to remove disease-causing agents from sugarcane when apical meristems of less than 2 mm are dissected out and used to initiate cultures (Fitch *et al.,* 2001; Ramgareeb *et al*., 2010). In addition, NovaCane® has been able to increase the multiplication rate for commercial sugarcane varieties, obtaining an average of 1,150 plants from one apical meristem after 11 weeks in culture (Ramgareeb *et al*., 2010). Consequently, this technique has the potential to propagate disease-free genotypes for use in the plant breeding programme.

Aims

The first aim of the study was to enable transfer of pre-release germplasm produced in the irrigated north to southern regions for further evaluation (Stage 5 of the Plant Breeding programme). A second aim was to identify other areas where NovaCane® could impact on the plant breeding programme.

NovaCane® facilitates north-south germplasm transfer

Tops of mature field-grown stalks of six pre-release genotypes (01F0085, 01F0152, 01F0797, 01F1920, 01F2143 and 86Z0104) selected in the northern irrigated region (Pongola Research Station) were collected and cultured using NovaCane®. Meristems of 2 mm or less were excised, shoots multiplied via direct organogenesis and rooted as described by Ramgareeb *et al*. (2010). Plants with well formed roots were transferred to seedling trays containing 1:1 vermiculite:peat moss and acclimated in a glasshouse as previously reported (Meyer *et al*., 2009). These plants have been planted in propagation plots at the Empangeni Research Station and will be included in Stage 5 trials (VT2) of the southern coastal programmes in 2011.

For each of the genotypes tested, an average of 800 to 2,400 plants per meristem were obtained in 20 weeks (Figure 1). Overall, this translates to a propagation ratio of one stalk producing 1,600 plants, similar to that obtained using NovaCane® on commercial cultivar NCo376 (Ramgareeb *et al*., 2010). Such results indicate the versatility of *in vitro* propagation and represent a significant propagation improvement on eight plants per stalk obtained conventionally.

The ability of NovaCane® to remove disease and propagate simultaneously is already impacting the plant breeding programme by enabling the transfer of high potential genotypes from northern to southern regions. Although taking these genotypes through the NovaCane® process increases

the time before they are tested in the southern region, without NovaCane® this transfer would not be possible.

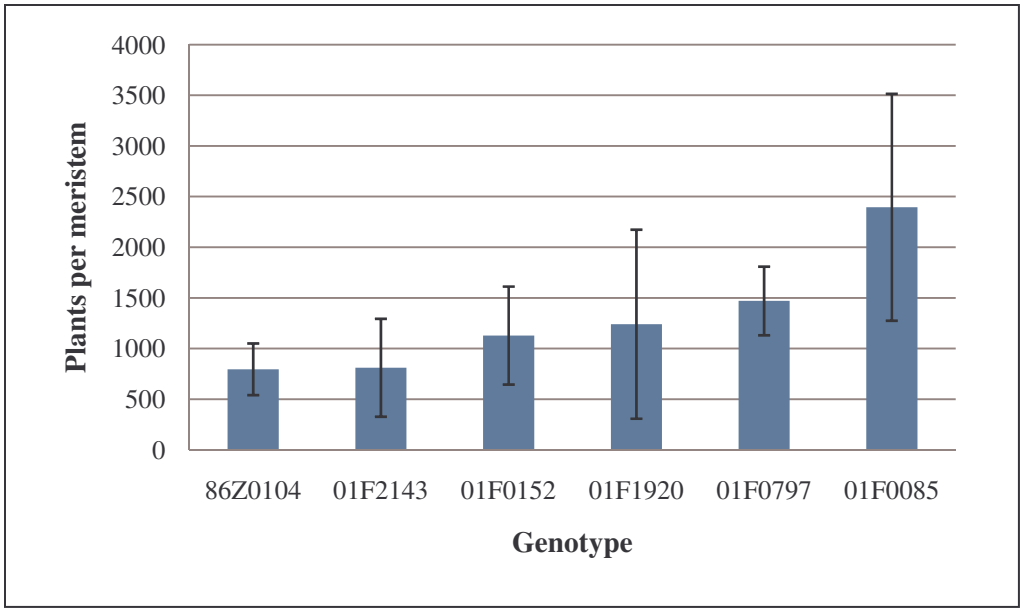

Figure 1. Mean number of plants produced per meristem for six pre-release plant breeding genotypes after five months in culture (n=3-4; ±SE).

Additional opportunities for NovaCane® application in the plant breeding programme

In the final evaluation stage of the breeding programme (Stage 5, VT2), approximately 100 outstanding genotypes from VT1 (Stage 4) are tested at six SASRI research stations as well as on co-operator farms. Plant crop assessment results in the best four to six genotypes from each region being selected and propagated in PP2 (Propagation Plot 2) (Figure 2). Subsequent ratoon crop data is used to select the most promising genotype from PP2 for further propagation in PP3 (Propagation Plot 3), before being distributed and propagated in the mill area bulking plots prior to release (Anonymous, 2003). NovaCane® is able to produce large numbers of plants from a single meristem in short periods of time. This technique could enable selection of VT2 genotypes without concurrent planting of PP2 or PP3. When the most promising genotype has been selected based on a statistical combination of plant and ratoon data, NovaCane® could be used to propagate sufficient material of the pre-release genotype to plant the mill area bulking plots in each region.

The best genotypes selected for PP2 are transferred to other regions for assessment. However, before they can be included in trials, sufficient material needs to be generated. At present, this takes between one and two years, depending on the region. NovaCane® has the potential to propagate these genotypes allowing them to bypass conventional propagation plots and be immediately included in trials. Consequently, much needed regional information on genotype performance will be available more rapidly than is currently possible.

Certain N cultivars developed in South Africa are distributed to African countries with whom SASRI has cultivar agreements (e.g. Zambia, Mali and Tanzania). As some of these countries receive only 10 x 3-budded setts per cultivar, land, labour and a substantial amount of time is required to propagate received cultivars prior to release to that country's industry. Because NovaCane® has a high propagation ratio, another potential use for this technique would be rapid introduction of cultivars for testing in trials and seedcane schemes in other countries.

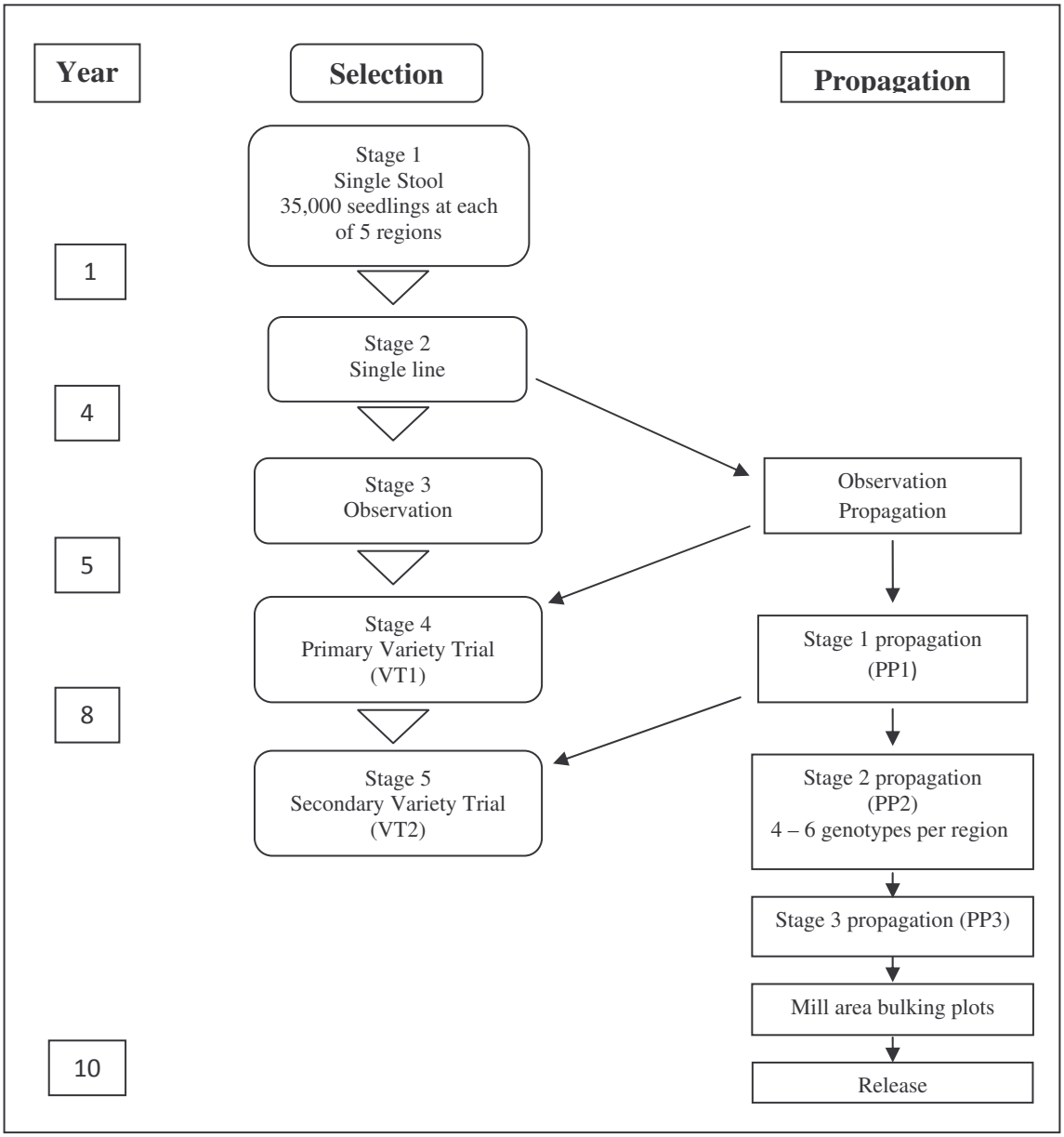

Figure 2. Abridged schematic diagramme of the plant breeding field selection programme at the Empangeni Research Station.

Another function of the plant breeding programme is to import cultivars from other countries to test their commercial application. A single pot of each cultivar is planted and monitored in the SASRI quarantine facility for two years. Those that are disease free are planted in open quarantine for a year before sufficient material is available to enter the Plant Breeding selection

programme at Stage 2. Should the plant breeders have confidence in the performance of an imported cultivar, it would be beneficial to have larger numbers to allow the new cultivar to enter the selection programme later on at Stage 4 or 5. This is possible with NovaCane® as large plant numbers can be generated within a five month period.

NovaCane® eliminates disease-causing organisms from propagated sugarcane material. Currently genotypes imported from other countries as parents for the Plant Breeding crossing programme are quarantined for two years before being used. If propagated using NovaCane® this material could be ready for use a year earlier.

Summary

NovaCane®, a rapid *in vitro* propagation technique, is being using to transfer genotypes with potential from the northern irrigated to the southern regions of the South African sugarcane industry. This is possible as meristem cultures of 2 mm or less have been shown to be free of disease-causing agents.

It is envisaged that rapid multiplication of disease-free sugarcane using NovaCane® can impact on the plant breeding programme in a number of ways. These would include: enabling regional information on cultivar performance to be more readily available; allowing selection of the best genotypes without requiring propagation stages PP2 or PP3; increasing the amount of material given to countries with which SASRI has cultivar transfer agreements; ensuring disease removal from imported genotypes thereby reducing quarantine times; and incorporating imported cultivars for screening in the selection programme at a later stage than is currently possible. Concomitantly, land and labour requirements for the programme may be reduced.

REFERENCES

Anon (2003). *Plant Breeding, Crossing and Selection Programmes*. Published by the South African Sugarcane Research Institute, Mount Edgecombe, South Africa.

Bailey RA and Bechet GR (1989). A comparison of seedcane derived from tissue culture with conventional seedcane. *Proc S Afr Sug Technol Assoc* 63: 125-129.

Fitch MMM, Lehrer AT, Komor E and Moore PH (2001). Elimination of sugarcane yellow leaf virus from infected sugarcane plants by meristem tip culture visualized by tissue blot immunoassay. *Plant Pathol* 50: 676-680.

Meyer GM, Banasiak M, Ntoyi TT, Nicholson TL and Snyman SJ (2009). Sugarcane plants from temporary immersion culture: Acclimating for commercial production. *Acta Hort* 812: 323-327.

Parfitt R (2005). Release of sugarcane varieties in South Africa. *Proc S Afr Sug Technol Assoc* 79: 63-71.

Ramgareeb S, Snyman SJ, van Antwerpen T and Rutherford RS (2010). Elimination of virus and rapid propagation of disease-free sugarcane (*Saccharum* spp. cultivar NCo376) using apical meristem culture. *Plant Cell Tiss Organ Cult* 100: 175-181.

Snyman SJ, Meyer GM, Banasiak M, Nicholson TL, van Antwerpen T, Naidoo P and Erasmus JD (2008). Micropropagation of sugarcane via NovaCane®: Preliminary steps in commercial application. *Proc S Afr Sug Technol Assoc* 81: 513-516.