BUREAU OF SUGAR EXPERIMENT STATIONS

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FINAL REPORT - SRDC PROJECT BSS209

MERISTEM TRANSFORMATION FOR SUGARCANE GENETIC ENGINEERING

by

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ABSTRACT

During initial investigations to re-establish the technology to transform and regenerate plants from sugarcane meristems, a new method of sugarcane tissue culture was invented. This method holds such potential that this project was concluded early so the full potential of the new system could be investigated. Due to the early completion of this project, this final report is brief and describes the start of the meristem transformation project and the discovery of the new tissue culture system.

SUMMARY

Meristems offer considerable advantages over callus for sugarcane transformation. These advantages include speed and a significantly lower potential for somaclonal variation. The major current disadvantage of a meristem transformation system, the production of chimaeric plants, was to be addressed in this project.

As part of the initial effort to re-establish meristem cultures to pursue this aim, an alternative tissue culture method was also investigated. This method showed considerable potential to retain the two major advantages of the meristem-based system. Further, this method also has considerable potential to realise a high multiplicative value (number of propagules produced in tissue culture per unit input tissue) and to limit chimaerism due to the speed and origin of the regenerated plants.

On the basis of these initial results it was proposed to terminate the current project after one year (instead of the original four) to concentrate on this new method of tissue culture. The research described in this report therefore effectively represents six months of a four year proposal, due to the shifting of resource to the new method and the mutually agreed early termination of BSS209 in favour of the new project.

1.0 BACKGROUND

Sugarcane genetic engineering is limited by a number of factors including somaclonal variation. Somaclonal variation is a major concern for sugarcane genetic engineering, as the transgenes are introduced into clones with agronomically elite characteristics, and the transformants themselves (the T0 generation) will be grown without further manipulation (eg backcrossing). The current callus-based tissue culture system appears to generate unacceptably high numbers of somaclonal variants, effectively negating the cultivar's existing elite characteristics, and minimising any benefits derived from the introduction of the transgene. Most of the lines of transgenic sugarcane in the field in Bundaberg and Gordonvale show somaclonal variation to some extent. All the lines have yields lower than the original parent clones. These clones, the result of a callus-based transformation system are a major achievement in the application of genetic engineering to sugarcane improvement, but are a technical failure because of the variation present in the transformed lines.

Previous research at BSES demonstrated that sugarcane meristems were suitable for transformation, but the system needed further development before practical application (Gambley *et al.* 1993). Unfortunately, this methodology was 'shelved' when the callus-based system became available. Meristematic regions were originally chosen for transformation because of reports of low somaclonal variation in plants derived from meristems compared to those derived from callus (eg Irvine *et al.* 1991). Recently, new techniques to address the technical problem of selection of transformed tissues within the meristematic region have been reported (Lowe *et al.* 1995). By combining previous experience in sugarcane meristem transformation with the new techniques for selection, we proposed to develop and deliver a working sugarcane meristem transformation system.

Somaclonal variation has long been noted in many tissue cultured plant species. The current sugarcane transformation system uses the synthetic plant growth regulator 2,4-D to induce and maintain embryogenic callus and requires a substantial amount of time in culture (six to seven months) before plantlets can be selected and regenerated. Both 2,4-D and the length of time spent in culture are considered to contribute to somaclonal variation. The meristem method has the considerable advantages that material only spends two months in tissue culture and 2,4-D is not required. However, the major problem with this method, which was not overcome at the time, was how to apply selection pressure to select transformed from non-transformed cells. Recent reports indicate that transformed sectors of maize embryos can be successfully excised and recovered on cytokinin-supplemented media (Lowe *et al.* 1995) Cytokinin-supplemented media enhances gene expression and regeneration of transformed sugarcane meristems (Gambley *et al.* 1994). With the recent introduction of the green fluorescent protein (GFP) visual selection gene to plant biotechnology research, excision and recovery of transformed sectors from sugarcane meristems and axillary buds is now a practical possibility.

Thus, there is considerable expectation that transgenic sugarcane plants regenerated from transformed meristems or buds would show minimal variation for three reasons, namely:

- meristem rather than callus material for transformation,
- no 2,4-D in the media, and
- a shorter time in the tissue culture system.

Recent developments in both plant molecular biology and work on the transformation of meristematic tissues in other crops have provided new tools and methods to readdress the problems in the sugarcane meristem system. The need for an alternative transformation system for sugarcane has been highlighted by the somaclonal variation evident in the transgenic lines in the field in Meringa and Bundaberg. Many of the lines have obvious phenotypic differences from the parental clone, and all transgenic lines had yields lower than the original parental clone. While these clones represent a significant achievement for sugarcane biotechnology research, the somaclonal variation present means that these plants are a technical failure in terms of providing a method to introduce genes into agronomically elite germplasm with minimal disruption to the existing superior phenotype. Whilst efforts are underway to address this problem, the central role of 2,4-D in this tissue culture/transformation system will make progress difficult.

2.0 OBJECTIVES

The aim of this proposal was to perfect a sugarcane meristem-based transformation system for application in sugarcane genetic engineering. This aim was to be achieved by addressing the following objectives:

- Establish meristem/axillary bud cultures using protocols previously developed.

- Adapt the existing micro-projectile bombardment protocol for the GFP marker system.

- In conjunction with CSIRO Project CC027 develop an Agrobacterium-mediated transformation system for meristems.

- Develop a strategy for the selection and regeneration of non-chimaeric transformants.

- Determine the extent of somaclonal variation induced by the meristem transformation system.

3.0 OUTCOMES

- Axillary bud and apical meristem cultures of Q117 and Q124 cultivars were established. Callus cultures of the same cultivars were also established.

- As isolation of intact shoot meristem in large numbers is labour-intensive, experiments to rapidly propagate large numbers of sugarcane shoots and plantlets were initiated.

- This new tissue culture has significant advantages over the proposed meristem-based system. Initial studies indicated:

- A regeneration system at least as fast, or faster than the meristem system.
- A low potential for somaclonal variation due to the speed of regeneration and the absence of 2,4-D in the media.
- A high multiplicate value (number of propagules produced per unit input tissue).
- Probable embryogenetic origin of the regenerated plants should prevent chimaerism.
- A provisional patent in Australia, describing the new system and initial transformation studies.

4.0 METHODOLOGY

4.1 Establish meristem/axillary bud cultures

The basic protocols for establishing cultures of sugarcane meristematic cultures were developed at BSES previously (Gambley *et al.* 1993, 1994) and were to be used to establish cultures of a range of cultivars including Q124 and Q117. Establishment involves aseptically removing axillary bud cultures and initiating growth on media supplemented with plant growth regulators such as kinetin and BAP. Initial conditions for buds of Q117 had already been established: concentrations and conditions for culture of buds from other cultivars may need to be investigated and optimised.

4.2 New tissue culture system

The basis of the new system is described in provisional patent specification ‘Plant Transformation’, application number PQ1531, 9 July 1999. Details of this method are contained in the provisional patent application. SRDC has a copy of this provisional application.

5.0 RESULTS AND DISCUSSION

The key result generated by BSS209 is described in the above provisional patent specification. This new method has considerable potential for sugarcane tissue culture and transformation. The system has only been tested on two varieties, namely Q117 and Q124. Both of these varieties responded to the system. This system has considerable potential for both mass propagation of new varieties, as well as the basis of a transformation system.

6.0 IMPLICATIONS AND RECOMMENDATIONS

The major recommendation arising from this project has already been accepted and enacted. This project has been terminated after one year, and the resources and funds of BSS209 and other projects diverted to the new program of work, detailed in SRDC project proposal BSS242. This new project proposal has the aim of developing this new sugarcane tissue culture system for application in both mass propagation and sugarcane genetic engineering. The system may be adaptable to the tissue culture and transformation of other monocot species: this option will also be investigated in this proposal. Full details can be found in the commercial-in-confidence project proposal BSS242.

7.0 PUBLICATIONS

Prakash Lakshmanan, Adrian Elliott, Christopher Grof and Grant Smith. (1999). Plant transformation. Provisional patent application in Australia. No. PQ1531. Fisher Adams Kelly, Brisbane. 22 pp and 1 figure.

8.0 REFERENCES

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