

**BUREAU OF SUGAR EXPERIMENT STATIONS  
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**FINAL REPORT  
SRDC PROJECT BS59S  
ESTABLISHMENT OF AN *IN VITRO* GERMPLASM  
COLLECTION OF BASIC SUGARCANE  
SPECIES AND RELATED GENERA**

**by**

**P W J Taylor**

**SD95006**

**Principal Investigator:**

**Dr P W J Taylor  
Research Officer  
BSES  
PO Box 86  
Indooroopilly Q 4068  
Phone (07) 371 6100**

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## 1.0 SUMMARY

An *in vitro* germplasm collection was established for basic species of sugarcane and the related genus *Erianthus*. *In vitro* plants were established from apical buds and/or embryogenic callus for *Saccharum officinarum* (19 cultivars out of 20 tested), *S. robustum* (5/5), *S. sinense* (1/1), *S. barberi* (2/2) and *E. arundinaceus* (9/10). Excised meristematic tissue was treated with bleach (1%) for 10 min, washed in sterile distilled water, then treated with a combination of rifampicin (3.125 µg/mL) and tetracycline (3.125 µg/mL) antibiotics for 5 min prior to culturing to eradicate contaminating saprophytic microorganisms. Plantlets of the *Saccharum* species and *E. arundinaceus* were transferred to storage medium and stored at 18°C. Establishing *in vitro* plantlets for *Erianthus* clones was difficult from both callus and apical buds. Improvement in shoot production occurred when apical buds were incubated under continuous high light intensity (130 µE/m<sup>2</sup>/s<sup>-1</sup>). This is the first report of the establishment of *in vitro* plants of *Saccharum robustum* and *Erianthus arundinaceus*. Random amplified polymorphic DNA (RAPD) analysis of plants of *S. officinarum*, *S. robustum* and *E. arundinaceus* clones, regenerated from callus and stored *in vitro* at 18°C for 3 and 6 months, revealed no polymorphisms between treatments. These results indicated that *in vitro* culturing and storage of up to six months have no effect on the genetic stability of these plants.

## 2.0 OBJECTIVES

- Adapt the *Saccharum* spp. hybrid *in vitro* meristem culture technique to species of *Saccharum* and *Erianthus arundinaceus*.
- Assess the genetic stability of these species under *in vitro* storage conditions.

## 3.0 BACKGROUND

Commercial sugarcane (*Saccharum* spp. hybrid) consists of six species, *S. officinarum*, *S. robustum*, *S. sinense*, *S. barberi*, *S. spontaneum* and *S. edule*. Species of *Erianthus* and *Miscanthus* are part of a closely related interbreeding group which formed part of the evolution of sugarcane (Daniels and Roach, 1987). Sugarcane cultivars contain disease resistance and high sugar yielding genes originating from these wild relative species and related genera of sugarcane.

A low-maintenance *in vitro* germplasm collection provides a cost-effective alternative to growing plants under field conditions. An *in vitro* collection provides security from extremes of environmental conditions, and pests and diseases; and also provides a method for conservation of species that are difficult to maintain under cultivated conditions. *In vitro* culture may also eradicate pathogens from imported germplasm. *In vitro* storage of sugarcane plants has been developed at the Sugarcane Breeding Institute, Coimbatore, India (Sreenivasan and Sreenivasan, 1985) and at the French international aid organisation CIRAD in Montpellier, France (Paulet *et al.*, 1991). At the Bureau of Sugar Experiment Stations in Brisbane, Australia, an *in vitro* germplasm collection was established for over 200 *Saccharum* spp. hybrid clones (Taylor and Dukic, 1993). However, there are very

few reports of *in vitro* collections of *Saccharum* species and the related genus *Erianthus*. Sreenivasan and Sreenivasan (1985) reported the establishment of *in vitro* plants of *S. officinarum*, *S. sinense* and *S. barberi* but they failed to note which cultivars of these were cultured and established *in vitro*.

The preservation of genetic integrity of each species can be evaluated using field trials to compare morphological and physiological characters of plants originating from *in vitro* and field grown material. Random amplified polymorphic DNA (RAPD) analysis has been developed for use with *Saccharum* spp. hybrids (Taylor *et al.*, 1995) to evaluate the effects of *in vitro* culture on plant genetic stability. If permanent changes in DNA profiles occur during *in vitro* culture and storage, then the frequency of such changes may be used to monitor and optimise culture and storage conditions. This paper describes the establishment of *in vitro* plants for cultivars of various *Saccharum* species and *E. arundinaceus*, and the assessment of the genetic integrity of these *in vitro* plants using DNA analysis.

#### 4.0 MATERIALS AND METHODS

*In vitro* plants were established from embryogenic callus and/or apical meristems as described by Taylor *et al.* (1992) and Taylor and Dukic (1993) for 20 cultivars of *S. officinarum*, five cultivars of *S. robustum*, one cultivar of *S. sinense*, two cultivars of *S. barberi* and 10 cultivars of *E. arundinaceus*.

##### 4.1 Establishment of embryogenic callus cultures and plant regeneration

Callus was initiated from immature leaf explants cultured on MS medium and embryogenic callus was selectively subcultured as described by Taylor *et al.* (1992). After two to four months of culture, embryogenic callus was transferred to solid MS medium without plant growth regulators and incubated at 28°C under continuous light at 40  $\mu\text{E}/\text{m}^2/\text{s}^{-1}$ . Regenerated plantlets were transferred to rooting medium (MS with 60 g/L sucrose and 1 mg/L NAA). *In vitro* plants were then transferred to storage medium and maintained at 18°C as described by Taylor and Dukic (1993).

##### 4.2 Establishment of *in vitro* plants from apical meristems

Apical meristems surrounded by two to three whorls of developing leaves were aseptically excised from stalks of six- to eight-month-old field grown plants and cultured on MS medium as described by Taylor and Dukic (1993). The apical meristems were cultured at 28°C under continuous light at 40  $\mu\text{E}/\text{m}^2/\text{s}^{-1}$ . Regenerated plantlets were transferred to rooting medium and *in vitro* plants were then transferred to storage medium and maintained at 18°C.

### 4.3 Decontamination of shoot apices

A large number of shoot apices died due to contaminating saprophytic microorganisms. The bacterium that caused the most frequent contamination, based on colony morphology, was isolated onto SPYA medium and identified as a *Bacillus* species. The efficacy of chemotherapy treatments, involving antibiotics and disinfectant, and thermootherapy treatments was assessed to eradicate the *Bacillus* sp. from shoot apices.

#### 4.3.1 Antibiotic bioassay

A sample (200  $\mu$ L) of an overnight SPY broth culture of *Bacillus* sp. was evenly spread onto SPYA plates. Sterile glass fibre filters (Millipore) were dipped into various concentrations of ampicillin, streptomycin, tetracycline and rifampicin antibiotics and placed onto the inoculated plates. After incubation overnight at 28°C the diameter of the zone of inhibition around the filters was measured.

Rifampicin and tetracycline, combined at the concentration of 3.125  $\mu$ g/mL, were assessed for efficacy to inhibit growth of *Bacillus* sp. in liquid tissue culture medium (MS with BAP and kinetin). Liquid tissue culture medium (50 mL), with and without antibiotics, was placed in 250 mL flasks and inoculated with 1 mL of an overnight culture of *Bacillus* sp. Cultures were incubated at 28°C at 170 rpm and growth measured using a spectrophotometer at 550 nm.

The efficacy of rifampicin and tetracycline to decontaminate *in vitro* plantlets contaminated with *Bacillus* sp. was assessed. Contaminated plants growing in MS BAP/kinetin medium were treated with rifampicin and tetracycline 3.125  $\mu$ g/mL by either growing in medium supplemented with the antibiotics for several subcultures or by being incubated in liquid medium and antibiotics for up to 24 hours before subculturing into solid culture medium.

#### 4.3.2 Disinfectant

The efficacy of commercial bleach (sodium hypochlorite, 4% active ingredient) to inhibit growth of *Bacillus* sp. was assessed. Commercial bleach was diluted with water to either 1% or 2%, 20 mL aliquots transferred to 100 mL flasks and inoculated with 1 mL of an overnight culture of *Bacillus* sp. After the cultures were incubated at 28°C for 10 or 15 min, samples (200  $\mu$ L) were evenly spread onto SPYA plates and colony growth assessed.

Apical meristems surrounded by two to three whorls of developing leaves were dissected from field-grown plants (five replicates), treated in 1% bleach with Tween 20 (0.1%) for 10 min, washed twice in sterile distilled water for 3 min per wash, and then treated with rifampicin and tetracycline combined (3.125  $\mu$ g/mL) for 5 min. The meristems were cut longitudinally in two, making sure that each half contained buds. One half was cultured on SPYA plates, incubated at 28°C and assessed for bacterial growth, while the other half was cultured on MS BAP/kinetin medium, incubated at 28°C and assessed for bud

germination. Five meristems were also dissected and cultured on SPYA and MS BAP/kinetin media without disinfecting.

### 4.3.3 Thermotherapy

The effect of temperature on growth of *Bacillus* sp. was assessed by adding 1 mL samples of an overnight culture of *Bacillus* sp. to 9 mL SPY medium and incubating in a water bath at 50, 52 or 55°C for 5 or 10 min. After treatment, the cultures were incubated at 28°C at 200 rpm and growth measured at 2, 4, 6 and 18 hours. For growth measurements, 300 µL samples were diluted with 2.7 mL distilled water, transferred to disposable cuvettes and optical density measured using a spectrophotometer at 550 nm. Three replicates were set up for each treatment. Further experiments assessed the efficacy of 55°C for 20 min and 65°C for 5 min to inhibit bacterial growth.

The effect of temperature on growth of apical meristems was assessed in two experiments by dissecting apical meristems surrounded by two to three whorls of developing leaves from field-grown sugarcane plants and placing these into 10 mL sterile distilled water. In the first experiment, the bottles containing the meristems (two replicates per treatment) were transferred to a water bath at 50°C or 60°C for 10, 20, 30 or 60 min. After treatment, the degree of necrosis of the meristems was noted and each meristem was then incubated on SPYA at 28°C and assessed for bacterial growth. In the second experiment the temperature was 55°C for 10 min (three replicates). The meristems were then cut longitudinally in two, one half was cultured on SPYA, incubated at 28°C and assessed for bacterial growth, while the other half was cultured on MS BAP/kinetin medium, incubated at 28°C and assessed for bud germination. Three meristems were also dissected and cultured on SPYA and MS BAP/kinetin media without temperature treatment.

### 4.4 *In vitro* culture of *Erianthus arundinaceus*

Leaf explants for establishing embryogenic callus and shoot apices of each cultivar of *E. arundinaceus* tested became necrotic and died after culture.

To enhance embryogenic callus development various vitamin regimes and synthetic auxins were tested. Leaf explants of *E. arundinaceus* cultivar IK48 were cultured on MS medium containing either MS, N6 or Gamborg's vitamins (Table 1) at both full and half strength concentrations. Leaf explants of IJ407, IK22 and IJ370 were cultured on MS medium containing the synthetic auxins 2,4-D or picloram at 3 and 7 mg/L. After five to six weeks incubation in the dark at 28°C the explants were rated for degree of browning and callus formation.

Apical meristems of *E. arundinaceus* cultivars IJ384, IJ389 and IK48 were dissected, treated with 1% bleach for 10 min, followed by washing in sterile distilled water, then treated with a mixture of tetracycline and rifampicin antibiotics (3.125 mg/L) for 5 min prior to culturing on either 10 mL of solid MS BAP/kinetin medium or 5 mL of liquid MS BAP/kinetin medium containing filter paper supports (Sorbarod, Jepson, Bolton and Co. Ltd, London) with or without 1% PVP in plastic bottles (2.5 cm diameter, 8 cm in

length) sealed with a screw-capped lid. The liquid media were replaced every week with fresh media. In a further experiment, apical meristems of IJ384 and IK48 were decontaminated and cultured in liquid MS BAP/kinetin medium with or without ascorbic acid (0.1 or 1.0 mM). Cultures were incubated under diffuse light at 28°C.

**Table 1**  
**Vitamin treatments added to MS micro and macro salts for culture of *E. arundinaceus* leaf explants**

	Vitamins mg/L		
	MS	N6	Gamborg
myo-Inositol	100.0	100.0	100.0
Thiamine HCl	0.1	0.5	10.0
Nicotinic acid	0.5	5.0	1.0
Pyridoxine HCl	0.5	0.5	1.0
Biotin		0.05	
Folic acid		0.5	

The effect of light intensity on bud germination and shoot growth was assessed by culturing apical meristems of *E. arundinaceus* cultivars IJ394 and IJ407 on solid MS BAP/kinetin medium and incubating under light intensities of 40, 90, and 130  $\mu\text{E}/\text{m}^2/\text{s}^{-1}$  (30 w fluorescent tubes, GEC, England). Shoot germination and multiplication from meristems were recorded.

#### 4.5 Assessment of genetic stability of *in vitro* plants using RAPD analysis

Four plants of selected cultivars of *S. officinarum* (Ashy Mauritius, E257, HQ409, IJ328, IJ469, IM248, IS226, MQ28-674), *S. robustum* (IJ417, NG57-208) and *E. arundinaceus* (IJ394), regenerated from embryogenic callus and stored *in vitro* for three and six months, were established in pots in the glasshouse. Sets of field-grown plants of the same cultivars were also planted in pots.

##### 4.5.1 DNA isolation

DNA was extracted from the first, fully expanded leaf of each plant using the modified CTAB method of Graham *et al.* (1994). Genomic DNA was quantified spectrophotometrically (Sambrook *et al.*, 1989).

#### 4.5.2 Polymerase chain reaction

Seven arbitrary 10-mer oligonucleotide sequences, OPB-11, OPC-14, OPC-15, OPD-01, OPM-01, OPM-05 and OPM-06 (Operon Technologies, USA), were used to amplify plant genomic DNA fragments. These primers have been used to amplify sugarcane hybrid genomic DNA (Taylor *et al.*, 1995). Each 25  $\mu$ L reaction volume contained 30 ng DNA template, 0.8 U *Taq* DNA polymerase (Boehringer Mannheim Biochemica), 0.24 mM each of dATP, dGTP, dTTP and dCTP (Promega Corporation), 0.2  $\mu$ M primer and PCR buffer with a final concentration of 10.0 mM Tris-HCl, pH 8.3, 50.0 mM KCl, 3.0 mM MgCl<sub>2</sub>, 0.1 mg/mL gelatin.

Amplification was performed in a Perkin Elmer 9600 GeneAmp PCR System and was initiated by a denaturation of 1 min at 94°C, followed by 33 cycles of 10 sec each at 94°C, 30 sec at 40°C and 1 min at 72°C. The amplification was completed with one cycle of 5 min at 72°C. Reaction products were resolved by electrophoresis on 1.5% (w/v) agarose gels, stained with ethidium bromide and revealed by UV illumination. PCR reactions were repeated to establish reproducibility of results.

### 5.0 RESULTS AND DISCUSSION

*In vitro* plants were established from apical buds and/or callus and then transferred to storage medium at 18°C for *Saccharum officinarum* (19 cultivars out of 20 tested), *S. robustum* (5/5), *S. sinense* (1/1), *S. barberi* (2/2) and *E. arundinaceus* (9/10) (Table 2). This is the first report of the establishment of *in vitro* plants of *Saccharum robustum* and *Erianthus arundinaceus*. Sreenivasan and Sreenivasan (1985) reported the establishment of *in vitro* plants of *S. officinarum*, *S. sinense* and *S. barberi* but they failed to note which cultivars of these were cultured and established *in vitro*.

In this study, *in vitro* plants of *S. barberi* cultivars could only be established from callus cultures (Table 2). The main function of a germplasm collection consisting of species and genera is to conserve genes rather than genotypes. Therefore, the induction of somaclonal variation in specific cultivars, that may result from prolonged callus culture (Scowcroft, 1984), is considered less important than conserving the genes that will be used in breeding programs to improve the agronomic base of new cultivars.



Table 2

Establishment of *in vitro* plants of *Saccharum* species and *E. arundinaceus* from callus and apical buds

Clone	Callus	Apical buds	Clone	Callus	Apical buds
<i>S. officinarum</i>			<i>S. robustum</i>		
Ashy Mauritius	+	+	IJ416	+	+
Black cheribon	+	+	IJ417	+	+
E257	+	+	IM229	+	+
HQ409	+	+	IS184	-	+
IJ317	+	+	NG57-208	+	+
IJ328	+	+	<i>S. barberi</i>		
IJ462	+	+	Agoule	+	-
IJ465	+	+	Mungo	+	-
IJ469	+	+	<i>S. sinense</i>		
IJ507	+	+	Manga sic	+	+
IM248	+	+	<i>E. arundinaceus</i>		
IM257	+	+	IJ365	-	+
IS146	+	+	IJ370	-	+
IS226	+	+	IJ384	-	-
Malabar	+	+	IJ389	-	+
MQ28-674	+	+	IJ394	+	+
Mol4943	-	-	IJ407	-	+
NG57-30	+	+	IK22	-	+
NG57-54	+	+	IK24	-	+
NG57-221	+	+	IK48	-	+
			IK103	+	+

### 5.1 Decontamination of shoot apices

Establishing *in vitro* plantlets for *Saccharum* clones from apical meristems was difficult due to contaminating saprophytic microorganisms that killed the tissue during culture. The reasons for these clones having high levels of contamination are unknown but may have been associated with the morphology of the surrounding leaf axils, allowing buds and leaf explants to be colonised by microorganisms. In contrast, the level of contamination in cultured leaf explants for establishing embryogenic callus was very low. Leifert *et al.* (1991) reported that losses due to bacterial contaminants of the initial explant cultured *in vitro* can be extremely high.

Various antibiotics were screened for efficacy to control contaminating bacteria (Table 3). A combination of rifampicin and tetracycline was effective against several bacteria, including a *Bacillus* sp. commonly isolated from contaminated tissue, and was not toxic to the plant tissue. Rifampicin and tetracycline combined, each at a concentration of

3.125  $\mu\text{g/mL}$ , inhibited *Bacillus* sp. growth in liquid tissue culture medium. This result indicated that the antibiotics were efficient against *Bacillus* sp. under nutrient conditions and pH optimum for plant tissue culture. Commercial bleach containing sodium hypochlorite diluted to 1% or 2% for 10 or 15 min also killed the *Bacillus* sp. and was not toxic to plant tissue.

**Table 3**  
Efficacy of antibiotics to inhibit growth of *Bacillus* sp.

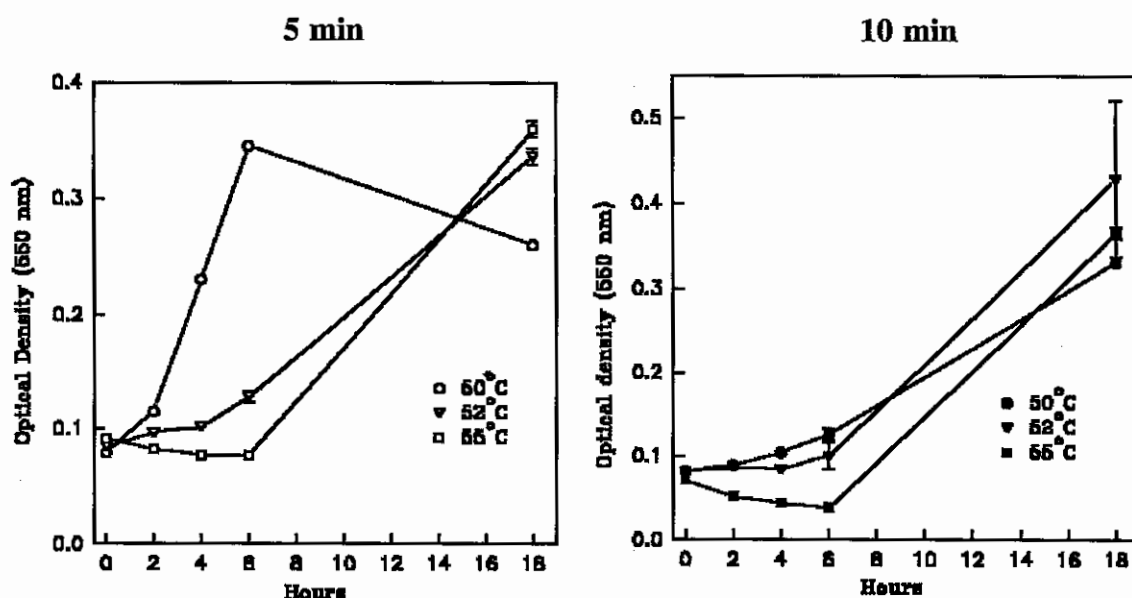
Antibiotic	Concentration (mg/L)	Zone of growth inhibition (mm)
Ampicillin	100	nil
Ampicillin	200	nil
Streptomycin	100	nil
Tetracycline	50	5.4
Tetracycline	25	4.8
Tetracycline	12.5	1.3
Rifampicin	50	3.6
Rifampicin	25	2.5
Rifampicin	12.5	1.9
Tetracycline + Rifampicin	25 + 25	3.3
Tetracycline + Rifampicin	12.5 + 12.5	1.9
Tetracycline + Rifampicin	6.25 + 6.25	1.8
Tetracycline + Rifampicin	3.125 + 3.125	1.0

However, antibiotic treatment of contaminated tissue culture plantlets growing in culture failed to totally eradicate the *Bacillus* sp. Treatments involving several subcultures on antibiotic supplemented medium or incubation in liquid medium with antibiotics failed to control the contaminant. Leifert *et al.* (1991) also found that *Pseudomonas* sp. could not be eliminated from *Delphinium* plants during tissue culture by any antibiotic treatment tested. They postulated that the failure could have been due to lower activity of the antibiotic due to high pH of the medium, antibiotic inactivation due to phenolics produced by the explant, or due to the bacteria surviving in or on plant tissues which the antibiotic failed to reach.

Decontamination of shoot apices of sugarcane plants using heat treatment was unsuccessful. Heat treatments of 50°C for 30 or 60 min, 55°C for 10 min and 60°C for 10, 15, 20 or 30 min resulted in necrosis of the meristematic tissue and inhibited shoot germination. Heat treatment of 50°C for 10 or 20 min caused some browning of the tissue. Bacterial contamination occurred on meristems up to and including treatments of 50°C for 30 min but did not occur at higher temperature treatments. Temperatures above 55°C for more than 10 min are required to effectively kill the contaminating *Bacillus* sp. (Fig 1), but at these temperatures the plant tissue would also be killed.

Figure 1

Growth of *Bacillus* sp. in SPY broth after various temperature treatments at 50, 52 and 55°C for either 5 or 10 min. Results are the mean  $\pm$  SE from 3 replicate treatments



As a result of these experiments, all meristematic tissue was treated with 1% bleach for 10 min, followed by washing in sterile distilled water, then treated with a mixture of tetracycline and rifampicin antibiotics (3.125 mg/L) for 5 min prior to culturing on MS medium containing BAP (0.2 mg/L) and kinetin (0.1 mg/L). Most apical meristems treated with bleach, washed in sterile water, treated with rifampicin and tetracycline antibiotics, then cultured on bacteriological or tissue culture media, remained free of contaminating bacteria and regenerated shoots. In contrast, over 50% of untreated meristems resulted in bacterial contamination growing on the medium and died. Chemotherapy treatments were effective in surface sterilising tissue, but bacteria or yeast that have colonised or infected subepidermal tissue are more difficult to control.

## 5.2 *In vitro* culture of *Erianthus arundinaceus*

Establishing *in vitro* plantlets for *Erianthus* clones was difficult from both callus and apical buds. Very little embryogenic callus developed on the leaf explants and non-embryogenic callus growth was slow resulting in a low efficiency of plant regeneration. There was no difference in the degree of browning of leaf explants and the formation of callus on leaf explants cultured with different vitamins after six weeks incubation. Leaf explants cultured on full strength salts and vitamins were less necrotic than on half strength media.

Various concentrations of synthetic auxins 2,4-D and picloram were tested but no improvement in embryogenic callus formation occurred compared to the standard 2,4-D at 3 mg/L. Picloram has been used by Fitch and Moore (1990) to culture embryogenic callus of sugarcane. No improvement in shoot production occurred when apical buds were cultured on filter paper supports in liquid medium compared to culturing on solid medium. The addition of insoluble PVP to the medium, to absorb phenolic compounds secreted by the meristematic tissue, or the addition of the antioxidant ascorbic acid at 0.1 and 1 mM, did not enhance establishment of *in vitro* plantlets.

The improvement in shoot regeneration and establishment of *in vitro* plants of *E. arundinaceus* was achieved after apical meristems were incubated under continuous light intensities of 90 or 130  $\mu\text{E}/\text{m}^2/\text{s}^{-1}$  compared to the standard light intensity of 40  $\mu\text{E}/\text{m}^2/\text{s}^{-1}$ . At 40  $\mu\text{E}/\text{m}^2/\text{s}^{-1}$ , the meristems of all *E. arundinaceus* became necrotic and died whereas, at 90 or 130  $\mu\text{E}/\text{m}^2/\text{s}^{-1}$  they remained healthy and produced multiple shoots after eight weeks of culture. Under light intensity of 130  $\mu\text{E}/\text{m}^2/\text{s}^{-1}$ , nine cultivars of *E. arundinaceus*, out of 10 tested, were established as *in vitro* plants. Cultivars of *E. arundinaceus* may have been unable to grow as heterotrophs at the low light intensity, whereas the high light intensity may have induced the green meristems to photosynthesis which in turn resulted in shoot germination. The reason cultivar IJ384 remained recalcitrant to *in vitro* growth is unknown.

### 5.3 RAPD analysis of *in vitro* plants

*In vitro* plants regenerated from embryogenic callus were assessed for genetic integrity. RAPD analysis of genomic DNA of cultivars of *S. officinarum*, *S. robustum* and *E. arundinaceus* resolved between 68 and 72 scorable markers from seven primers. This compares to 73 markers resolved for *Saccharum* hybrid cultivars (Taylor *et al.*, 1995) using the same seven primers. Primers produced between 5 and 13 amplification products, which ranged in size between approximately 400 and 3800 bp.

Although RAPD analysis efficiently differentiated *Saccharum* species and *E. arundinaceus*, it failed to reveal any polymorphism between plants that had been stored *in vitro* for three and six months, and field-grown plants for selected cultivars of *S. officinarum*, *S. robustum* and *E. arundinaceus* regenerated from callus. Taylor *et al.* (1995) also reported the failure of RAPD analysis to reveal any polymorphism in sugarcane cultivars regenerated from apical meristems and stored *in vitro* for up to 12 months, although many polymorphisms were detected using RAPDs in protoplast-derived callus of Q63. As discussed by Taylor *et al.* (1995), RAPDs may lack the sensitivity to reliably detect small genetic changes due to somaclonal variation. Nevertheless, these results indicated that no major genetic changes had occurred in plants of *Saccharum* species and *E. arundinaceus* regenerated from callus, cultured *in vitro* and stored for up to six months.

## 6.0 RECOMMENDATIONS

Apply the *in vitro* culture technique to imported germplasm of basic sugarcane species for screening and eradication of viruses, especially Bacilliform virus (SRDC Project BS94S addresses this recommendation).

## 7.0 PUBLICATIONS ARISING FROM PROJECT

Taylor, P W J, Fraser, T A and Dukic, S (1994). *In vitro* culture of *Saccharum* species and *Erianthus arundinaceus*. Proceedings of the 34th Australian Society of Plant Physiologists Conference, Gold Coast, no. P20.

## 8.0 PUBLICATIONS IN PREPARATION

Taylor, P W J and Birch, R G (1995). Micropropagation of sugarcane. In: *Biotechnology in Agriculture and Forestry. High-Tech and Micropropagation*, ed. Y P S Bajaj, Springer Verlag. (In preparation).

Taylor, P W J (1995). *In vitro* germplasm conservation of sugarcane clones, basic sugarcane species and related genera. In: *Conservation of Genetic Resources In vitro*. Ed. M K Razdan, M/s Science Publishers, Inc., USA. (In preparation).

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Taylor, P W J and Fraser, T A (1995). Effect of *in vitro* storage conditions on genetic stability of sugarcane.

## 9.0 ACKNOWLEDGMENTS

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