USE OF PCR-BASED MARKERS FOR IDENTIFICATION OF *ERIANTHUS* GENETIC MATERIAL IN PUTATIVE INTERGENERIC HYBRIDS (*SACCHARUM* X *ERIANTHUS*)

M HARVEY[1], A D'HONT[2], K ALIX[2], AND BI HUCKETT[1]

[1]*South African Sugar Association Experiment Station, Private Bag X02, Mount Edgecombe, 4300*
[2]*Agretrop, CIRAD, BP 5035, 34032 Montpellier, France*

Abstract

Modern sugarcane varieties arose from interspecific crosses between *Saccharum officinarum* and *S. spontaneum* varieties, and subsequent backcrossing to *S. officinarum* cultivars. Due to the lack of further genetic input, there has been concern about an overall reduction in the pool of genes available for improvement of sugarcane varieties. Therefore, breeders have looked to related genera such as *Erianthus* for introgression of novel genetic material into the germplasm. Little success has been achieved in introgression programmes carried out in the past few years, largely due to the difficulties encountered in the identification of true *Saccharum* x *Erianthus* hybrids using morphological traits. As a solution to this problem, PCR-based primers have been developed which target 5s rDNA sequences shown to be polymorphic between the *Saccharum* and *Erianthus* genomes. This paper describes the use of these primers to detect the presence of *Erianthus* genetic material in various putative hybrids produced in breeding programmes at the Experiment Station. These results, confirmed with Restriction Fragment Length Polymorphism (RFLP) analysis, allow identification of true hybrids of *Saccharum* and *Erianthus*, which may be used in the breeding programme for improving traits such as ratooning ability, vigour and tolerance to drought and flooding.

Introduction

The '*Saccharum* complex' includes species from five genera: *Saccharum, Erianthus* (sect *Ripidium*), *Miscanthus* (sect *Diandra*), *Sclerostachya* and *Narenga*. It is thought that modern sugarcane varieties arose from crosses within the *Saccharum* genus, largely between *S. officinarum* and *S. spontaneum* clones (Daniels and Roach, 1987). Subsequent breeding strategies involved backcrossing to the sugar producing *S. officinarum* varieties, with no contribution from the related in-breeding genera of the '*Saccharum* complex'. As a result of this lack of further genetic input, and the present slow progress encountered in the production of better sugarcane varieties (D'Hont *et al.*, 1995; Garside, 1997), concern has been expressed about an overall reduction in the pool of genes available for improvement of sugarcane. As a result, breeders have begun to look to other genera such as *Erianthus* for introgression of traits such as tolerance to drought and flooding, ratooning ability, vigour and resistance to diseases and pests.

Over the past few years, several programmes have been initiated to introduce *Erianthus* genetic material into the sugarcane breeding germplasm. Little success has been achieved thus far, mostly due to difficulties encountered in the identification of true *Erianthus* x *Saccharum* hybrids based on their morphological appearance. In an attempt to overcome this problem, molecular techniques have been developed to detect the presence of *Erianthus* genetic material in putative hybrids (D'Hont *et al.*, 1995). By designing PCR-based primers which target the highly variable 5s rRNA spacer region of the genome, those authors were able to distinguish between pure *Saccharum* and hybrid clones based on the size of the PCR fragment produced.

This paper describes the use of these 5s rRNA PCR primers to detect the presence/absence of *Erianthus* chromosomes in F1 progeny from various intergeneric crosses made at the Experiment Station. Results were then confirmed using Restriction Fragment Length Polymorphism (RFLP) analysis with an *Erianthus* specific repeat sequence, EaCIR1 (Alix *et al.*, 1998).

Material and methods

DNA extraction

DNA was extracted from 21 putative hybrid clones produced in intergeneric crosses at the Experiment Station (Table 1). In addition, DNA was isolated from 10 individuals thought to be *Erianthus* clones (Table 1). In all cases the extraction methodology used was as described by Harvey and Huckett (1998).

PCR Amplification

The sequences of the PCR primers were as follows: 5' GTG-ACC-TCC-TGC-GAA-GTC-CT 3' and 5' CCC-ATC-CGT-GTA-CTA-CTC-TC 3' (D'Hont *et al.*, 1998). PCR reaction mixtures contained 5 ng template DNA, 100 µM dNTPs, 0,2 µM of each primer, 10 mM Tris-HCl, 50 mM KCl, 1,5 mM $MgCl_2$, 0,1 mg/ml gelatine (pH 8,3) and 1 U Taq Polymerase (Boehringer Mannheim). Amplification was carried out using a Gene Amp 9700 thermocycler (Perkin Elmer, Applied Biosystems) with the following cycling profile: one cycle of 94°C for 3 min, 35 cycles of 94°C for 3 min, 55°C for 1 min and 72°C for 1 min and a last cycle of 72°C for 7 min.

Table 1. Results of 5s rRNA PCR diagnostic test indicating the presence (Y) or absence (N) of *Erianthus* and *Saccharum* genetic material in putative hybrids.

Genotype	Origin	*E. arundinaceus* band (Y/N)	*S. officinarum* band (Y/N)
N64/1232	Putative *S. officinarum* x *Erianthus* hybrids	N	Y
76E0467		N	Y
76E0514		N	Y
76E0536		N	Y
76E0649		N	Y
9E31089		N	Y
93E1201		N	Y
93E1222		N	Y
93E1224		N	Y
93E1239		N	Y
93E1258		N	Y
93E1275		N	Y
93E1285		N	Y
93E1331		N	Y
93E1350		N	Y
93E1412		N	Y
93E1419		N	Y
93E1440		N	Y
93E1441		N	Y
93E1444		N	Y
93E1455		N	Y
Unknown 1	*Erianthus* clones	Y	N
Unknown 2		Y	N
Unknown 3		Y	N
E. arundinaceus		Y	Y
IJ 76-332		Y	Y
IJ 76-407		Y	Y
E. ravennae		Y	Y
US 82-31		N	Y
US 82-36		N	Y
US 82-41		N	Y

After amplification PCR products were separated on 1% (w/v) agarose gels and visualised after staining with ethidium bromide.

RFLP analysis

DNA samples (10 µg) from a subset of clones (*E. arundinaceus*, US82-41, US82-36, IJ76-407, unknown 1, unknown 2, 93E1224 and 76E467) were digested with the enzyme HindIII (Boehringer Mannheim), separated on 0,8% (w/v) agarose gels and blotted on to nylon membrane as described previously (Chomczynski, 1992). This blot was hybridised, using the methodology of Hoisington (1992), to an *Erianthus* specific satellite sequence (EaCIR1) (Alix *et al.*, 1998).

Results and Discussion

The spacer region of the 5s rRNA gene has been characterised by rapid evolutionary change, with the result that its heterozygosity has been exploited in various phylogenetic studies (McIntyre *et al.*, 1992; Moran *et al.*, 1992). D'Hont *et al.* (1995) used this variability to design PCR primers which could be used to distinguish species within the *Saccharum* complex from one another. Amplification in the presence of these primers resulted in the production of a major band of 230 bp for *S. officinarum* clones, a fragment between 190 and 230 bp for *S. spontaneum* individuals and a 370 bp product for *Erianthus* (D'Hont *et al.*, 1995). Using this methodology those authors were able to identify hybrid progeny in a cross conducted between the *S. officinarum* clone BNS3066 and the *E. arundinaceus* clone IK 7648 (D'Hont *et al.*, 1995).

In the past few years a number of crosses have been made at the Experiment Station in an attempt to introgress *Erianthus* chromosomes into the breeding germplasm and introduce genes for important traits. Although the resulting progeny appeared phenotypically to be hybrids, studies conducted by other authors have shown physical appearance to be a poor measure of the success of introgression (D'Hont *et al.*, 1995). As a result, it was decided to analyse these putative F1 hybrids at the molecular level using the PCR methodology developed by D'Hont and co-workers.

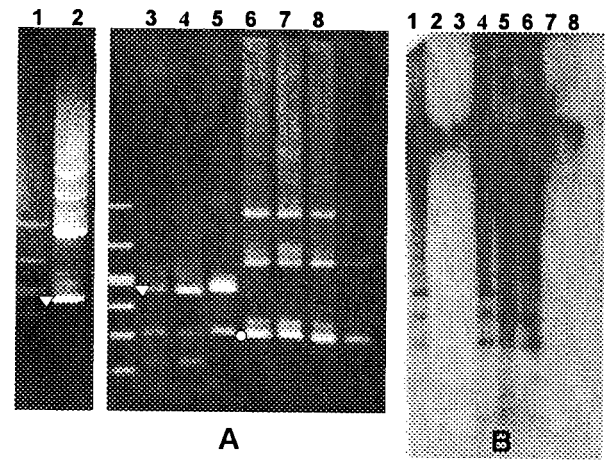

Figure 1. Representative portions of an agarose gel showing the product obtained after PCR amplification with the 5s rRNA primers (A) and an autoradiograph produced after Southern hybridisation with the *Erianthus* repeat probe (B).

(A) size markers (1), *E. arundinaceus* (2), unknown 1 (3), unknown 2 (4), IJ76-407 (5), US82-41 (6), 93E1224 (7) and 76E0467 (8).

(B) *E. arundinaceus* (1), US82-41 (2), US82-36 (3), IJ76-407 (4), unknown 1 (5), unknown 2 (6), 93E1224 (7) and 76E0467 (8). *Erianthus* specific bands are shown with (∇) and the *S. officinarum* band with (O).

As can be seen from Table 1 and Figure 1, none of the putative hybrids contained the *Erianthus* specific band, although all produced the fragment originating from *S. officinarum*. Although disappointing, this result was not unexpected, as difficulties have been encountered by other breeders attempting to introgress *Erianthus* material (D'Hont *et al.*, 1995). In contrast, all the *Erianthus* clones, with the exception of the US clones, displayed the larger *Erianthus* band (Table 1).

The PCR result was confirmed using RFLP analysis. DNA from two putative hybrids, two unknown clones and *E. arundinaceus* was restricted, blotted on to a membrane and probed with the EaCIR1 repeat sequence (Alix *et al.*, 1998). Figure 1 shows that the results obtained with the 5s PCR test (Figure 1A) were echoed on the Southern blot (Figure 1B). Those clones such as *E. arundinaceus*, IJ76-407 and the 2 unknown clones, which gave a typical *Erianthus* 5s PCR product, also show strong hybridisation to the *Erianthus* specific probe, whereas the putative *Erianthus* hybrids do not have a comparative profile (Figure 1). The ladder pattern observed in Figure 1B occurs due to the tandem organisation of the EaCIR1 repeat sequence.

This paper demonstrates the utility of the simple and rapid 5s rRNA PCR test to screen putative F1 hybrids from crosses between *Saccharum* and *Erianthus*. It is suggested that all potentially interesting F1 progeny from intergeneric crosses be tested in this way. The 5s genes are present at only one locus in the genome of *Erianthus*. Other markers, which cover the entire genome, should be used in conjunction with this PCR test to monitor further introgression when only a few *Erianthus* chromosomes remain in the hybrids.

Acknowledgements

The authors would like to thank the staff of the Plant Breeding department at SASEX for relevant information and provision of putative *Erianthus* hybrids and other clones.

REFERENCES

Alix, K, Baurens, FC, Paulet, F, Glaszmann, JC and D'Hont, A (1998). Isolation and characterisation of a satellite DNA family in the *Saccharum* complex. *Genome* (in press).

Chomczynski, P (1992). One-hour downward alkaline capillary transfer for blotting of DNA and RNA. *Anal Biochem* 201: 134-139.

Daniels, J and Roach, BT (1987). Taxonomy and evolution. pp 7-84 In: DJ Heinz (ed) *Sugarcane Improvement Through Breeding.* Elsevier Press, Amsterdam.

D'Hont, A, Rao, PS, Feldmann, P, Grivet, L, Islam-Faridi, N, Taylor, P and Glaszmann, JC (1995). Identification and characterisation of sugarcane intergeneric hybrids, *Saccharum officinarum* x *Erianthus arundinaceus*, with molecular markers and DNA in situ hybridisation. *Theor Appl Genet* 91: 320-326.

D'Hont, A, Ison, D, Alix, K, Roux, C and Glaszmann, JC (1998). The genus *Saccharum* by physical mapping of ribosomal RNA genes. *Genome* (in press).

Garside, AL (1997). Yield decline research in the Australian sugar industry. *Proc S Afr Sug Technol Ass* 71: 3-8.

Harvey, M and Huckett, BI (1998). Preliminary genetic analysis of the sugarcane population AA40. *Proc S Afr Sug Technol Ass* 72 (in press).

Hoisington, D (1992). *Laboratory Protocols.* CIMMYT Applied Molecular Genetics Laboratory, Mexico.

McIntyre, CL, Winberg, B, Houchins, K, Appels, R and Baum, BR (1992). Relationships between *Oryza* species (*Poaceae*) based on 5s DNA sequences. *Pl Syst Evol* 183: 249-264.

Moran, GF, Smith, D, Bell, JC and Appels, R (1992). The 5s RNA genes in *Pinus radiata* and the spacer region as a probe for relationships between *Pinus* species. *Pl Syst Evol* 183: 209-221.