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Australian Journal of Agricultural Research

Volume 49, 1998

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Australian Academy of Science

Aust. J. Agric. Res., 1998, **49**, 915–21

Isozyme markers in *Saccharum* spp. hybrids and *Erianthus arundinaceus* (Retz.) Jeswiet

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Abstract. Verification of the authenticity of the hybrid origin of progeny from interspecific or intergeneric crossing in introgression studies in plant improvement is essential before usage of such progeny. This study undertook to determine whether isozyme phenotypes verified the hybrid origin of apparent crosses between a clone of *Erianthus arundinaceus* (Retz.) Jeswiet and several *Saccharum* spp. hybrid clones. Starch gel electrophoresis was used to resolve 18 isozyme systems for markers that would distinguish *E. arundinaceus* from *Saccharum* spp. hybrid clones. Eight isozyme systems revealed 16 bands that were present in *E. arundinaceus* but absent from the sugarcane parents. When a population of putative *E. arundinaceus*×*Saccharum* spp. hybrid progeny was screened using these isozyme systems, none of the clones expressed the bands characteristic of *E. arundinaceus*. Thus, their intergeneric nature was disproven.

Additional keywords: starch gel electrophoresis, germplasm maintenance, introgression, allozyme.

Introduction

The genetic base of sugarcane is narrow, with only 31 original parents contributing to the current commercial sugarcane clones (Roach 1989), all of which are derived from interspecific crosses (Stevenson 1965). This narrowness may explain why improvement from conventional breeding has been slow. Efforts have been made at Bureau of Sugar Experiment Stations (BSES) to broaden the genetic base of sugarcane by using *Erianthus arundinaceus* (Retz.) Jeswiet, a member of the '*Saccharum* complex'. This complex comprises 5 interbreeding genera (*Saccharum* L., *Erianthus* sect. *Ripidium* Henrard, *Miscanthus* sect. *Diantra* Keng, *Narenga* Bor, and *Sclerostachya* (Hack.) A. Camus) that are assumed to be involved in the origin of sugarcane (Mukherjee 1957). *Erianthus arundinaceus* was chosen for this study because it has useful agronomic characters including disease resistance, drought and waterlogging tolerance, and good ratooning ability (Lee *et al.* 1993). A population of putative first introgression progeny (I_1) had been obtained using the *E. arundinaceus* clone IK76–41M as the female parent in crosses with 4 *Saccharum* spp. hybrid clones as sources of pollen. Circumstantial rather than conclusive evidence existed for the intergeneric nature of these progeny. This included:

(i) breeding records [however, as excised panicles (Heinz and Tew 1987) are used in all sugarcane crosses in Australia, there is potential for error, as panicles from the wrong parents could be placed in a breeding lantern (large pollination bag)];
(ii) the presence of the aerenchyma in the stalks;
(iii) high ash and phenolic contents in the extracted juice;
(iv) the progeny had a 'wild' appearance;
(v) the expression of the dewlap, the transitional zone between leaf sheath and lamina, of *Saccharum* appeared to be dominant over the dewlaplessness of *Erianthus*.

Extensive field evaluation trials of the putative intergeneric hybrids had commenced because of confidence about their status as true hybrids. This research sought

10.1071/AR96163 0004-9409/98/060915$05.00

to confirm the intergeneric nature of the introgression progeny by using starch gel electrophoresis of isozymes. Nine isozyme systems were known that distinguished *E. arundinaceus* from *Saccharum* (Waldron and Glasziou 1971; Glaszmann *et al.* 1989; Wood and Strand 1989). This research was based on the premise that the occurrence in the progeny of isozyme banding patterns indicative of each parent would provide evidence of introgression (Crawford 1989).

Material and methods

Plant material

Sixty-seven *E. arundinaceus* clones collected from throughout the Indonesian archipelago (Berding and Koike 1980) were screened using all enzyme systems examined in this study. The number of *Saccharum* species hybrid clones and putative I_1 progeny screened* varied for each enzyme system studied, as indicated in Fig. 1*a–h*.

A range of plant materials was screened for isozyme bands. Samples from either the lamina excised from midpoint of the last fully expanded leaf (+ 1 leaf, Moore 1987), or the non-chlorophyllous leaf spindle from 10 mm above the growing point, gave the clearest banding patterns.

Isozyme electrophoresis

Eighteen isozymes were assayed in the study (Table 1), including the 9 systems mentioned above, that characterise *E. arundinaceus*. Starch gels were prepared at least 12 h before use. Each gel contained 11·9% w/v starch. Hydrolysed potato starch was added to 100 mL of the required gel buffer (Table 1) and prepared according to Wendel and Weeden (1989). The prepared starch was poured into Perspex trays (6×162×205 mm). The gels were covered with glass plates and allowed to set overnight. Two hours before the gels were loaded with samples, they were trimmed and cooled to 4°C. Extraction of the samples then commenced.

The extraction buffer for tissues with high levels of interfering substances (Wendel and Weeden 1989) was used for all isozymes evaluated in this study except alcohol dehydrogenase (ADH), for which optimal banding occurred when sodium metabisulfite and sucrose were not added to the extraction buffer. Two drops of chilled extraction buffer were added to approximately 200 mg of fresh plant material in a 7-mL weigh boat. The sample was crushed with a Perspex rod, pushed to the side of the vessel, and pressed so that the extract was expressed. Wicks of 4 by 6 mm cut from Whatman No. 3 chromatography paper were saturated in the extract, and stored on ice in Petri dishes while other extracts were prepared. Excess moisture was removed from the wicks by blotting with tissue paper. Twenty wicks were inserted in a gel along a vertical cut made approximately 30 mm from the cathodal end. A 4-mm gap was left between wicks. Four gels were run in parallel in a 4°C cabinet at a constant current (75 A) and variable voltage (peaking at 250 V) and power, using a Biorad model 3000Xi powerpack. Band definition was optimal if wicks were removed after 20 min. Electrophoretic runs varied from 3·5 h to 15 h. Prior to staining, starch gels were sliced horizontally into three 2-mm slices with a steel guitar string held taut in a hacksaw frame. Aspartate aminotransferase (AAT), esterase (EST), and malic enzyme (ME) were assayed using the methods of Soltis *et al.* (1983). Peptidase (PEP) and phosphoglucoisomerase

Table 1. Isozymes assayed to discriminate between *Saccharum* spp. hybrid and *E. arundinaceus* (E) clones, and to provide evidence of hybridity in the progeny from crosses between them

Enzyme	Abbreviation	E.C. No.	Buffer system[A]	Tissue[B]	E markers[C]
Acid phosphatase	ACP	E.C. 3·1·3·2	3	Spindle	−
Alcohol dehydrogenase	ADH[D]	E.C. 1·1·1·1	3	Spindle	+
Aminopeptidase[E]	AMP[D]	E.C. 3·4·11·1	6–7	−	−
Amylase	AMY[D]	E.C. 3·2·1·1/2	5–7	−	−
Aspartate aminotransferase	AAT[D]	E.C. 2·6·1·1	5	Spindle	+
Catalase	CAT	E.C. 1·11·1·6	6	−	−
Diaphorase	DIA	E.C. 1·6·99.−	6	Spindle	+
Esterase	EST[D]	E.C. 3·1·1.−	6	Spindle	+
Isocitrate dehydrogenase	IDH	E.C. 1·1·1·42	1–3	Spindle	−
Malate dehydrogenase	MDH	E.C. 1·1·1·37	1–2	Spindle	−
Malic enzymes	ME[D]	E.C. 1·1·1·40	6	Leaf	+
Peptidase	PEP	E.C. 3·4.−.−	5–6	−	−
Peroxidase	PRX[D]	E.C. 1·11·1·7	6	Leaf	+
Phosphoglucoisomerase	PGI[D]	E.C. 5·3·1·9	6	Spindle	+
Phosphoglucomutase	PGM	E.C. 5·4·2·2	1	Spindle	+
Phosphogluconate dehydrogenase	PGD	E.C. 1·1·1·44	1	Leaf	+
Triosephosphate isomerase	TPI	E.C. 5·3·1·1	6	−	−
Shikimate dehydrogenase	SKDH[D]	E.C. 1·1·1·25	1	−	−

[A]Gel and tank buffer systems from Wendel and Weeden (1989).
[B]Minus indicates no banding observed with tissues surveyed.
[C]Minus indicates systems could not be optimised to run on starch gel, or did not reveal bands characteristic of *E. arundinaceus* at BSES Meringa.
[D]Isozyme systems identified in previous studies that have zymograms characteristic of *E. arundinaceus* (Roughan *et al.* 1971; Waldron and Glasziou 1971; Glaszmann *et al.* 1989; Wood and Strand 1989; Nagai *et al.* 1991).
[E]Including leucine aminopeptidase (LAP).

* Information about the identity of these clones is available from the corresponding author.

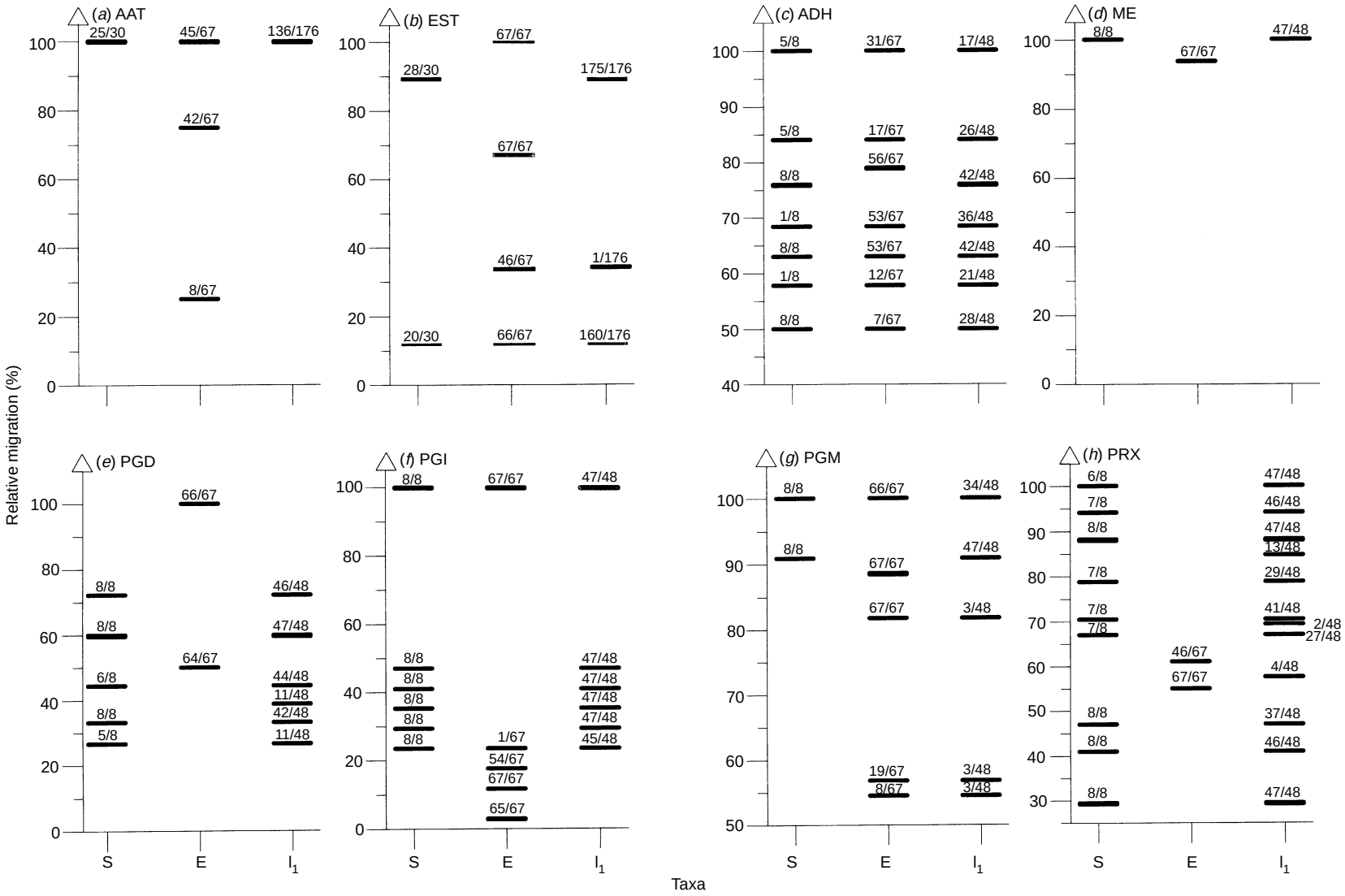

Fig. 1. (*a–h*) Banding patterns of the 8 enzyme systems with bands characteristic of *E. arundinaceus* (E) compared with the bands observed in *Saccharum* spp. hybrids (S) and putative introgression progeny (I_1). The fastest band is at 100% migration. The proportion of the population that expressed the band is written on top of the band, if possible, otherwise to the right-hand side. Enzyme systems: AAT, aspartate aminotransferase; EST, esterase; ADH, alcohol dehydrogenase; ME, malic enzymes; PGD, phosphogluconate dehydrogenase; PGI, phosphoglucoisomerase; PGM, phosphoglucomutase; PRX, peroxidase.

(PGI) were assayed using the methods of Richardson *et al.* (1986) and Wood and Strand (1989), respectively. All other enzyme systems were assayed as recommended by Wendel and Weeden (1989). Each clone was screened twice for each enzyme system. If the banding was inconsistent between independent runs, the clone was re-screened. When staining was complete, the relative migration (RM), which was the distance migrated relative to the fastest band observed, and the intensity of each band were recorded. However, analysis of isozyme bands was carried out on a presence/absence basis.

Results and discussion

Potential markers for E. arundinaceus

Bands that distinguished *E. arundinaceus* from *Saccharum* spp. hybrids were detected in 8 isozyme systems: AAT, EST, ADH, ME, phosphogluconate dehydrogenase (PGD), PGI, phosphoglucomutase (PGM), and peroxidase (PRX) (Fig. 1*a–h*). Of these, PGM and PGD had not been identified previously as having bands that characterised *E. arundinaceus*. Glaszmann *et al.* (1989) observed bands characteristic of *E. arundinaceus* in the other 6 enzyme systems, but found that polyacrylamide gels were required for clear separation of AAT, EST, and PRX isozymes.

The AAT isozyme zymogram had a band that was common to both *Saccharum* and *Erianthus* at 100% RM, whereas *E. arundinaceus* was distinguished from *Saccharum* by the presence of 1 band at 25% and 1 band at 75% RM. These 2 bands were expressed in 8 of 67 (11·9%) and 42 of 67 (62·7%) of the *E. arundinaceus* population, respectively, but in none of the *Saccharum* spp. hybrid parents (Fig. 1*a*). However, the AAT bands that characterised *E. arundinaceus* were not detected in IK76–41(M), the *E. arundinaceus* clone used as the female parent in the introgression process. This system was therefore unable to confirm the hybrid origin of progeny reportedly bred from IK76–41(M). Three bands in the EST zymogram distinguished *E. arundinaceus* from *Saccharum* spp. hybrids (Fig. 1*b*). The fastest band (100% RM) and a band at 66·6% RM (Fig. 1*b*) were observed in all *E. arundinaceus* clones screened ($n = 67$). The third band (at 33·3% RM, Fig. 1*b*) was observed in 46 of the 67 (68·6%) *E. arundinaceus* clones.

Eight bands were usually observed in the ADH zymogram (Fig. 1*c*). A band at 79% RM distinguished *E. arundinaceus,* occurring in 56 of the 67 (83·6%) clones examined. This band was not observed in any of the *Saccharum* spp. hybrid parents, and is a potential marker for introgression. A slower band at 68·4% RM (Fig. 1*c*) was characteristic of *E. arundinaceus*, occurring in 53 of the 67 (79·1%) clones. However, this band also occurred in one of the *Saccharum* spp. hybrid parents (76N1772, Fig. 1*c*), and so could not be used as a reliable marker for *E. arundinaceus*.

The ME banding pattern (Fig. 1*d*) substantiated the findings of Glaszmann *et al.* (1989) and Gallacher *et al.* (1995), with 1 fast band always present in *Saccharum* spp. hybrids (100% RM) and a slightly slower band at 93·7% RM characterising all the *E. arundinaceus* clones.

Two bands characteristic of *E. arundinaceus* were observed in the PGD zymogram (Fig. 1*e*). The fastest moving band (100% RM) was observed in 66 of the 67 (98·5%) *E. arundinaceus* clones, and the other band (at 75% RM) was observed in 64 of 67 (95·5%; Fig. 1*e*) *E. arundinaceus* clones. There have been no previous reports of characteristic bands for *E. arundinaceus* in the PGD enzyme system. Five distinct PGD bands (Fig. 1*e*) were observed regularly in the *Saccharum* spp. hybrid parents.

The PGI isozyme system had 3 distinct zones. A single band common to *Saccharum* spp. hybrids and *E. arundinaceus* always occurred at 100% RM (Fig. 1*f*), which conflicts with the results of Glaszmann *et al.* (1989), who observed no bands common between *Saccharum* spp. hybrids and *E. arundinaceus*. In the middle zone (23–47% RM), 5 bands occurred frequently in the *Saccharum* spp. hybrid parents (Fig. 1*f*). The lowest of these bands occurred in 1 of the 67 (1·5%) *E. arundinaceus* clones. Three bands characteristic of *E. arundinaceus* occurred in the zone of 2·9–17·6% RM (Fig. 1*f*). As shown in Fig. 1*f*, these *Erianthus* bands (slowest to fastest) occurred in 97%, 100%, and 80·6%, respectively, of the *E. arundinaceus* clones ($n = 67$). Only 1 PGI band characteristic of *E. arundinaceus* was detected by Glaszmann *et al.* (1989).

In the PGM zymogram (Fig. 1*g*), the fastest migrating band occurred in 8 of 8 clones in *Saccharum* spp. hybrid and in 66 of 67 *E. arundinaceus* clones. A band characteristic of the *Saccharum* spp. hybrid clones (in 8 of 8) occurred at 90·9% RM. Two PGM bands characteristic of *E. arundinaceus* (88·6 and 81·8% RM, Fig. 1*g*) were expressed in all *E. arundinaceus* clones examined ($n = 67$). This is the first report of the 2 PGM bands that characterise *E. arundinaceus*. Two other bands (54·5 and 56·8% RM) were observed in some *E. arundinaceus* clones, occurring in 8 (11·9%) and 19 (28·3%) of the 67 clones, respectively, but were not observed in any of the *Saccharum* spp. hybrid parents. As these 2 lower bands were not detected in IK76–41(M), they were not considered useful markers for the putative introgression progeny. Neither Wood and Strand (1989) nor Nagai *et al.* (1991) found any bands that characterised *E. arundinaceus* when they assayed the PGM enzyme system.

In the PRX isozyme zymogram (Fig. 1*h*), 11 different bands were observed: 9 characterising *Saccharum* and 2 characterising *E. arundinaceus*. The 9 bands

in *Saccharum* consisted of 6 in the zone of fastest migration and 3 in the zone of slowest migration. The 2 bands characterising *E. arundinaceus* were observed in the intermediate migration zone (55–61% RM). Glaszmann *et al.* (1989), using polyacrylamide gels, detected banding patterns for *Saccharum* spp. hybrid clones similar to those seen here. However, Glaszmann *et al.* (1989) reported only a single band characterising *E. arundinaceus*.

The remaining isozyme systems examined during the isozyme study (Table 1) either did not reveal bands characteristic of *E. arundinaceus* or the enzyme systems could not be optimised, and consequently the bands were indistinct.

Isozyme marker inheritance in introgression progeny

In progeny from *E. arundinaceus*×*Saccharum* spp. hybrid crosses, the number of isozyme bands was expected to increase, as 8 isozyme systems with 16 bands characterising *E. arundinaceus* had been found (Fig. 1*a*–*h*). Some of these bands should have been expressed in the introgression progeny. However, the bands that characterised *E. arundinaceus* were not expressed in the putative introgression progeny, with the exception of 3 bands observed in the PGM zymogram that were expressed at a low incidence (Fig. 1*a*–*h*).

The bands in the AAT (Fig 1*a*), ADH (Fig. 1*c*), ME (Fig. 1*d*), and PGI (Fig. 1*f*) zymograms that characterised *E. arundinaceus* were not observed in any of the introgression progeny. Similarly, the bands characteristic of *E. arundinaceus* at 100% and 66% RM in the first zymogram were not expressed in any of the introgression progeny (Fig. 1*b*). However, the EST band characteristic of *E. arundinaceus* at 33% RM was observed in an I_1 clone (1 out of 176; Fig. 1*b*). The presence of this band in an I_1 clone, however, was not taken as an indication of hybridity. The reason for this is that the EST enzyme system tends to be a complex multilocus system, with the number of loci varying from taxon to taxon (Richardson *et al.* 1986). Problems associated with examining a system such as EST include: isozymes encoded by separate loci often overlap on the gel, thus bands scored as single entities may have multiple constituents; protein products from different loci can hybridise and form heteropolymeric isozymes with very complex banding patterns; and heterozygous isozymes may exhibit atypical band positions (Richardson *et al.* 1986). Interpretation of the EST isozyme system is therefore difficult, especially when the genetic basis of the EST variation is unknown (Richardson *et al.* 1986). Esterase bands have, however, been used to identify interspecific progeny from crosses of *S. officinarum*×*S. spontaneum* (Waldron and Glasziou 1971). This system was used in the hope of identifying intergeneric hybrids from *E. arundinaceus*×*Saccharum* spp. hybrid crosses.

The 2 PGD bands characteristic of *E. arundinaceus* were not observed in any of the I_1 population (Fig. 1*e*); however, the faster band (100% RM) was observed in 3 of the 38 third-generation introgression clones (I_3, data not shown).

The PGM band characteristic of *E. arundinaceus* at 88·6% RM was not inherited by any of the introgression progeny (Fig. 1*g*). The *E. arundinaceus* band at 81·8% RM was observed in 3 of the 48 I_1 clones examined (Fig. 1*g*). This low incidence in the introgression progeny was interpreted as stain artefacts, which occur regularly in PGM zymograms (Richardson *et al.* 1986).

In the PRX isozyme system, the 2 bands characterising *E. arundinaceus* were not inherited by the introgression progeny (Fig. 1*h*). Additive inheritance was expected in this system, as Nagai *et al.* (1991) observed that progeny from a *Saccharum* spp. hybrid×*E. arundinaceus* cross possessed bands characteristic of the *E. arundinaceus* parent. The PRX enzymes may be encoded by many loci and often are subject to post-translational modifications (Acquaah 1992). Thus, this enzyme is often considered unreliable as the number of glycosyl groups attached to different copies of the enzyme in an individual often varies (Acquaah 1992). If the unmodified enzymes coded in *Saccharum* spp. hybrid and *E. arundinaceus* clones are the same, then bands found to be characteristic of *E. arundinaceus* would be due to glycosyl groups added during post-translational modifications, and not to differences in the nuclear genome.

De novo *isozyme bands in introgression progeny*

A number of isozyme zymograms (particularly PRX and PGD) revealed *de novo* bands in introgression progeny that were not observed in any of the *Saccharum* spp. hybrid or the *E. arundinaceus* parents (Fig. 1*e* and *h*). *De novo* isozymes have been observed in a number of studies in the *Saccharum* complex. Tung *et al.* (1973) observed *de novo* PRX and EST bands in progeny of intraspecific crosses. As clear additive inheritance was observed for parental bands, the *de novo* bands did not interfere with identification of the parents of the progeny. Similarly, Roughan *et al.* (1971) observed *de novo* bands in an amylase zymogram of progeny from an intergeneric cross *S. officinarum* L.×*N. porphyrocoma* (Hance ex Trimen) Bor. *De novo* bands, resulting from unique combinations of the peptide subunits from each parent, are typical in polyploids (Gottlieb 1982). They cannot, however, be considered indicative of successful intergeneric crosses here, because inheritance of bands from both parents was not observed.

Parentage of introgression progeny

The banding patterns of the I_1 clones resembled their respective *Saccharum* spp. hybrid parents but not the *E. arundinaceus* parents (Fig. 1*a–h*). The results of the isozyme analysis consistently indicated that *E. arundinaceus* clones are not involved in the parentage of the I_1 clones and therefore of the introgression population. However, the results substantiate the view that the *Saccharum* spp. hybrid clones are parents of the introgression progeny. Subsequent work by A. D'Hont (pers. comm. 1994) obtained similar results when analysing the hybridisation patterns of 6 nuclear probes of the putative I_1 families. Bands indicative of the male (*Saccharum* spp. hybrid) parent were detected in the progeny; however, only very few bands present in the female (*E. arundinaceus*) parent were detected. A. D'Hont (pers. comm. 1994) also observed many novel bands that were not characteristic of either parent in the I_1 progeny. Similarly, 2 mitochondrial probes indicated that the clone IK76–41(M), the putative *E. arundinaceus* female parent, was not the cytoplasmic parent. D'Hont proposed that the female parent could have been a *S. spontaneum* clone, as its mitochondrial probe hybridisation patterns were similar to those previously observed in *S. spontaneum* (D'Hont *et al.* 1993). Historical, flavonoid, and morphological evidence (G. Sukarso, pers. comm. 1993) indicated that mislabelling of an *E. arundinaceus* clone as the putative parent occurred after the crosses for the introgression population were made in 1983.

Recommendations and conclusion

Sixteen isozyme bands characteristic of *E. arundinaceus* were detected in 8 enzyme systems. This is the first report that bands of the enzyme systems PGM and PGD characterise *E. arundinaceus*. The other 6 isozyme systems with *E. arundinaceus* bands had banding patterns similar to those reported by Glaszmann *et al.* (1989). None of the bands characteristic of *E. arundinaceus* were inherited by the putative introgression progeny. The isozyme evidence indicated that *E. arundinaceus* was not the parent of the introgression progeny as first thought (N. Berding, pers. obs. 1989), even though there was morphological and circumstantial evidence to the contrary. If the clone IK76–41(M) is not the female parent of the introgression progeny then mislabelling of either the progeny or the female parent must have occurred. Historical records and information from Indonesia indicated that the real parent of the introgression progeny, IK76–41, was lost from the collection at Meringa as a result of mislabelling after the crosses were made in 1983 (Lee 1994).

Grassl (1962) and Stevenson (1965) recommended that progeny should be examined cytologically to ensure that they are hybrids. However, the putative *Erianthus–Saccharum* introgression was refuted by the absence of *E. arundinaceus* isozyme markers. It is critical that the identity and parentage of putative introgression progeny should be substantiated before field trials and yield evaluations commence. This could be achieved by screening the parents and progeny for expression of markers *viz*:

(*i*) using a range of isozyme systems with markers characteristic of both parents, then testing the putative progeny for expression of these markers. As specific parental isozyme bands may not be expressed in putative progeny, a minimum of 6 isozyme systems which characterise both parents should be examined to ensure that all hybrids are detected. In the case of *Erianthus* introgression, at least 6 of the 8 isozyme systems with bands characteristic of *E. arundinaceus* (Fig. 1*a–h*) should be chosen;

(*ii*) by screening the putative progeny with nuclear and cytoplasmic probes that distinguish between the parents.

Verification of clonal identity should be given a higher priority in sugarcane germplasm management. The present study has revealed problems in relying on 'location' of a plot in a field as verification of the identity of a clone *viz*:

(*i*) the original clone IK76–41 was lost from BSES Meringa through mislabelling of an *E. arundinaceus* clone as IK76–41;

(*ii*) the progenies from crosses were accepted as intergeneric hybrids between *E. arundinaceus* and *Saccharum* spp. hybrids because of the above mislabelling.

To prevent similar events occurring in future, all sugarcane germplasm introduced into Australia, and all clones used as parental germplasm at BSES Meringa, should be described or finger-printed, using molecular or isozyme markers. Clones should be routinely rescreened at set intervals to verify clonal identity, as the frequency of clonal misidentification should not be underestimated (Eksomtramage *et al.* 1992). Gallacher *et al.* (1995) were able to detect mislabelling with 97% confidence, using starch gel electrophoresis for 3 isozyme systems (PRX, ADH, and PGM). Similarly, the use of nuclear and cytoplasmic molecular markers, as well as the high level resolution for isozyme discrimination provided by cellulose acetate electrophoresis (S. Dukic, pers. comm. 1994) or polyacylamide gel electrophoresis, (Glaszmann *et al.* 1989; Eksomtramage *et al.* 1992) may provide reliable techniques for clonal identification.

References

Acquaah, G. (1992). 'Practical Protein Electrophoresis for Genetic Research.' (Dioscorides Press: Portland, OR.)

Berding, N., and Koike, H. (1980). Germplasm conservation of the *Saccharum* complex: A collection from the Indonesian archipelago. *Hawaiian Planters' Records* **59**, 131–76.

Crawford, D. J. (1989). Enzyme electrophoresis and plant systematics. *In* 'Isozymes in Plant Biology'. (Eds D. E. Soltis and P. S. Soltis.) pp. 146–64. (Dioscorides Press: Portland, OR.)

D'Hont, A., Lu, Y. H., Feldmann, P., and Glaszmann, J. C. (1993). Cytoplasmic diversity in sugarcane revealed by heterologous probes. *Sugarcane* **1**, 12–15.

Eksomtramage, T., Paulet, F., Noyer, J. L., Feldmann, P., and Glaszmann, J. C. (1992). Utility of isozymes in sugarcane breeding. *Sugar Cane* 14–21.

Gallacher, D. J., Lee, D. J., and Berding, N. (1995). Use of isozyme phenotypes for rapid discrimination among sugarcane clones. *Australian Journal of Agricultural Research* **46**, 601–9.

Glaszmann, J. C., Fautret, A., Noyer, J. L., Feldmann, P., and Lanaud, C. (1989). Biochemical genetic markers in sugarcane. *Theoretical and Applied Genetics* **78**, 537–43.

Gottlieb, L. D. (1982). Conservation and duplication of isozymes in plants. *Science* **216**, 373–80.

Grassl, C. O. (1962). Problems and potentialities of intergeneric hybridization in a sugar cane breeding programme. *Proceedings of the International Society of Sugar Cane Technologists* **11**, 447–56.

Heinz, D. J., and Tew, T. L. (1987). Hybridization procedures. *In* 'Sugarcane Improvement through Breeding'. (Ed. D. J. Heinz.) pp. 313–42. (Elsevier: Amsterdam.)

Lee, D. J. (1994). IK76–41: *Erianthus arundinaceus* or a intergeneric hybrid? *Proceedings of the Australian Society of Sugar Cane Technologists* **16**, 357.

Lee, D. J., Berding, N., and Bielig, L. M. (1993). *Saccharum*×*Erianthus* intergeneric breeding: pollination studies. *Proceedings of the Australian Society of Sugar Cane Technologists* **15**, 244–50.

Moore, P. H. (1987). Anatomy and morphology. *In* 'Sugarcane Improvement through Breeding'. (Ed. D. J. Heinz.) pp. 85–142. (Elsevier: Amsterdam.)

Mukherjee, S. K. (1957). Origin and distribution of *Saccharum*. *Botanical Gazette* **119**, 55–61.

Nagai, C., Ahloowalia, B. S., and Tew, T. L. (1991). Somaclonal variants from an intergeneric hybrid: *Saccharum*×*Erianthus*. *Euphytica* **53**, 193–9.

Richardson, B. J., Baverstock, P. R., and Adams, M. (1986). 'Allozyme Electrophoresis.' (Academic Press: Sydney.)

Roach, B. T. (1989). Origin and improvement of the genetic base of sugarcane. *Proceedings of the Australian Society of Sugar Cane Technologists* **11**, 34–47.

Roughan, P. G., Waldron, J. C., and Glasziou, K. T. (1971). Starch inheritance in *Saccharum*. Enzyme polymorphism for β-Amylase in interspecific and intergeneric hybrids. *Proceedings of the International Society of Sugar Cane Technologists* **14**, 257–65.

Soltis, D. E., Haufler, C. H., Darrow, D. C., and Gastony, G. J. (1983). Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers and staining schedules. *American Fern Journal* **73**, 9–27.

Stevenson, G. C. (1965). 'Genetics and Breeding of Sugarcane.' (Longmans: London.)

Tung, L. Y., Se, C. S., Song, L., and Chao, L. H. (1973). Studies on isozymes of sugarcane I. Peroxidase and esterase isoenzymes in major Taiwan sugarcane varieties and their parents. *Report of the Taiwan Sugar Research Institute* **62**, 63–81.

Waldron, J. C., and Glasziou, K. T. (1971). Isoenzymes as a method of varietal identification in sugarcane. *Proceedings of the International Society of Sugar Cane Technologists* **14**, 249–56.

Wendel, J. F., and Weeden, N. F. (1989). Visualization and interpretation of plant isozymes. *In* 'Isozymes in Plant Biology'. (Eds D. E. Soltis and P. S. Soltis.) pp. 5–45. (Dioscorides Press: Portland, OR.)

Wood, B., and Strand, A. (1989). Characterization of the world collection of sugarcanes and related grasses by isozyme analysis. Report to ISSCT Breeding Committee.

Manuscript received 27 November 1996, accepted 16 March 1998