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P. Besse · C. L. McIntyre · N. Berding

Ribosomal DNA variations in *Erianthus*, a wild sugarcane relative (*Andropogoneae-Saccharinae*)

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Abstract Variation at the 18S + 26S and 5S ribosomal DNA loci was assessed on 62 *Erianthus* Michx. clones, representing 11 species, and 15 clones from two *Saccharum* L. species used as a reference. Genus-specific markers for *Erianthus* Michx. sect. *Ripidium* Henrard (Old World species) were identified. Ribosomal DNA units in *Erianthus* sect. *Ripidium* exhibited an additional *Bam*HI site compared to *Saccharum*, and 5S units showed length and restriction-site differences between *Erianthus* and *Saccharum*. These markers will be useful to follow introgression in *Saccharum* × *Erianthus* hybrids. Six ribosomal units (for 18 + 26S genes) were revealed in *Erianthus* sect. *Ripidium*, differing by restriction-site positions and/or length. These results provided new information on species relationships and evolution within the genus *Erianthus*. The Indonesian and Indian forms of *E. arundinaceus* (Retz.) Jeswiet gave different restriction patterns, which were similar to those of *E. bengalense* (Retz.) R. C. Bharadwaja and *E. procerus* (Roxb.) Raizade, respectively. The two 2n = 20 species, *E. ele-phantinus* Hook.f. and *E. ravennae* (L.) P. Beauv., could also be differentiated at this locus. Two of the New World *Erianthus* species studied, *E. rufipilus* (Steud.) Griseb. and *E. longisetosus* Andersson, appeared more like *Erianthus* sect. *Ripidium*, whereas *E. trinii* Hack. and *E. brevibardis* Michx. showed patterns consistent with *Miscanthus sinensis* Andersson and *S. spontaneum* L., respectively. Finally, the comparison of rDNA restriction maps among *Erianthus* sect. *Ripidium*, *Saccharum*, sorghum and maize, led to unexpected conclusions concerning the relationships between the different genera and the position of *Erianthus* in the "*Saccharum* complex".

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P. Besse · C. L. McIntyre (✉)
CSIRO Division of Tropical Crops and Pastures, 306 Carmody Road, St Lucia, Qld 4067, Australia

N. Berding
BSES Meringa Experimental Station, Gordonvale, Qld 4865, Australia

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Introduction

Modern cultivated sugarcanes are derived mostly from interspecific crosses between *Saccharum officinarum* L. (the "noble cane") and *Saccharum spontaneum* L. More recently, introgression between *Saccharum* species and other related genera within the *Saccharinae* Benth. subtribe, such as *Erianthus*, has been achieved. Although they are characterised by a higher fibre content, attempts are being made to use *Erianthus* species, and *E. arundinaceus* in particular, in breeding programs in Australia, as a germplasm source of improved ratooning and vigour, drought and flooding tolerance, and resistance to *Pachymetra chaunoriza* (Croft and Dick) root rot.

From a taxonomic point of view, *Erianthus* sect. *Ripidium* is considered part of the "*Saccharum* complex", which includes five genera [*Saccharum*, *Erianthus* sect. *Ripidium*, *Miscanthus* sect. *Diandra* Keng, *Sclerostachya* (Hack.) A. Camus, and *Narenga* Bor] representing a closely related interbreeding group suggested to be involved in the origin of sugarcane (Daniels and Roach 1987). Of these five genera, *Erianthus* and *Miscanthus* are presumed to be the most primitive forms (Daniels et al. 1975).

As opposed to *Erianthus* sect. *Ripidium* (including seven Old World species), the rest of the genus *Erianthus* (New World species) is not considered to belong to the "*Saccharum* complex" (Grassl 1972). However, this classification is still under review and some New World species, such as *E. rufipilus*, are in the process of being transferred to sect. *Ripidium* (Daniels and Roach 1987).

Molecular markers from *Erianthus* would be useful in order to: (1) define genus-specific markers to follow introgression of *Erianthus* chromosomes in intergeneric hybrids, (2) examine the relationships among the different *Erianthus* species, (3) assess the difference between

New and Old World *Erianthus* species, and (4) examine the relationship between *Erianthus* and related genera such as sugarcane, maize and sorghum.

Nuclear ribosomal DNA loci (including 26 + 18S, and 5S loci) have been widely described (Flavell 1980; Rogers and Bendich 1987; Hemleben et al. 1988, 1992; Lapitan 1992), and have been employed to assess phylogenetic relationships among related species and genera. Indeed, due to their relatively rapid rates of concerted evolution, these sequences provide restriction fragment length polymorphism (RFLP) patterns that are generally highly homogeneous within groups, and show characteristic heterogeneity between groups (Dover et al. 1982; Zimmer et al. 1988). Study of these markers includes detailed sequencing, especially for 5S genes (Scoles et al. 1988; Dvorak et al. 1989; Lagudah et al. 1989; Reddy and Appels 1989; McIntyre et al. 1990), as well as RFLP studies. The latter have already proved to be successful in revealing length or sequence variations in rDNA repeat units that have been used in a large number of plant species to assess genetic diversity (Saghai Maroof et al. 1984; Flavell et al. 1986; Cordesse et al. 1990; Sano and Sano 1990; Chalmers et al. 1992; Lanaud et al. 1992; Besse et al. 1993; Laurent et al. 1993) or phylogenetic relationships (Jorgensen et al. 1987; Zimmer et al. 1988; Delseny et al. 1990; Dubcovsky et al. 1992; Faivre-Rampant et al. 1992; Havey 1992; Hilu and Johnson 1992; Kim et al. 1992; Terauchi et al. 1992; Borisjuk et al. 1993). Previous studies in *Saccharum* using rDNA markers (Springer et al. 1989; Glaszmann et al. 1990) suggested that they would be appropriate molecular markers in *Erianthus* to help resolve the previously mentioned issues.

Materials and methods

Materials

Sixty two *Erianthus* individuals were studied, representing 11 different species (see Table 1). Fifteen *Saccharum* representatives (nine *S. spontaneum* and six *S. officinarum*) were also studied as references (see Table 1).

The majority of the clones studied were obtained from the Australian Sugarcane Genetic Resource Center located at the Bureau of Sugar Experimental Station (BSES) at Gordonvale (Queensland, Australia). Most of these clones (especially for *E. arundinaceus*) were collected in Indonesia (IJ, IS, IK) (Berding and Koike 1980), and a few others in India (SES). Some clones from a USDA collection (Canal Point, USA) were kindly supplied by Dr David Burner, USDA ARS, Houma, Louisiana (see Table 1).

Leaves were collected, freeze dried, ground, and kept at −20 °C before use. Four nuclear ribosomal probes were used: pTA71, a complete rDNA sub-repeat from wheat (Gerlach and Bedbrook 1979) was used to study variations at the 5.8 + 18 + 26S RNA gene level. For more detailed investigations, two pTA71 subclones (kindly provided by Prof. Quetier, Paris XI University, France) were used, consisting of the 4.4-kb *Eco*RI-*Bam*HI fragment covering the intergenic spacer (IGS) and parts of the 18 and 26S regions, and the 3.7-kb *Bam*HI-*Bam*HI fragment located within the coding region. The clone pScT7, isolated from rye (Lawrence and Appels 1986), was used for 5S ribosomal gene investigations.

Methods

Genomic DNA was extracted according to Hoisington (1992) using CetylTrimethyl Ammonium Bromide methodology. Ten micrograms of DNA were restriction-digested according to the supplier's instructions (Promega). Electrophoresis of restricted DNA was performed in 0.8% agarose gels, with a Tris Borate EDTA buffer, and run for 10 h at 1.7 V/cm. After treatment in 0.25 N HCl for 15 min, gels were Southern blotted onto Hybond™ N^+ (Amersham) membranes in 0.4 N NaOH. Probes were separated from the plasmid by digestion using the appropriate restriction enzymes, electrophoresis in a low-melting-point agarose gel, and excision from the gel (Hoisington 1992). Probes (30 ng) were ^{32}P-labelled by random priming using the Megaprime™ (Amersham) labelling system. Hybridisation and washing procedures were performed as described previously (Besse et al. 1993), but at 68 °C without formamide.

Results and discussion

Ribosomal DNA variations in *Erianthus* and *Saccharum*

18 + 26S rRNA genes

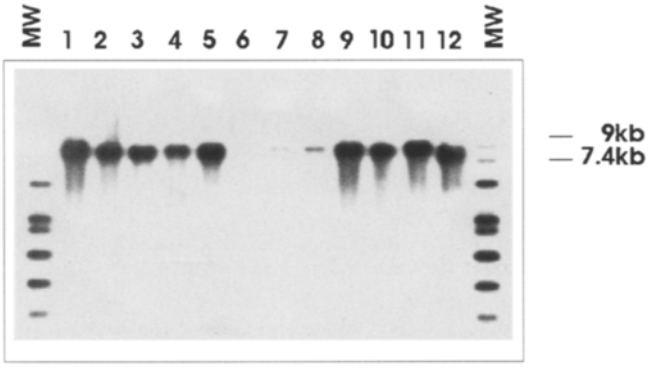
A subset of the collection (composed of at least one representative of each species) was digested with different restriction enzymes to assess the level of polymorphism obtained with each enzyme.

The enzymes *Eco*RV, *Bst*EII, *Xba*I and *Eco*RI (Fig. 1) cut only once per repeat unit. The size of ribosomal units can be evaluated and lies roughly between 7.4 and 9 kb. These enzymes were assumed to cut only once per repeat unit across the entire experimental population.

The enzymes *Bam*HI and *Sac*I revealed restriction fragment length polymorphism, and the entire collection was thus digested with them to assess fragment variation.

Based on the RFLP profiles revealed with *Bam*HI and *Sac*I, *Bam*HI × *Sac*I double-restriction digests, and by hybridisation with pTA71 and its two subclones, restriction mapping of the ribosomal DNA units was

Fig. 1 *Eco*RI digests hybridised with pTA71. *Lanes 1 to 12* are: (*1*) IK 76-48, (*2*) IS76-120, (*3*) IS76-140, (*4*) IS76-188, (*5*) IJ76-502, (*6*) E. bengalense, (*7*) SES 311, (*8*) US 67-8-1, (*9*) SES 273, (*10*) SES 305, (*11*) Mandalay, (*12*) POJ 213. Molecular-weight-marker (*MW*) Raoul (Appligene, France) fragment sizes are: 9, 7.4, 5.6, 4.4, 3.9, 3.6, 2.9, 2.3, 1.8 kb. Relevant fragment sizes are shown



performed. Twelve different types of units were determined (Fig. 2).

Typical profiles obtained with *Bam*HI and *Sac*I digests for Type-I units are shown in Fig. 3. *Bam*HI profiles show a constant 3.8-kb fragment (Fig. 3 a), corresponding to the presence of two *Bam*HI sites in the coding region. *Sac*I profiles show 0.5-, 0.7-, 1.1- and 1.65-kb constant fragments (Fig. 3 b), resulting from the presence of five *Sac*I sites. Length variations are revealed (5.6-, 5.5-, 4.7- and 4.2-kb *Bam*HI variants, see Fig. 3), defining Type-Ia, -Ib, -Ic and -Id units (Fig. 2).

As opposed to Type-I units, Type-II and -III units are characterised by the occurrence of an additional *Bam*HI site in the IGS (Fig. 2), leading to a characteristic 2.7-kb restriction fragment on the *Bam*HI blots (Fig. 4 a)

Type-II units are drawn in Fig. 2. They show sites in the coding region that are very similar to that of Type-I units, but the *Sac*I site (presumably located in the small spacer) is available, or present, in only a fraction of the subunits, since a 1.8-kb fragment is also present, in addition to the 1.1-kb and 0.7-kb fragments (lanes 1–3, Fig. 4b). From these experiments, it cannot be determined whether this variation is due to a real presence/absence or to differential methylation of the site. Length variations are revealed in Type-II units, and define Type-IIa, b, c, d, and e units corresponding to IGS length variants (*Bam*HI fragments) of 3.5, 3.1, 2.7, 2.6, and 2.5 kb, respectively (Fig. 2). These variations can be detected on *Sac*I and *Bam*HI digests (lane 1, Fig. 4a, b). However, the occurrence of these length variations was more precisely investigated using *Bam*HI × *Sac*I double digests and hybridisation with the 4.4-kb spacer fragment probe (Fig. 5). Corresponding major variants of 1.8 kb (IIc unit, lanes 1–7), 1.6 kb (IId units, lane 8) and 1.5 kb (IIe units, lanes 8–9) are revealed. In addition to the major 1.8-kb variant (IIc units), some individuals exhibit minor length variants of 2.2 kb (IIb units) and 2.6 kb (II a units) (lanes 2–5 on Fig. 5).

Type-III units are of the same length as Type-IIc (Fig. 2, lane 7 Fig. 5). However, they are characterised by the total absence of the *Sac*I site in the small spacer (no 0.7- nor 1.1-kb fragments were detected, see lanes 4–6 on Fig. 4b). The possible occurrence of an additional *Sac*I site, leading to the presence of an additional faint band at 1.2 kb on the hybridisation profiles, has not yet been elucidated.

Type-IV units are characterised by the presence of a different *Bam*HI site in the IGS (Fig. 2), leading to the occurrence of a new 1.8-kb *Bam*HI fragment (lane 2, Fig. 6a). Two length-variants of 5.0 and 3.0 kb were revealed, defining Type-IVa and -IVb units, respectively (Fig. 2). These length-variants were investigated using *Eco*RI × *Bam*HI double digests (Fig. 6c). In Type-I or -IV units, these digests reveal, constant fragments of 3.8 and 1.0 kb, together with one for 4.8 kb resulting from a non-availability or methylation of the central *Bam*HI site. An additional 0.8-kb fragment is revealed in the Type-IV units (lane 1 Fig. 6c), as well as spacer fragments of 3.0 kb (lane 1 Fig. 6c, Type IVb) and 4.0 kb (Type IVa), the latter corresponding to the 5.0-kb length-variant characterised by non-digestion (or absence) of the additional *Bam*HI site. Both variants are revealed within the same individual.

The overall structure for rDNA units obtained for *Erianthus* species is consistent with those previously described in *Saccharum* and its relatives (Springer et al. 1989; Glaszmann et al. 1990). As far as the coding region is concerned, restriction sites described for *Erianthus* and sugarcane (especially those for *Xba*I, *Eco*RV, *Bam*HI and *Eco*RI) appear highly conserved within the family Poaceae, including rice, wheat, barley, maize, sorghum, rye, finger millet, *Tripsacum*, *Aegilops*, and

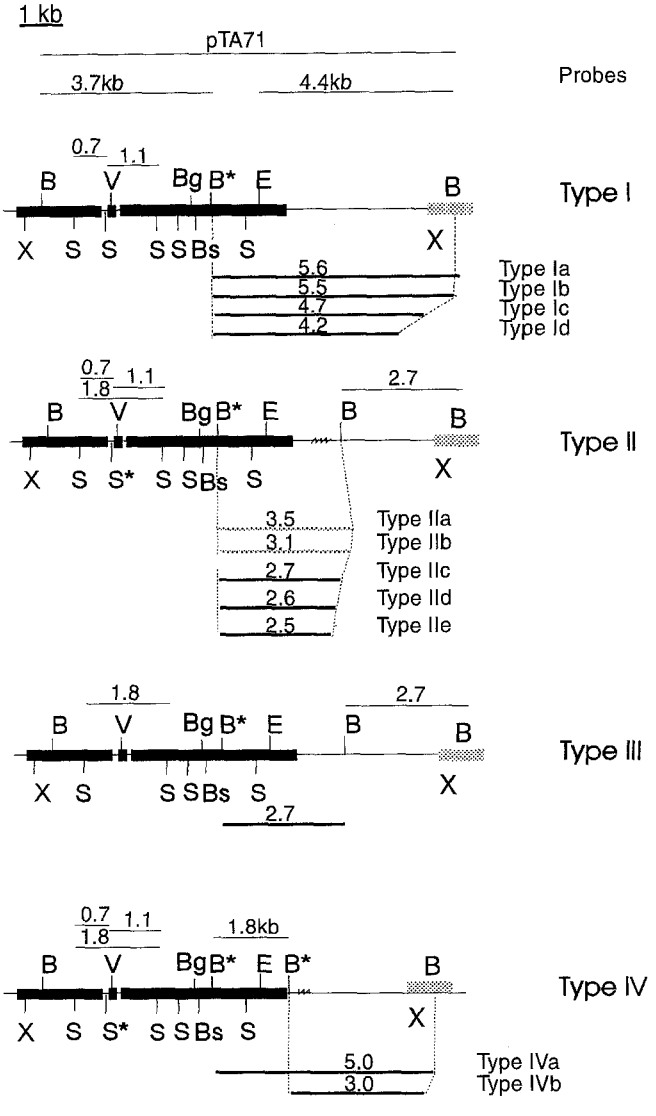


Fig. 2 Restriction map of ribosomal DNA units (18 + 26S) in *Erianthus* and *Saccharum* for the restriction enzymes *Bam*HI(*B*), *Bgl*II (*Bg*), *Bst*EII (*Bs*), *Eco*RI (*E*), *Eco*RV (*V*), *Sac*I (*S*), *Xba*I (*X*). Restriction sites labelled * are absent or non available in a portion of the sub-units. Diagnostic specific fragments, as well as *Bam*HI intergenic spacer length variants, are drawn. The *shaded area* of the gene represents the adjacent ribosomal unit

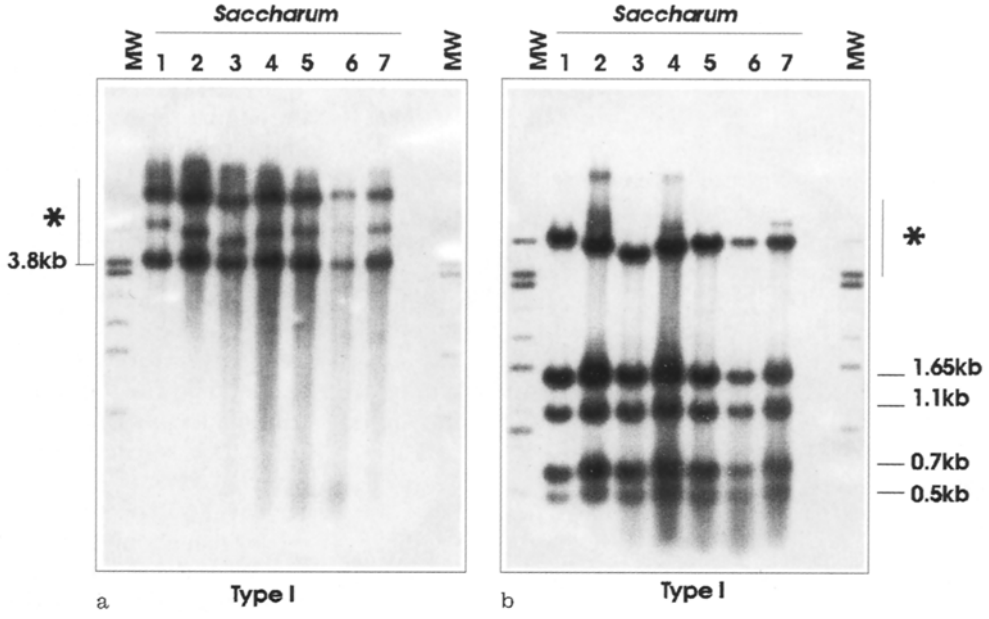


Fig. 3 *Bam*HI (**a**) and *Sac*I (**b**) digests hybridised with pTA71 showing profiles characteristic of Type-I units. *Lanes 1 to 7* are (*1*) Mandalay, (*2*) SES11, (*3*) Coimbatore, (*4*) Glagah 1286, (*5*) Mol 5801, (*6*) Mol 5903, (*7*) Mol 5904. Molecular-weight-marker (*MW*) Raoul (Appligene, France) fragment sizes are: 5.6, 4.4, 3.9, 3.6, 2.9, 2.3, 1.8, 1.4, 1.2, 1.0, 0.9, 0.55, 0.37 kb. Sizes of constant fragments are indicated, as well as length variants (*)

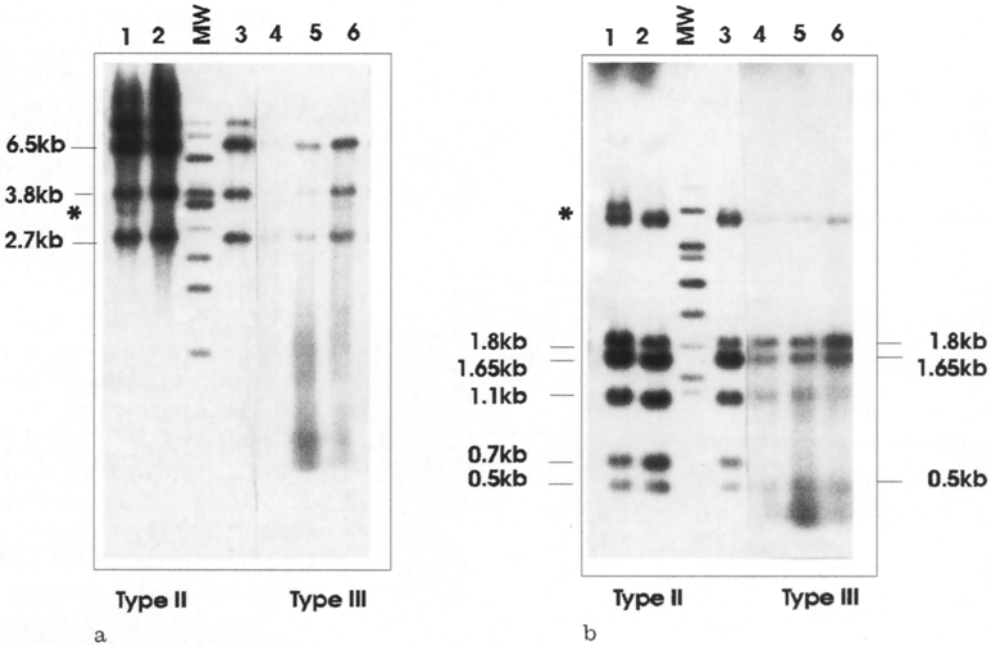


Fig. 4 *Bam*HI (**a**) and *Sac*I (**b**) digests hybridised, (**a**) with pTA71, (**b**) with the 3.7-kb fragment, showing profiles characteristic of Type-II and -III units. *Lanes 1 to 6* are (*1*) IJ76-502, (*2*) IJ76-508, (*3*) IJ76-408 (Type-II), (*4*) *E. procerus*, (*5*) SES 311, (*6*) SES 314 (Type-III). Molecular-weight-marker (*MW*) Raoul (Appligene, France) fragment sizes are: 9, 7.4, 5.6, 4.4, 3.9, 3.6, 2.9, 2.3, 1.8, 1.4, 1.2 kb. Sizes of constant fragments are indicated, as well as length variants (*)

Elymus (Gerlach and Bedbrook 1979; Appels et al. 1980; Zimmer et al. 1988; Springer et al. 1989; Sano and Sano 1990; Dubcovsky et al. 1992; Hilu and Johnson 1992; Kim et al. 1992). The site variations revealed are located in non-coding regions, which is consistent with the known evolutionary process of ribosomal RNA genes (Appels and Honeycutt 1986; Zimmer et al. 1988; Delseny et al. 1990).

Based on the variations revealed for the *Bam*HI and *Sac*I digests, the entire experimental population was assigned ribosomal unit types. The results are given in Table 1.

5S ribosomal RNA genes

The entire population was digested with *Bam*HI and hybridised with the 5S ribosomal probe pScT7 (Fig. 7). Hybridisation profiles show a characteristic ladder pattern (Fig. 7). Two length-variants of 500 and 370 bp are

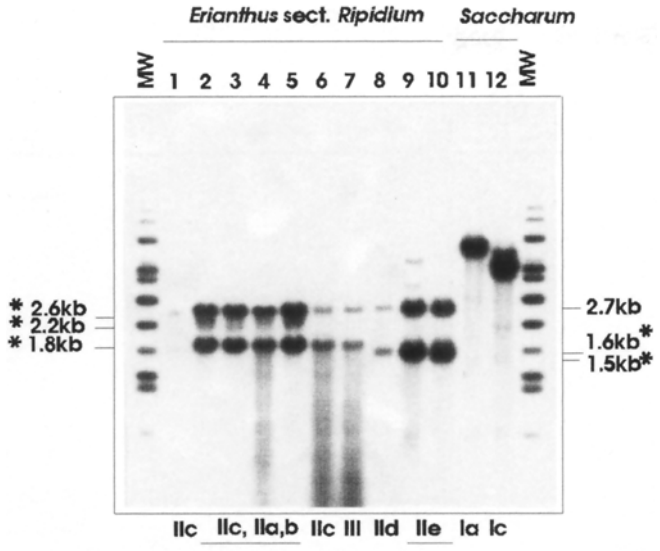


Fig. 5 *Bam*HI × *Sac*I double digests hybridised with the 4.4-kb probe, showing spacer length variations characteristic of Type-II and -III units. *Lanes 1 to 12* are: (*1*) IK 76-48, (*2*) IK 76-120, (*3*) IK 76-140, (*4*) IK76-188, (*5*) IK76-502, (*6*) E. bengalense, (*7*) SES 311, (*8*) US67-8-1, (*9*) SES 273, (*10*) SES 305, (*11*) Mandalay, (*12*) POJ 213. Molecular-weight-marker (*MW*) Raoul (Appligene, France) fragment sizes are: 9, 7.4, 5.6, 4.4, 3.9, 3.6, 2.9, 2.3, 1.8, 1.4, 1.2, 0.75 kb

revealed. Restriction-site variations at the 5S level were also evaluated. The results (only the minimum number of restriction sites detected can be given, due to the RFLP nature of our study and the high methylation pattern of 5S units) are presented in Table 2. They show that the 500-bp length and 370-bp length 5S units also differ in the number of *Taq*I restriction sites.

The distribution of 5S *Bam*HI length variations (370 and 500 bp) in the entire experimental population is given in Table 1.

Genus-specific markers for *Erianthus* sect. *Ripidium*

18 + 26 S rRNA genes

The 58 *Erianthus* sect. *Ripidium* clones tested show either Type-II or -III units (Table 1), both characterised by the presence of an additional *Bam*HI in the spacer. None of the *Saccharum* representatives tested had this site, all of them exhibited Type-I units (Figs. 2 and 3). The occurrence of this additional *Bam*HI site leads to the detection of an additional and *Erianthus* sect. *Ripidium*-specific 2.7-kb *Bam*HI fragment (Figs 4 a, 5). This result is consistent with previous work by Glaszmann et al. (1990) involving one *Erianthus* representative. The absence of this site in *Saccharum* has been demonstrated in two *Saccharum* species. By looking at *Bam*HI-site localisation in the ribosomal unit types described for other *Saccharum* species (Glaszmann et al. 1990), it can be seen that this site is also absent from *S. robustum* Brandes and Jeswiet ex Grassl, *S. sinense* Roxb., *S. barberi* Jeswiet and even *Miscanthus* Anderson. Thus, the *Erianthus* sect. *Ripidium* specificity of this *Bam*HI site is clearly demonstrated.

5S ribosomal RNA genes

The length of the 5S unit differs between *Erianthus* sect. *Ripidium* and *Saccharum*. All the *Erianthus* sect. *Ripidium* individuals show 5S units that were 500 bp in length, whereas all the *S. spontaneum* and *S. officinarum* accessions studied exhibit 370-bp length 5S units (Fig. 7, Table 1).

Genus-specific markers were identified at both the ribosomal DNA and 5S levels. This was expected due to the important morphological differences between *Erian-*

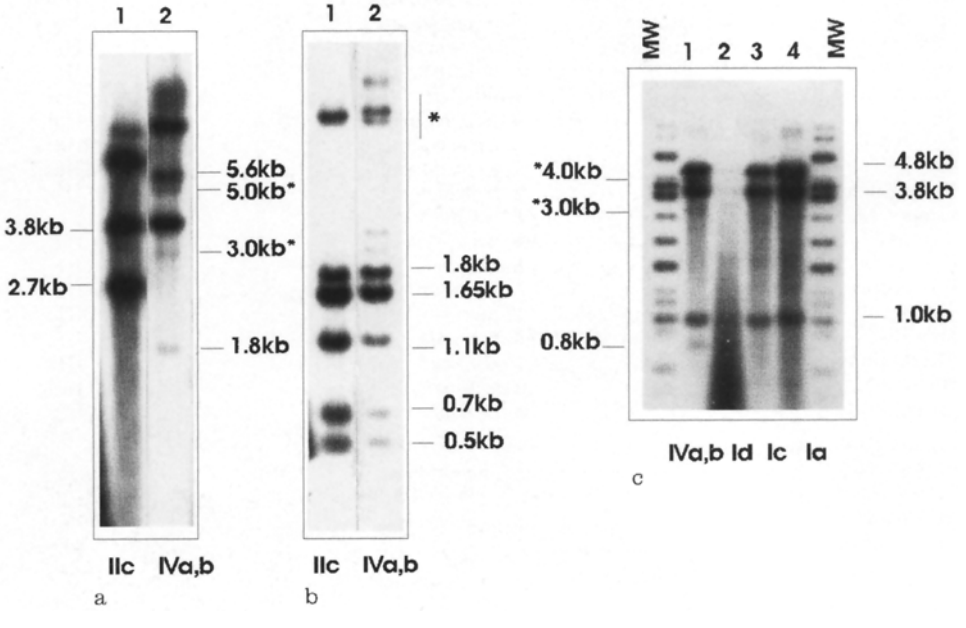


Fig. 6 *Bam*HI (**a**) and *Sac*I (*b*) digests hybridised with pTA71 showing a comparison of Type-II and -IV units. *Lanes 1–2* are: (*1*) US5711-2, (*2*) US63-6. *Eco*RI × *Bam*HI digests (**c**) hybridised with pTA71 showing a comparison of Type-I and -IV units. *Lanes* 1–4 are (*1*) US63-6, (*2*) US68-01-4, (*3*) Coimbatore, (*4*) Mol 5801. Molecular-weight-marker (*MW*) Raoul (Appligene, France) fragment sizes are: 9, 7.4, 5.6, 4.4, 3.9, 3.6, 2.9, 2.3, 1.8, 1.4, 1.2, 1.0, 0.9, 0.55, 0.37 kb. Length of relevant constant fragments are indicated, as well as IGS length variants (*)

Table 1 List of the clones studied and corresponding ribosomal unit types and 5S unit length. Individuals come from the Meringa B.S.E.S. collection, except * from the U.S.D.A. collection. When clones in the collection are named after the species name, their name is printed in plain to avoid confusion

Clone		Species	2n[a]	Ribosomal unit type	5S length (bp)
Erianthus sect *Ripidium* (Old World species)					
IJ 76-332	IJ =	*E. arundinaceus*	60	IIc	500
IJ 76-334	Irian Jaya	*E. arundinaceus*		IIc	500
IJ 76-346		*E. arundinaceus*		IIc	500
IJ 76-357		*E. arundinaceus*		IIc	500
IJ 76-365		*E. arundinaceus*		IIc	500
IJ 76-378		*E. arundinaceus*		IIc	500
IJ 76-384		*E. arundinaceus*	60	IIc	500
IJ 76-388		*E. arundinaceus*		IIc	500
IJ 76-394		*E. arundinaceus*	60	IIc	500
IJ 76-397		*E. arundinaceus*		IIc	500
IJ 76-400		*E. arundinaceus*	60	IIc	500
IJ 76-404		*E. arundinaceus*		IIc IIab	500
IJ 76-407		*E. arundinaceus*	60	IIc	500
IJ 76-408		*E. arundinaceus*	60	IIc IIab	500
IJ 76-410		*E. arundinaceus*		IIc	500
IJ 76-476		*E. arundinaceus*		IIc IIab	500
IJ 76-485		*E. arundinaceus*		IIc	500
IJ 76-502		*E. arundinaceus*	60	IIc IIab	500
IJ 76-508		*E. arundinaceus*	60	IIc	500
IK 76-20	IK =	*E. arundinaceus*		IIc	500
IK 76-24	Kalimantan	*E. arundinaceus*		IIc	500
IK 76-41		*E. arundinaceus*		IIc	500
IK 76-45		*E. arundinaceus*		IIc	500
IK 76-48		*E. arundinaceus*		IIc IIab	500
IK 76-63		*E. arundinaceus*	60	IIc IIab	500
IK 76-79		*E. arundinaceus*		IIc	500
IK 76-88		*E. arundinaceus*	60	IIc	500
IK 76-101		*E. arundinaceus*	60	IIc	500
IK 76-111		*E. arundinaceus*		IIc IIab	500
IS 76-120	IS =	*E. arundinaceus*		IIc IIab	500
IS 76-126	Sulawesi	*E. arundinaceus*	60	IIc	500
IS 76-133		*E. arundinaceus*		IIc	500
IS 76-140		*E. arundinaceus*		IIc IIab	500
IS 76-150		*E. arundinaceus*		IIc IIab	500
IS 76-162		*E. arundinaceus*		IIc IIab	500
IS 76-172		*E. arundinaceus*	60	IIc	500
IS 76-188	IS =	*E. arundinaceus*		IIc IIab	500
IS 76-202		*E. arundinaceus*	60	IIc	500
IS 76-220		*E. arundinaceus*	60	IIc	500
MINDINAO		*E. arundinaceus*		IIc	500
USDA 2798		*E. arundinaceus*		IIc	500
NG 28-7	New Guinea	*E. arundinaceus*	60	IIc	500
SES 181	SES = India	*E. arundinaceus*	40	III	500
SES 300		*E. arundinaceus*	30	III	500
Hep. Kwa-1546		Unknown		IIc	500
SES 273		*E. elephantinus*	20	IIe	500
SES 305		*E. elephantinus*	20	IIe	500
SES 372		*E. elephantinus*	20	IIe	500
E. bengalense		E. bengalense		IIc	500
*Imp 9751		*E. bengalense*		IIc	500
E. kanal		*E. kanal*		IId	500
E. sarpet		*E. sarpet*		III	500
E. procerus		*E. procerus*		III	500
SES 311	India	*E. procerus*	40	III	500
SES 314		*E. procerus*	40	III	500
*Kalimpong		*E. procerus*	40	III	500
US 67-8-1		*E. ravennae*	20	IId	500
US59-27-11		*E. ravennae*		IId	500
Erianthus (New World species)					
*US 57-11-2		*E. longisetosus*	60	IIc	500
*US 57-60-2		*E. rufipilus*	20	IId	500
*US 63-6		*E. brevibarbis*	72	IVab	370
*US 68-01-4		*E. trinii*	38	Id	370

Table 1 (Continued)

Clone	Species	2n[a]	Ribosomal unit type	5S length (bp)
Saccharum species				
MANDALAY	*S. spontaneum*	96	Ia	370
SES 11	*S. spontaneum*	64	Ib	370
COIMBATORE	*S. spontaneum*	64	Ic	370
GLAGAH 1286	*S. spontaneum*	112	Ib	370
MOL 5801	*S. spontaneum*	80	Ia	370
MOL 5903	*S. spontaneum*	80	Ia	370
MOL 5904	*S. spontaneum*	80	Ia	370
NG 28-101	*S. spontaneum*	80	Ia	370
NG 51-2	*S. spontaneum*	80	Ia	370
POJ 213	*S. officinarum*	80	Ic	370
OTAMITE	*S. officinarum*	80	Ic	370
CHITTAN	*S. officinarum*	80	Ic	370
BADILA	*S. officinarum*	80	Ic	370
ASHY MAURITIUS	*S. officinarum*	80	Ic	370
BLACK CHERIBON	*S. officinarum*	80	Ic	370

[a] Chromosome numbers for *Erianthus* are according to Mohan and Sreenivasan (1983) and Burner (1991). Chromosome numbers for *S. officinarum* are according to Sreenivasan and Nair (1991) and for *S. spontaneum* according to Panje and Babu (1960) and Kandasami et al. (1983)

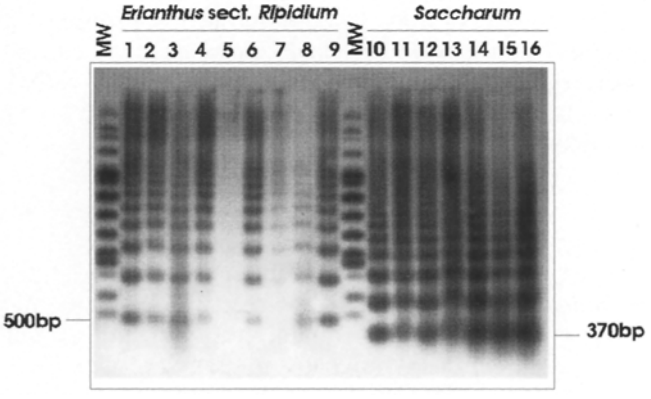


Fig. 7 *Bam*HI digests hybridised with 5S probe pScT7. *Lanes 1–9* are: (*1*) IK76-41, (*2*) IK 76-45, (*3*) IK76-48, (*4*) IK76-63, (*5*) IK76-79, (*6*) IK76-88, (*7*) IK76-101, (*8*) IK76-111, (*9*) IJ76-408; *Lanes* 10–16 are (*10*) Mandalay, (*11*) SES11, (*12*) Coimbatore, (*13*) Glagah 1286, (*14*) Mol 5801, (*15*) Mol 5903, (*16*) Mol 5904. Molecular-weight-marker (*MW*) Raoul (Appligene, France) fragment sizes are: 9, 7.4, 5.6, 4.4, 3.9, 3.6, 2.9, 2.3, 1.8, 1.4, 1.2, 1.0, 0.75, 0.55 kb

Table 2 5S ribosomal unit site variation in *Erianthus* and *Saccharum*

Enzymes	Minimum number of detectable restriction sites in 5S units	
	500 bp *Erianthus*[a]	370 bp *Saccharum*[a]
*Bam*HI	1	1
*Bgl*II	0	0
*Bst*EII	1	1
*Eco*RI	0	0
*Eco*RV	0	0
*Sac*I	0	0
*Sca*I	1	1
*Taq*I	2	0
*Xba*I	0	0

[a] For a detailed description of 5S unit length in the collection, see Table 1

thus sect. *Ripidium* and *Saccharum*. These genera were also easily differentiated using flavonoids (Williams et al. 1974), isozymes (Waldron and Glaziou 1972), lipoids (Vijayalakshmi and Rao 1963), and cytoplasmic DNA markers (D'Hont et al. 1993). Ribosomal DNA markers have already proven successful in checking putative hybrids in intergeneric crosses involving *Erianthus* (data not shown). The usefulness of PCR amplification of 5S spacer length variations for this purpose has also been demonstrated (D'Hont et al. 1995).

Species-specific rDNA variations within *Erianthus* sect. *Ripidium*

Species-specific variations were detected within *Erianthus* and will provide reliable tools to assist germplasm characterisation. Information concerning the relationships and grouping of the different species surveyed can be obtained by comparing the ribosomal DNA variations detected.

E. arundinaceus, *E. bengalense*, *E. procerus*

All of the *E. arundinaceus* clones from Indonesia (39 individuals) show Type-IIc units (Table 1, lanes 1–3 Fig. 4, lanes 1–5 Fig. 5). Interestingly, NG 28-7, known as the only *E. arundinaceus* specimen collected in New Guinea (Berding and Roach 1987), shows similar profiles to that of Indonesian *E. arundinaceus* clones, as do Mindinao (presumably collected from the Philippines), USDA 2798, and Hep Kwa 1546 (unclassified) (Table 1). The two Indian *E. bengalense* clones studied exhibit Type-IIc units (Table 1, lane 6 Fig. 5). The four *E. procerus* individuals, as well as the two *E. arundinaceus* Indian clones SES 181 and 300, exhibit Type-III units (Table 1, lanes 4–6 Fig. 4, lane 7 Fig. 5).

Thus, the Indonesian forms of *E. arundinaceus* differ from the Indian forms. Moreover, the *E. arundinaceus* clones from India are identical to *E. procerus* individuals. This result is in accordance with the suggestion that *E. procerus* is closely related to *E. arundinaceus* (Daniels and Roach 1987), but only as far as Indian forms of *E. arundinaceus* are concerned, and can be related to the common Indian origin of these individuals. This result is also consistent with the cytotype, as the Indian *E. arundinaceus* and *E. procerus* clones studied show chromosome numbers of $2n = 30–40$, whereas all the Indonesian *E. arundinaceus* clones are $2n = 60$. However, this contrasts with the flavonoid studies which showed that *E. arundinaceus* (either from India or Indonesia) and *E. procerus* were different (flavonoid F8: Daniels et al. 1975, 1980).

On the other hand, the *E. arundinaceus* individuals from Indonesia appear very similar to the Indian *E. bengalense* clones at the rDNA level, though they are very different morphologically. *E. arundinaceus* is a large plant with well-differentiated stalks, while *E. bengalense* has very small vegetative stalks (Daniels and Roach 1987).

In both cases, discrepancies with flavonoid or morphological markers can be explained by the fact that the variations revealed in rDNA reflect more ancient variations than the other markers. The *E. procerus* and Indian *E. arundinaceus* (Type III) clones are the only ones in *Erianthus* sect. *Ripidium* differentiated by a restriction-site variation in the rDNA unit. Due to the evolutionary pattern of ribosomal genes, this indicates that these Indian *Erianthus* species might have a different origin from the *E. arundinaceus* individuals from Indonesia and from the Indian *E. bengalense* clones also. The present results also indicate that Indian *E. arundinaceus* and *E. procerus* on one hand, and Indonesian *E. arundinaceus* and Indian *E. bengalense* on the other hand, might have a common origin (as revealed by rDNA variations and chromosome number), but have then evolved differently (as revealed by flavonoid or morphological studies).

Intraspecific variation in E. arundinaceus

Among the 39 *E. arundinaceus* individuals from Indonesia studied, some exhibit two other minor units (fainter hybridisation of the corresponding IGS length-variants), Type IIa and IIb, in addition to the major Type-IIc unit (Table 1, lane 1 Fig. 4, lanes 2–5 Fig. 5).

The rapid divergence of IGS length-variants implies that they generally become rapidly specific to a species, to sub-groups within a species, or even to individuals from geographically different locations. Within *E. arundinaceus*, no correlation between IGS length-variants and geographical origin can be revealed. This could be related to the fact that the geographical areas surveyed (Sulawesi, Kalimantan and Irian Jaya) are not sufficiently climatically or geographically different to allow differentiation. This indicates that all the *E. arundinaceus* individuals surveyed represent a very narrow genetic pool, and that this species shows low levels of polymorphism, at least at their rDNA level. *E. arundinaceus* clones collected from Kalimantan to Jaya are described as being virtually uniform as far as morphological markers are concerned (Berding and Roach 1987). This is in accordance with the present results on rDNA IGS length variations.

E. ravennae, E. elephantinus

The two *E. ravennae* representatives exhibit Type-IId rDNA units (Table 1, lane 8 Fig. 5), and the three *E. elephantinus* individuals exhibit Type-IIc units (Table 1, lanes 9–10 Fig. 5). *E. ravennae* and *E. elephantinus* are very similar at both cytotype ($2n = 20$) and floral character levels. They are differentiated only by vegetative characters (Daniels and Roach 1987). Vijayalakhmi (1967) suggested that *E. elephantinus* is probably a specialised race of *E. ravennae*. However, they can be easily differentiated, as they exhibit different rDNA units, in accordance with a previous flavonoid study (Daniels et al. 1980).

E. kanal, E. sarpet

E. kanal and *E. sarpet* are not recognized taxonomic designations (Berding, personal communication), but represent previously assigned unrecognized classifications. As far as ribosomal DNA is concerned, *E. sarpet* can be considered as *E. procerus* (since it shows Type-III units, see Table 1) and *E. kanal* is very similar to *E. ravennae* (Type-IId units, see Table 1). Chromosome counts are needed to confirm this classification.

As already demonstrated in a large number of plant species, the present study showed the usefulness of ribosomal DNA markers for inferring phylogenetic relationships among *Erianthus* species. Indeed, the levels of intraspecific sequence variation appear to be very low at the rDNA level. This contrasts with isozymes (Waldron and Glaziou 1972) and flavonoid markers (Daniels et al. 1975, 1980) which revealed as much intraspecific as interspecific polymorphism in *Erianthus* and related genera. These markers will be useful in assigning new accessions or unclassified individuals to particular *Erianthus* species.

New World *Erianthus* species

E. longisetosus, E. rufipilus

E. longisetosus and *E. rufipilus* exhibit all the *Erianthus* sect. *Ripidium* specific markers. They show the specific

*Bam*HI site in the rDNA units and have 500-bp length 5S genes (Table 1). Moreover, *E. longisetosus* clone US57-11-2 shows rDNA units very similar to that of *E. arundinaceus* (Type-IIc units, lane 1 Fig. 6 a, b, c). Interestingly, this clone exhibits 60 chromosomes (Burner 1991), as do all the Indonesian *E. arundinaceus* clones (Mohan and Sreenivasan 1983), and has a stalk morphology typical of *E. arundinaceus* (D. Burner, personal communication). US57-60-2 *E. rufipilus* also shows rDNA profiles identical to those of *E. ravennae* (Type-IId units, data not shown). They both have a $2n = 20$ cytotype (Mohan and Sreenivasan 1983; Burner 1991) and show typical stalk morphology and an awned floret (D. Burner, personal communiction). It is unlikely that all these facts result from coincidence; rather they suggest that *E. rufipilus* and *E. longisetosus* may be better classified as *Erianthus* sect. *Ripidium*, since they exhibit all the *Erianthus* sect. *Ripidium* specific markers (at the rDNA and 5S levels). The possible re-classification of *E. rufipilus* to sect. *Ripidium* has already been suggested based on morphological evidence (Daniels and Roach 1987).

E. trinii, *E. brevibardis*

On the other hand, *E. trinii* and *E. brevibardis* do not appear to be part of sect. *Ripidium* as they do not exhibit any of the sect. *Ripidium* specific markers. Their 5S units are of the same length (370 bp, Table 1) as *Saccharum*.

E. brevibardis clone US63-6 exhibits Type-IVab units (Fig. 2, lane 2 Fig. 6 a, b, lane 1 Fig. 6 c, Table 1). These rDNA units are similar (same additional *Bam*HI site in the IGS) to those of some *S. spontaneum* clones from India described in a previous work (Glaszmann et al. 1990). The co-migration of RFLP fragments (and hence the similarity of rDNA units) was checked by running the New World species on the same gel as SES106B (data not shown), a *S. spontaneum* clone from India included in Glaszmann et al. (1990). Although this similarity could be an artefact due to the limited number of restriction sites surveyed, with-in the limits of the present study this *E. brevibardis* clone appears similar to Indian *S. spontaneum* clones. *E. brevibardis* has $2n = 72$ chromosomes, an atypical number for *Erianthus*, but normal for *S. spontaneum*, and its floret is unawned, like *S. spontaneum* (D. Burner, personal communication). These results suggest that this may be a clone of *S. spontaneum*.

The *E. trinii* clone US 68-1-4 shows Type-Id units, atypical of *Erianthus*. These units are of a similar length to those described by Glaszmann et al. (1990) for a *Miscanthus* individual. The chromosome number of US 68-1-4 is $2n = 38$ (D. Burner, personal communication), typical of *Miscanthus sinensis* (Daniels and Roach 1987). Moreover, the inflorescence of *E. trinii* clone US68-1-4 resembles that of *M. sinensis* (D. Burner, personal communication). This suggests that the US68-1-4 *E. trinii* clone may be better classified as *M. sinensis*. A similar result was obtained by Sobral et al. (1994) based on chloroplastic DNA analysis.

Comparison between *Erianthus* sect. *Ripidium*, *Saccharum*, *Zea* and *Sorghum*

A comparison of these different genera based on rDNA variations can be done using rDNA restriction maps previously defined for sorghum and maize by Springer et al. (1989). They showed that sorghum and sugarcane were closer to each other than either is to maize. By comparing ribosomal restriction maps in the coding sequences proposed for maize, sorghum (Springer et al. 1989), sugarcane (Springer et al. 1989; this study) and *Erianthus* (this study), the genus *Erianthus* can be classified with the sugarcane sorghum group (presence of the *Bst*EII site). A further comparison of these genera can be made by comparing restriction-site variations in non-coding sequences. The presence of an additional *Sac*I site near the *Eco*RV site was specific to sugarcane as opposed to sorghum and maize (Springer et al. 1989). In that sense, *E. sarpet*, *E. procerus* and *E. arundinaceus* clones from India (Type-III units) appear much closer to sorghum than to sugarcane, at least at the rDNA level, as they lack this additional *Sac*I site. All other *Erianthus* species (*E. arundinaceus* from Indonesia, *E. bengalense*, *E. kanal*, *E. procerus*, *E. elephantinus*, *E. ravennae*, *E. rufipilus*, and *E. longisetosus*) appear much closer to sugarcane in this respect. Sobral et al. (1994), based on a chloroplast study, and A1 Janabi et al. (1994), based on mitochondrial DNA analysis, demonstrated surprising evidence for a maternal lineage between *Erianthus* and *Sorghum*. The present study also tends to show the existence of nuclear relationships between sorghum and *Erianthus*, but only for *E. arundinaceus* from India and *E. procerus*. The present study also provides evidence of a nuclear lineage between sugarcane and all the other *Erianthus* species studied.

Due to their concerted mode of evolution, rDNA genes are informative markers to examine relationships between species and genera, as already proved in maize and *Tripsacum* (Zimmer et al. 1988), pea, pumpkin and wheat (Jorgensen et al. 1987), *Brassica* spp. (Delseny et al. 1990) and *Solanum* spp. (Borisjuk et al. 1993). These markers have also demonstrated their usefulness in the present study, since their analysis allowed the definition of *Erianthus* genus-specific markers that have already been used effectively to assess the legitimacy of *Saccharum* × *Erianthus* introgression populations. Although some of the species were only represented by a few clones, due to the non-availability of more representatives in the collections, useful information on the relationships among the different *Erianthus* species studied, as well as among *Erianthus* and related genera, has been obtained. Although very powerful as molecular markers, ribosomal DNA genes only represent a small part of the genome. Therefore, in order to complement

this analysis, the present *Erianthus* germplasm collection is in the process of being evaluated by means of single-copy RFLP markers. This should help resolve some of the questions raised in this study, and give an accurate description of the diversity and relationship in the genus *Erianthus* and its position within the *Saccharum* complex.

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