GENETIC BASE BROADENING OF SUGARCANE (*SACCHARUM* SPP.) BY INTROGRESSION OF GENES THROUGH INTERGENERIC HYBRIDISATION

By

K. SHANMUGHA SUNDARAM, M.KRISHNAMURTHI
S. RAJESWARI , SHINI SEKAR and M. SHANMUGANATHAN
R&D Centre, E.I.D. Parry (I) Ltd.,
Pugalur-639113, India
ShanmughaSundaramK@parry.murugappa.com

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Abstract

SUGARCANE (*Saccharum* spp.) is one of the crops for which interspecific hybridisation has provided a major breakthrough for its improvement. However, intergeneric hybridisation has been gaining importance to broaden the genetic base and to obtain commercially useful traits and simultaneously increase the total biomass. The benefits derived from the use of wild species like *S. spontaneum* have been realised since the early 1900s, and the compatibility of *Saccharum officinarum* with *Erianthus* is now being explored to incorporate disease-resistance genes. This genus also has a great potential for vigour, better ratoonability and tolerance to environmental stresses. In E.I.D Parry (India) Ltd, R & D Centre, attempts were made through a conventional breeding program to introduce these traits into commercial sugarcane cultivars. The true nature of these hybrids is confirmed by morphological features like leaf orientation, leaf striping, stalk length, stalk thickness, internode length, stalk colour and bio-chemical features like sucrose, fibre content and analysis through molecular techniques. Selected intergeneric hybrids were used for backcrosses to develop commercial cultivars.

Introduction

Sugarcane breeding has come a long way from the time it was started by the Dutch in Indonesia. The importance of breeding was realised by many industries and thus sugarcane breeding was commenced in earnest with *Saccharum officinarum* clones collected from Papua New Guinea, Irian Jaya and the Pacific Islands. Badila and Korpi, along with striped Singapore, Malabar Red etc. (Deer, 1921) remained under commercial cultivation until the 1920s when hybrids started to overtake these *S. officinarum* clones. During the initial period of sugarcane breeding, the improvement of sugarcane relied on the selection of naturally occurring variants of *S. officinarum* obtained by expeditions to its centre of origin in New Guinea (Ramdoyal and Badaloo, 2002).

In the late 1800s and the early 1900s, interspecific hybridisation with wild species (*Saccharum spontaneum* L.) provided a major break-through in cane yield and sugar improvement. The Dutch used *S. spontaneum* clone Kassoer in their breeding program and produced POJ2878, the Java wonder cane. This was followed by the Indians who used a *S. spontaneum* from Coimbatore and released a nobilised cane, Co205. It was not until after the Second World War that serious effort went into the use of *S. spontaneum*. A concentrated and systematic effort was made by the CSR Company in Australia and Fiji to nobilise superior *S. spontaneum* clones with *S. officinarum* (Roach, 1971; Krishnamurthi, 1987). Some of these F_1 populations are available in Fiji and perhaps Macknade Queensland. In Canal Point USA, another program was undertaken to nobilise Thai *S. spontaneum* (Miller *et al.*, 2005).

It was generally considered that the noble canes would contribute the ability to store sucrose, while the vegetative vigour, good ratooning and disease resistance would come from *S. spontaneum*. Although the progress was initially good, by the late 1960s it was realised that the exceedingly narrow genetic base of the existing clones was beginning to impede further progress (Kennedy, 2001). To break this yield barrier, sugarcane breeders attempted to generate intergeneric hybrids between *S. officinarum* and *Erianthus*. Among the several species of *Erianthus* present in both the old and new world, *E. arundinaceus* and *E. procerus* have a wider distribution in India, China, Myanmar, Thailand, Philippines, Indonesia and New Guinea, while the other species have a restricted distribution (Nair and Praneetha, 2006).

Mukherjee (1958) assigned the origin of the species to the Indo-Myanmar-China region where the species is extensively distributed. The species subsequently spread to the adjoining areas in South and South-East Asia. This is the only cane forming species among *Erianthus* and has enormous potential as a contributor of germplasm to current cultivars for better ratoonability, vigour and tolerance to environmental stresses such as drought and flooding.

Numerous attempts have been made to cross *E. arundinaceus* with sugarcane to introduce these characters into modern cultivars. The need to broaden the genetic base, given the spectra of yield stagnation, and the need to introgress specific characters from wild and associated genera, raised the interest in cane breeding. Intergeneric hybridisation provides the required genetic variability by incorporating useful genes from wild relatives into commercial sugarcane. Further, back crossing with high-sucrose commercial cultivars was essential to develop commercial varieties.

During the 1960s, two clones i.e. one of *Erianthus procerus* and another *E. bengalense* were exported from Coimbatore into Fiji (courtesy T.V. Sreenivasan and M. Naidu) and extensive studies were undertaken with regard to flowering, anthesis, pollen and compatibilities. Crosses were made using *S. officinarum*, Korpi and Badila, with *E. procerus* in Fiji. A series of hybrids i.e. LF 63-51, LF 63-55, LF 63-70, LF 63-71 were selected and used. These hybrids were identified by plant type, internode length and inflorescence shape.

Apart from researches in Fiji, Sreenivasan (pers. comm., 1993) was one of the first to attempt crosses with *Erianthus* and obtained good progenies. He also indicated the chromosome elimination effect in *Erianthus* progenies. One of the major obstacles in the past has been the identification of true hybrids using morphological characters. Sometimes this method of identification appears to be misleading the breeders. To overcome this problem, molecular diagnostic tools have recently been developed for use in sugarcane to identify the putative inter-generic hybrids (D'Hont *et al.*, 1995; Alix *et al.*, 1998, 1999).These tools include sequence-tagged PCR to identify true hybrids at the seedling stage, and genomic *in situ* hybridisation (GISH) to characterise the chromosome complement of hybrids (Piperidis *et al.*, 2000).

A planned intergeneric hybridisation program was started in E.I.D Parry (India) Ltd. to exploit the potential of *Erianthus arundinaceus* to develop commercial cultivars (Krishnamurthi *et al.*, 2004).

Materials and methods

Materials used

The female parents used were 16 pure *S. officinarum* and 4 commercial cultivars. Four accessions of *Erianthus arundinaceus* were used as males (from Thailand and Indonesia) in 2000–2001 crossing seasons at Bangalore. Clones with less than 5% viable pollen were chosen as females in the crossing program. 63 cross combinations were made and 1625 seedlings were produced. Out of these cross combinations, the three cross combinations viz., Badila x *E. arundinaceus*, Korpi x *E. arundinaceus* and CoC 671 × *E. arundinaceus* produced 111 seedlings subjected to selection based on morphological traits. Twelve putative hybrids were selected for further evaluation.

Flowering and synchronisation of flowering

Sugarcane is an intermediate day plant that will flower in response to gradually increasing night length. Most *E. arundinaceus* clones in our germplasm collection flower together with *S.*

officinarum cultivars. Based on the need, flowering was synchronised using the techniques developed by Daniels and Krishnamurthi (1967) and Krishnamurthi (1987). The flowering was arrested with night lighting and induction achieved by reducing the day length.

Crossing techniques

Crossing with *Erianthus arundinaceus* was not easy as it had to be carried out with precision to overcome many hurdles. The pollen from *Erianthus* was collected at 5.30 am and pollinated before the pollen shedding in the female parent. The pollen collection, pollen viability testing and handling, pollination, fuzz collection, fuzz processing, seedling raising, selection and further crossings were carried out as per Krishnamurthi *et al.* (2006).

Field evaluation

The hybrid seedlings were sown in trays and transplanted into pro-trays prior to field planting. The field planting was in rows 1.2 m apart spaced at 60 cm. The selection was carried out based on morphological traits coupled with hand refractometer brix %. The selected hybrids were multiplied in 5.0 m × 1 row plots for seedcane purposes.

The selected intergeneric hybrids were planted in a plot size of 4 rows x 5 m x 4 replications along with female and male parents using RBD design in the 2005–06 season at Pugalur, Tamil Nadu, India. Regular agricultural operations were carried out for this trial. The field characters viz., stalk length (cm), number of internodes, internode length (cm), internode thickness (cm), stalk number per square metre, were collected 10 months after planting. The stalk weight (kg) and cane yield data were collected at the time of harvest (Rajeswari, 2004).The quality parameters viz., brix %, pol %, purity %, fibre % and POCS % were estimated in the laboratory using CSR methods. The analysis was performed on five stalk samples, which were collected at the age of 12 months.

Molecular analysis

For the confirmation of the hybrid nature of the F_1 populations, molecular analysis was carried out at the Sugarcane Breeding Institute (SBI), Coimbatore, India. The hybrids were screened along with their male and female parents using sugarcane SSR primer pairs. The amplified products were analysed on PAGE gels with silver staining.

Results and discussion

Sugarcane flowering in Bangalore, India is between November and mid-January. The *in vitro* pollen germination test indicated that the pollen germination was ranging between 30% and 90%. The same was found to be true for the *in-vivo* test. It was found that *Erianthus* shed pollen at least three times a day i.e 5.30 am, 9.00 am and 3.00 pm. The pollen produced at 9.00 am and 3.00 pm showed low viability when cultured *in vitro*. The various hybrids produced viable pollen in abundance.

One thousand six hundred twenty five seedlings were produced from 63 cross combinations. Out of these, twelve putative hybrids from the following three cross combinations were used for the field and quality analysis (Table 1). No genuine hybrids were identified from other crosses.

Table 1—The number of seedlings and number of putative hybrids generated from crosses between *Saccharum spp.* and *E. Arundinaceus.*

Female parent	Male parent	No. of seedlings produced	No. of selected putative hybrids
Badila	*E. arundinaceus*	23	4 (PIH 01-0010, PIH 01-3246, PIH 01-3315, PIH 01-3317)
Korpi	*E. arundinaceus*	32	3 (PIH 01-3231, PIH 01-3227, PIH 01-3228)
CoC 671 cultivar	*E. arundinaceus*	56	5 (PIH 01-0135, PIH 01-0080, PIH 00-0082, PIH 01-0127, PIH 01-0480)

The metric field traits like stalk length (cm), number of internodes, internode length (cm), internode thickness (cm), stalk number per square metre, stalk weight (kg) and cane yield (t/ha) observed from parents and hybrids are presented in Table 2. The selected hybrid progenies exhibit early vigour, recorded higher cane yield and were better in all morphological traits when compared to parents in the research plots.

Table 2—Field data of parents and intergeneric hybrids.

Parents and hybrids	Stalk length (cm)	No.of inter nodes	Internode length (cm)	Internode thickness (cm)	Stalk no/ sq.m	Stalk weight (kg)	Yield (t/ha)
Badila	175.3	18	8.9	3.9	6	3	88.0
E. arundinaceus	240.4	13	18.9	1.9	30	1.1	100.3
PIH 01-0010	179.9	21	12.2	2.8	12	2.2	107.8
PIH 01-3246	195.8	20	15.1	2.6	15	2.3	113.8
PIH 01-3315	203.4	23	10.8	3	13	2.2	113.0
PIH 01-3317	190.5	21	14.1	3.1	17	2.4	118.0
Korpi	165.4	17	9.4	3.5	8	2.5	100.5
E. arundinaceus	240.4	13	18.9	1.9	30	1.1	100.3
PIH 01-3231	205.9	19	11.2	3.1	15	2.2	146.3
PIH 01-3227	200.4	22	10.9	3	18	2.4	155.3
PIH 01-3228	209.9	23	12.8	2.9	17	2.5	139.3
CoC 67-1	195.5	22	10.6	3.3	14	2.2	121.3
E. arundinaceus	240.4	13	18.9	1.9	30	1.1	100.3
PIH 00-0135	210.4	24	14.2	3.1	18	2.3	146.0
PIH 00-0080	230.7	26	12.4	2.9	22	2.5	162.8
PIH 00-0082	205.2	25	11.9	2.9	26	2.5	155.3
PIH 00-0127	200.7	24	15.1	3.2	21	2.3	169.8
PIH 00-0480	235.5	28	14.9	3.1	25	2.1	171.0

The ANOVA (Table 3) revealed that there are significant differences among the entries tested which indicates the difference among the parents and hybrids. All hybrids tested here showed intermediate values between the parents for almost all traits studied.

Table 3—ANOVA of metric field data**.**

SV	df	Mean squares						
		Stalk length	No.of internodes	Internode length\	Internode thickness	Stalk no/ sq.m	Stalk weight	Yield
Rep	3	49.92	0.69	2.98	0.04	5.62	0.01	7.0
Treat	15	1687.97**	5.83**	26.36**	0.71**	164.12**	0.58**	2920.6**
Error	45	13.38	2.24	0.47	0.09	2.11	0.04	6.9
Total	63	413.83	14.93	6.75	0.23	40.85	0.17	700.7
Mean	–	202.80	21.66	12.71	3.02	17.31	2.29	131.8
SEd	–	2.59	1.06	0.48	0.21	1.03	0.15	1.88
CD(0.01)	–	6.96	2.85	1.30	0.56	2.76	0.40	5.03
CV%	–	1.80	6.92	5.39	9.85	8.40	9.28	2.00

(** Significance at 1% level)

The quality data (Brix %, Pol % in juice, purity %, fibre % and pure obtainable cane sugar (POCS %)) were collected for all parents (except *Erianthus*) and hybrids. Since there is no extractable juice in the *E. arundinaceus* stalks, only fibre data were recorded.

The mean values of the replicated samples are presented in Table 4. Most of the intergeneric hybrids showed higher fibre % and lower POCS % compared to *S. officinarum* clones.

Table 4—Quality data of parents and intergeneric hybrids.

Parents and Hybrids	Brix %	Pol %	Purity %	Fibre %	POCS %
Badila	18.2	16.1	88.46	11	12.46
E. arundinaceus	–	–	–	38.4	–
PIH 01-0010	14.6	12.5	85.62	17.2	8.76
PIH 01-3246	15.4	13.2	85.71	19.1	9.03
PIH 01-3315	14	11.8	84.29	17.5	8.15
PIH 01-3317	16.3	13.4	82.21	18.4	8.99
Korpi	19.2	17	88.54	12	13.01
E. arundinaceus	–	–	–	38.4	–
PIH 01-3231	16.2	14.2	87.65	17.4	10.08
PIH 01-3227	15.9	13.5	84.91	18.3	9.28
PIH 01-3228	16.3	13.3	81.60	19.4	8.76
CoC 67-1	18.0	17.3	96.11	12.5	13.80
E. arundinaceus	–	–	–	38.4	–
PIH 00-0135	16.1	13.7	85.09	18.4	9.41
PIH 00-0080	15.8	14.2	89.87	17.8	10.19
PIH 00-0082	15.8	14.1	89.24	20.1	9.77
PIH 00-0127	16.3	13.5	82.82	19.7	8.95
PIH 00-0480	15.8	14.6	92.41	18.6	10.54

The ANOVA (Table 5) revealed that all entries tested are significantly different for these traits.

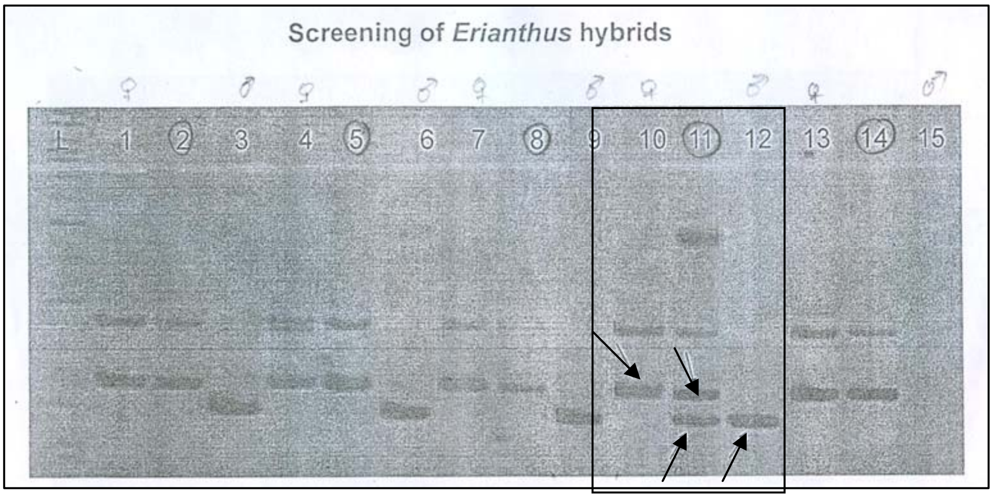
Table 5—ANOVA of quality data.

SV	df	Mean squares				df	MS
		Brix %	Pol %	Purity %	POCS %		Fibre %
Rep	3	0.07	0.01	2.66	0.04	3	0.10
Treat	14	7.10**	9.58**	62.52**	11.49**	15	144.04**
Error	42	0.03	0.05	2.32	0.08	45	0.09
Total	59	1.71	2.31	16.63	2.78	63	34.36
Mean	–	16.26	14.16	86.97	10.08	–	18.49
SEd	–	0.12	0.16	1.07	0.19	–	0.21
CD(0.01)	–	0.33	0.48	2.91	0.53	–	0.57
CV%	–	1.06	1.66	1.75	2.75	–	1.62

(** Significance at 1% level)

The molecular work was carried out at the Sugarcane Breeding Institute (SBI), Coimbatore, Tamil Nadu, India. Until now, one hybrid PIH 01-3228 was confirmed by SSR markers specific to *Saccharum* and *Erianthus* (Figure 1).

In Figure 1, hybrids other than those reported in this paper are also shown. The confirmed hybrid has both *Saccharum* and *Erianthus* bands. The molecular characterisation of all hybrids is currently in progress.



Lane 10—Korpi, Lane 11—PIH 01-3228 (Korpi x *E. arundinaceus)*, Lane 12—*E. arundinaceus*
Fig. 1—Results of putative hybrids identification with SSR primer pairs.

In the process of *Erianthus* hybridisation, breeders have to handle issues such as anthesis of male parents which posed a major problem. *Erianthus* is unique in the time for anthesis which takes place at 5.30 am and the viability is more than 50–60%, whereas subsequent anthesis on the same day produces enough quantity of pollen but the viability is less than 10%; hence, pollination is ineffective. Moreover, the pollen has the habit of coagulation. Hence, viability is greatly reduced if not handled carefully. The second major problem encountered was incompatibility with most true *S. officinarum*. The few which give good progeny are *S. officinarum* clones, Korpi, Badila and a few NG clones. The incompatibility might be due to incongruity between the pollen and pistil of the two genera (Lee, 1995). Our experience indicates using commercials like CoC 67-1 and Co 86-032 as females produces a good number of hybrid seedlings.

Molecular techniques paved a new way for introgression of desirable genes from *Erianthus* to *Saccharum.* Other than *E. arundinaceus,* another species, *E. rockii,* has been identified by Chinese sugarcane breeders, and attempts were made to introgress with *S. officinarum*. Cai *et al.* (2005a) reported that *E. rockii* was distinct from other species of *Erianthus* and *Saccharum* species through SSR and AFLP markers. Verification of intergeneric hybrids of *E. rockii* and *Saccharum* using molecular markers was reported by Aitken *et al.* (2007) and they observed n+n transmission of chromosomes. Molecular confirmation of hybrids was reported by many scientists in the last few years. Cai *et al.* (2005b) confirmed the introgression of *E. arundinaceus* into sugarcane by identification of genuine BC_1 progeny from an F_1 intergeneric hybrid x *Saccharum* clone with molecular markers.

Chromosome elimination was also observed in intergeneric hybrids when analysed using genomic *in situ* hybridisation (GISH). Most of these intergeneric hybrids were sterile and unable to use for backcross (Piperidis *et al.,* 2000) whereas the hybrids produced from this project are showing hybrid vigour and produce more than 30 to 40% viable pollen. One hybrid PIH 01-3315 (*Badila* x *Erianthus*) became male sterile and does not produce any viable pollen. Among all the hybrids, PIH 01-3228 (*Korpi* x *Erianthus*) was subjected to molecular analysis and proved to be a putative intergeneric hybrid (Figure1). These hybrids are being used for further backcrosses to develop commercial cultivars.

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ELARGISSEMENT DE LA BASE GÉNÉTIQUE DE LA CANNE À SUCRE (*SACCHARUM* SPP.) PAR INTROGRÉSSION DES GÈNES À TRAVERS L'HYBRIDATION INTERGÉNERIQUE

Par

K. SHANMUGHA SUNDARAM, M. KRISHNAMURTHI
S. RAJESWARI, SHINI SEKAR et M. SHANMUGANATHAN

R&D Centre, E.I.D. Parry (I) Ltd.,
Pugalur-639113, Inde.
ShanmughaSundaramK@parry.murugappa.com

MOTS CLÉS: *Erianthus*, Nobilisation, Hybride Intergénérique,

Résumé

LA CANNE À SUCRE (*Saccharum* spp.), est une des cultures chez laquelle l'hybridation interspécifique a fourni des avancées majeures pour son amélioration. Cependant, l'hybridation intergénérique a gagné en importance pour élargir la base génétique et obtenir des traits utiles au niveau industriel et, augmenter la biomasse totale conjointement. Les avantages dérivés de l'utilisation des espèces sauvages comme *S. spontaneum* ont été réalisés depuis le début des années 1900s. Maintenant, la compatibilité du *Saccharum officinarum-Erianthus* est explorée pour incorporer les gènes de résistance aux maladies. Le genre a également un grand potentiel pour la vigueur, une meilleure repousse et la tolérance aux stresses environnementaux. Au E.I.D Parry (Inde) Limitée, R & D Centre, des tentatives ont été effectuées à travers un programme d'amélioration conventionnel afin d'introduire ces caractères dans des cultivars commerciaux de canne à sucre. L'authenticité de ces hybrides a été vérifiée sur la base des caractères phénotypiques notamment, l'orientation des feuilles, le dépaillage, la longueur de la tige, le diamètre de la tige, la longueur des entrenœuds, la couleur de la tige et les propriétés biochimiques telles que la teneur en saccharose, la teneur en fibre et des analyses par des techniques moléculaires. Des hybrides intergénériques sélectionnés ont été employés dans des programmes de rétro-croisements pour développer des cultivars commerciaux.

AMPLIANDO LA BASE GENÉTICA DE LA CAÑA DE AZÚCAR (*SACCHARUM* SPP.) MEDIANTE INTROGRESSIÓN DE GENES A TRAVÉS DE LA HIBRIDIZACIÓN INTERGENÉTICA

Por

K. SHANMUGHA SUNDARAM, M. KRISHNAMURTHI
S. RAJESWARI, SHINI SEKAR et M. SHANMUGANATHAN
R&D Center, E.I.D. Parry (I) Ltd.,
Pugalur-639113, India.
ShanmughaSundaramK@parry.murugappa.com

PALABRAS CLAVES: *Erianthus*, Nobilización, Híbridos Intergenéricos.

Resumen

LA CAÑA DE AZÚCAR (*Saccharum* spp.) es uno de los cultivos en el que mediante la hibridización interespecífica ha proporcionado un importante avance en el mejoramiento de variedades. Sin embargo, la hibridización intergenérica ha ganado importancia para ampliar la base genética y obtener caracteres comerciales útiles y al mismo tiempo incrementar la biomasa. Los beneficios derivados del uso de especies silvestres como *S.spontaneum* fueron considerados desde los inicios de 1900, y la compatibilidad de *Saccharum officinarum* con *Erianthus* se ha explorado para obtener genes de resistencia a enfermedades. Este género también tiene un gran potencial para vigor, mejorar el rebrote y tolerancia al estrés ambiental. En el Centro de Investigaciones E.I.D Parry (India) Ltd., se han realizado varios programas de cruzas convencionales para introducir estos caracteres en los cultivares comerciales. La verdadera naturaleza de estos híbridos se confirman mediante los caracteres morfológicos como orientación de las hojas, rayado de la hoja, largo de tallo, grosor del tallo, largo del entrenudo, color del tallo, y caracteres bioquímicos como contenido azucarero, contenido de fibra y análisis con técnicas moleculares. Los híbridos intergenéricos son usados como cruzas recurrentes para el desarrollo de cultivares comerciales.