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Morphology and cytology of a novel intergeneric hybrid *Saccharum* x *Tripsacum* and its derivatives

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Abstract: *The present day commercial sugarcane varieties are derivatives of man made interspecific hybrids between cultivated Saccharum officinarum and wild S. spontaneum of the family Gramineae. Interspecific and intergeneric hybrids with wild related species are being developed for the genetic base broadening of sugarcane. The present study is on the morphology and cytology of a novel hybrid between sugarcane and Tripsacum, a robust perennial grass native of the western hemisphere. The sugarcane variety CoC671 (2n= 108) when pollinated with Tripsacum sps. hybrid (2n= 54) gave rise to hybrids with chromosome number ranging from 2n= 108 to 116 and resembling sugarcane in morphology. In pollen mother cells there were predominance of bivalents and a few multivalents at metaphase I, meiotic abnormalities such as lagging chromosomes and disoriented spindles in anaphase I and asynchronous division and disoriented spindles in anaphase II. These hybrids were fertile and were further crossed with sugarcane and Tripsacum to obtain backcross progeny. The Saccharum x Tripsacum hybrid backcrossed with the sugarcane variety Co 775 gave rise to two distinct morphological categories of plants, one with high tillering (Mean = 41) and thin canes (1.44 cm) and another with low tillering (13.39) and thick canes (2.39 cm). Their leaf width also varied significantly. Most of the broad leaved plants were non-flowering. The back cross hybrids with Tripsacum were with less cane height and with broad and long leaves. Among the backcross hybrids a few plants had the Tripsacum morphological features on leaf and stem. The inflorescence of all the hybrids had resemblance with that of Saccharum. Fertility of the hybrids and variability in the backcross progeny had shown that these hybrids can be further used in genetic base broadening of sugarcane.*

INTRODUCTION

Sugarcane is an important agro industrial crop of tropical and subtropical regions and is cultivated on close to 20 million hectares in more than 90 countries. Sugarcane belongs to the grass family (Gramineae), an economically important seed plant family that includes cereals such as maize, wheat, rice, and sorghum as well as many forage crops. The modern commercial cultivars of sugarcane are interspecific hybrids between cultivated *Saccharum officinarum* and wild *S. spontaneum.* In order to widen the genetic base for its diversified use for production of sugar, ethanol, fibre and electricity and to improve the productivity interspecific and intergeneric hybridization with wild related species is being performed (Sreenivasan *et al.*, 1987). Intergeneric hybrids between *Saccharum* with members of Gramineae such as *Erianthus* (Janaki Ammal, 1941; D'Hont *et al*., 1995; Premachandran and Lalitha, 2007), *Imperata* (Janaki Ammal, 1941), *Miscanthus* (Li *et al*., 1948), Bambusa (Venkatraman, 1937), *Sorghum* (Thomas and Venkatraman 1930; Nair, 1999), *Zea* (Janaki Ammal, 1938) have been reported. The wide hybrids in sugarcane can be maintained by clonal propagation and due to the high ploidy level many of the hybrids are fertile also, thereby it can be used in gene introgression studies.

Tripsacum is a perennial grass native to the western hemisphere, belonging to the Maydeae sub tribe of Andropogoneae tribe, closely related to *Zea mays*. The *Tripsacum* species are with predominantly diploid (2n = 2x = 36) or tetraploid (2n = 4x = 72) ploidy levels. The diploids reproduce sexually whereas tetraploids reproduce by diplosporous apomixis. Sexual allotriploids (2n =3x=54) were also developed from crosses of sexual diploid *Tripsacum dactyloides* (2n = 36), and apomictic tetraploid (2n = 72) *T. maizar* (Li *et al*, 2000). The present study is on the hybrid between a commercial sugarcane variety (*Saccharum* spp. hybrid) and the triploid *Tripsacum* species hybrid.

MATERIALS AND METHOD

The plant materials used in this study consist of sugar cane commercial varieties CoC 671 and Co 775 and *Tripsacum* sps. hybrid being maintained at Sugarcane Breeding Institute, Coimbatore which was originally received from Genetics Division, Indian Agricultural Research Institute, New Delhi. The inflorescence of field grown sugarcane variety CoC 671 was covered with a cloth bag before the start of

spikelet opening and was pollinated with *Tripsacum* pollen for five days from the start of spikelet opening. The seedlings were raised in the glass house and were transplanted to poly bags after 25 days. These seedlings were transferred to field after 45 days. The hybrids obtained were pollinated with the sugarcane variety Co 775 or was selfed and seedlings were raised. One of the (sugarcane x *Tripsacum*) x sugarcane hybrids was pollinated with *Tripsacum* and the backcross progenies were raised. All the hybrids were maintained clonally in the field. The tillering data on the plants were taken at the age of three months and leaf length, width as well as juice brix were recorded at ten months after planting. The somatic chromosome number of parental as well as the hybrid clones were determined by root tip squash technique. Single budded sets were planted for rooting in poly bags with river sand. After 5-7 days young growing roots were collected and pretreated in alpha bromo naphthalene for 1 hr. After washing thoroughly in water the roots were transferred to 3:1 ethanol: acetic acid and stored at 4°C for at least 16 hrs. The roots were kept in 1N HCl at 60°C for hydrolysis, for about 12 minutes. After hydrolysis the roots were transferred to leuco basic fuschin and kept for half an hour in dark. The well stained root tips were cut and squashed in 1% aceto carmine. Well spread chromosomes were counted and photographed under microscope.

For meiotic studies immature inflorescences at short blade stage were fixed in 3:1 ethanol-acetic acid with ferric acetate for 14 to 18 hrs. Then they were stored in 70% ethanol at 4◦C. Anthers from individual spikelets were smeared on glass slide in a drop of 1% acetocarmine. Chromosome behaviour at different meiotic stages of pollen mother cells was observed. For determining pollen fertility mature anthers were teased out in a 1:1 mixture of acetocarmine and glycerol and observed under microscope after one hour. Well stained pollens were counted as fertile and incompletely stained or unstained pollens were counted as sterile.

PCR amplification of 5s rDNA region of the parental clones and the hybrids was done. PCR reactions were performed on total DNA extracted from leaf tissues of the parental as well as the hybrid progeny samples. The primer sequences reported by D'Hont *et al*. (1995) were used. The amplification reaction mix consisted of 5ng of genomic DNA, 0.2µM of each primer P1 (5'TGGGAAGTCCT(C/T)GTGTTGCA3') and P2 (5'-(T/G)T(A/C)G(T/C)GCTGGTATGATCGCA-3'), 200µM of dNTP mix, 1X PCR buffer and one unit of Taq polymerase in a 25µl final volume. The PCR was carried out for one 3-min cycle at 95◦C, 30 cycles of 55s at 93◦C, 15s at 55◦C and 30s at 72◦C. The amplification products were separated by electrophoresis in 2% agarose gels in TAE buffer at 70V.

RESULTS AND DISCUSSION

Fifteen hybrids were raised from the cross between the sugarcane variety CoC 671 and the *Tripsacum* sps. All the hybrids resembled sugarcane in general plant morphology. Data on tillering, stalk diameter, leaf length and leaf width data of the parental sugarcane clone and the hybrids are given in Table 1. The mean stalk diameter, leaf length and leaf width of the hybrid were 2.71cm, 127.6 cm and 5.34 cm, respectively. The inflorescence was an open panilcle as that in sugarcane in all the hybrids. All the hybrids observed were fertile and their floral morphology also resembled sugarcane. The chromosome number of the sugarcane variety CoC 671 is 2n = 108 and that of the *Tripsacum* clone was determined to be 2n = 54. The chromosome number of the five hybrid clones determined ranged from 2n = 108 to 114 (Fig. 1a, 1b). In pollen mother cells of the hybrids at metaphase I predominant bivalent formation was observed. In the hybrid CYM 06-1416 with 2n = 108 there were 54 bivalents in many cells. The anaphase segregation was

PCR amplification of 5s rDNA region of the parents and the hybrid progeny revealed the hybridity of the progeny studied. *Tripsacum* showed two bands of size ~200bp and 450 bp. CoC 671 showed a band of 250 bp while the hybrids showed bands of ~250 bp and 500 bp (Fig. 3). Polymorphism of 5s rDNA region is useful for the identification of intergeneric hybrids (D'Hont *et al*., 1995). PCR amplification of the 5s rDNA region of the parental as well as F1 clones proved the hybridity of the progenies. *Tripsacum* distorted due to lagging chromosomes and disoriented spindles (Fig. 2).showed two bands of size 200bp and 450bp and CoC 671 is having a single band of size 250bp. The amplified fragment sizes of the hybrids were different from that of the parental clones which may be due to the modification of the 5s rDNA region in hybrids when the genomes of two distant species are brought together.

The *Saccharum* x *Tripsacum* hybrid CYM 06-1416 was crossed with sugarcane variety Co775. The backcross hybrids obtained were with two distinct morphological categories, with considerable

difference in leaf width. The narrow leafed plants were with 2.4 to 3.3 cm leaf width whereas the broad leaved ones were having leaf width from 4.5 to 7.5 cm. Those with narrow leaves were with thin canes, less leaf length and high tillering compared to that of broad leaved category (Table 1). Most of the broad leaved plants were non-flowering, whereas the narrow leaved were early and profusely flowering. The somatic chromosome numbers of four narrow leaved and four broad leaved BC1 plants were determined. The broad leaved plants had the chromosome number ranging from 2n=104 to 106, whereas the narrow leaved plants were with 2n= 84 to 87 (Table 2). The two distinct morphological categories of plants which differ in their chromosome number also drastically indicate that these two categories of plants are with different genome constitution.

The {[(sugarcane x *Tripsacum)*] x sugarcane} x *Tripsacum* hybrids were also with two categories of plants, with narrow leaves and profuse tillering and with broad leaves and less tillering. Some of these plants had tuft of hairs at the base of the leaf sheath as that in *Tripsacum*, which was not observed in *Saccharum* clones. The selfed progeny of the sugarcane x *Tripsacum* hybrid CYM 06-1417 were with broad leaves only.

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Intergeneric hybridization is being done in sugarcane with related genera of grasses in order to introgress economically important characters such as disease resistance, ratoonability, high biomass production, etc. Distant hybridization of sugarcane with members outside Saccharastrae such as *Bambusa* (Venkatraman, 1937; Rao *et al*., 1967) and *Zea* (Janaki Ammal, 1938) was also reported. *Tripsacum* belongs to the subtribe Maydeae and there are many reports of intergeneric hybridization of *Tripsacum* with its related genus *Zea mays* (deWet and Harlan, 1974; deWet *et al*., 1972; Stalker et al., 1977). In the present study the hybrids from the cross between *Saccharum* variety CoC 671 (2n= 108) and triploid *Tripsacum* (2n=54) were similar to the female parent in plant morphology. The chromosome number 2n= 108 to 114 observed in the hybrids varied from the expected chromosome number of 2n= expected from n+n 81 (*ie*. 54 +27) transmission. In interspecific and intergeneric hybrids of *Saccharum* functioning of 2n gametes was well documented (Sreenivasan *et al*., 1987; Lalitha and Premachandran, 2007). In *Zea* x *Tripsacum* crosses also the 2n gametes function generally (deWet and Harlan, 1974).

The chromosome number of the progenies ranges from 108-114. When an F1 hybrid was back crossed with sugarcane variety Co775 the progeny could be put in two distinct morphological categories. One type of progenies is having broad leaves and the other group is having narrow leaves. The broad leaved progenies were nonflowering and the narrow leaved were early flowering .There is a considerable variation in their tillering as well as cane. The two distinct morphological categories of plants differ in their chromosome number also drastically indicating that these two categories of plants are with different genome constitution.

Saccharum is able to tolerate alien genome in hybrid combinations as seen from the large number of intergeneric hybrids reported by various authors. The fertility of the hybrids between the genera *Saccharum* and *Tripsacum* as found in the present study and the variability in the backcross progeny had shown that such hybrids can be used for gene introgression from the distantly related *Tripsacum* to sugarcane.

Table 1. Comparison of leaf width, number of tillers, stalk diameter and leaf length of *Saccharum* x *Tripsacum* hybrids and its back cross progenies

Category of hybrid	No. of plants	Leaf width (cm)		Number of tillers		Stalk diameter (cm)		Leaf length (cm)	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
F1 progeny	8	3.5-6.2	5.34	8-65	27.75	1.4-3.3	2.71	100-142	127.63
Narrow leaved BC1	9	2.4-3.3	2.7	20-56	41	1.3-1.6	1.4	100-142	116.1
Broad leaved BC1	18	4.5-7.5	6.4	2-26	13.4	1.8-3.1	2.4	105-193	153.7

Table 2. Chromosome number of some (*Saccaharum x Tripsacum*) x *Saccharum* hybrids

Clone	Morphological category	Chromosome number (2n)
CYM 07-766	Narrow leaved	87
CYM 07-768	Narrow leaved	84
CYM 07-789	Narrow leaved	85
CYM 07-792	Narrow leaved	85
CYM 07-767	Broad leaved	104
CYM 07-770	Broad leaved	105
CYM 07-791	Broad leaved	108
CYM 07-832	Broad leaved	106

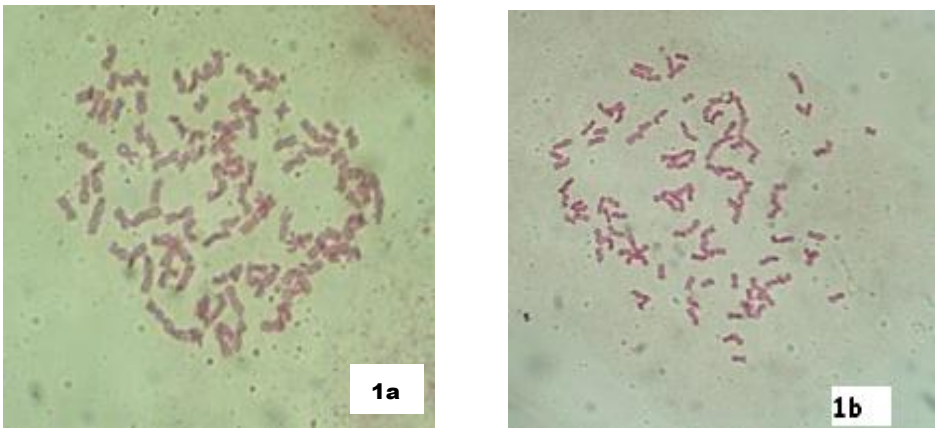


Fig 1. Somatic chromosome number in *Saccharum* x *Tripsacum* hybrids.
1a.CYM 06-1416 (2n = 108) 1b. CYM 06-1429 (2n = 114)

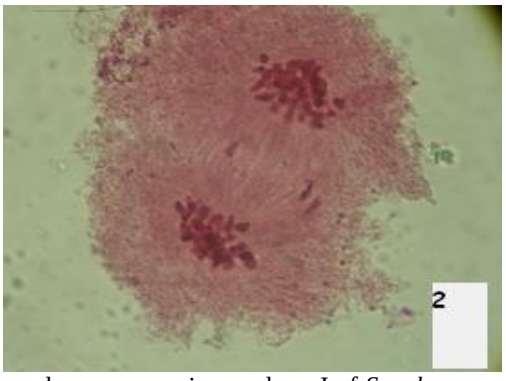
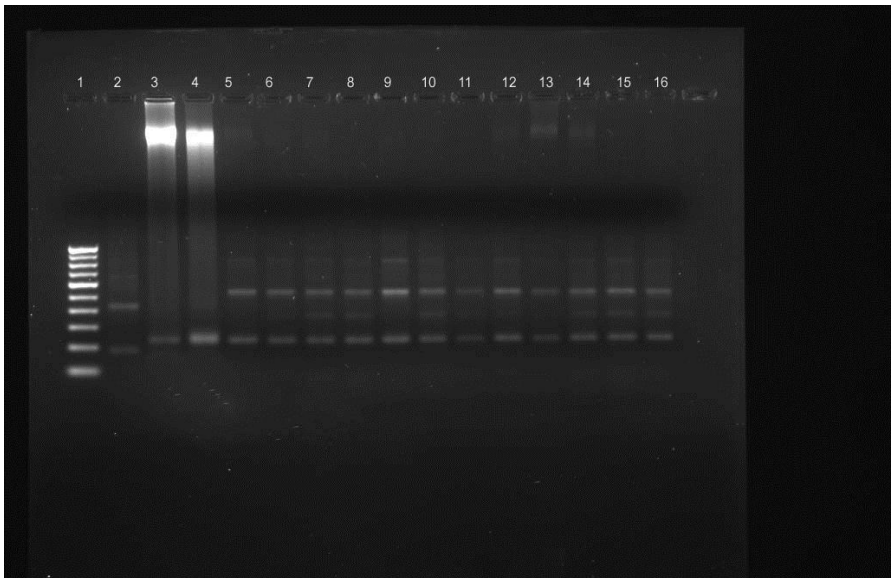


Fig 2. Lagging chromosomes in anaphase I of *Saccharum* x *Tripsacum* hybrid



Lane 1: 100bp marker, lane 2: *Tripsacum*, lane 3: CoC 671 lane 5-16 *Tripsacum* x CoC671 progenies
Fig 3. 5s r DNA amplification of parental and hybrid clones

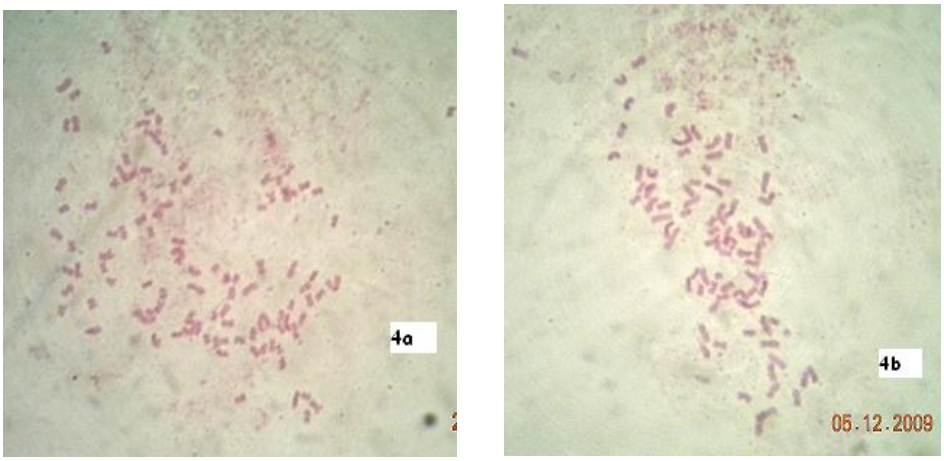


Fig. 4. Somatic chromosome number of (*Saccharum* X *Tripsacum*) X *Saccharum* backcross progenies.4a.CYM 07 – 791 (2n=108) 4b. CYM 07 – 768 (2n = 84)

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