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Genomic *in situ* hybridization (GISH) and performance analysis in intergeneric hybrids from five consecutive generations of *Erianthus* x *Saccharum*

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Abstract. *Erianthus arundinaceus,* one of the species of '*Saccharum* complex' has a number of important agronomic traits including good ratooning ability, tolerance to both drought and waterlogging, disease resistance and vigor and is of interest as a potential source of parental germplasm to sugarcane breeders. We report here for the first time the chromosome composition, *Erianthus* chromosome transmission patten and agronomical trait evaluation of *Erianthus* addition lines in five consecutive generations of *E. arundinaceus* x *Saccharum*. The hybridity of randomly selected clones could confirm with *Erianthus* Specific Tandem Repeat (ESTR) sequences. The results of classical cytology revealed that the mode of transmission of gametes, except in the second generation, followed n+n pattern whereas in second generation (CYM 07-971) it was showing 2n+n with elimination of few chromosomes. Progressive elimination of *Erianthus* chromosomes is observed in consecutive generations where the F1 showed 30 *Erianthus* chromosomes and it was ranged from 0-2 in fifth generation. Agronomical trait analysis indicates that further backcrossing with commercial clones with high juice quality or intercrossing among the selected progenies would improve both juice quality and cane traits in *E. arundinaceus* x *Saccharum* hybrids.

Keywords: *Erianthus*, *Saccharum*, introgression, cytology, genomic *in situ* hybridization, intergeneric hybrid, sugarcane.

Sugarcane belongs to the genus *Saccharum* and it consists of six species namely *S. officinarum* L., *S. barberi* Jesw., *S. sinense* Roxb., *S. robustum* Brandes and Jesw. ex Grassl, *S. spontaneum* L. and *S. edule* Hassk. The cultivated sugarcane varieties are derivatives of interspecific hybridization between *S. officinarum* and *S. spontaneum*. Critical analysis of pedigree of the cultivated varieties revealed that, only a limited number of basic species clones have been contributed to the parental material in sugarcane breeding programmes (Roach, 1989; Hemaprabha et al., 2022). The cytoplasmic variability among major sugarcane varieties under cultivation are not much as only limited *S. officinarum* clones were used as female parent in early breed-

ing programmes (Hemaprabha et al., 2022). This may result in vulnerability to disease epidemics and abiotic stresses. In order to widen the cytoplasmic base of sugarcane cultivars, the wild related species with diverse chloroplast and mitochondrial genomes have been utilized in breeding programmes. The "*Saccharum* complex" includes the genera *Erianthus* Michx, *Miscanthus* Anderss., *Sclerostachya* (Hack.) A. Camus and *Narenga* Bor., besides the *Saccharum* species and constitute a closely related inter breeding group (Mukherjee 1957; Daniels *et al.* 1975).

The genus *Erianthus* has established by Michaux in 1803, based on the Greek word 'Erion' meaning wool

and 'anthos' meaning flower, referring to its woolly glumes. It is considered a primitive genus of 'Saccharum complex' (Mukherjee 1957). It is wide in distribution occurring in America (New World species), Mediterranean, India, China, South East Asia, New Guinea (Old World species). The Old-World species generally placed under section *Ripidium*, are only important in the evolution and improvement of sugarcane. Among the species of the genus *Erianthus* sect. *ripidium*, *E. arundinaceus* (Retz.) Jesw. (2n = 30, 40, 60) distributed in India, China, Indonesia and New Guinea and has many desirable agronomic traits for sugarcane breeding such as disease resistance, drought resistance, high biomass and broad adaptability. Efforts are under way in many research stations to introgress *Erianthus* germplasm into sugarcane to develop more productive and better adapted sugarcane varieties.

Fertile and sterile hybrids were reported from crosses involving *Saccharum* and *E. arundinaceus* (D' Hont *et al.* 1995, Besse *et al.* 1997, Piperidis *et al.* 2000, 2010, Cai *et al.* 2005, Wu *et al.* 2014, Huang *et al.* 2015) in which either *S. officinarum* or sugarcane variety used as female and was further back crossed with sugarcane. The major difficulty in transferring the desirable characters from *Erianthus* into sugarcane is the incompatibility among the genera which prevents further improvement through hybridization. When crosses between two species are not successful due to hybrid sterility and genome elimination bridge crosses were found to be effective in certain cases. The successful use of *S. spontaneum* as a bridge species for transferring characters from *Erianthus* to *Saccharum* was reported by Premachandran *et al.* 2011. In this case *Erianthus* has been used as female parent. The production and molecular cytogenetic characterization of intergeneric F1 progeny and back cross progenies has been reported earlier (Lekshmi *et al.* 2016, Premachanran *et al.* 2017). Later the backcross progenies were developed by crossing with sugarcane commercial varieties to develop near commercial sugarcane clones. Nuclear and cytoplasmic contribution from *Erianthus* was confirmed in second and third generation hybrids (Lekshmi *et al.* 2016).

Here we aimed to determine the somatic chromosome number of different backcross progenies of *Erianthus* x *Saccharum*. We also selected two different sets of BC1, BC2, BC3 and BC4 progenies and used GISH to clarify the pattern of *E. arundinaceus* chromosome transmission. The aim of the study was to find out the clone that contain 1-2 *Erianthus* chromosomes which can be further used for sequencing the *Erianthus* chromosomes and also to tag the trait specific *Erianthus* chromosomes by correlation studies. This work will provide a basis for subsequent genome research as well as trait specific breeding programmes in sugarcane.

MATERIALS AND METHODS

Plant material

The materials used for the study consist of two sets of progenies from BC2, BC3 and BC4 generations. In the first set, the BC 3 progeny, Co 15015, derived from a cross combination of CYM 08-903 and the Sugarcane cultivar Co 94008 (Lekshmi *et al.* 2016). BC4 progenies (GI 18-1, GI 18-2, GI 18-3, GI 18-4) were from a crossing combination between Co 15015 (female) and sugarcane cultivar, Co 11012 (male). In another set of clones used for the study BC2, BC3 and BC4 progenies were developed by a parallel set of hybridization process. A BC2 clone, CYM 08-922 was selected as female parent and BC3 progeny TWC 82, was derived from a cross combination CYM 08-922 x Sugarcane variety BO91. Four BC4 progenies were developed (FWC 28, FWC 29, FWC 39 and FWC 2) from a cross between TWC 82 x Sugarcane hybrid. The details of the clones used for the study are given in Table 1. To understand the flow of chromosome transmission pattern in successive generations of *Erianthus* x *Saccharum*, the clones from *Erianthus* (female parent), F1 and BC1 were also included in the study.

The somatic chromosome number in the F1 and back cross progenies was determined by root tip squash technique (Sobhakumari and Asmita Dutta, 2014). For GISH analysis the mitotic slide preparation was performed as per D'Hont *et al.* 1996 with minor modifications. Single budded cuttings from the hybrids were collected at 1.30pm. Excised root tips of about 1 cm were treated with 2mM 8-hydroxy quinolone at room temperature for 2h, washed in water and fixed in Ethanol: Acetic acid (3:1) for overnight at 4°C. The washed root tips were hydrolyzed in 0.25N HCl and digested at 37°C for 75min in the enzyme solution containing 2.0% cellulase

ONOZUKA R-10 (Himedia) and 20% pectinase (Himedia) in citrate buffer. After washing the meristematic tissues of the root tips were squashed in a drop of fixative. Cells can be separated by gentle pressing over the coverslip with filter paper. Slides were then freezed by dipping it in liquid nitrogen. After removing the coverslip, the frozen slide was immediately dehydrated in absolute ethanol and stored in moisture free slide box.

Genomic in situ *hybridization*

Total genomic DNA of the *Erianthus* clone, IK 76-62, was extracted from young leaves using CTAB method (Doyle and Doyle, 1990). Fragmentation (500bp – 1000bp) has been done by sonication. The fragmented DNA of *Erianthus* labeled with biotin 11-duTP using random primed labeling method as described by manufacturer (Thermo Scientific, USA) was used as probe. The 30µl hybridization mixture containing 5µlof labeled probe, 15µl deionized formamide, 6 µl 50% dextran sulphate, 2.25µl 20xSSC, 0.5 µl salmon sperm DNA and 1.25 µl double distilled water was denatured at 100°C for 10min, and then placed immediately in ice. Each chromosomal slide was denatured at 72°Cfor 2min in denaturation solution containing 70% deionized formamide and dehydrated in a series of precooled ethanol solutions (75%, 95%and 100%). After adding 30µl of hybridization solution to the slide it was incubated in a humid box with 2xSSC and at 37°C for overnight. Post hybridization washes in 2xSSC and 50% formamide at 42°C slides were dried. Biotin labeled probe was detected using avidin-FITC (Fluorescein iso thio cynate) (Vector laboratories, Burlingane, CA) by 1h incubation at 37°C. After washing and drying the chronosomes were counter stained with a vectasheild vibrance anti-fading medium with DAPI (4,6 diamino-2-phenylindole) (Vector laboratories, Burlingane, CA). GISH signals were captured using a ProgRes Capture Pro image capturing software attached to AxioScope A1fluorescent microscope (CarlZeiss, Gottingen, Germany). Images were processed using Adobe Photoshop.

Screening for drought tolerance and red rot resistance

The experimental trial was conducted at East Chithirai Chavadi farm of ICAR-Sugarcane breeding institute, Coimbatore, Tamil Nadu, India (110 N, 770E, 427 MSLaltitude) during 2021-22. The backcross hybrids involving Erianthus were planted in split plot desgn, treatments as main plots and genotypes as subplots. The drought treatment was insisted by withholding water during the formative phase of the crop (60-150 DAP). The yield and juice quality data were recorded at 10th month of crop.

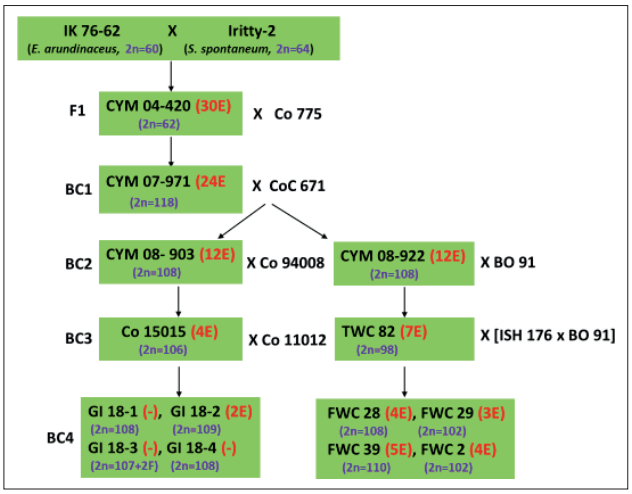

Figure 1. *Erianthus arundinaceus* introgression pattern in five generations of *Erianthus arundinaceus* x *Saccharum officinarum.*

The drought tolerance indices were calculated as follows:

Stress tolerance index (STI) STI = (Yp * Ys) / ($\bar{Y}$p)2

Yp and Ys are the average yield under normal and moisture stress conditions, respectively. $\bar{Y}$p is the average yield of all genotypes under normal moisture conditions, Screening for red rot resistance under controlled condition testing. The clones were screened for red rot resistance against the virulent inoculum of cf 671 (*Colletotricum falcatum*) under controlled condition testing (CCT) and the disease reaction was scored as per Mohanraj et al., (1997).

RESULTS AND DISCUSSION

Chromosome composition of the F1 hybrid

GISH analysis of the F1 hybrid CYM 04-420 from the cross between IK 76-78 (*Erianthus arundinaceus*, 2n=40) x Iritty-2 (*Saccharum spontaneum*, 2n= 64) revealed that a total of 62 chromosomes (Table 1) of which 30 were from *E. arundinaceus* and 32 were from *S. spontaneum* (Fig. 3a), as expected from a classical n+n chromosome transmission (Table. 1) (Lekshmi *et al.* 2016; Premachandran *et al.* 2017). The result was contradictory to the report of Wu *et al.* (2014) and Piperidis *et al.* (2000) where they have reported the presence of anueploids in F1 generation.

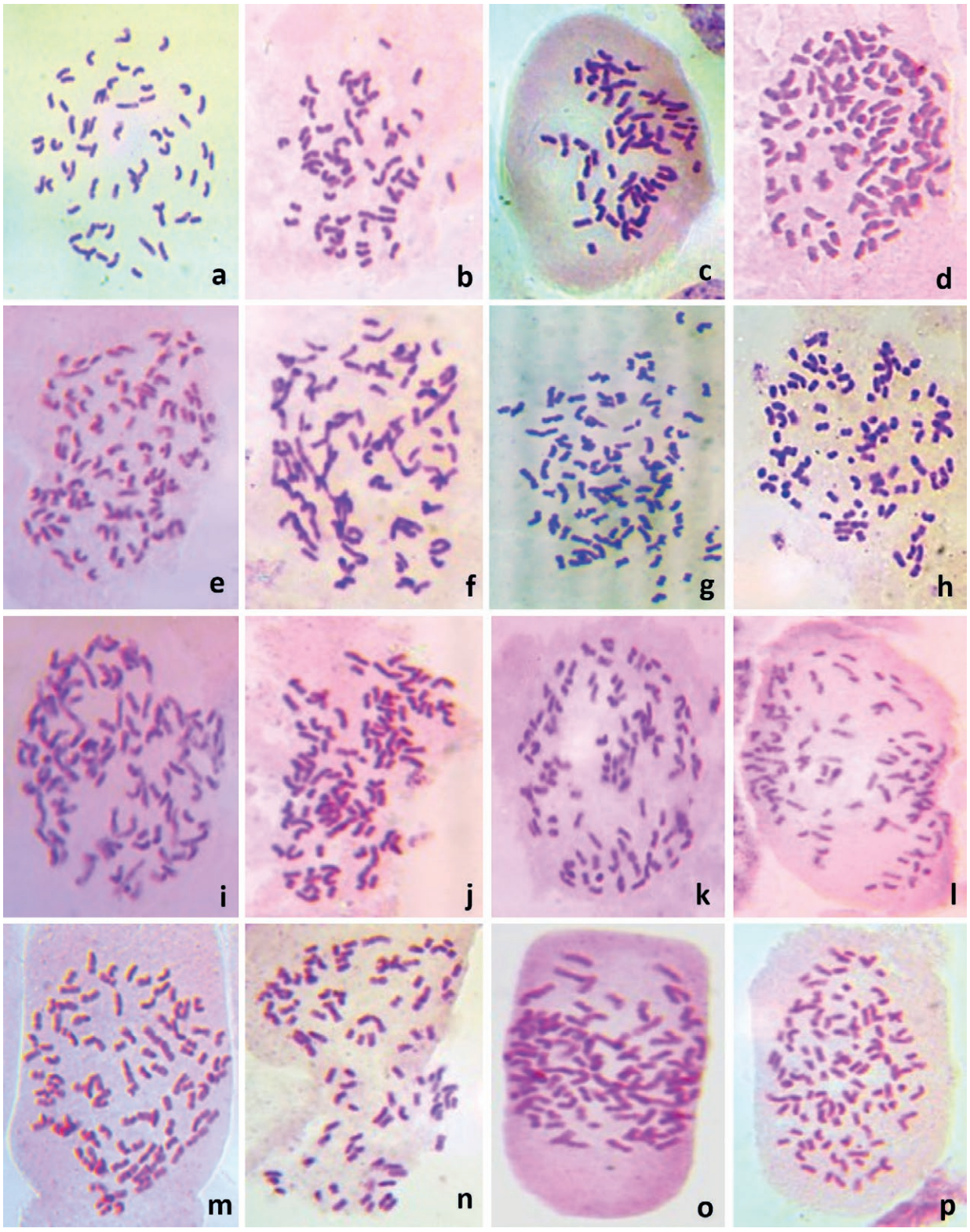

Figure 2. Somatic chromosome number of different clones in F1 to BC4 generations: a) IK 76-78 (2n=60), b) Iritty-2 (2n=64), c) CYM 04-420 (2n=62), d) CYM 07-971(2n= 118), e) CYM 08-903 (2n=108), f) CYM 08-922 (2n=108), g) Co 15015 (2n=106), h)TWC 82 (2n=98), i) GI 18-1(2n=108), j) GI 18-2 (2n=109), k)GI 18-3 (2n=107+2F), l) GI 18-4 (2n=108), m) FWC-2 (2n=102), n) FWC-28, (2n=108), o) FWC-29 (2n=102), p) FWC-39 (2n=110).

Table 1. Chromosome composition of parents and hybrids of different generations involving *Erianthus arundinaceus*.

S.No	Generation	Clone Name	Total 2n	From *Saccharum*	From *Erianthus*	Recombinant	No of cells observed
1	Female parent	IK76-62	60	-	60	0	10
2	Male parent	Iritty-2	64	-	-	0	10
3	F1	CYM 04-420	62	32	30	0	15
4	BC1	CYM 07-971	118	94	24	0	12
5	BC2	CYM 08-903	108	96	12	0	16
6	BC2	CYM 08-922	108	96	12	0	15
7	BC3	Co 15015	106	102	4	0	20
8	BC3	TWC 82	98	91	7	0	15
9	BC4	GI 18-1	108	108	-	0	10
10	BC4	GI 18-2	109	107	2	0	15
11	BC4	GI 18-3	108	108	-	0	10
12	BC4	GI 18-4	108	108	-	0	12
13	BC4	FWC-28	108	104	4	0	15
14	BC4	FWC-29	102	99	4	0	15
15	BC4	FWC-39	110	105	5	0	15
16	BC4	FWC-2	102	98	4	0	15

2n+ n chromosome transmission in BC1 progeny

Due to nonsynchronous flowering of the sugarcane hybrid the fundamental principles of backcross breeding are not appropriate in the case of this crop. During intergeneric hybridization in F1 the chromosome inherited from divergent parents of different genera are often unable to pair with each other during meiosis which leads to male sterility in F1 hybrid. In order to obtain BC1 generation the F1 hybrid (CYM 04-420) was used as a female parent and a commercial variety Co 775 was used as a male parent. From this cross many BC1 progeny were generated. We considered one BC1 progeny, CYM 07-971 for further analysis. The total chromosome compliment for this hybrid was 2n=118 of which 94 chromosomes were derived from *Saccharum* and 24 chromosomes were derived from *E. arundinaceus* (Table 1, Fig. 3b). These results indicated that the BC1 progeny was a product of 2n+n transmission. Piperidis *et al.* (2000), Piperidis *et al.* (2010) and Wu *et al.* (2014) were reported similar results. 2n gametes were originated from fusion of two megaspore (Megaspore Tetrad Cell Fusion) or due to chromosome doubling after second meiotic division (Post Meiotic Restitution) (Narayanaswami (1940), Bremer (1961).

n+n chromosome transmission in BC2 and BC3 progeny

GISH analysis of two BC2 (CYM 08-903 and CYM 08-922) revealed that these clones were with a total of chromosome compliment of 2n=118 of which 96 chromosomes were derived from *Saccharum* species and 12 chromosomes were from *E. arundinaceus* (Table 1, Fig. 3 c & d). It was found that the number of *E. arundinaceus* chromosomes in BC1 parent was 24 and in BC2 progeny it was reduced by half. This indicate that BC2 progeny (CYM 08-903 and CYM 08-922) were the product of n+n transmission. Parallel back crosses were conducted with these BC2 progenies. i. e., CYM 08-903 x Co 94008 and CYM 08-922 X Bo 91. A nearly commercial cane, Co 15015 with 2n=108 was generated as BC3 progeny from CYM 08-903 x Co 94008. GISH experiment revealed that 102 chromosomes were derived from *Saccharum* species and 4 from *E. arundinaceus*. Another BC3 progeny, TWC 82, from the cross CYM 08-922 X BO 91 was with 2n=98 of which 91 chromosomes were from *Saccharum* and 7 chromosomes were from *E. arundinaceus*. Our results indicate that the BC3 progeny were the product of n+n transmission. Piperidis *et al.* (2010, 2013) and Huang *et al.* (2014) reported that the similar transmission was in BC2 and Bc3 progeny between different species clones of *S. officinarum* and *E. arundinaceus*.

In this study, both the BC3 progeny, Co 15015 and TWC 82, were derived from BC2 clones CYM 08-903 and CYM 08-922 respectively. Both the BC2 parents were having 12 *E. arundinaceus* chromosomes. In TWC 82 is seven *Erianthus* chromosomes were observed. It is found that more than half of the *E. arundinaceus* chromosomes in CYM 08-922 was transmitted to TWC-82. Where as in the case of Co 15015 only four (less than half) *E. arundi-*

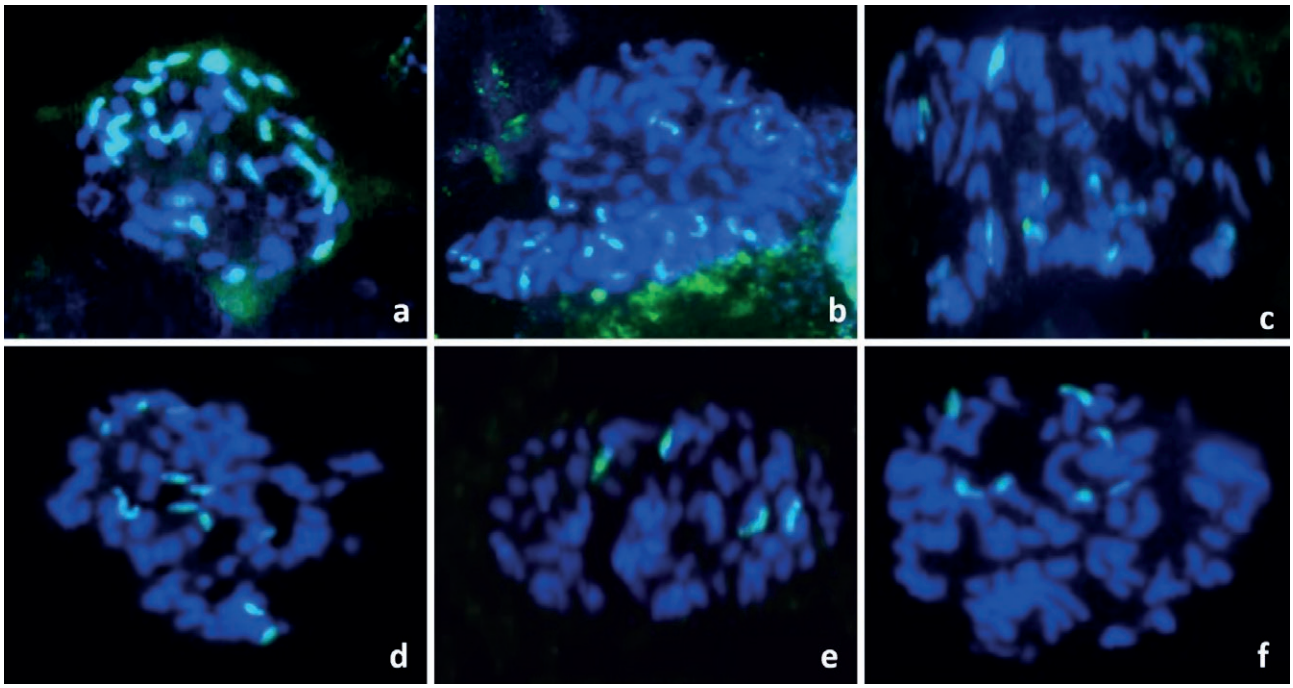

Figure 3. Genomic *in situ* hybridization with *E. arundinaceus* probe: a) *E. arundinaceus* x *S. spontaneum* hybrid, CYM 04-420, with 30 *E. arundinaceus* chromosomes, b) CYM 07-971 with 24 *E. arundinaceus* chromosomes, c) CYM 08-903 with 12 *E. arundinaceus* chromosomes, d) CYM 08-922 with 12 *E. arundinaceus* chromosomes, e) Co 15015 with four *E. arundinaceus* chromosomes, f) TWC- 82 with seven *E. arundinaceus* chromosomes.

naceus chromosomes from BC2 (CYM 08-903) was transmitted. Though there was difference in the number of *E. arundinaceus* chromosomes transmitted from female BC2 parents, the transmission pattern was n+n only with addition/deletion of few chromosomes.

During the nobilization of *S. officinarum* X *S. spontaneum* 2n+n chromosome segregation happened in the early generations like F1 and BC1. Whereas in the nobilization with *E. arundinaceus* 2n+n segregation happened during later stages and this slows down the progress of nobilization. In order to develop BC4 progenies the BC3 progeny, Co 15015, crossed with another improved Co cane, Co 11012. Cytological analysis of the four BC4 progeny revealed plants with a total chromosome compliment ranging from 107-109 chromosomes. GISH analysis revealed that out of these four BC4 progeny only one clone was having 2 *E. arundinaceus* chromosomes whereas three clones were not having any *Erianthus* chromosomes. In the parallel back crossing programme TWC 82 (BC3) crossed with an interspecific hybrid and four BC4 progeny were generated. The 2n chromosome number ranged from *Saccharum* spp. and 3-5 from *E. arundinaceus* respectively (Fig. 4).

Interspecific hybridization or intervarietal hybridization providing frequent utilization of limited number of parental clones resulted in the narrow genetic base of modern sugarcane cultivars and subsequently showing susceptibility to biotic and abiotic stresses. It has become necessary to include wild relatives of *Saccharum* in breeding programme to broaden the genetic diversity for increased productivity and better adaptability. As one of the most important wild relatives of sugarcane, *E. arundinaceus* has agronomically important genes for sugarcane breeding. At ICAR-Sugarcane breeding Institute we are regularly utilizing *E. arundinaceus* in breeding programmes and a series of genuine progeny have been developed in different back crossed generations.

Generally, the cytological methods and molecular markers are widely used to specifically detect the alien chromosomes and chromosome segments in the putative back cross progenies. It is found that Genomic *in situ* hybridization (GISH) is a powerful cytological tool for identifying the introgression pattern of alien chromosomes in sugarcane background. This study clearly indicates the number of back cross generations a breeder has to be developed to incorporate the alien chromosomes. In our study we found that in the BC4 progeny from a cross Co 15015 x Co 11012 only one hybrid was with two *Erianthus* chromosomes whereas the other progenies were without *E. arundinaceus* chromosomes. Hence

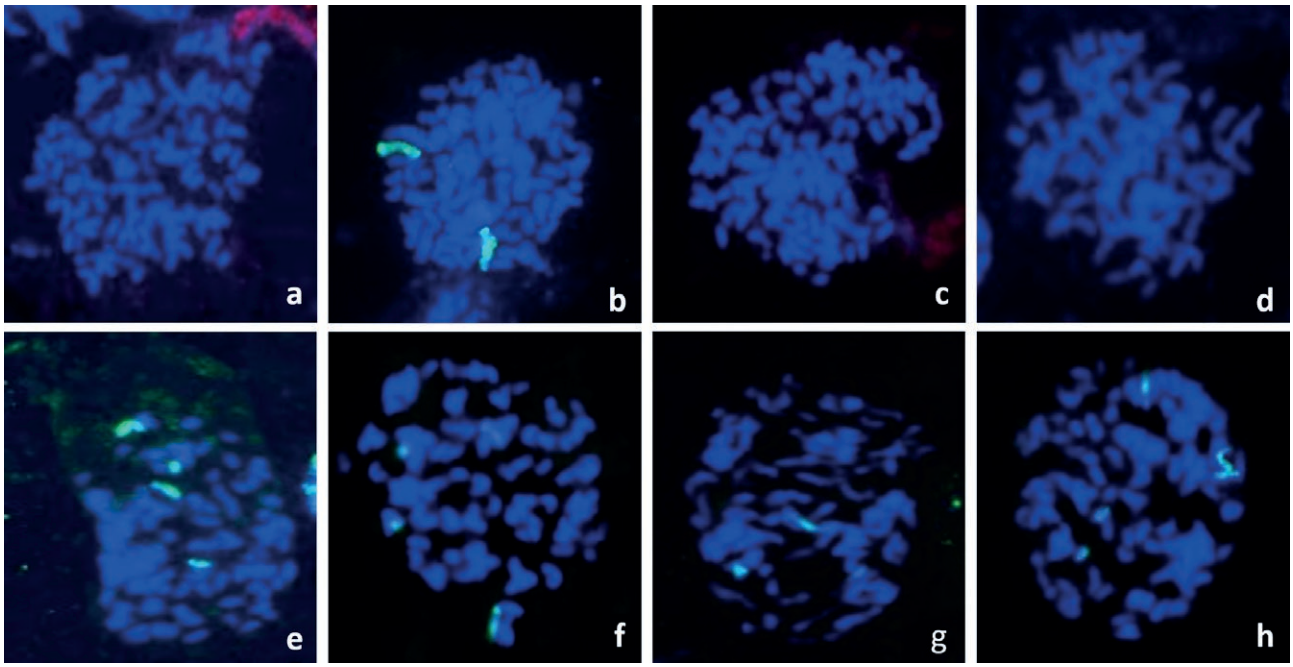

Figure 4. Genomic *in situ* hybridization with *E. arundinaceus* probe in 5th generation hybrids: a) GI 18-1 – Nil *E. arundinaceus*, b) GI 18-2 with two *E. arundinaceus* chromosomes c) GI 18-3 – Nil *E. arundinaceus*, d) GI-18-4 – Nil *E. arundinaceus*, e) FWC-2 with four *E. arundinaceus* chromosomes f) FWC-28 with *four E. arundinaceus* chromosomes g) FWC-29 with three *E. arundinaceus* chromosomes g) FWC-29 with three *E. arundinaceus* chromosomes.

this is the stage where the introgression breeding can be stopped as further transmission of *Erianthus* chromosomes is not possible in successive generations. Whereas in the parallel cross BC4 progenies from TWC 82 x ISH hybrid 3-5 *E. arundinaceus* chromosomes were there in which we can go for one more back crossing to get improved clone with minimum *Erianthus* chromosomes.

The agronomic performance of the backcross hybrids involving *Erianthus* for cane yield quality and red rot resistance is presented in the Table 2. Three hybrids recorded significantly higher cane height than the commercial hybrid Co 86032 (225.0 cm). The sucrose in juice ranged from 8.44% in TWC 82 to 19.98% in Co 15015. Three hybrids recorded significantly higher yield than the commercial check Co 86032 (121.8 t/ha). For red rot resistance, eight were moderately resistant and only one was susceptible. The clones were also screened for water stress during 2021-22. The results showed that the commercial check had a stress tolerance index of 0.761 and five clones recorded significantly higher STI (Table 3). The hybrid TWC 82 recorded the highest STI of 1.678 followed by FWC-2. The entry TWC combined both red rot resistance and water stress tolerance. The juice quality of the hybrids showed significantly lower than commercial check Co 86032 and only one Co 15015 had 19.98% of juice sucrose. Further backcrossing with commercial clones with high juice quality would further improve both juice quality and cane traits. Similarly, Nair et al. 2017 reported backcrossing of *E. procerus* hybrids with commercial varieties to obtain commercially acceptable levels of agronomic traits.

Intergeneric hybrid population between *Saccharum* spp. and *E. arundinaceus* often resulted in false hybrids due to selfing. In order to avoid this, it is highly encouraged to integrate efficient molecular markers with GISH or FISH. Deng *et al.* (2002) used isozyme markers because of similar banding pattern could not identify the genuine hybrids. Later 5Sr marker used to identify the true hybrid progeny with *Erianthus* specific 5SrDNA sequences. It was found that amplification was not obtained beyond BC2 generation. As 5Sr DNA had one locus per set of chromosomes it presents only on a few chromosomes in each genome. Due to unequal segregation of *Erianthus* chromosomes and also its elimination at different stages the advanced back cross progenies may not inherit the chromosomes that carry the 5SrDNA loci. Hence *Erianthus* specific 5Sr DNA sequences may not be reliable for the identification of true hybrid progeny. The *Erianthus* specific Tandem Repeat sequence (ESTR) reported by Yang *et al.* (2019) was used as a marker to confirm the hybridity of back cross progeny. In our study true intergeneric hybrids between *Saccha-*

Table 2. Performance of Backcross hybris involving *Erianthus* for cane yield, juice quality traits and red rot resistance.

	Clone Name	Cane Ht (cm)	Cane dia (cm)	SCW (Kgs)	Brix (%)	Pol (%)	Purity (%)	CCS %	NMC ('000/ha)	Cane yield (t/ha)	RR
1	CYM 07-971	210.00	2.61	1.09	14.72	12.06	81.93	8.03	89.00	96.71	MR
2	CYM 08-903	205.00	2.91	1.20	19.07	16.94	88.83	11.74	75.00	89.75	MR
3	CYM 08-922	255.00	2.75	1.38	14.89	12.09	81.20	8.01	101.50	140.07	MS
4	Co 15015	240.00	2.80	1.25	21.63	19.98	92.37	14.10	88.10	110.13	MR
5	TWC 82	265.00	2.95	1.68	11.88	8.44	71.04	5.16	98.30	165.14	MR
6	GI 18-1	220.00	2.46	0.82	19.67	17.60	89.48	12.24	85.00	69.98	MR
7	GI 18-2	235.00	2.57	1.15	15.61	13.17	84.37	8.90	89.00	102.35	MS
8	GI 18-3	235.00	2.71	1.22	19.41	17.11	88.15	11.82	91.50	111.63	MR
9	FWC-28	275.00	2.67	1.15	16.19	13.66	84.37	9.23	94.44	108.61	MS
10	FWC-29	205.00	2.83	1.23	15.47	13.28	85.84	9.05	86.11	105.92	MR
11	FWC-39	255.00	2.51	1.25	15.85	13.48	85.05	9.15	60.19	75.23	MR
12	FWC-2	235.00	2.65	1.45	13.51	10.51	77.79	6.80	100.00	145.00	S
	Co 86032	225.00	2.75	1.45	19.91	17.79	89.35	12.37	84.00	121.80	MS
	CD (P>0.05)	16.23	0.32	0.18	1.12	1.05	4.56	0.98	9.56	11.36	

Table 3. Stress tolerance Index (STI) of Backcross hybris involving Erianthus.

Sl.No.	Clone	Cane yield (t/ha)		STI
		Control	Stress	
1	CYM 07-971	96.71	72.3	0.558
2	CYM 08-903	89.75	68.5	0.490
3	CYM 08-922	140.07	115.5	1.290
4	Co 15015	110.13	75.34	0.662
5	TWC 82	165.14	127.35	1.678
6	GI 18-1	69.98	52.2	0.291
7	FWC-28	108.61	82.3	0.713
8	FWC-29	105.92	78.3	0.662
9	FWC-39	75.23	48.75	0.293
10	FWC-2	145.00	117.35	1.357
11	Co 86032	121.80	78.35	0.761
12	Co 06022	127.41	89.35	0.908
13	CoM 0265	126.00	86.54	0.870
14	Co 775	85.80	37.3	0.255
	Overall mean	111.97	80.67	0.771
	Treatments (P>0.05)	9.85		
	Clones (P>0.05)	16.34		0.34

rum spp. and *E. arundinaceus* could be rapidly identified using PCR with *Erianthus* Specific Tandem repeat primer pair (Fig. 5). It was found that PCR detection results highly coincides with GISH results.

Due to the genetic distance between the two genera the chromosome pairing and chiasma formation during meiosis is not taking place in intergeneric hybrids of *Saccharum* and *Erianthus*. From our study it has revealed that *E. arundinaceus* genome introgressed into *Saccharum* as whole chromosome by traditional breeding. The approaches like QTL mapping and marker assisted breeding in the advanced generations of back crosses (BC3 and BC4) will help to determine the agronomic value of individual *E. arundinaceus* chromosomes. Though *E. arundinaceus* clones are with many desirable agronomic traits for sugarcane genetic improvement. We have limited knowledge on the complex genome of this hexaploid species. Development and determination of *Saccharum – Erianthus* introgression lines with one or two *E. arundinaceus* chromosomes is a necessary step to simplify the genome analysis by dissecting out the alien chromosomes. In this study we identify a clone, GI 18-2, with two *Erianthus* chromosomes that can be segregated to much lower level in the next generation. In these population identifying genuine hybrid clones with 1-2 *E. arundinaceus* chromosomes without any recombination or translocation using GISH could be used for dissecting out and sequencing these alien chromosomes.

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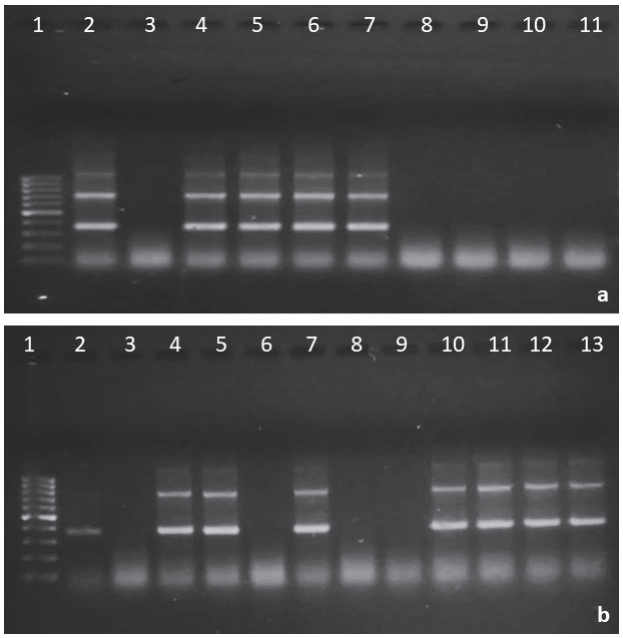

Figure 5. a) Electrophoretogram of F1, BC1 and BC2 progenies with their parents for amplification of ESTR primer in genomic DNA: 1) 100bp ladder, 2) IK 76-62 (Female parent). 3) Iritty-2 (Male parent), 4) CYM 04-420, 5) CYM 07-971, 6) CYM 08-903, 7) CYM 08-922, 8) Co 775, 9) CoC 671, 10) Co 94008, 11) Bo 91. b) Electrophoretogram of BC3 and BC4 progenies for amplification of ESTR primer in genomic DNA: 1) 100bp ladder, 2) IK 76-62 (Female parent). 3) Iritty-2 (Male parent), 4) TWC 82, 5) Co 15015, 6) GI 18-1, 7) GI 18-2, 80 GI 18-3, 9) GI 18-4, 10) FWC-2, 11) FWC-28, 12) FWC-29, 13) FWC-39

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