Meiotic Abnormalities in Intergeneric Hybrids between *Saccharum spontaneum* and *Erianthus arundinaceus* (Gramineae)

Raghavan Lalitha and Mylottazhikam N. Premachandran*

Crop Improvement Division, Sugarcane Breeding Institute, Coimbatore-641 007 India

Received July 24, 2007; accepted August 20, 2007

Summary Cytological studies were made in hybrids between *S. spontaneum* L clone Iritty-2 ($2n=64$) and *Erianthus arundinaceus* (Retz.) Jesweit clone IK 76-99 ($2n=60$). The chromosome number of 19 hybrids ranged from $2n=78$ to 86. Predominantly bivalent formation was observed at diakinesis and metaphase I stages in pollen mother cells of all the hybrids. At later meiotic stages abnormalities such as lagging chromosomes, multipolar spindles, abnormal planes of cytokinesis and micronuclei were present. In one hybrid CYM 04-391 with chromosome number $2n=80$, the pollen mother cells had cytomixis and syncyte formation in the prophase stage. Certain syncytes had very high number of chromosomes in the form of bivalents at diakinesis and metaphase I, with nearly 80 to more than 600 bivalents. Fertile pollen was observed in few anthers of CYM 04-391 and the pollen size variation was high in it compared to that in the other similar hybrids.

Key words Sugarcane, *Saccharum spontaneum*, *Erianthus arundinaceus*, Intergeneric hybrid, Meiotic abnormalities, Syncyte.

Sugarcane (*Saccharum* spp. hybrid) is a major crop in the tropical and subtropical regions of the world grown mainly for sugar and ethanol. The commercial sugarcane varieties are complex derivatives of man made-interspecific hybrids involving the ‘noble’ cane *Saccharum officinarum* L. and the wild grass *S. spontaneum* L. in which other *Saccharum* species *S. barberi* Jesweit, *S. sinense* Roxb. and *S. robustum* Jesweit and Brandes ex Grassl. were also incorporated (Daniels and Roach, 1987). The chromosome number of the commercial sugarcane clones range between $2n=100$ and 130, higher than that of the basic species *S. officinarum* ($2n=80$) and *S. spontaneum* ($2n=64$ or 80) involved in their origin (Sreenivasan *et al.* 1987). This is due to the phenomenon of $2n+n$ transmission in crosses involving *S. officinarum* and *S. spontaneum* and also in the first back cross when *S. officinarum* is used as female parent. The ‘*Saccharum* complex’, proposed by Mukherjee (1957) includes the genera *Saccharum*, *Erianthus* Michx. sect. *Ripidium* Henrad, *Scleorostachya* (Hack.) A. Camus and *Narenga* Bor. which constitute a closely related interbreeding group. Later, Daniels *et al.* (1975) suggested to include *Miscanthus* also to the *Saccharum* complex. The origin of *S. officinarum* is still debated and the most plausible path of evolution was given by Daniels and Roach (1987) that it was originated in the East Indonesian/New Guinea area from *S. spontaneum*, *Miscanthus sinensis* and *Erianthus arundinaceus* (Retz.) Jesweit.

S. spontaneum with wide eco-geographical distribution has many cytotypes with chromosome numbers ranging from $2n=40$ to 128 (Panje and Babu, 1960; Sreenivasan *et al.* 1987). Janaki Ammal (1941) crossed *S. spontaneum* ‘Glagha’ ($2n=112$) with *Erianthus ravennae* ($2n=20$) and obtained hybrids having chromosome number $2n=66$, resulting from $n+n$ transmission. The *S. spontaneum* ‘SES 248’ ($2n=40$) $\times$ *E. ciliaris* ($2n=40$) gave rise to hybrids with $n+n$ transmission

* Corresponding author, e-mail: premsbi@rediffmail.com

(Kandasami 1961). The hybrids of *S. spontaneum* 'Coimbatore' ($2n=64$) $\times$ *E. ciliares* ($2n=40$) were with $2n=84$, from $2n+n$ transmission. *Erianthus arundinaceus* is a large grass with tall and thick stalks resembling sugarcane and having useful traits such as growth vigour, wide ecological adaptability, extensive root system, drought and water logging resistance, good ratoonability, high biomass and disease resistance (D'Hont *et al.* 1995). *E. arundinaceus* is being used in the sugarcane breeding programmes for introgression of favourable agronomic characters from *E. arundinaceus* to sugarcane but the non-flowering or sterility of many of the hybrids with *S. officinarum* was a limiting factor (Piperidis *et al.* 2000). The wide crosses of sugarcane with the wild species *S. spontaneum* and *E. arundinaceus* are being done to improve the biomass yield of sugarcane as an energy crop (Ming *et al.* 2006). Although *S. spontaneum* and *E. arundinaceus* were considered as major players in the origin of *S. officinarum*, no detailed cytogenetic study of hybrids between *S. spontaneum* and *E. arundinaceus* was reported. The present study was taken up to understand the cytogenetic behaviour of the hybrids between the 2 wild species *S. spontaneum* and *E. arundinaceus* which are considered to be involved in the origin of cultivated sugarcane.

Material and methods

The *Saccharum spontaneum* L. clone Iritty-2 was pollinated with *Erianthus arundinaceus* (Retz.) Jesweit clone IK 76-99 and the seedlings raised from the hybrid seeds were clonally maintained in the field. The somatic chromosome number of the parental clones and the hybrids were determined by root tip squash technique. Actively growing root tips from cane cuttings planted in pots with river sand were excised and treated in a saturated solution of α -bromo naphthalene for about 90 min. After thorough washing the root tips were fixed in 3 : 1 ethanol–acetic acid at least for 16 h. The root tips were hydrolyzed in 1 N hydrochloric acid at 60°C for 12 min and after washing in water were stained in leuco-basic fuchsin under dark for 30 min. Deeply stained root tips were excised and squashed in 1% acetocarmine. Well spread mitotic chromosomes were counted and photographed.

For meiotic studies immature inflorescence at the boot stage was fixed in 3 : 1 ethanol–acetic acid. The anthers from individual florets were smeared on glass slides using 1% acetocarmine. Chromosome behaviour at different meiotic stages in pollen mother cells was observed. The pollen fertility was determined by stainability of pollen in 1 : 1 mixture of 1% acetocarmine–glycerol. Uniformly stained pollen was scored as fertile, while partially stained and unstained pollens were considered as sterile. The pollen size was determined by measuring the diameter of the uniformly stained pollen under a light microscope, using ocular micrometer, calibrated with a standard stage micrometer.

Results

The chromosome numbers of the *S. spontaneum* clone Iritty-2 and *E. arundinaceus* clone IK 76-99 were determined to be $2n=64$ and $2n=60$, respectively. The meiosis was normal in both clones, having 32 bivalents in Iritty-2 and 30 bivalents in IK 76-99. The anaphase segregation in them was also devoid of any abnormalities. Out of 20 seedlings raised from the cross between these clones, 19 plants were found to be hybrids with chromosome number ranging from $2n=78$ to 86 (Table 1), and the remaining one the selfed progeny of the female parent *S. spontaneum* with $2n=64$.

The *S. spontaneum* $\times$ *E. arundinaceus* hybrids which had chromosome number ranging from $2n=78$ to 86 studied were having predominantly bivalent formation at diakinesis and metaphase I stages in pollen mother cells. More than 50% pollen mother cells had only bivalents. The univalents and multivalents observed were few, with a maximum of 6 univalents. At anaphase I, in all the hy-

Table 1. Chromosome number, pollen fertility and pollen size of hybrids between *Saccharum spontaneum* and *Erianthus arundinaceus*

S. No.	Clone number	Chromosome number (2n)	Pollen fertility (%)	Pollen diameter		
				Mean (μm)	Standard deviation	Coefficient of variation (%)
1	CYM 04-385	78	3.5	33.29	3.17	9.53
2	CYM 04-386	80	43.8	32.63	4.71	14.44
3	CYM 04-387	84	55.7	28.82	3.36	11.65
4	CYM 04-388	82	87.3	44.19	6.64	15.02
5	CYM 04-390	80	3.2	29.26	2.15	7.34
6	CYM 04-391	80	7.4	48.68	14.51	29.81
7	CYM 04-392	82	49.5	34.29	5.52	16.09
8	CYM 04-393	86	1.2	49.96	6.24	12.49
9	CYM 04-394	86	81.5	43.11	7.44	17.26
10	CYM 04-395	78	35.9	39.32	8.01	20.36
11	CYM 04-396	82	18.9	26.61	2.01	7.56
12	CYM 04-397	80	80.2	37.92	3.33	8.77
13	CYM 04-398	82	37.5	34.14	5.00	14.64
14	CYM 04-399	80	87.6	47.80	6.87	14.37
15	CYM 04-401	82	32.7	38.68	5.45	14.09
16	CYM 04-402	82	24.2	42.72	5.52	12.93
17	CYM 04-403	80	59.1	35.08	6.95	19.83
18	CYM 04-404	78	39.0	37.72	3.54	9.39
19	CYM 04-405	78	69.5	41.76	5.41	12.96

brids varying numbers, up to 12, lagging chromosomes were present in the pollen mother cells. The data on the frequency of pollen mother cells with laggards at anaphase I and II are presented in Table 2. The frequency of pollen mother cells with more than 5 laggards was the highest in CYM 04-399 at anaphase I and anaphase II. Other hybrids CYM 04-391, CYM 04-397 and CYM 04-404 also had high frequency of pollen mother cells with more than 5 laggards. The tetrads were having micronuclei in all the hybrids.

In one *S. spontaneum*×*E. arundinaceus* hybrid clone, CYM 04-391 with chromosome number $2n=80$ (Fig. 1a), many meiotic abnormalities were observed from pachytene stage onwards (Fig. 1b to j). Cytomixis and the fusion of pollen mother cells were very common, along with few normal pollen mother cells. In the fused pollen mother cell the chromatin of the component cells was independent with nuclear envelopes at pachytene stage, appearing as multinucleate syncytes. At diakinesis, large number of bivalents was seen in the syncytes. The number of bivalents was from 80 to more than 650 in certain syncytes. At metaphase, some syncytes were with up to 5 metaphase plates with multipolarity. There was variation in number of chromosomes arranged in different metaphase plates in the same syncyte. In certain pollen mother cells few chromosomes were far apart from the spindles. At anaphase there were cells with large number of scattered chromosomes, having appearance of highly condensed mitotic chromosomes. Absence of cytokinesis at telophase I and II resulted in some large sized restitution nuclei. There were anucleate cells adjacent to normal pollen mother cells at prophase to telophase II. In pollen mother cells with normal size also there were abnormalities such as multipolar spindles and disoriented spindles at metaphase I, lagging chromosomes at anaphase I, absence of cytokinesis, and micronuclei at telophase I and II. The number of pollen mother cells that could be observed for meiotic abnormalities were less in CYM 04-391 compared to that in other hybrids (Table 1).

In most of the anthers of the hybrid clone CYM 04-391 unstained, empty pollen only was observed, whereas in few anthers stainable pollen was present, up to 7.4%. The size of such stained

Table 2. Number of laggards in pollen mother cells at anaphase I and anaphase II

Sl. No.	Clone number	Frequency of pollen mother cells with laggards																
		Anaphase I								Anaphase II								
		No. of cells		0	1	2	3	4	5	>5	No. of cells		0	1	2	3	4	5
1	CYM 04-385	166	0.41	0.21	0.10	0.28	0.00	0.00	0.00	187	0.30	0.00	0.06	0.40	0.25	0.00	0.00	0.00
2	CYM 04-386	167	0.39	0.00	0.17	0.28	0.07	0.08	0.01	155	0.38	0.00	0.25	0.21	0.12	0.05	0.00	0.00
3	CYM 04-387	152	0.11	0.00	0.24	0.20	0.15	0.21	0.09	166	0.23	0.10	0.17	0.16	0.14	0.08	0.11	0.00
4	CYM 04-388	173	0.27	0.36	0.17	0.08	0.13	0.00	0.00	188	0.34	0.00	0.08	0.09	0.39	0.10	0.00	0.00
5	CYM 04-390	184	0.09	0.12	0.05	0.09	0.23	0.28	0.13	190	0.12	0.09	0.16	0.28	0.34	0.00	0.00	0.00
6	CYM 04-391	107	0.12	0.00	0.04	0.07	0.12	0.13	0.52	62	0.00	0.00	0.00	0.06	0.00	0.00	0.94	0.00
7	CYM 04-392	148	0.38	0.06	0.07	0.20	0.12	0.18	0.00	186	0.16	0.05	0.15	0.20	0.45	0.00	0.00	0.00
8	CYM 04-393	104	0.14	0.00	0.13	0.45	0.17	0.11	0.00	149	0.21	0.13	0.42	0.08	0.10	0.06	0.00	0.00
9	CYM 04-394	184	0.34	0.21	0.24	0.21	0.00	0.00	0.00	159	0.30	0.04	0.06	0.13	0.47	0.00	0.00	0.00
10	CYM 04-395	139	0.37	0.14	0.09	0.12	0.10	0.18	0.00	166	0.28	0.11	0.07	0.13	0.22	0.19	0.00	0.00
11	CYM 04-396	173	0.15	0.00	0.00	0.17	0.25	0.36	0.09	172	0.19	0.00	0.00	0.18	0.36	0.17	0.11	0.00
12	CYM 04-397	136	0.39	0.02	0.02	0.04	0.07	0.18	0.28	156	0.37	0.00	0.08	0.10	0.14	0.30	0.00	0.00
13	CYM 04-398	171	0.14	0.14	0.16	0.23	0.32	0.00	0.00	150	0.13	0.11	0.24	0.42	0.09	0.00	0.00	0.00
14	CYM 04-399	99	0.28	0.00	0.00	0.00	0.00	0.00	0.72	161	0.21	0.00	0.00	0.00	0.09	0.14	0.56	0.00
15	CYM 04-401	151	0.12	0.27	0.25	0.14	0.09	0.00	0.13	153	0.19	0.00	0.12	0.11	0.20	0.27	0.11	0.00
16	CYM 04-402	150	0.21	0.14	0.13	0.13	0.28	0.11	0.00	193	0.16	0.19	0.07	0.21	0.22	0.15	0.00	0.00
17	CYM 04-403	164	0.16	0.07	0.18	0.13	0.32	0.14	0.00	168	0.27	0.14	0.17	0.15	0.19	0.08	0.00	0.00
18	CYM 04-404	169	0.22	0.04	0.08	0.06	0.07	0.23	0.30	182	0.20	0.08	0.05	0.06	0.08	0.18	0.35	0.00
19	CYM 04-405	150	0.44	0.27	0.10	0.09	0.10	0.00	0.00	164	0.45	0.12	0.18	0.26	0.00	0.00	0.00	0.00

Fig. 1. Cytology of *Saccharum spontaneum*×*Erianthus arundinaceus* hybrid CYM 04-391. a) Somatic chromosomes ($2n=80$), b) pollen mother cell with 38 bivalents and 4 univalents, c) disoriented spindle at metaphase II with micronuclei, d) syncyte with two nuclei, e) pollen mother cells with size difference at telophase I. f) syncyte with abnormal metaphase plates. g) syncyte with seven nuclei h) syncyte with nearly 280 bivalents i) syncyte with more than 500 bivalents j) large nuclei at telophase in a syncyte, k) size difference in pollen. Scale bar: 10 μm for a to j and 100 μm for k.

pollen was highly variable (Fig. 1k) ranging from 34 μm to 67 μm in diameter with a high coefficient of variation 29.8% in the hybrid CYM 04-391, compared to the coefficient of variation ranging from 7.56 to 20.36% in other *S. spontaneum*×*E. arundinaceus* hybrids studied (Table 2). This particular hybrid and other seven hybrids either selfed or crossed with *S. spontaneum*, *E. arundinaceus* or commercial sugarcane clones were female fertile and seedlings were obtained.

Discussion

Among the cytotypes of *S. spontaneum* octaploids with chromosome number $2n=64$, with a basic chromosome number $x=8$, is prevalent in the Indian subcontinent and were reported to be having normal meiosis with regular bivalent formation, high pollen fertility and good seed set (Sreenivasan *et al.* 1987). The *S. spontaneum* clone Iritty-2 from south India was with $2n=64$. The *E. arundinaceus* clone IK 76-99 was collected from Kalimantan province in Indonesia and is with chromosome number $2n=60$ as that in other clones of this species collected from this region. They are hexaploids with basic chromosome number $x=10$ (Besse and McIntyre 1998). The chromosome

number in *S. spontaneum* ($2n=64$) $\times$ *E. arundinaceus* ($2n=60$) hybrids were higher than $2n=62$ expected from $n+n$ chromosome transmission, but with less than $2n=92$ or 94 , that was expected from functioning of a $2n$ gamete from either male or female parent. These hybrids with $2n=78$ to 86 could be the products of n gamete from one of the parents and $2n$ gamete from the other parent and subsequent elimination of few chromosomes from the hybrid.

In *S. officinarum* $\times$ *S. spontaneum* crosses the $2n+n$ transmission is the norm rather than an exception (Sreenivasan *et al.* 1987). In the *S. officinarum* $\times$ *E. arundinaceus* hybrids studied by fluorescent *in situ* hybridization by Piperidis *et al.* (2000) 6 hybrids had fewer than 70 chromosomes expected from $n+n$ transmission and 1 hybrid had approximately 78 chromosomes, 50 of which were contributed by *S. officinarum* and 28 by *E. arundinaceus*, was attributed to $2n+n$ transmission. Chromosome elimination occurred in all the hybrids studied by them. In the present study of *S. spontaneum* $\times$ *E. arundinaceus* hybrids resulted from $2n+n$ or $n+2n$ transmission also chromosome elimination had occurred. If we consider that a $2n$ gamete from *S. spontaneum* functioned in the formation of the hybrids, the number of chromosomes eliminated ranged from 8 to 16, whereas if $2n$ gamete from *E. arundinaceus* was functioning, the number of chromosomes eliminated ranged from 6 to 14. The elimination of certain chromosomes from *S. spontaneum* or *E. arundinaceus* will be negating the genomic imbalances created by coexisting 2 diverse genomes in the hybrid whereas in the *S. officinarum* $\times$ *S. spontaneum* hybrids the $2n+n$ transmission provide the genome balance without any chromosome elimination.

All the *S. spontaneum* $\times$ *E. arundinaceus* hybrids studied were having predominantly bivalent formation and abnormalities in chromosome segregation. The high frequency of lagging chromosomes in these hybrids indicated the instability of the contributing genomes of *S. spontaneum* and *E. arundinaceus* in the hybrids and the non inclusion of many chromosomes in the meiocytes may result in gametes with varying chromosome constitution. In the interspecific hybrid between *Brachiaria ruziziensis* and *B. brizantha*, with predominant bivalent formation, the meiotic abnormalities such as precocious migration of chromosomes to poles, laggards, micronuclei, abnormal planes of cytokinesis, *etc.* was reported by Risso-Pascotto *et al.* (2005).

The syncyte formation in certain clones of *Saccharum* and *Erianthus* was first reported by Price (1956) and suggested that functional $2n$ and $3n$ pollen grains may be produced from these syncytes. Syncytes with 2 to 10 nuclei per pollen mother cell were observed in commercial sugarcane varieties CP 61-37, CP 70-1133, CP 77-1776 and other 3 clones by Burner and Legendre (1993). There was variation in frequency of syncytes between these sugarcane clones and CP 70-1133 had nearly 25% pachytene cells as syncytic. They have also observed syncytes at metaphase I with bivalent pairing of the chromosomes as observed in the present study. The syncyte formation in CYM 04-391 could be gene controlled as in the case of *Hordeum vulgare* (Smith, 1942) and *Penisetum americanum* (Rao and Koduru 1978) and elimination of certain chromosomes in the *S. spontaneum* $\times$ *E. arundinaceus* hybrid CYM 04-391 might have favoured the expression of this recessive trait.

The formation of pollen with varying size in the *S. spontaneum* $\times$ *E. arundinaceus* hybrids indicated that the pollen may differ in ploidy level and in the number of chromosomes. The female fertility of the hybrids studied had shown that they are not evolutionary dead ends. Cytomixis and syncyte formation was reported in many plants and gametes with higher ploidy levels could be formed by these mechanisms. The genome reshuffling in the *S. spontaneum* $\times$ *E. arundinaceus* hybrids resulted by elimination of certain chromosomes at the zygotic level itself as well as the allosyndetic pairing, abnormalities in anaphase segregation, irregular cytokinesis, cytomixis and syncyte formation could lead to progeny with novel chromosome combinations.

References

- Besse, P. and McIntyre, C. L. 1998. Chromosome *in situ* hybridization of ribosomal DNA of *Erianthus* sect. *Ripidium* species with varying chromosome numbers confirms $x=10$ in *Erianthus* sect. *Ripidium*. *Genome* **42**: 270–273.
- Burner, D. M. and Legendre, B. 1993. Chromosome transmission and meiotic stability of sugarcane (*Saccharum* spp.) hybrid derivatives. *Crop Sci.* **33**: 600–606.
- Daniels, J. and Roach, B. T. 1987. Taxonomy and evolution. *In*: Heinz, D.J. (ed.) *Sugarcane Improvement through Breeding*. Elsevier, Amsterdam, The Netherlands. pp. 7–84.
- , Smith, P., Paton N. and Williams, C. A. 1975. The origin of the genus *Saccharum*. *Sugarcane Breed. Newsl.* **36**: 24–39.
- D'Hont, A., Rao, P. S., Feldman, P., Grivet, L., Islam-Faridi, N., Taylor, P. and Glaszmann, J. C. 1995. Identification and characterisation of sugarcane intergeneric hybrids, *Saccharum officinarum*×*Erianthus arundinaceus*, with molecular markers and DNA *in situ* hybridization. *Theor. Appl. Genet.* **91**: 320–326.
- Janaki Ammal, E. K. 1941. Intergeneric hybrids of *Saccharum*. *J. Genet.* **41**: 217–253.
- Kandasami, P. A. 1961. Interspecific and intergeneric hybrids of *Saccharum spontaneum* L. 1. Functioning of gametes. *Cytologia* **26**: 117–123.
- Ming, R. Moore, P. H., Wu, K. K., D'Hont, A., Glaszmann, J. C., Tew, T. L., Mirkov, T. E, da Silva, J., Jifon, J., Rai, M., Schnell, R. J., Brumbley, S. M., Lakshmanan, P., Comstock, J. C. and Paterson, A. H. 2006. Sugarcane improvement through biotechnology. *Plant Breed. Rev.* **27**: 15–118.
- Mukherjee, S. K. 1957. Origin and distribution of *Saccharum*. *Bot. Gaz.* **119**: 55–61.
- Panje, R. R. and Babu, C. N. 1960. Studies in *Saccharum spontaneum*. Distribution of geographical association of chromosome numbers. *Cytologia* **25**: 152–172.
- Piperidis, G, Christopher, M. J., Carol, B. J., Berding, N. and D'Hont, A. 2000. Molecular contribution to selection of intergeneric hybrids between sugarcane and the wild species *Erianthus arundinaceus*. *Genome* **43**: 1033–1037.
- Price, S. 1956. Cytological studies in *Saccharum* and allied genera. I. Syncyte formation in certain clones of *Saccharum* and *Erianthus*. *Cytologia* **21**: 21–37.
- Rao, M. K. and Koduru, P. K. R. 1978. Cytogenetics as a factor for syncyte formation and male sterility in *Pennisetum americanum*. *Theor. Appl. Genet.* **3**: 1–7.
- Risso-Pascotto, C., Pagliarini, M. S. and do Valle, C. B. 2005. Meiotic behaviour in interspecific hybrids between *Brachiaria ruziziensis* and *Brachiaria brizantha* (Poaceae). *Euphytica* **145**: 155–159.
- Smith, L. 1942. Cytogenetics as a factor for multiploid sporocytes in barley. *Amer. J. Bot.* **29**: 451–456.
- Sreenivasan, T. V., Ahloowalia, B. S. and Heinz, D. J. 1987. Cytogenetics. *In*: Heinz, D. J. (ed.) *Sugarcane Improvement through Breeding*. Elsevier, Amsterdam, The Netherlands. pp. 211–253.
-