RESEARCH ARTICLE

MICROSPOROGENESIS IN A FERTILE *SACCHARUM OFFICINARUM* X *ERIANTHUS ARUNDINACEUS* HYBRID WITH FLORAL ABNORMALITIES

M. N. Premachandran* and R. Lalitha[1]

Abstract

Saccharum officinarum is the basic species involved in the origin of commercial sugarcane varieties and Erianthus arundinaceus is a related wild species with biotic and abiotic stress resistance. The chromosome number, meiotic behaviour, floral abnormalities and fertility of the S. officinarum (2n=80) x E. arundinaceus (2n = 60) hybrid 96 GUK 578 was studied. The somatic chromosome number of the hybrid was 2n = 110, with 2n + n transmission from the parental clones. The meiotic metaphase was generally normal with 55 bivalents, whereas the subsequent meiotic stages exhibited abnormalities such as precocious separation of bivalents at late metaphase I, distorted spindles, lagging chromosomes, failure of cytokinesis, abnormal planes of cytokinesis, etc. The first division and second division restitution of nucleus resulted in 2n gametes along with the normal n gametes and aneuploid gametes from elimination of chromosomes due to lagging chromosomes. Even though pistillody was present in most of the spikelets, up to 16.4 % fertile pollen was found in anthers. The female fertility was confirmed by obtaining progeny by pollinating the hybrid with E. arundinaceus, S. spontaneum and a commercial sugarcane variety Co 1148. The chromosome number of backcross hybrid with E. arundinaceus was 2n = 85, and that of the hybrid with Co 1148 was 2n = 110, both resulting from n + n transmission. From the cytological and floral studies the hybridity of 96 GUK 578 could be confirmed and this fertile hybrid can be used in introgression of genes from E. arundinaceus to sugarcane.

Key words : *Intergeneric hybrid, meiotic abnormalities, microsporogenesis, pistillody, Saccharum officinarum x Erianthus arundinaceus, sugarcane*

M. N. Premachandran and R. Lalitha[1]
Crop Improvement Division, Sugarcane Breeding Institute
Coimbatore- 641 007, India
[1]Present address: Department of Biotechnology
Achariya Art's and Science College
Villianur – 605 110, Pondicherry, India
email: premsbi@rediffmail.com

Introduction

Saccharum officinarum L. (Gramineae) is the basic species involved in the development of commercial varieties of sugarcane, a major crop in the tropical and subtropical regions of the world grown mainly for producing sugar and ethanol. The man made hybrids involving the 'noble' cane *S. officinarum* (2*n* = 80) and the wild grass *S. spontaneum* L. (2*n* = 40 to 128) were 'nobilized' by backcrossing to *S. officinarum*. *Saccharum barberi* Jesweit, *S. sinense* Roxb. and *S. robustum* Jesweit and Brandes clones were also incorporated into the sugarcane breeding programme later, thereby resulting in the complex interspecific nature of the present day sugarcane varieties (Daniels and Roach 1987). *Saccharum officinarum* clones were seen only under domesticated conditions in Melanesian native gardens and a large number of clones were collected through organized expeditions to New Guinea and adjacent Indonesian islands in the 19th and 20th centuries. Besides the species of the genus *Saccharum* L., many species of the related genera such as *Erianthus* Michx. sect. *Ripidium* Henrad, *Miscanthus* Anderss, *Sclerostachya* (Hack.) A. Camus and *Narenga* Bor., which constitute the closely related interbreeding *Saccharum* complex group (Mukherjee 1957; Daniels et al. 1975), can also form hybrids with sugarcane. The close morphological similarity of *Erianthus* to *Saccharum*, the primitiveness of the former in relation to the latter

and the evidence from biochemical studies suggested the contribution of *Erianthus* to the origin of *Saccharum* (Daniels and Roach 1987).

Erianthus arundinaceus (Retz.) Jesw. is a wild grass with tall and thick stalks resembling sugarcane. The *S. officinarum* x *E. arundinaceus* hybrids were studied by D'Hont et al. (1995), Besse et al. (1997), Piperidis et al. (2000), Cai et al. (2005) and Piperidis et al. (2010). Even though many reports of the hybrids were available, *E. arundinaceus* was not utilized much in introgression breeding of sugarcane due to non-flowering nature or lack of fertility of the hybrids. Roach (1989) pointed out that *E. arundinaceus* "nobilizes" more readily than *S. spontaneum*, because the F1 hybrids are near to commercial types, have hybrid vigour and possess drought tolerance and disease resistance. The *S. officinarum* x *E. arundinaceus* hybrids were further crossed with sugarcane cultivars and the chromosome constitution of advanced generation hybrids was studied by Piperidis et al. (2010). Premachandran et al. (2011) reported the use of *S. spontaneum* x *E. arundinaceus* and *E. arundinaceus* x *S. spontaneum* hybrids in repeated backcrosses with commercial sugarcane varieties for introgression of *E. arundinaceus* traits to sugarcane. The present study is on the meiotic abnormalities in microsporogenesis and the floral abnormalities of an intergeneric hybrid between *S. officinarum* and *E. arundinaceus* clones.

Material and methods

The plant materials used in the study were *Saccharum officinarum* L. clone 28 NG 210, *Erianthus arundinaceus* (Retz.) Jesweit clones IK 76-99 and IK 76-91, *S. spontaneum* L. clone SES 106B and the commercial sugarcane variety Co 1148, which are clonally maintained at Sugarcane Breeding Institute, Coimbatore.

The hybridization was made under controlled conditions after covering the inflorescence of female parent with a cloth bag supported by aluminium cage before the start of spikelet opening in them. The pollination of the female inflorescence was done in the morning with the pollen collected from the male parent. The pollination was done for 5-6 days from initial spikelet opening. The fluff was collected and sowed in glasshouse and the seedlings were initially transplanted to poly bags and later to field. The plants were further multiplied by planting cane cuttings in the field and maintained clonally.

The somatic chromosome number of the clones studied was determined by root tip squash technique (Lalitha and Premachandran 2007). The microsporogenesis was studied from pollen mother cells undergoing meiosis in young florets. Immature inflorescences at the boot stage were fixed in 3:1 ethanol: acetic acid mixture and kept for a minimum of one day at room temperature. The anthers from individual florets were smeared on glass slides using 1% acetocarmine. Chromosome behaviour at different meiotic stages of pollen mother cells was observed and photographed.

The pollen fertility was determined by stainability of pollen in 1:1 mixture of 1% acetocarmine: glycerol. Uniformly stained pollen was scored as fertile and partially stained and unstained pollen were considered sterile. The pollen size was determined by measuring the pollen diameter under light microscope using ocular micrometer calibrated with standard stage micrometer.

For studying the floral abnormalities, mature spikelets were individually excised under a dissection microscope and the florets were dissected. The details of the numbers of normal anthers, anthers with stigmatic lobes and number of carpel in each floret were observed from sessile and pedicellate spikelets.

Results and discussion

From the cross between *S. officinarum* clone 28 NG 210 and *E. arundinaceus* clone IK 76-99, one seedling was obtained when fluff from two inflorescences was sown. The vigorous hybrid was maintained clonally as 96 GUK 578. It flowered regularly in the second week of November. When the hybrid was pollinated with *E. arundinaceus* clone IK 76-91, two seedlings were obtained. From the crosses 96 GUK 578 x *S. spontaneum* SES 106B and 96 GUK 578 x sugarcane variety Co 1148, more than 200 seedlings each were obtained.

The chromosome numbers of the *S. officinarum* clone 28 NG 210 and *E. arundinaceus* clone IK 76-99 were $2n = 80$ and $2n = 60$, respectively. The

hybrid between these clones, 96 GUK 578, had the somatic chromosome number *2n* = 110 (Fig. 1a) which was expected from the 2*n* + *n* gamete transmission. The *S. officinarum* x *E. arundinaceus* hybrids studied by D'Hont et al (1995) and Piperidis et al. (2000) were aneuploids either with less than 2n=70 chromosomes, expected from *n* + *n* transmission, or with 2*n* = 78 chromosomes, more than that from *n* + *n*. Through genomic *in-situ* hybridization analysis, they found that the chromosome elimination could be from either of the parental species. The *S. officinarum* x *E. arundinaceus* hybrids studied by Besse et al. (1997) and Piperidis et al. (2010) had the chromosome number 2*n* = 70, expected from *n* + *n* transmission. In the present study, the *S. officinarum* x *E. arundinaceus* hybrid had complete chromosome complement expected from 2*n* + *n* transmission. Bremer (1961) and Sreenivasan et al. (1987) observed *n* + *n* transmission in intraspecific crosses of *S. officinarum* and *2n* + *n* transmission in interspecific crosses between *S. officinarum* and *S. spontaneum* as the norm with a very strong balance in *S. officinarum* for euploid chromosome combinations. In *S. spontaneum* x *E. arundinaceus* hybrids studied by Lalitha and Premachandran (2007), the chromosome elimination occurring in the hybrids resulted from *2n* + *n* transmission and all the hybrids were female fertile. The hybrids in which chromosome elimination was confirmed by molecular cytogenetic studies (D' Hont et al. 1995; Piperidis et al. 2000) involved *E. arundinaceus* clones IK76-48 and IK 76-79, which belonged to a small subgroup segregated from the rest of the Indonesian *E. arundinaceus* as reported by Besse et al. (1997). The genotypic difference in the parental clones, especially the *E. arundinaceus* clone IK 76-99, would have resulted in the genomic compatibility in the hybrid 96 GUK 578 to prevent chromosome elimination.

From the *S. officinarum* x *E. arundinaceus* hybrid 96 GUK 578 backcrossed with *E. arundinaceus* clone IK 76-91, two hybrids 05-461 and 05-462 were obtained. These plants had 2*n* = 85 chromosomes (Fig. 2a), indicating that the female gamete with 55 chromosomes functioned in fertilization with male gamete having 30 chromosomes, i.e. n + n transmission. When 96 GUK 578 was pollinated with the commercial sugarcane variety Co 1148 (2*n* = 110) and *S. spontaneum* clone SES 106B, a large number of plants could be obtained. The chromosome number of six plants in the progeny of 96 GUK 578 x Co 1148 cross studied was 2*n* = 110. The fertility of the *S. officinarum* x *E. arundinaceus* hybrids may depend on the parental genotypes as well as the chromosome constitution of the hybrid. The hybrid 96 GUK 578 was fertile similar to that reported by Cai et al. (2005) and Piperidis et al. (2010), when *S. officinarum* clone Badila was crossed with *E. arundinaceus* clone HN92-105 or HN92-77 and Luohanzzhe was crossed with HN92-84. The other *S. officinarum* x *E. arundinaceus* crosses such as IJ 76-455 x IK 76-22, IJ 76-455 x IK 76-79 and NG 57-16 x IS 76-172 produced hybrids which were sterile (Piperidis et al. 2000).

In 96 GUK 578, majority of the pollen mother cells at metaphase I had 55 bivalents and less than 5% had two to four univalents. Subsequent stages after metaphase I exhibited many abnormalities such as precocious migration and irregular segregation of chromosomes in most of the pollen mother cells. Pollen mother cells with precocious chromosome migration to the poles in late metaphase I and metaphase II, lagging chromosomes at anaphase I and II, distorted spindles at anaphase II, failure of cytokinesis, abnormal planes of cytokinesis and micronuclei in the telophase of the first and second divisions were frequently noticed (Fig. 1b to 1i). In certain pollen mother cells, due to asynchronization at metaphase II, one of the sister cells underwent normal cytokinesis, whereas in other cell, the lack of cytokinesis resulted in restitution nucleus. At metaphase II, restitution nuclei were frequently seen in one or both the dividing daughter cells of each pollen mother cell (Fig. 1g). The absence of cytokinesis or abnormal planes of cytokinesis at telophase II were seen in most of the PMCs resulting in the production of various sized microspores. Due to the failure in the reductional wall, a dyad is formed which will give rise to 2*n* microspores. A triad is formed when the reductional wall is partially formed or one of the equational walls has failed to form, with which two *n* microspores and one 2*n* microspore could be formed. The distorted spindles at anaphase II affected the movement of chromosomes to the poles and also resulted in restitution nucleus at telophase II. Various

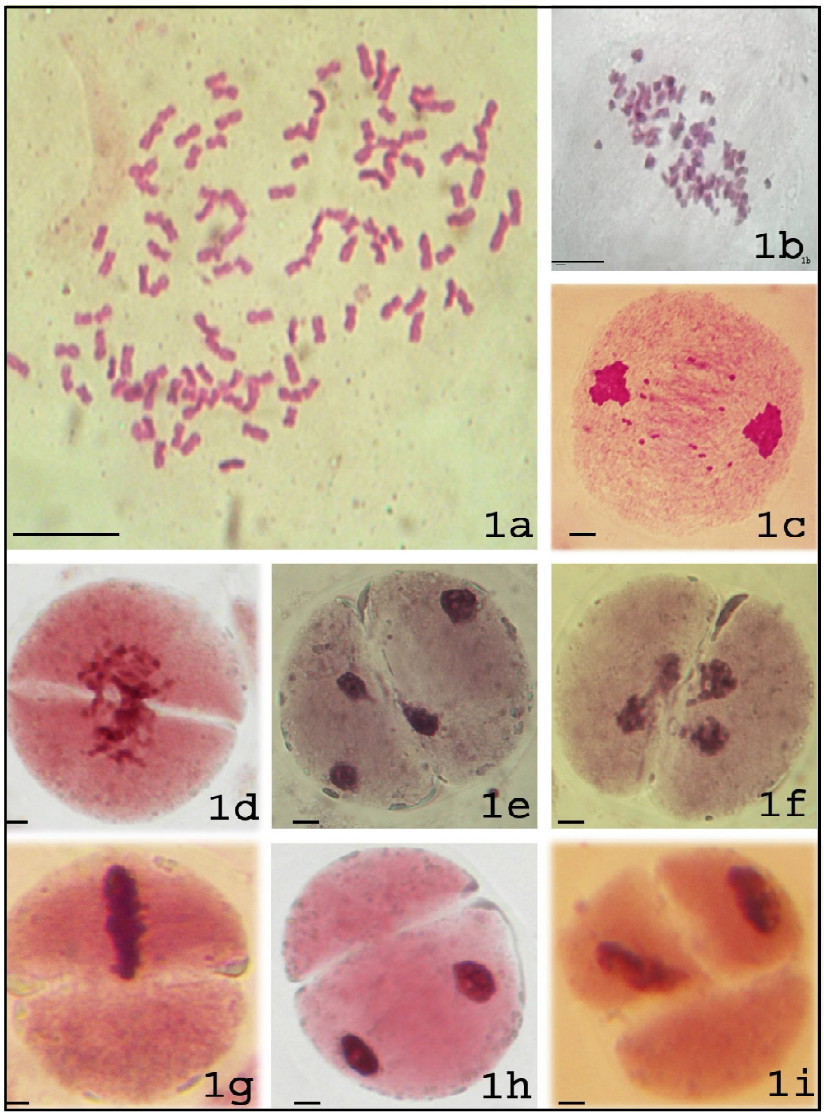

Fig. 1. Cytology of *Saccharum officinarum* x *Erianthus arundinaceus* hybrid 96 GUK 578

(a) Somatic chromosomes (2*n* = 110). (b) Pollen mother cell at metaphase I showing precocious segregation of few bivalents. (c) Lagging chromosomes at anaphase I. (d) Sticky chromosome group which lead to restitution nucleus. (e and f) Orientation of spindles at different planes at anaphase II. (g) One daughter cell remains empty without any chromatin material; other cell shows restitution nuclei at metaphase II. (h) At telophase II, one cell remains empty whereas other cell undergoes normal division. (i) In triad, one cell without nuclear material and two cells with restitution nucleus in each cell at telophase II. (Scale bar = 10μ).

planes of cytokinesis (Fig. 1d) and absence of cytokinesis also led to restitution nucleus in the pollen mother cells during telophase I and telophase II. These irregularities contributed to the formation of abnormal microspores with different combinations of *n* and 2*n* nuclei. The meiotic abnormalities leading to 2n gamete formation in sugarcane cultivar x *S. spontaneum* hybrids was reported by Belig et al. (2003). Similar meiotic abnormalities were reported in other crop plants also. Failure of cytokinesis was found in maize genotypes (Caetano-Pereira et al. 1998) and in many accessions of *Paspalum* species (Pagliarini et al. 1999). Distortion in meiotic spindles responsible for unreduced gamete formation in soybean varieties was reported by Bione et al. (2000).

The present study had shown that the different meiotic abnormalities observed in the *S. officinarum* x *E. arundinaceus* hybrid lead to production of 2*n* gametes by both first division and second division restitution resulting in large variability among such gametes, along with *n* gametes. The precocious segregation of few bivalents, occurrence of lagging

chromosomes and chromosomes not migrating to the poles due to distorted spindles can contribute to gametes with varying chromosome number. The normal pairing and segregation in the pollen mother cells lead to normal *n* gametes also. These aneuploid and euploid gametes involving chromosomes from the genomes of both *S. officinarum* and *E. arundianceus* result in enormous variability that can have selective advantage in fertilization with gametes from other clones with varying genome constitution. The chromosome number of the hybrids from crosses with *E. arundinaceus* IK 76-91 and sugarcane variety Co 1148 had indicated that the female gametes with *n* = 55 only functioned in these crosses. Even though *n*, 2*n* and aneuploid gametes were produced in 96 GUK 578, selective functioning of gametes may occur in different crosses due to the endosperm balance number in the parental clones involved, as was suggested by Burner et al. (1993). The chromosome constitution of the first generation and second generation hybrid of *S. officinarum* x *E. arundinaceus* hybrid with commercial sugarcane cultivars was studied by Piperidis et al. (2010). The first generation hybrid with sugarcane had 2*n* + *n* transmission whereas the second generation hybrids had *n* + *n* transmission as that in the F1 hybrid. The presence of many *S. spontaneum* chromosomes along with haploid genome of *E. arundinaceus* would have altered endosperm balance in the first generation hybrid leading to functioning of 2*n* gamete in cross with sugarcane.

Various degrees of pistillody, the floral abnormality having modification of stamen into pistil, were observed in the spikelets of the *S. officinarum* x *E. arundinaceus* hybrid 96 GUK 578. In both pedicellate and sessile spikelets, variation among the florets in the number of well developed anthers, pistilloid anthers and number of carpel was observed (Fig. 2b and 2c). Out of the 204 sessile spikelets observed, 122 were normal with three anthers and one carpel with bifid stigma in florets, whereas out of 204 pedicellate spikelets, 172 were with normal florets. The frequency of various floral abnormalities observed is presented in Table 1. The basal portion of the styles was swollen in many florets as was observed in certain sugarcane hybrids by Dutt and Rao (1948). Normal florets were more in pedicellate spikelets than in sessile ones. The number of anthers in florets varied from one to six and the number of carpels varied from one to three. The number of pistilloid anthers also varied from one to six. Malformed anthers with stigmatic lobes also were found to have well developed pollen. The pollen fertility as determined in different anthers by stainability in acetocarmine was up to 16.4 %. The pollen diameter of 96 GUK 578 varied from 43.2 ìm to 69.6 ìm with a mean of 52.01 ìm, compared to mean diameter 54.6 ìm in *S. officinarum* 28 NG 210 and 34.9 ìm in *E. arundinaceus* IK 76-99.

In sugarcane hybrids, pistillody was reported previously when *S. spontaneum* was used as female parent in crosses with commercial varieties (Dutt and Rao 1948; Krishnaswamy 1951). In *Arabidopsis* and *Antirrhinum*, loss-of-function mutants of class B genes displayed homeotic alterations in both stamens and petals (Bowman et al. 1991). Pistillody, the homeotic transformation of floral organs to pistil, was reported in wide hybrids of wheat with nucleo-cytoplasmic incompatibility (Murai et al. 2002). The pistillody in *S. officinarum* x *E. arundinaceus* hybrid 96 GUK 578 in the present study may also be due to the nuclear cytoplasmic interactions, as in

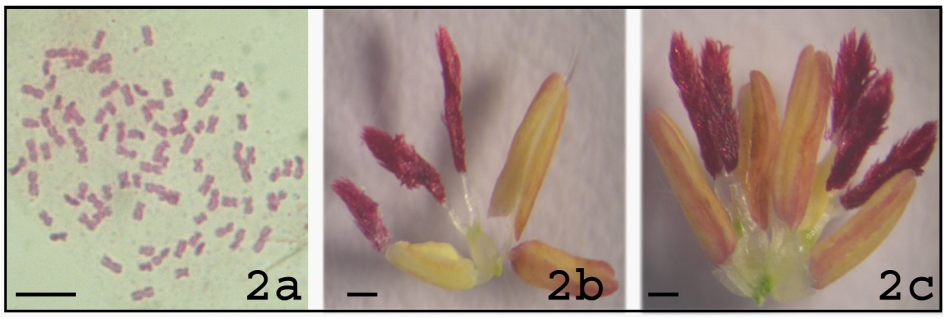

Fig. 2. Pistillody in *S. officinarum* x *E. arundinaceus* hybrid 96 GUK 578

(a) Somatic chromosomes (2*n* = 85) in (*S. officinarum* x *E. arundinaceus*) x *E. arundinaceus* backcross hybrid CYM 04-461. (b) Pistillody in 96 GUK 578 - with stigmatic lobes in one anther. (c) Double floret with pistillody in 96 GUK 578. (Scale bar = 10 μ for 2a and 2 mm for 2b & 2c).

Table 1. Floral abnormalities in *S. officinarum* x *E. arundinaceus* hybrid, 96 GUK 578

S. No.	Floral category[#]	No. of florets in sessile spikelets	No. of florets in pedicellatespikelets
1	1C + 3A (Normal floret)	123	172
2	1C + 3A + 1P	16	6
3	1C + 3A + 2P	1	-
4	1C + 3A + 1P	1	-
5	1C + 2A	-	4
6	1C + 2A + 1P	2	1
7	1C + 2A + 2P	1	-
8	1C + 2A + 3P	1	-
9	1C + 4A	5	1
10	1C + 4A + 1P	-	1
11	1C + 4A + 2P	-	1
12	1C + 5A	4	1
	Subtotal having 1 carpel	154	185
13	2C + 1A + 1P	1	-
14	2C + 2A + 2P	-	1
15	2C + 2A + 3P	2	-
16	2C + 3A	9	1
17	2C + 3A + 1P	7	3
18	2C + 3A + 2P	2	1
19	2C + 4A	7	4
20	2C + 4A + 1P	3	5
21	2C + 4A + 2P	1	1
22	2C + 5A	10	1
23	2C + 5A + 1P	3	-
24	2C + 6A	4	1
	Subtotal having 2 carpels	49	18
25	3C + 1A	1	-
26	3C + 3A	1	-
	Subtotal having 3 carpels	2	-
	Total	205	205

[#] A - Normal anther; C - Carpel; P - Pistilloid anther with stigmatic lobe

wheat, by the presence of *E. arundinaceus* chromosomes along with *S. officinarum* cytoplasm. The hybrid nature of 96 GUK 578 was evident from the pistillody, chromosome number and the meiotic chromosome behaviour. The partial pistillody was not affecting the female fertility of the hybrid and hence the hybrid could be further used in introgression breeding.

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