RESEARCH ARTICLE

CYTOGENETICS AND PERFORMANCE ANALYSIS OF PRE-BREEDING HYBRIDS OF *SACCHARUM OFFICINARUM* AND *SACCHARUM SPONTANEUM*

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Abstract

In sugarcane the genetic base of modern varieties has been reported to be narrowdue to the limited number of species clones used in primary crosses. This necessitates exploration of new possibilitiesby sugarcane breeders to increase genetic variability through utilization ofclones from the germplasm. In the present investigation, pre-breeding hybrids were developed from the crosses between atypical forms of *Saccharum officinarum* and *Saccharum spontaneum*, which include original species and improved clones developed through intraspecific crosses. Meiotic studies of the parents revealed the abnormalities, occasional univalents and rare multivalent pairing in atypical *S. officinarum* clones whereas normal meiosis was observed in *S. spontaneum* clones. The analysis of the progenies has been done for cytological behaviour and quantitative and qualitative parameters. The somatic chromosome number of 87 interspecific hybrids derived from seven biparental crosses involving 5 *S. officinarum* clones (IJ 76-501, IJ 76-315, IJ 76-418, IJ 76-159, 57 NG 136) and 7 *S. spontaneum* clones (SES 407, IND 81-151, IND 81-111, IND 95-530, 92-233, 92-112, 93-243) was determined through root tip mitosis. All the hybrids were found to be the resultant of 'n+n' transmission from the parental clones. In all the crosses anueploids were obtained with chromosome elimination ranging from 1-7 and addition of 1-4 chromosomes. Maximum loss of chromosomes was found in the progenies of the cross IJ 76-418 x 92-233 whereas maximum number of progenies (33%) with chromosome balance was obtained in the crosses 51 NG 159 x 92-112 and 51 NG 159 x 93-243. Detailed meiotic study of the randomly selected progenies confirmed its contribution to heterozygosity in the successive generation through abnormalities during chromosome segregation, multivalent formation and high chiasma frequency. Analysis of agronomical characters like plant height, internode length, number of inter nodes, stalk diameter, number of millable canes, brix % and sucrose % revealed significant variability among the crosses and within the crosses. Red rot screening of 114 progenies resulted in 52 resistant clones with greater number of resistant progenies in the cross IJ 76-501 x IND 81-151 (63%). The cultivated and wild species used in this study were not used so far in sugarcane breeding programmes and they provided the much needed genetic diversity in the progenies for qualitative and quantitative parameters. The diverse variability and identified clones can be exploited as donors in future breeding programmes.

Key words: Pre-breeding, *Saccharum officinarum, Saccharum spontaneum,* interspecific hybrids, chromosome, red rot analysis

Introduction

The present day sugarcane cultivars are hybrids derived from a few interspecific crosses performed a century ago between *Saccharum officinarum* and *Saccharum spontaneum* and back crossed to *S. officinarum*. These hybrids derived their ability to produce cane and sugar from *S. officinarum*, disease resistance, hardiness and ability to withstand adverse conditions from *S. spontaneum*. The

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sugarcane improvement programmes all over the world have been closely linked with the interspecific hybridization involving cultivated and wild species. Among the different species of the genus *Saccharum*, *S. officinarum* is by far the most important for its role in early sugarcane agriculture and later in the evolution of modern sugarcane varieties. This species comprises a group of clones which have chromosome number 2n=80. These are considered typical clones and those deviating from this number are regarded as 'atypical'. Atypical clones have originated in nature as a result of natural hybridization with other species or related genera. (Price 1960; Nair 1973). India is considered to be a major centre of diversity for *S. spontaneum*, the wild species of sugarcane. During the sugarcane improvement programme this wild species has contributed to resistance to disease and pests, improvement in cane yield through increase in number of millable canes and better ratoonability, and expansion of area under sugarcane cultivation by imparting wider adaptability. The first Indian commercial hybrid cane Co 205 was an interspecific hybrid between *S. officinarum* Vellai and *S. spontaneum* Coimbatore (Barber, 1920). In India subsequently *S. barberi*, *S. sinense* and wild species *S. spontaneum* were utilized, along with *S. officinarum* for evolving commercial hybrids (Venkatraman 1938). After the initial interspecific crosses inter se crossing of their derivatives has been done by the breeders for further improvement of yield and quality of commercial hybrid cane varieties.

Because of the small number of clones/ species used in primary crosses, genetic base of modern sugarcane varieties has been reported to be narrow and is thought to be the reason for the present slow in sugarcane breeding (Arceneaux 1965; Natarajan 1984; Naidu and Sreenivasan 1987; D'Hont et al. 1995). Concern has also been expressed in this regard about the overall reduction of the gene pool available for sugarcane improvement. This necessitates exploration of new possibilities by sugarcane breeders to increase genetic variability by identifying and effecting crosses between diverse parents from the germplasm collection.

Under a genetic base broadening program Sugarcane Breeding Institute, Coimbatore undertook a massive interspecific hybridization work involving different species of *Saccharum*, starting from early 1980's. As a result of this evaluation programme several elite interspecific hybrids were identified in all major zones of the country. In the present study pre breeding hybrids have been developed through crosses between atypical forms of *S. officinarum* (cultivated species) and *S. spontaneum* (wild species). These hybrids were subjected to cytogenetic analysis and evaluated for their qualitative and quantitative parameters.

Materials and methods

Plant material: The experimental materials for this study comprised 162 progenies obtained from seven biparental crosses involving five *S. officinarum* clones (IJ 76-501, IJ 76-315, IJ 76-418, 51 NG-159, 57 NG 136) as pistil parents and seven *S. spontaneum* clones (SES 407, IND 81-151, IND 81-111, IND 95-530, 92-233, 92-112, 93-243) as pollen parents. The crossing programme was conducted at Sugarcane Breeding Institute and its regional Research Centre, Agali, Kerala.

Cytological Studies: The somatic chromosome number of the parents and hybrids was determined through mitotic studies. Root tips were pre-treated with a saturated solution of á-bromo naphthalene for 2h, fixed in alcohol : acetic acid (3:1) solution, hydrolysed in 1N HCl at 60 C for 13 minand stained in leuco basic fuchsin for 30 min. Squashes were prepared in 1% acetocarmine. Ten to 15 well spread metaphase plates were examined to determine the somatic chromosome number.

Meiotic studies were conducted in pollen mother cells of the hybrids. The young panicles were collected and fixed in carnoy's fluid over night and later stored in 70% alcohol. The anthers were teased out from the florets and pollen smear was prepared in 1% acetocarmine solution. Different stages of meiosis, namely diakinesis, metaphase, anaphase and telophase were observed to study the chromosome behaviour during microsporogenesis.

Analysis of agronomical characters: Data were collected at 12^{th}month from the progenies of the crosses for the agronomical characters like plant height, internode length, number of internodes, stalk diameter, number of millable canes, brix% and sucrose%.

The hybrids were subjected to screening against red rot disease under Controlled Condition Testing (CCT) method as suggested by Mohanraj et al. (1997) and Viswanathan et al. (1998).

Data collected were statistically analysed by one way ANOVA for significant variation of agronomical characters within the clones of the crosses and among the crosses, and comparison were made with Duncan's Multiple Range Test using SPSS 11.5 version.

Results and discussion

Cytological studies in parents and hybrids

The clones of *S. officinarum* are characterised by the absence of fourth glume (Rao, 1949) and this is considered an important character to distinguish *S. officinarum* from *S.spontaneum*, *S. barberi* and *S. sinensis*. The atypical clones of *S. officinarum*are reported to have the fourth glume, indicating the genetic difference from the noble canes with 2n=80 chromosomes (Nair 1973). Root tip mitosis has been done in parents to determine

Table 1. Parents used in pre breeding programme with their somatic chromosome number

Female Parent (*Saccharum officinarum*)		Male parent (*Saccharum spontaneum*)	
Clone	**2n**	**Clone**	**2n**
IJ 76- 501	116	SES 407	64
IJ 76- 501	116	IND 81- 151	64
IJ 76-315	110	IND 81- 111	64
IJ 76- 418	114	92-233	64
51 NG- 159	114	92-112	64
51 NG -159	114	93- 243	64
51 NG- 136	114	95-530	64

the somatic chromosome number in parents and hybrids (Table 1). All the *S. officinarum* clones which were used as female parents were atypical ones with 2n chromosome number > 100. The *S. spontaneum* clones which were used as males showed 2n=64.

Differences have been observed between *S. officinarum* (2n=80) and the atypical noble canes regarding their gametic contribution in crosses with *S. spontaneum*. While functioning of female gametes with chromosome numbers other than 2n was an exception in *S. officinarum* (2n=80) when crossed with *S. spontaneum* (Bremer 1923; Dutt and Subba Rao 1933; Nishiyama 1956; Price 1964; Nair 1968). The somatic chromosome number of 87 interspecific hybrids derived from seven biparental crosses was determined through mitosis. The hybrids showed n+n transmission from the parental clones. Chromosome elimination was observed in almost all the progenies studied. In all the crosses aneuploids were obtained with chromosome elimination range of 1-7 and addition range of 1-4 chromosomes. Maximum loss of chromosomes was found in the progenies of the cross IJ 76- 418 x 92-233. Chromosome balance with expected number was observed in 36% of progenies of 51 NG 159 x 92-112 followed by 30% progenies of 51NG 159 x 93-243. Considering the normal meiotic behaviour of the pollen parent *S. spontaneum*, it can be concluded that the gamete contributed by it had n=32 only. The aneuploids may be a result of irregular meiotic behaviour of atypical *S. officinarum* parent. This view has been supported by the result obtained by Nair (1973) where in some interspecific hybrid eggs of *S. officinarum* with n=4-11 have functioned and formed aneuploids progenies.

During meiosis the frequency of bivalents (42.29 - 43.63) was more than the univalents or trivalents (0.25 - 2.47) in all the PMCs studied (Table 2). Trivalents occurred rarely in PMCs of only two crosses, IJ 76-501 x SES 407 and 51 NG 136 x 95-530. Higher association of chromosomes like quadrivalents was not present in the hybrids. Highest

Table 2. Data on meiosis of the hybrid clones

Cross	No. of bivalents (Mean)	No. of trivalents (Mean)	No. of univalents (Mean)	Chiasma /cell (Mean)	Chiasma / bivalent (Mean)	Observed Chromosome Nos.
IJ 76- 501 x IND 81- 151	43.01	-	1.23	55.55	1.27	86, 88, 87, 92, 95
IJ 76-315 x IND 81- 111	42.75	-	0.94	54.25	1.26	86, 88, 84
IJ 76- 418 x 92-233	42.29	-	1.79	55.8	1.30	82, 84, 89, 86, 92
51 NG- 159 x 92-112	43.32	-	2.42	52.75	1.29	88, 86, 89,90, 92
51 NG- 159 x 93- 243	43.12	-	2.47	45.02	1.30	89, 86, 90, 92
51 NG- 136 x 95-530	43.63	0.25	1.75	52.88	1.23	84, 87 84, 90, 93, 94
IJ 76-501 x SES 405	43.47	0.5	0.25	57.56	1.34	86, 88, 90, 92, 94

values for chiasmata per cell (57.56) and chiasmata per bivalent (1.34) were obtained in the progenies of IJ 76-501 x SES 407. Meiotic abnormalities like asynchrony in chromosome separation, laggards, bridges and micronuclei were observed in progenies of all crosses. Different stages of meiosis observed in IJ 76-501 x IND 81-151are shown in Fig. 1.

of each cross. Duncan's Multiple Range Test showed that HR brix of 57 NG 136 x 95-530 was significantly different from that of other crosses.

Red rot analysis

A total of 114 progenies from seven crosses which were showing good field stand and better

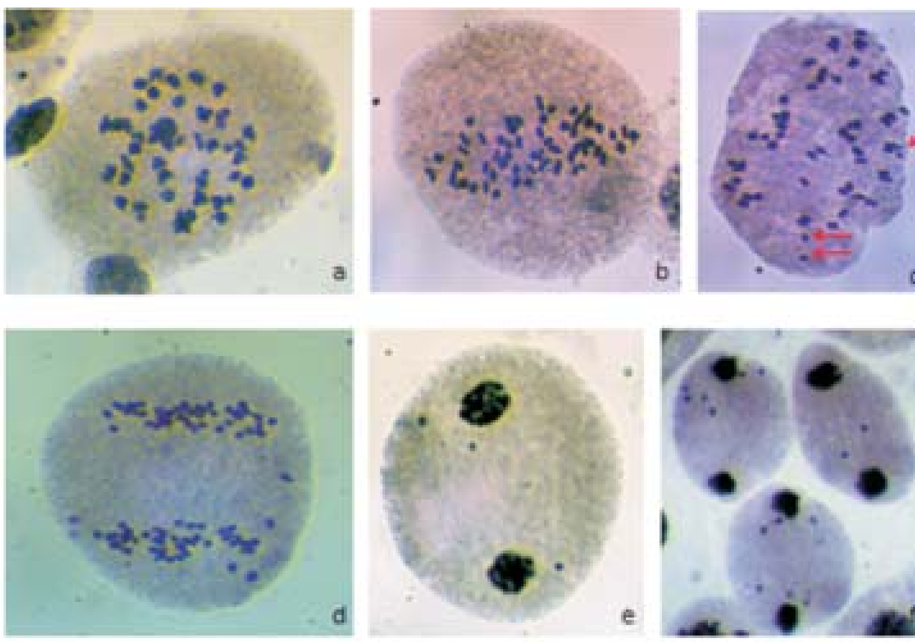

Fig. 1. Meiotic analysis of IJ 76-501 x IND 81-151- (a) Diakinesis (b) Metaphase (c) Metaphase with univalents (arrows) (d) Anaphase (e) Telophase (f) Telophase with micronuclei

agronomical characters were subjected to red tot analysis through CCT method. In the crosses IJ 76 – 501 x IND 81-151 and 51 NG- 159 x 92-112 eight clones were red rot resistant. In total 29 resistant and 23 moderately resistant types were obtained (Table 4).

From the present hybridization programme, 29 F1 hybrids were selected based on yield and quality characters with red rot resistance and are clonally propagated to further nobilize them to commercial status. The cultivated and wild species used in this

Morphological studies

Quantitative and qualitative parameters were studiedin 162 progenies of seven crosses (Table 3). When the agronomical characters and phenotypic appearances were considered, better progenies were obtained from the cross 51 NG 159 x 92-112 which produced more number of progenies with chromosome stability. Two clones each from IJ 76-315 x IND 8-111 and 51 NG 136 x 95 – 530 showed more than 16% sucrose. Analysis showed significant variation for different characters among the clones

Table 3. Analysis of important economic traits in hybrids of different crosses

Cross	HR brix	Stalk diameter	Internode length	Internode number	Plant height
IJ76 -501 x SES 407	8.472[a]	1.3549[a]	13.39[ab]	11.84[a]	131.96[a]
IJ76 -501 x IND 81-151	12.037[c]	1.4983[ab]	11.83[ab]	14.98[bc]	135.33[a]
IJ76 315 x IND 81-111	9.730[ab]	1.6136[bc]	11.32[a]	14.35[abc]	160.14[b]
IJ76 418 x 92-233	12.886[c]	1.7452[cd]	11.86[ab]	14.29[abc]	149.670[ab]
51 NG 159 x 92-112	11.247[bc]	1.5537[b]	14.04[b]	16.07[c]	192.18[c]
51 NG 159 x 93-243	12.060[c]	1.5193[ab]	13.30[ab]	12.43[ab]	151.37[ab]
57 NG 136 x IND 95-530	16.133[d]	1.8200[d]	12.00[ab]	16.33[c]	169.00[b]
SE (mean)	0.331	0.035	0.453	0.531	4.31

Table 4. Status of red rot resistance in progenies of hybrids

Cross	R	MR	S	MS	HS	Total
IJ 76- 501 x SES 407	2	6	10	-	9	27
IJ 76-501 x IND 81- 151	8	6	3	-	5	22
IJ 76-315 x IND 81- 111	3	3	8	-	7	21
IJ 76- 418 x 92-233	4	2	2	-	-	8
51 NG- 159 x 92-112	8	4	2	1	10	25
51 NG- 159 x 93- 243	3	2	3	-	2	10
51 NG- 136 x 95-530	1	-	-	-	-	1
TOTAL	29	23	28	1	33	

study were not used so far in sugarcane breeding programmes and they provided the much needed genetic diversity in the progenies for qualitative and quantitative parameters.

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