

## RESEARCH ARTICLE

**CYTOLOGICAL STUDIES ON *SACCHARUM SPONTANEUM*L.  
FROM ANDHRA PRADESH, INDIA****M. Praneetha and N.V. Nair\*****Abstract**

Chromosome number of 34 *Saccharum spontaneum* accessions from the Godavari and Krishna river basins of Andhra Pradesh, India, was determined. There were four cytotypes among the 34 accessions with  $2n=48, 64, 66$  and  $80$ . Types with  $2n=64$  and  $48$  were more common among the accessions studied while  $2n=80$  was found to be rare. In meiotic studies on 16 accessions, meiosis was found to be largely regular with mostly bivalent formation and occasional univalents or multivalents. Anaphase and telophase stages were found to be normal.

**Key Words:** *Saccharum spontaneum*, Andhra Pradesh, chromosome number, cytotypes

**Introduction**

Occurrence of *Saccharum spontaneum* had been reported from different parts of the world. In India the species is distributed almost throughout the country (Sreenivasan et al. 2001; Sreenivasan and Sreenivasan 1994; Kandasami et al. 1983). However the maximum diversity is reported from North-East India, particularly in Arunachal Pradesh (Nair and Senthil Kumar 2006). India is considered to be the center of origin and diversity for the species (Mukherjee 1950; Roach and Daniels 1987). The species exhibits considerable variability in gross morphology and in terms of cytotypes. Dwarf forms of less than 1m height to tall forms growing to over 7m in height had been recorded in the species. Over 40 cytotypes of *S. spontaneum* in the range of  $2n=40-128$  had been reported. Over 30 cytotypes including the lowest cytotype of  $2n=40$  have been reported from India.

*Saccharum spontaneum* is considered to be the most important genetic resource for varietal improvement in sugarcane. The major breakthroughs in sugarcane breeding had been achieved through interspecific hybridization involving *S. officinarum* and *S. spontaneum* followed by back crossing of the hybrids to the cultivated forms. The sugarcane varieties grown world over today thus have *S. spontaneum* derivation and the high productivity, tolerance to abiotic stresses and resistance to pests and diseases of the modern sugarcane varieties have been attributed to *S. spontaneum*. In view of the importance of the species in sugarcane breeding, considerable attention had been given to the collection, conservation and characterization of *S. spontaneum*. Under the National Agricultural Technology Project (NATP) on biodiversity, Sugarcane Breeding Institute organized several explorations to the distributional areas of the species

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in the country to collect the *Saccharum* germplasm during 1999-2004. The present study pertains to the cytological evaluation of 34 *S. spontaneum* accessions collected from the southern state of Andhra Pradesh under the NATP.

### Materials and methods

Thirty four *S. spontaneum* accessions from the Godavari and Krishna river basins of Andhra Pradesh, India, formed the material for the present study (Table 1). For mitotic studies, young vegetative buds were planted in pots and roots were collected

**Table 1. Place of collection and chromosome number of 34 *Saccharum spontaneum* accessions from Andhra Pradesh**

S. No.	Clone	Place of collection	District	River basin	Chromosome No. (2n)
1	IND02-1160	Narasapally	Nizamabad	Godavari	64
2	IND02-1162	Rupalli Gate	Nizamabad	Godavari	64
3	IND02-1163	Edupallipeddavaru	Nizamabad	Godavari	64
4	IND02-1164	Manjira	Nizamabad	Godavari	64
5	IND02-1166	Tirlapuram	Nizamabad	Godavari	64
6	IND02-1167	Mohammed Nagar	Nizamabad	Godavari	64
7	IND02-1168	Nizamsagar	Nizamabad	Godavari	64
8	IND02-1170	Pothangal	Nizamabad	Godavari	66
9	IND02-1171	Chikli	Nizamabad	Godavari	64
10	IND02-1172	Aaloor	Nizamabad	Godavari	64
11	IND02-1173	Doodikagama	Adilabad	Godavari	64
12	IND02-1175	Gudipet	Adilabad	Godavari	64
13	IND02-1176	Gudipet	Adilabad	Godavari	64
14	IND02-1178	Kallera	Adilabad	Godavari	64
15	IND02-1179	Kallera	Adilabad	Godavari	66
16	IND02-1180	Arugunuru	Karimnagar	Godavari	64
17	IND02-1181	Arugunuru	Karimnagar	Godavari	64
18	IND02-1187	Parnasala	Khamma	Godavari	48
19	IND02-1188	Devarpalli	Khammam	Godavari	48

Table 1 Continued

S. No.	Clone	Place of collection	District	River basin	Chromosome No. (2n)
20	IND02-1189	Cherla	Khammam	Godavari	64
21	IND02-1194	Rekapalli	Khammam	Godavari	66
22	IND02-1196	Walamur	E. Godavari	Godavari	64
23	IND02-1205	Bobberlanka	E. Godavari	Godavari	48
24	IND02-1206	Bobberlanka	E. Godavari	Godavari	48
25	IND02-1207	Boberlanka	E. Godavari	Godavari	48
26	IND02-1211	Lanka Bridge	E. Godavari	Godavari	48
27	IND02-1213	Nayakangudem	Khammam	Godavari	48
28	IND03-1267	Vijayawada	Krishna	Krishna	48
29	IND03-1269	Kandipadu Velangonda	Guntur	Krishna	48
30	IND03-1270	Thummakacherru	Guntur	Krishna	64
31	IND03-1277	Karnool Rd.	Mahbubnagar	Krishna	66
32	IND03-1280	Karnool Rd.	Mahbubnagar	Krishna	80
33	IND03-1281	Gollenpalli	Prakasam	Krishna	64
34	IND03-1286	Vundavele	Krishna	Krishna	48

at 1.30 pm and pre-treated with a saturated solution of  $\mu$ bromo-naphthalene for 2 h at room temperature. The roots were then washed in running water and fixed in Ostergren and Heneen fixative (1962) and kept refrigerated overnight. The roots were washed and hydrolyzed in 1 N HCl for 13 minutes at 60°C and stained in Leucobasic Fuchsin for 30 min and squashed in 1% aceto-carmine. Well spread metaphase plates were studied and photographed. A minimum of 20 well spread metaphase plates were studied to determine the chromosome number. Meiotic studies were made on 16 of the accessions that flowered during the season. Spikelets were collected between 10-11am and fixed in Carnoy's

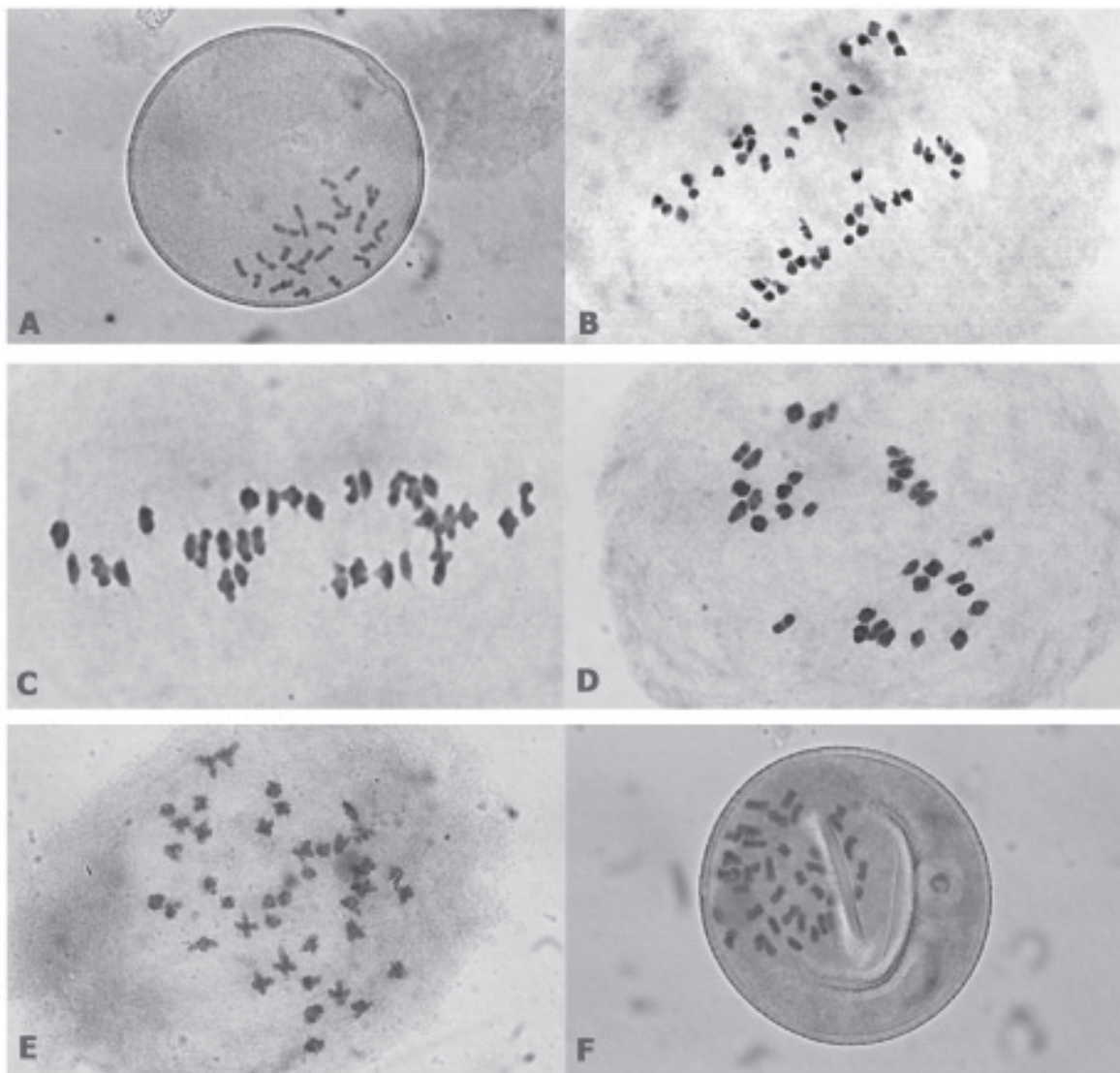
fixative containing 60 ml ethyl alcohol, 30 ml chloroform, 8 ml acetic acid and 2 ml ferric acetate and kept at room temperature for 24-48 h. Spikelets were washed in water and stored in 70% ethyl alcohol. Anther smears were prepared in 1% aceto-carmine and appropriate stages were studied and photographed. Pollen grain squash was done as per Jagathesan and Sreenivasan (1966).

## Results

The somatic chromosome number of 34 *S.spontaneum* accessions was determined (Table 1). There were four cytotypes among the 34 accessions with chromosome numbers  $2n=48, 64,$

66 and 80 (Fig. 1). The major cytotype was  $2n=64$  observed in 19 accessions followed by  $2n=48$  observed in 10 accessions. Four accessions were of  $2n=66$  and one accession recorded  $2n=80$ . The distribution of the cytotypes revealed that the  $2n=80$  form was present only in the Krishna river basin while the other three cytotypes were present in both the river basins.

Meiosis was studied in 16 accessions (Table 2) representing different cytotypes. Meiosis was largely regular with predominant bivalent formation (Fig. 1) and occasional presence of univalents and multivalents. Twenty four bivalents were observed in  $2n=48$  forms, most of which were rod bivalents with less number of ring bivalents. Univalents and trivalents were absent in  $2n=48$  forms, though



**Fig. 1.** (A) Pollen mitosis in IND02-1206 ( $n=24$ ); (B) Anaphase in IND02-1188 ( $2n=48$ ); (C) Metaphase I in IND02-1167 showing 32ii; (D) Metaphase I in IND02-1170 showing 33ii; (E) Diakinesis in IND03-1280 showing 40ii; (F) Pollen mitosis in IND03-1280 showing  $n=40$

**Table 2. Meiotic behaviour of 16 *Saccharum spontaneum* accessions from Andhra Pradesh**

S. No.	Clone	Bivalents			Multivalents		Chiasma/ Bivalent
		Univalents	Ring	Rod	Trivalents	Quadri- valents	
1	IND02-1160	-	14.8	17.2	-	0.5	1.48
2	IND02-1162	-	11.1	20.9	-	2.7	1.43
3	IND02-1164	1.8	8.3	22.7	0.2	0.6	1.25
4	IND02-1167	0.4	7.6	23.2	-	0.6	1.25
5	IND02-1170	0.2	11.4	21.5	-	0.4	1.36
6	IND02-1173	0.2	12.1	19.8	-	-	1.38
7	IND02-1176	-	15.3	15.8	-	-	1.48
8	IND02-1179	0.4	14.1	18.7	-	-	1.42
9	IND02-1180	-	16.1	15.9	-	0.1	1.51
10	IND02-1181	0.1	14.0	17.9	0.1	0.4	1.44
11	IND02-1188	-	10.4	13.6	-	-	1.43
12	IND02-1189	0.2	11.6	20.3	-	-	1.36
13	IND02-1196	0.2	11.1	20.8	-	-	1.34
14	IND02-1205	-	9.7	14.3	-	0.3	1.44
15	IND02-1206	-	12.1	11.9	-	0.5	1.52
16	IND03-1280	-	15.7	24.3	-	2.5	1.46

occasional quadrivalents were present. The mean number of chiasma/bivalent in  $2n=48$  forms was 1.43-1.52.

In  $2n=64$  forms, bivalents ranged from 30.8 to 32.0. Here again the number of rod bivalents was higher than the ring bivalents. The number of ring bivalents ranged from 7.6 to 16.1 while rod bivalents ranged from 15.8 to 23.2. Univalents were present in six of the  $2n=64$  forms and the highest number of univalents was recorded in the clone IND-02-1164 (1.8). Trivalents and quadrivalents were present in two

and six accessions respectively. Variation in chiasma frequency was also observed among the accessions studied. The mean value of chiasma/bivalent ranged from 1.25 to 1.51.

The mean number of bivalents was 32.8 and 32.9 respectively for the two  $2n=66$  types, with more number of rod bivalents. The mean number of univalents present in the two accessions was 0.2 and 0.4 respectively; multivalents ranged from zero to 0.4. The range of chiasma/bivalent was 1.36-1.42.

Rod bivalents were predominant (24.3) in the  $2n=80$  form compared to ring bivalents (15.7) and chiasma/bivalent was 1.46; univalents and trivalents were absent. The mean number of multivalents was 2.5. Anaphase and telophase were normal in all the accessions studied.

## Discussion

During the present study of 34 accessions collected from the two major river basins in Andhra Pradesh, four cytotypes viz.  $2n=48$ , 64, 66 and 80 were recorded; of these,  $2n=64$  and 48 were predominant. A wide range of chromosome numbers has been reported for *S. spontaneum* from Andhra Pradesh. Panje and Babu (1960) reported seven cytotypes with  $2n=48$ , 54, 60, 62, 64, 72 and 86 among the 36 accessions of *S. spontaneum* collected from Krishna, Bhadrachalam, Godavari, Chintapalle and Vizianagaram regions in Andhra Pradesh. The predominant cytotypes in the state are  $2n=48$ , 60 and 64. Thus with the inclusion of the present report, altogether nine cytotypes have been recorded so far from the state. Cytotypes with  $2n=48$ , 64, 66 and 80 were observed among the accessions collected from the Krishna river basin of which  $2n=48$  type was the predominant one. The cytotypes present in the Godavari river basin were  $2n=48$ , 64 and 66. The cytotype with  $2n=64$  was predominant in the Godavari basin while  $2n=48$  forms were relatively less frequent and were mostly present in the lower reaches of the river.  $2n=48$  is the lowest chromosome number reported from tropical India and this has not been recorded from other southern states.  $2n=80$  forms present in the Krishna river basin were conspicuously absent in the Godavari river basin.

Meiosis was regular with largely bivalent formation in all the four cytotypes; only a few univalent and multivalents occurred in some of the accessions. But anaphase and telophase were regular resulting in viable pollen grains. The tendency of the chromosomes to pair only as bivalents at meiosis irrespective of the ploidy level is common in different species of *Saccharum* and related genera (Sreenivasan 1981; Sreenivasan and Sreenivasan 1984; Burner 1991). Janaki Ammal (1939) observed high frequency of bivalent formation even in a triplopolyploid of *S. spontaneum*. These reports suggest a natural tendency for bivalent formation in *Saccharum*, irrespective of the ploidy level, possibly under genetic control (Riley and Law 1965; Burner and Legendre 1994). Univalents were present in many accessions at low frequency in the accessions studied, particularly in  $2n=64$  types. Sreenivasan (1975) observed that the univalents present in *S. spontaneum* are most likely due to the precocious separation of rod bivalents and attributed this to the reduced homology of the chromosomes of the constituent bivalents arising from structural changes. Precocious separation of bivalents is common in plants with small chromosomes (Swaminathan and Howard 1953), as in *Saccharum* species.

The number of chiasma ranged from 1.25 to 1.52 in the accessions studied. Chiasma frequency in *S. spontaneum* from different parts of the world has been reported to be in the range of 1.412 - 1.649 (Sreenivasan 1975; Nair 1968). The present results are also close to this range, though it is lower than the chiasma/bivalent reported in some of the other grass species (Koul and Nagpal 2002). The number of chiasma is dependent on the length of the chromosome and longer chromosomes are likely to

have more number of chiasma. The *Saccharum* chromosomes are relatively small in size and consequently the number of chiasma per homologue is also less. The number of chiasma present is correlated to the recombination frequency, i.e. higher the number of chiasma, higher the recombination frequency.

Thus, *S. spontaneum* present in Andhra Pradesh is diverse with respect to cytotypes compared to other southern states wherein largely  $2n=64$  types have been reported. The presence of low chromosome forms with  $2n=48$  not found in other southern states also makes the *S. spontaneum* diversity in the state all the more important from the point of view of cytotype evolution in the species.

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