RESEARCH ARTICLE

CYTOLOGICAL STUDIES IN HYBRIDS BETWEEN A SUGARCANE CULTIVAR AND THREE CYTOTYPES OF WILD *SACCHARUM SPONTANEUM* L.

A. Suganya*, R. Nithiyanantham, M.N. Premachandran, N. Balasundaram, P. Govindaraj, A. Selvi and N. Vijayan Nair

Abstract

The role of the wild species Saccharum spontaneum L. in sugarcane varietal improvement is well documented. S. spontaneum is a highly polymorphic species with different cytotypes including 2n=40, 48, 56, 60, 64, 72, 80, 96, 112, 120 and 128. The somatic chromosome number from root tips of 30 interspecific hybrids, derived from the crosses between the commercial sugarcane cultivar Co 8371 (2n=108) and S. spontaneum clones with different chromosome numbers (2n=64, 72 and 80), were determined from root tips. All the hybrids were found to be resultants of 'n + n' transmission from the parental clones. Comparison of chromosome number of progeny from three crosses involving three cytotypes (2n=64, 72 and 80) of S. spontaneum revealed chromosome balance in 2n=64 involving hybrids with the presence of expected chromosome number 2n=86 in six out of 10 hybrids studied. In the progeny of Co 8371 x S. spontaneum (SH 216, 2n=72), the chromosome loss ranged from 2 to 10 with 2n=80-88, whereas the expected number was 2n=90. None of the hybrids from Co 8371 x S. spontaneum (2n=80) group showed the expected number 2n=94 and the chromosome number ranged from 2n=84 to 2n=93. The clone 04-897 with 2n=84 exhibited the maximum loss of 10 chromosomes. The present study indicated that chromosome loss in the sugarcane x S. spontaneum hybrids depended on the cytotypes of S. spontaneum. Among the three cytotypes, the 2n=80 types had induced maximum chromosome loss. Though chromosomal loss in the progeny was higher when 2n=72 and 80 cytotypes of S. spontaneum were used as male parent, a careful selection of genotypes with desired traits can be used in introgression programme, irrespective of the chromosome number of S. spontaneum. They can be further used in backcrossing with sugarcane.

***Key words**: Saccharum spontaneum, cytotypes, chromosome number, hybrids*

Introduction

The present day commercial cultivars of sugarcane are complex hybrids of the genus *Saccharum* L. Utilization of wild species in sugarcane breeding dates back to 1885 (Daniels and Roach 1987). Interspecific hybrid varieties that resulted from early nobilization efforts in Indonesia (PoJ 2725 and PoJ 2878) and India (Co 205 and Co 213) formed the foundation on which all sugarcane breeding programmes in the world have been built. The first hybrid developed in India was 'Co 205' which was evolved from *S. officinarum* (Vellai, 2n=80) x *S. spontaneum* (Coimbatore, 2n=64). The wild species *S. spontaneum* has contributed about 10% of the genome to the cultivated sugarcane as evidenced from *in situ* hybridization studies (D'Hont et al. 1996) and is credited to impart the needed pest and disease resistance, abiotic tolerance, high tillering and biomass producing potential (Kandasamy et al. 1983). *S. spontaneum* is a highly polymorphic species existing in various cytotypes including 2n=40, 48, 56, 60, 64, 72, 80, 96, 112, 120 and 128 (Sreenivasan et al. 1987). The progeny of commercial cultivars x *S. spontaneum* with greater genetic diversity is ideal for the incorporation of new

A. Suganya, R. Nithiyanantham, M.N. Premachandran, N. Balasundaram, P.Govindaraj, A. Selvi and N. Vijayan Nair
Division of Crop Improvement, Sugarcane Breeding Institute
Coimbatore 641007, Tamil Nadu, India
email: harimuns@rediff.com

variation into the commercial background and it reduces the breeding cycle in the selection programme (Shang et al. 1968 and Walker 1971). In interspecific and intergeneric hybrids involving *S. spontaneum,* the chromosome number may not be the expected n + n of the parents. A cytologically stable type is preferable for effective selection of genotypes to utilize them further (Roach 1977). Hence in the present study, 30 hybrids derived from the crosses involving Co 8371, a commercial cane, and *S. spontaneum* with three different chromosome numbers, i.e. 2n=64, 72 and 80 were examined to understand the pattern of transmission of gametes through mitosis and to know whether the different cytotypes influence definite pattern of chromosomal additions and deletions.

Materials and methods

Crosses were made between a commercial variety *viz*. Co 8371 and three different *S. spontaneum* clones *viz*. SES 410 (2n= 64), SH 216 (2n=72) and IND 84-415 (2n= 80) under controlled conditions. The seedlings raised from the crosses were clonally maintained. Ten hybrids from each cross were included for the study. A total of 34 clones consisting of 30 hybrids and four parents were studied. Details of the crosses and the hybrids are presented below.

Co 8371 (2n=108) x *S. spontaneum* (SES 410, 2n=64) : Hybrids- 04-836, 04-839, 04-850, 04-856, 04-860, 04-864, 04-1931, 04-1933, 04-1940 and 04-1947.

Co 8371 (2n=108) x S. *spontaneum* (SH 216, 2n=72) : Hybrids- 04-868, 04-870, 04-885, 04-1016, 04-1954, 04-1957, 04-1959, 04-1964, 04-2087 and 04-2092.

Co 8371 (2n=108) x *S. spontaneum* (IND 84-415, 2n=80) : Hybrids- 04-891, 04-897, 04-903, 04-910, 04-1969, 04-1975, 04-1985, 04-1993, 04-2101 and 04-2105.

Mitotic studies: The chromosome numbers of the parents and the hybrids were determined through mitosis by root tip squash method. Single-budded cane setts were cut, planted and germinated in small pots containing river sand. Healthy root tips were collected during mid-day (1.00 p.m.) from 30-45 days old settlings, pretreated with a saturated solution of á-bromonaphthalene for 2 h at 4ºC and fixed in Oestrogen and Heneen's fixative (methanol - 60 ml, chloroform – 30 ml, distilled water – 20 ml, picric acid – 1 g) overnight at 4ºC. After several washes with distilled water, the root tips were hydrolysed with 1N HCl for 13 min at 60ºC, again washed and transferred to pectinase solution for 30 min under dark, again washed and transferred to leucobasic fuchsin stain for 30 min. After removing the root cap the deeply stained meristematic region was squashed with a drop of 1% acetocarmine and slides were examined. A minimum of 10 cells were counted for each clone under microscope (Leica Microstar IV) at 100 X magnification.

Meiotic studies: Meiosis was studied in pollen mother cells using pollen smear technique. The young panicles were collected and tested for the appropriate stage and the inflorescence branches were fixed in Carnoy's fluid overnight. These were washed repeatedly with 70% alcohol and stored in the same. The anthers were teased out from the florets and smear was prepared with a drop of 1% acetocarmine stain. The chromosome behavior at diakinesis, metaphase, anaphase and telophase was observed.

Results and discussion

Mitotic and meiotic studies in the parents

The chromosome number of the sugarcane variety Co 8371 was found to be 2n=108. Meiosis of the female parent exhibited meiotic abnormalities such as laggards, irregular polarity and micronuclei. Diakinesis showed 51-54 bivalents and 0-6 univlents. In anaphase I, 2-6 laggards and 1-2 bridges were observed (Fig.1a). About 61.95% of tetrads had micronuclei with a range from 1-6. *S. spontaneum* clones SES 410, SH 216 and IND 84-415 were with 2n=64, 2n=72 and 2n=80 chromosomes respectively. In SES 410 (2n=64), meiosis was normal with 32 bivalents in majority of the cells and anaphase was regular (Fig.1b). Rarely few laggards were observed. In IND 84-415 (2n=80), diakinesis indicated 38-42 bivalents and 0-2 univalents. Anaphase I was with 3-9 laggards and 1-4 bridges (Fig. 1d). Micronuclei (1-6) were noticed in 77.5% of the cells examined. Similar abnormalities were observed in SH 216 also (Fig. 1c; Table 1).

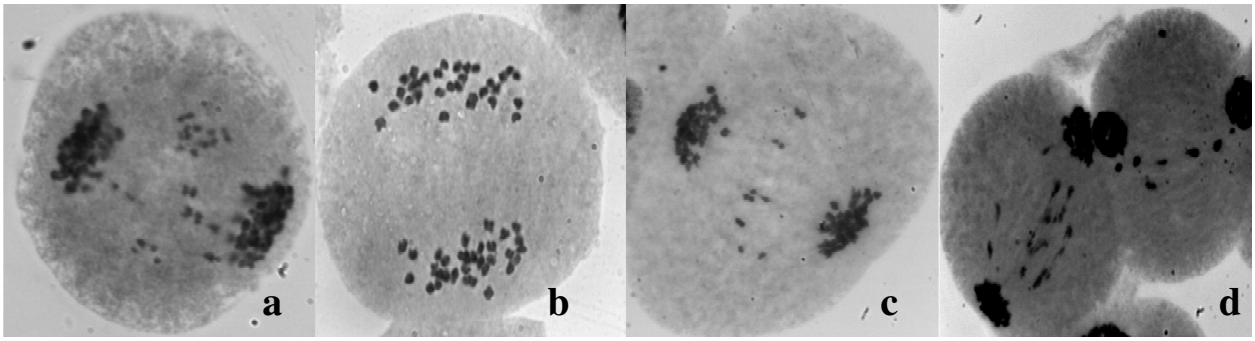


Fig.1. Meiosis of the parents: (a) laggards at anaphase I of Co 8371.(b) regular anaphase in SES 410 (2n=64). (c) lagging chromosomes in SH 216 (2n=72) at anaphase I. (d) bridges in IND 84-415 (2n=80) (100 x)

Table1. Meiotic abnormalities in parents

Clone	Bi valents	Uni valents	Number of *			Tetrads with micronuclei (%)
			Laggards	Bridges	Micro nuclei	
Co 8371	51-54	0-6	2-7 (4.3)	1-2 (1.3)	1-6 (1.1)	61.9
(2n=108)	(54.2)	(1.54)				
SES 410	31-32	0-2	-	-	1-2 (1.5)	6.6
(2n=64)	(31.6)	(rare)				
SH 216	34-36	0-4	3-8 (5.2)	-	3-6 (4.8)	-
(2n=72)	(34.9)	(2.1)				
IND 84-415	39-40	0-2	3-9 (5.8)	1-4 (3.2)	1-6 (2.8)	77.5
(2n=80)	(39.4)	(1.7)				

Observed chromosome number in the hybrids

Co 8371 x *S. spontaneum* (SES 410, 2n = 64): The chromosome numbers of hybrids derived from Co 8371 (2n=108) crossed with SES 410 (2n=64) varied from 2n=84 to 88. The expected number of 2n=86 was observed in six out of 10 hybrids studied (Fig 2a). These hybrids were 04-836, 04-839, 04-850, 04-864, 04-1940 and 04-1931. The hybrids 04-856 and 04-1933 with 2n=84 were with a loss of two chromosomes while the other two hybrids *viz.* 04-1947 and 04-860 had addition of one or two chromosomes with 2n=87 and 2n=88, respectively (Table 2).

Co 8371 x *S. spontaneum* (SH 216, 2n = 72): In the hybrids of Co 8371 x *S. spontaneum* (SH 216, 2n=72), the chromosome numbers ranged from 2n=80 to 88 and the expected number was 2n=90.The chromosome loss was from 2-10. The clone 04-1016 had 2n=80 with the elimination of maximum of 10 chromosomes (Fig. 2b). Deletion of seven chromosomes was noticed in the hybrid 04-868 with 2n=83. In four hybrids 04-870, 04-885, 04-1954, deletion of six chromosomes with 2n=84 was observed at higher frequency. The hybrids 04-2087, 04-2092, 04-1957 had shown 2n=88, 87 and 86 with loss of two, three and four chromosome respectively (Table 3; Fig.2c).

Co 8371 x *S. spontaneum* (IND 84-415, 2n = 80): In the hybrids from Co 8371 x *S. spontaneum* (2n=80), none of the clones had shown the expected number 2n=94 and the chromosome numbers ranged from 24=84 to 2n=93.The chromosome elimination was from 1-10. The clone 04-897 exhibited the maximum chromosomal loss of 10, with 2n=84. The hybrids 04-1993 and 04-2101 possessed 2n=91 with loss of three chromosomes, whereas the clone 04-1985 had 2n=93, losing four chromosomes.

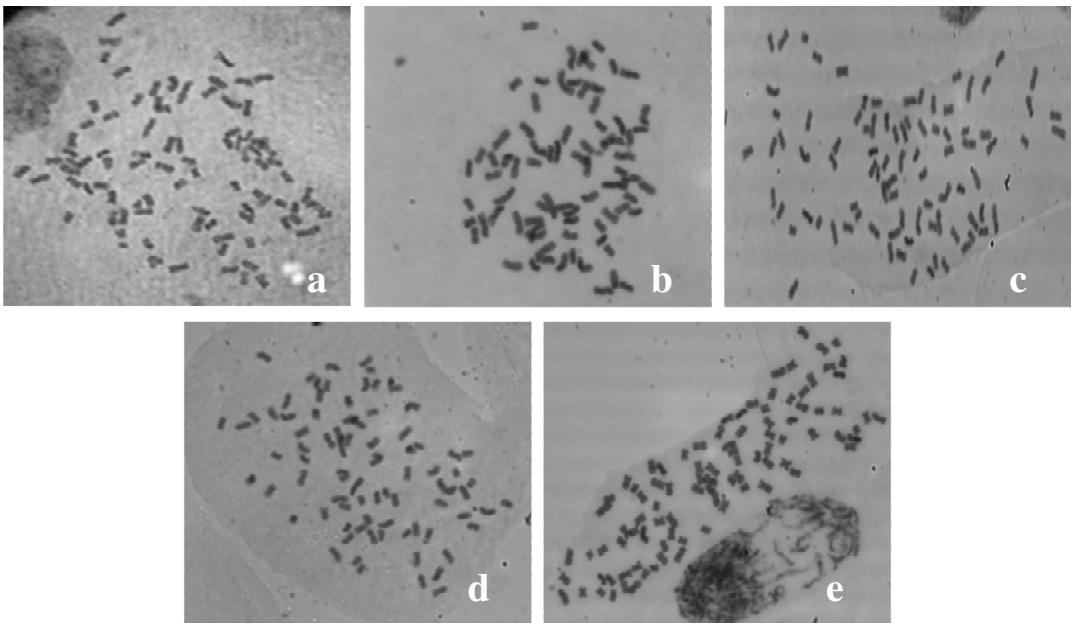


Fig. 2. Somatic chromosomes of the hybrids : a) 04-850 with the expected number, 2n=86. (b) 04-1016 (2n=80) with elimination of 10 chromosomes.(c) 04-2087 (2n=88) with (n+n)-2. (d).04-897 (2n=84) with deletion of 10 chromosomes.(e) 04-891 with 2n=93 (100x)

Elimination of six chromosomes was noticed in the hybrids 04-903 and 04-1969 and deletion of seven chromosomes was observed in 04-1975 and 04-2105 with 2n=87. In 04-910, the number of chromosomes eliminated was eight, with 2n=86. Elimination of 6-10 chromosomes was at a higher frequency in the hybrids from this cross (Table 4; Fig.2d and 2e).

Chromosome counts are necessary to understand the pattern of chromosome transmission in introgression of wild genome in S*accharum.* This leads to identification of cytologically stable and preferable euploid material for effective utilization. The studies on chromosome number in hybrids involving various species in the *Saccharum* complex revealed the occurrence of n + n, 2n + n, n + 2n and 2n + 2n gametic contribution from the parents (Sreenivasan 1987; Stevenson 1965; Price 1957; 1961). The cytological peculiarity of '2n' egg gamete transmission in interspecific crosses of *S. officinarum* with *S. spontaneum* was discovered by Bremer as early as 1922 and by others later on (Bremer 1949; Raghavan 1952). Transmission of 'n + n' gametes had also been reported (Price 1957; Kandasamy 1961; Nair 1975). However, the commercial varieties transmit their gametic chromosomes (n) regularly when intercrossed with *S. spontaneum* (Sreenivasan et al. 1987).

In the present study also, all the hybrids exhibited 'n + n' transmission and the aneuploid number resulted due to the elimination of one or a few chromosomes

Table 2. Chromosome number in the hybrids of Co 8371(2n=108) x *S. spontaneum* (SES 410, 2n = 64)

Cross	**Chromosome number (2n)**										
		Observed number in hybrids									
Co 8371 (2n=108) X	Expected number (n+n)	04-836	04-839	04-850	04-856	04-860	04-864	04-1931	04-1933	04-1940	04-1947
SES 410 (2n=64)	86	86	86	86	84	88	86	86	84	86	87

Table 3. Chromosome number in the hybrids of Co 8371(2n=108) x *S. spontaneum* (SH 216, 2n =72)

Cross	Chromosome number (2n)										
		Observed numbers in the hybrids									
Co 8371(2n=108) X	Expected number (n+n)	04-868	04-870	04-885	04-1016	04-1954	04-1957	04-1959	04-1964	04-2087	04-2092
SH 216 (2n =72)	90	83	84	84	80	84	86	84	86	88	87

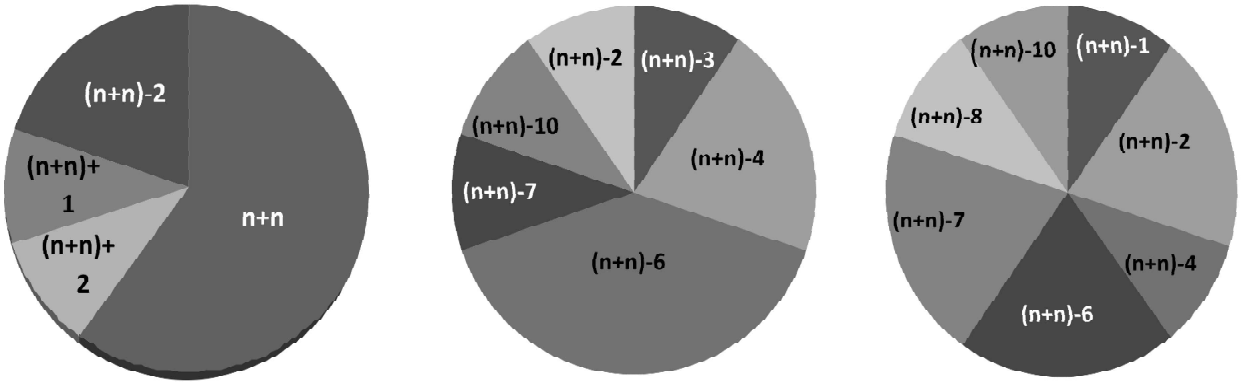
from the gametes. Presence of high frequency of hybrids with other than the expected chromosome number with n + n in the progeny from sugarcane x *S. spontaneum* crosses indicated the functioning of pollen grains with chromosome numbers other than the haploid number of the clone. The meiotic studies in the parental sugarcane clones and the three cytotypes of *S. spontaneum* indicated that such gametes are possible due to meiotic abnormalities. It could give rise to a new clone with an entirely different chromosome number due to their complex polyploidy. Comparison of chromosome numbers of three crosses involving three cytotypes (2n=64, 72 and 80) of *S. spontaneum* revealed chromosome balance in the hybrids involving 2n=64 cytotype, with the presence of expected chromosome number of 2n=86 in 60 % of the progeny studied and the magnitude of loss was only up to two chromosomes (Fig. 3a). The other hybrids derived with 2n=72 and 2n=80 had loss of 1-10 chromosomes. Most of these transmissions of aneuploid chromosome number are due to the irregular meiotic behavior and subsequent development of gametes with altered chromosome numbers in the parents (Nair 1975).

Meiotic studies in the parents in the present study also showed meiotic abnormalities in IND 84-415 (2n=80) with bridges and 4"8 laggards. Micronuclei were also observed. SES 410 (2n=64) meiosis was normal and rarely few laggards were observed. Spindle abnormalities accompanied by unequal numerical distribution of chromosomes and elimination of univalents have been observed in the meiosis of *S. spontaneum* (Nair 1973) to support this view. Roach (1977) also found meiosis to be more irregular in hybrids of *S. officinarum* with *S. spontaneum* (with 80 chromosomes) than in hybrids with *S. spontaneum* with 64 and 96 chromosomes.

Among the three variants (cytotypes) used, the cytotypes 2n=72 and 2n=80 had induced maximum chromosome losses at higher frequency (Fig.3b and 3c). The chromosome loss seemed to depend on the variants of *S. spontaneum*. Kandasamy (1958) noted differential functioning of PoJ 2725 with *S. spontaneum*, Coimbatore (2n=64) and Glagah (2n=112). He concluded that the male parent influenced the functioning of the gamete of the female parent. Similarly, the different cytotypes

Table 4. Chromosome number in the hybrids of Co 8371(2n=108) x *S. spontaneum* (IND 84-415, 2n =80)

Cross	Chromosome number (2n)										
		Observed numbers in the hybrids									
Co 8371 (2n=108) X	Expected number (n+n)	04-891	04-897	04-903	04-910	04-1969	04-1975	04-1985	04-1993	04-2101	04-2105
IND 84-415 (2n=80)	94	93	84	88	86	88	87	90	91	91	87



a.Co 8371(2n=108) x SES 410 (2n=64)

b.Co 8371(2n=108) x SH216 (2n=72)

c.Co 8371(2n=108) x IND 84-415 (2n=80)

Fig. 3. Frequency of chromosome elimination in hybrids

contributed to the differential elimination of chromosome in the hybrids used in the present study.

Though chromosome loss in progeny from crosses involving sugarcane with 2n = 72 and 80 cytotypes of *S. spontaneum* was higher than that in progeny of cross involving 2n = 64 cytotype, a careful selection of hybrid clone with near balanced chromosome number and vigor of clone will be highly advantageous in further nobilization programme. The loss of one or a few chromosomes from the *S. spontaneum* parent in the hybrid with sugarcane and the chromosomal variability in the progeny from different cytotypes can be effectively used in selection of clones with desired traits.

References

Bremer G (1923) A cytological investigation of some species and species hybrids with in the genus *Saccharum.* Genetica (The Hague): 597–148.

Bremer G (1949) Increase in chromosome numbers in species hybrids of *Saccharum.* Proc. VIII. Int. Cong. Genetics Heriditas 541.

Bremer G (1961) Problems in breeding and cytology of sugarcane III. The cytological crossing research of sugarcane. Euphytica 10:229-243.

D'Hont A, Feldmann I, Rao S, Berding N, Glasszmann JC (1996) Characterization of double genome structure of modern sugarcane cultivars – *Saccharum* sp. by cytogenetics. Mole. gene. Genetics 250:405-413

Kandasami PA (1958) Morphological and cytogenetical studies on interspecific and intergeneric hybrids of *S. spontaneum* L. M. Sc. Thesis.

Kandasami PA (1961) Studies in the interspecific and intergeneric hybrids of *Saccharum.* II. Staminal sterility in certain F_1 hybrids with *S. spontaneum* as the pistil parent. Indian J Genet 21(1) :75-76.

Kandasami PA, Sreenivasan TV, Ramana Rao TC (1983). Catalogue on sugarcane genetic resources. Vol.1. *Saccharum spontaneum* L. Sugarcane Breeding Institute. Coimbatore.

Nair MK (1975) Cytogenetics of *Saccharum officinarum* L. and *S. spontaneum* L. IV. Chromosome number and meiosis in *S. officinarum* x *S. spontaneum* hybrids. Caryologia 28:1–14.

Nair MK (1973) Cytogenetics of *Saccharum officinarum* and *S. officinarum* x *S. spontaneum* hybrids II.The probable origin of a typical noble canes. Cytologia 38:35-43.

Nair MK (1978) Cytogenetics of *S. officinarum.* Cytologia 28 (1):1-14.

Price S (1957) Cytological studies in *Saccharum* and allied genera II. Geographic distribution and chromosome numbers in *S. robustum.* Cytologia 22:40-52.

Price S (1961) Cytological studies in *Saccharum* and allied genera.VII. Maternal chromosome transmission by *S. officinarum* in intra and interspecific crosses. Bot Gazette 122:298-305.

Raghavan TS (1952) Genetical behaviour of *Sclerostachya* x *Narenga* hybrid and their backcrosses. Curr Sci 21(2): 35-36.

Roach BT (1977) Utilization of *S. spontaneum* in sugarcane breeding. Proc Int Soc Sug Cane Technol. 16:43-58.

Roach BT, Daniels J (1987) A review of the origin and improvement of sugarcane. Proc Copersucar Intern Sugarcane Breeding Workshop Brazil pp.1-32.

Shang KC, Juang PY, Huang ST (1968) A study on transmission of some important characteristics of Taiwan originated wild cane (*S. spontaneum*). Proc Int Soc Sug Cane Tech 19: 968-974.

Sreenivasan T.V (1987) Cytogenetics. In: Sugarcane improvement through breeding (Ed D.J. Heinz) Elsevier Amsterdam: 211-254.

Sreenivasan TV, Sreenivasan J (1994) Chromosome numbers of *Saccharum* and related gresses from India. Sugarcane 1:16-22.

Sreenivasan TV, Amalraj VA, William Jebadhas A (2001). Catalogue on sugarcane genetic resource. Vol.V. *Saccharum spontaneum*. Sugarcane Breeding Institute, Coimbatore.

Walker DIT (1971) Utilization of noble and *S. spontaneum* in the West Indies. Proc Int Soc Sug Cane Tech 14 (5): 224–232.