Cytogenetics of *Saccharum officinarum* L., *Saccharum spontaneum* L. and *S. officinarum* × *S. spontaneum* Hybrids
I. Chromosome mosaics

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Detailed morphological and cytological studies of the two species of *Saccharum*, namely *S. officinarum* and *S. spontaneum* and *S. officinarum* × *S. spontaneum* hybrids were undertaken to investigate the differences in karyotype between the two species, meiotic behavior of the parents and the hybrids, the basic chromosome numbers, the nature of gametes contributed by the species in hybridization and the phylogenetic relationship between the two species (Nair 1968). In the course of the study, frequent occurrence of intraplant variation of chromosome numbers were observed in the root tip cells of *S. officinarum*, pollen grains of *S. spontaneum* and pollen mother cells of *S. officinarum* × *S. spontaneum* hybrids. Since Frankhauser (1945) first used the term 'chromosome mosaic' to describe the variation in chromosome numbers within an individual, several cases have been reported on the occurrence of somatic cells, pollen grains or pollen mother cells with intraplant variation of chromosome numbers (Emsweller 1949, Vaarma 1949, Sachs 1952, Britton and Hull 1957, Shahare and Shastry 1963 and Jagathesan and Sreenivasan 1967). Detailed data on the chromosome mosaics observed in *S. officinarum*, *S. spontaneum* and their F₁ hybrids are presented in this paper and significance of this in the evolution of the species of *Saccharum* is discussed.

Materials and methods

In the present investigation, fifteen varieties of *S. officinarum* (vide Table 1), twelve varieties of *S. spontaneum* (vide Table 2) and 191 interspecific hybrids of *S. officinarum* × *S. spontaneum* have been utilised. For the study of somatic chromosomes of *S. officinarum*, the schedule developed by Jagathesan and Ratnambal (1967) was followed. The chromosome numbers in the pollen grains of *S. spontaneum* were determined by squash method reported by Jagathesan and Sreenivasan (1966). For the study of the meiosis, the young inflorescences were fixed in Carnoy's fluid (6 absolute alcohol: 3 chloroform: 1 acetic acid saturated with ferric acetate) for 24 hours. From the fixative, the materials were transferred to 70% alcohol before dissecting the anthers and squashing in aceto-carmin.

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Results

Variation in chromosome numbers in the root tip cells of S. officinarum varieties

Chromosome number other than the diploid number has been observed in the somatic (root tip) cells of eight varieties of *S. officinarum* with $2n=80$, among eleven varieties studied. The remaining varieties namely Manjri Red, Soerat Soembawa Wit and Teboe Pohina had only diploid numbers in their somatic cells. Aneuploid chromosome numbers ranging from 73 to 85 have been observed with varying percentage in the eight varieties (Table I and Figs. 1-7). In these

Table 1. Variation in chromosome numbers in the somatic (root-tip) cells of 15 varieties of *S. officinarum*

S. no.	Variety	Number of cells observed	2n	Number of numerically aberrant cells	Percentage of numerically aberrant cells	Extreme of chromosome numbers observed in the numerically aberrant cells	
						Lower	Upper
1.	Chittan	40	80	8	20.0	77	85
2.	Kaludai Bothan	40	80	6	15.0	75	82
3.	Manjri Red	40	80	—	—	—	—
4.	N.C. 20	40	80	6	15.0	75	83
5.	28 N.G. 224	40	80	5	12.5	79	82
6.	51 N.G. 50	40	80	5	12.5	78	81
7.	51 N.G. 143	40	80	3	7.5	79	82
8.	Soerat Soembawa Wit	40	80	—	—	—	—
9.	Striped Mauritius	40	80	5	12.5	78	85
10.	Teboe Pohina	40	80	—	—	—	—
11.	Zwart Cheribon	40	80	4	10.0	79	85
12.	Koelz 10534	40	88	4	10.0	86	89
13.	57 N.G. 77	40	90	6	15.0	84	99
14.	Kham	40	120	10	30.0	104	123
15.	28 N.G. 287	40	120	1	2.5	116	—

cases, the chromosome number which occurs in the highest frequency was considered as the diploid number. *S. officinarum* Chittan ($2n=80$) had the maximum percentage (20%) of such chromosome mosaics with the chromosome numbers varying from 77 to 85. The intraplant variation of somatic chromosome numbers has also been observed in the 'atypical' *S. officinarum* varieties Koelz 10534 ($2n=88$), 57 NG 77 ($2n=90$) (Fig. 8), Kham ($2n=120$) (Fig. 9) and 28 NG 287 ($2n=120$). Among these varieties, Kham had 30 percent cells with chromosome numbers varying from 104 to 123, while the percentage of cells with chromosome mosaic was less in the other three varieties (Table 1).

Variation in chromosome number in the dividing pollen grains of S. spontaneum varieties

A number of pollen grains with chromosome number different from the number for the variety were observed in the eleven out of twelve varieties of *S. spontaneum* studied (Figs. 10–15), the exception being *S. spontaneum* S. H. 248. The frequencies of such abnormal pollen grains and the range of chromosome

Figs. 1–9. Somatic chromosomes in *S. officinarum* varieties. 1–2, Chittan showing 80 and 79 chromosomes. 3, N.C. 20 showing 77 chromosomes. 4, 51 N.G. 50 showing 79 chromosomes. 5–6, Striped Mauritius showing 80 and 82 chromosomes. 7, Zwart Cheribon showing 81 chromosomes. 8, 57 N.G. 77 showing 88 chromosomes. 9, Kham showing 106 chromosomes.

numbers found are given in Table 2. To ensure accuracy, these chromosome counts were taken only from well-spread metaphase plates, which had retained the regular cell outline without any damage. The percentage of such abnormal cells varied from 6.7 in *S. spontaneum* Coimbatore to 72.0 in *S. spontaneum* Imp. 212. In the case of varieties with high frequency of variation in chromosome number in the pollen grains, such as Imp. 212, the correct numbers were determined

Figs. 10-15. Chromosomes at pollen mitosis in *S. spontaneum* varieties. 10, Imp. 212 showing 30 chromosomes. 11, SES. 565 showing 31 chromosomes. 12-13, Tabongo showing 40 and 37 chromosomes. 14, SES. 128-A showing ca. 54 chromosomes. 15, SES. 559 showing 65 chromosomes.

from the pollen mother cells and root tip squashes. *S. spontaneum* S.H. 248 had only haploid chromosome number in its pollen grains. In *S. spontaneum* SES. 128-A, the maximum chromosome number observed in the pollen grains almost neared the diploid number (Fig. 14). It is interesting to note that those varieties of *S. spontaneum* with a high percentage of pollen grains having aneuploid chromosome numbers, such as SES.205-A, SES.128-A also showed high meiotic irregularities (Nair, 1968).

Table 2. Variation in chromosome numbers in the dividing pollen grains of 12 varieties of *S. spontaneum*

S. no.	Variety	Number of pollen grains observed	n	Number of numerically aberrant pollen grains	Percentage of numerically aberrant pollen grains	Extreme of chromosome numbers observed in the numerically aberrant pollen grains	
						Lower	Upper
1. SES. 248		25	20	6	24.0	—	21
2. Holes-I		30	27	7	23.3	24	30
3. S.H. 248		30	28	—	—	—	—
4. Imp. 212		25	31	18	72.0	28	32
5. SES. 565		30	32	5	16.7	27	33
6. Coimbatore		30	32	2	6.7	33	34
7. SES. 205-A		30	32	10	33.3	30*	36
8. SES. 128-A		50	33	22	44.0	20	60
9. Tabongo		40	40	4	10.0	38	41
10. Glagah		30	56	2	6.7	55	57
11. SES. 552		30	62	5	16.7	61	64
12. SES. 559		30	64	6	20.0	61	65

* Excluding one B-chromosome in 6 out of 30 pollen grains.

Mosaicism in microsporocytes of S. officinarum × *S. spontaneum* hybrids

Meiotic study of the varieties of *S. officinarum* and *S. spontaneum* did not reveal the intraplant variation of chromosome numbers in the microsporocytes. However, frequent occurrence of pollen mother cells with chromosome mosaics have been observed in seven out of 191 interspecific hybrids between the two species studied. The percentage of such 'off-type' pollen mother cells varied from 1.8 in *S. officinarum* Soerat Soembawa Wit × *S. spontaneum* Glagah, (2n=136) to 9.0 in *S. officinarum* Manjri Red × *S. spontaneum* SES.559, (2n=86). The details of chromosome mosaics observed in the seven F₁ hybrids are given in Table 3.

Discussion

Intraplant variation in chromosome numbers in the somatic (root tip) cells of *S. officinarum* has been observed in eight out of eleven clones with 2n=80 and all the four 'atypical' forms studied. Jagathesan and Ratnambal (1967) have also recorded "a number of somatic cells with chromosome numbers varying

from 78 to 83" in *S. officinarum* $2n=80$ forms. In the present study such chromosome mosaics have also been observed in the dividing pollen grains of *S. spontaneum* varieties and in the microsporocytes of *S. officinarum* $\times$ *S. spontaneum* hybrids. Similar situations have been reported in several species and hybrids of Gramineae and other families. Variation in chromosome numbers in the root tips of several vegetatively propagated plants have been reported by Sharma (1956). The term 'chromosome mosaic' was used by Frankhauser (1945) to describe the variation in chromosome numbers within an individual arising as a result of spindle abnormalities or chromosome non-disjunction. Britton and Hull (1957) described chromosome mosaic in the somatic cells of *Rubus*. Intraplant variation of chromosome numbers has also been reported in the somatic cells of *Nicotiana* hybrids (Shung-Jan-Yung 1965).

Chromosome mosaics in the pollen mother cells of amphidiploid in *Triticum*, *Aegilops* and *Agropyron* were reported by Sachs (1952), in *Secale* by Rees and Thompson (1955), in intraspecific hybrids of *Triticum* by Hollingshed (1932), in *Datura* by Bhaduri and Sharma (1946), in *Corchorus* by Panda (1961), in tetraploid garden roses by Shahare and Shastry (1963) and in intraspecific hybrids of *Festuca arundinacea* by Malik and Thomas (1966). Sachs (1952) thought that the chromosome mosaics are due to defects in spindle mechanism dependent on certain gene combinations. According to Shahare and Shastry (1963) chromosome mosaics result from elimination of chromosomes due to instability in mitosis. The absence of chromosome mosaics in the microsporocytes of *S. officinarum* and *S. spontaneum* clearly indicates that the origin of this abnormality in the two species is not premeiotic. The origin of pollen grains with varying chromosome numbers met with in the clones of *S. spontaneum* are traceable to the unequal numerical distribution or elimination of univalents during meiosis as reported in *Festuca-Lolium* hybrids, (Darlington and Thomas 1937), *Gossypium* (Brown 1950) and *Narenga* (Jagathesan and Sreenivasan 1967). Spindle abnormalities accompanied by unequal numerical distribution of chromosomes and elimination of univalents have been observed in the meiosis of the varieties of *S. spontaneum* (Nair 1968) to support this view.

The view that the genetic factors are responsible for the chromosome mosaics is supported by the presence of pollen mother cells with altered chromosome numbers in one of the strains of autotetraploid rye (O'Mara 1942) and their absence in another strain (Müntzing 1951). The spindle abnormalities probably caused by genetic factors as in Triticales (Sachs 1952) were observed in the *S. officinarum* clones and this may be the cause for the mosaic cells observed in them.

The chromosome mosaics in microsporocytes have been observed only in the interspecific hybrids of *S. officinarum* $\times$ *S. spontaneum*. Secretion of tapetal cells has been reported to be responsible for initiation of meiosis in aneuploid cells of experimental amphiploids of *Triticinae* (Sachs 1952) resulting in chromosome mosaics. The presence of pollen mother cells with more or less bivalents than the expected seven in *Secale cereale* has been explained by Müntzing and Prakken (1941) as due to the exchange of chromosomal material between archesporial cells in the premeiotic mitosis. In the complex polyploid cells of the interspecific hybrids *S. officinarum* $\times$ *S. spontaneum* reported herein, probably similar mechanism operates

Table 3. Mosaicism in the pollen mother cells of *S. officinarum* × *S. spontaneum* hybrids

S. no.	<i>S. officinarum</i> × <i>S. spontaneum</i> hybrids	2n	Chromosome association	Number of pollen mother cells	Off type pollen mother cells		
					Chromosome number	Chromosome association	Number of PMCs
1.	Chittan × S.H. 248	108	54 _{II} to 48 _{II} + 12 _I	44	72	36 _{II}	2
2.	51 N.G. 143 × Glagah	136	67 _{II} + 2 _I to 62 _{II} + 12 _I	80	86	43 _{II}	5
3.	Soerat Soembawa Wit × Glagah	136	68 _{II} to 60 _{II} + 16 _I	111	120	60 _{II}	2
4.	Striped Mauritius × SES. 552	142	1 _{III} + 66 _{II} + 7 _I to 62 _{II} + 18 _I	54	112, 124	56 _{II} , 6 _{II} + 4 _I	4
5.	Manjri Red × SES. 559	86	43 _{II} to 35 _{II} + 16 _I	100	78, 80	39 _{II} , 40 _{II}	9
6.	Koelz 10534 × Coimbatore	109	54 _{II} + 1 _I to 47 _{II} + 15 _I	120	100 to 102	50 _{II} to 50 _{II} + 2 _I	8
7.	Kham × Coimbatore	88	43 _{II} + 2 _I to 37 _{II} + 14 _I	26	84	41 _{II} + 2 _I	1
							3.5
							6.3
							1.8
							7.4
							9.0
							6.7
							3.9

to give rise to the pollen mother cells with unexpected chromosome numbers.

The role of intraplant variation in speciation of vegetatively propagated plants have been discussed in a series of papers by Sharma and his collaborators (Sharma 1956, Sharma and Bhattacharya 1956, 1959, Sharma and Sharma 1959, Sharma and Mukhopadhyaya 1963). According to them speciation in such vegetatively propagated plants is affected through the entrance of a nucleus with altered chromosome number into the young growing shoots resulting in new shoots with different chromosome numbers. The occurrence of chromosome mosaics in *Saccharum* species and hybrids reveals that the alteration in chromosome numbers in somatic cells followed by vegetative propagation might have played a role in the evolution of at least some of the aneuploid forms of *S. spontaneum* and to a less extent in the evolution of *S. officinarum* from its putative ancestor *S. robustum*.

The pollen grains with different chromosome numbers within the same variety of *S. spontaneum* are also of evolutionary significance. Functioning of pollen grains with chromosome numbers other than the haploid number of the variety could give rise to a new variety with an entirely different chromosome number. There is no reason to doubt the functioning of such pollen grains at least in the high polyploid forms of *S. spontaneum*. Intensive selfing and intervarietal hybridization combined with the functioning of pollen grains with varying chromosome numbers may account for the extensive euploid-aneuploids reported in this species.

Summary

Intraplant variation in chromosome numbers have been reported in the somatic cells of *S. officinarum*, pollen grains of *S. spontaneum* and microsporocytes of *S. officinarum* × *S. spontaneum* hybrids. The origin of the chromosome mosaics has been traced to the spindle abnormalities caused by genetic factors in the case of *S. officinarum*, unequal numerical distribution or elimination of univalents during meiosis in *S. spontaneum* and disturbances in the pre-meiotic mitosis in the interspecific hybrids. It is concluded that the alteration in chromosome numbers in the cells of individual plants followed by vegetative propagation and the functioning of pollen grains with varying chromosome numbers might have played a significant role in the evolution of the extensive euploid-aneuploid series reported in *S. spontaneum*.

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