INTERGENERIC HYBRIDS OF *SACCHARUM*

IV. *SACCHARUM-NARENGA*

By E. K. JANAKI-AMMAL

John Innes Horticultural Institution, Merton, London

(With Seven Text-figures)

CONTENTS

	PAGE
1. History of the cross	23
2. Material and methods	24
3. General characters of parents and F_1 hybrids	24
4. Cytology of parents and F_1 hybrids	27
(*a*) Somatic chromosomes	27
(*b*) Meiosis and male sterility in *S. officinarum* (Vellai)	28
(*c*) Meiosis in *Narenga porphyrocoma*	29
(*d*) Chromosome behaviour in F_1 hybrids	29
(*e*) Behaviour of univalents	29
5. Summary	32
References	32

1. History of the cross

In Parts I–III of this series I have described what happens when high polyploid species of *Saccharum*—*S. officinarum* ($2n=8x=80$), *S. spontaneum* 'Glagah' ($2n=112$) or their derivatives, like POJ 2725 ($2n=106$)—are crossed with diploid species of *Erianthus* ($2n=20$), *Imperata* ($2n=20$) and *Zea* ($2n=20+2B$). I now come to an intergeneric hybrid of *Saccharum* in which the male parent, *Narenga porphyrocoma* Hance (Bor.), is a hexaploid. This cross was made under controlled conditions by the late C. A. Barber at Coimbatore in 1913. Its low sucrose content as compared with others of Barber's crosses made it worthless as a substitute cane, while its complete sterility prevented any further use of it as a parent. Its propagation as a possible economic cane was therefore stopped after a detailed recording of 100 F_1 seedlings had been made (Barber, 1916).

About a score of these seedlings were, however, grown at the Imperial Sugar Cane Station as 'an interesting demonstration and in the hope that at some time their fuller examination may be taken up', to quote Barber (1920). The present paper is the outcome of such a study, made on a few of these surviving hybrids and their parents.

2. Material and methods

Narenga porphyrocoma Hance (Bor.), until recently known as *Saccharum Narenga*, is a tall perennial grass found widely in north-east India. I collected many clones of it in 1937 from Assam, where I have seen it flowering profusely on the banks of the Brahmaputra. According to Barber (1916) the male parent he used for crossing with *S. officinarum* was raised from seeds collected in north Bihar. This plant was being propagated from cuttings at Coimbatore, so that I was able to examine the identical clone used by Barber in 1913. I have also examined clones I collected in Assam, as well as the herbarium sheets at Kew. Six of these clones proved to have 30 chromosomes.

The *S. officinarum* clone studied was the clone used by Barber in his cross. It was the same clone of Vellai which I used for crossing with *Zea Mays*.

Material for cytological studies was grown and collected at Coimbatore. The technique was the same as that described in previous papers. Permanent acetocarmine smears were used for meiotic studies.

3. General characters of parents and F_1 hybrids

According to Bor (1940) the retention of '*Saccharum Narenga*' in the genus *Saccharum* was anomalous, owing to its possessing morphological characters quite distinct from those species accepted as members of that genus. The glumes are more coriaceous, there are no non-flowering stems; the general appearance of the plant is flimsier; the inflorescence, which in *S. officinarum* is a large panicle, is very reduced in *Narenga* (Fig. 1), and only the lowest lateral axis bears secondary branches. There is a fourth glume which is absent in *Saccharum officinarum*.

All the hybrids between *Saccharum* and *Narenga* are very cane-like. Unlike the *Saccharum-Zea* cross they are extremely vigorous, and Barber (1916) reported that they flowered at 10 months from germination.

In quantitative characters such as diameter of stem, width of leaves, size and branching of inflorescence and length of callus hairs, the F_1 plants were mostly intermediate between the parents (Figs. 1, 2). But it would appear from Barber's analysis of 100 seedlings that they showed considerable variation among themselves. This is especially marked in his photograph of stems (1916), where he has a class of seedlings which equalled the *Saccharum* parent in stem diameter (Fig. 3). This type was not represented in the hybrids I examined cytologically. Barber's analysis also shows considerable variation in the percentage of sucrose

present in the 100 seedlings. The majority had about 11%, though some had as little as 6%.

In Table 1 I have summarized the general qualitative characters of taxonomic value noted in the parents and the hybrids. It will be seen

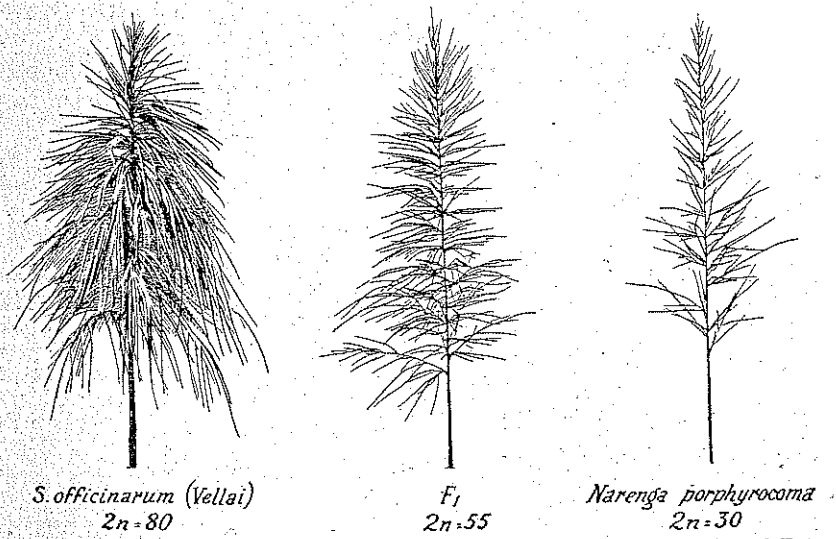

Fig. 1. Inflorescence of *Saccharum officinarum* (Vellai), *Narenga porphyrocoma* and F_1.

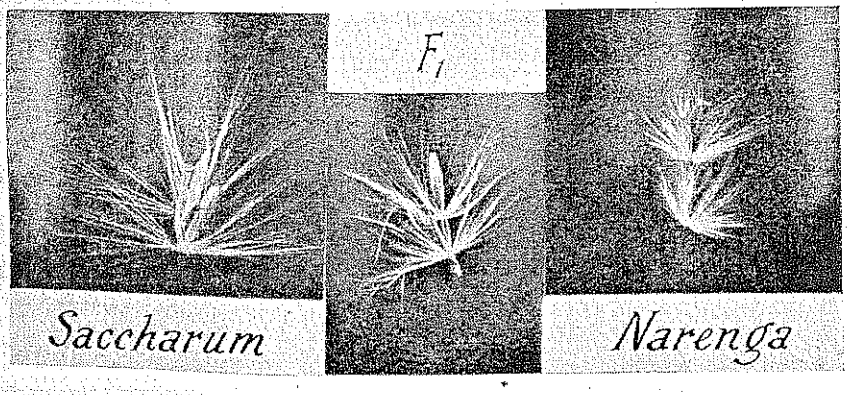

Fig. 2. Spikelets of *Saccharum*, *Narenga* and F_1.

that the hybrids resembled *Saccharum* in five and *Narenga* in five of the contrasting characters, one character, the shape of the nodal buds, being intermediate. One character is not intermediate: the minute cilia on the lodicules, lacking in *Saccharum* but present in *Narenga*, were very much exaggerated in all the F_1 hybrids. In the dominance of the epidermal hairs on the leaf and the ligular process (Fig. 4) the

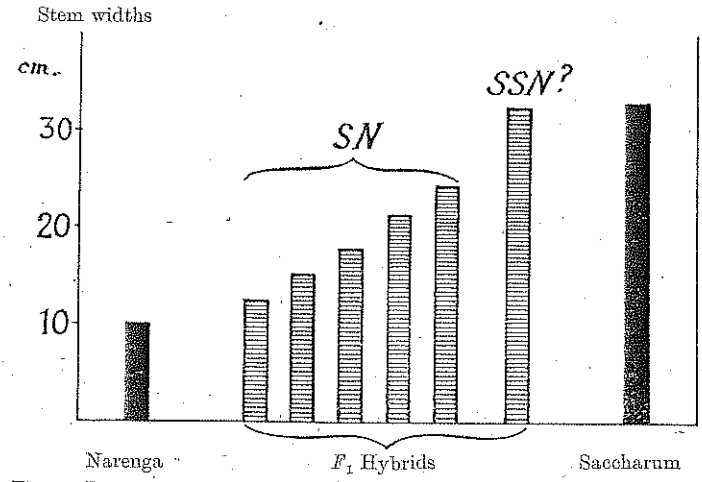

Fig. 3. Relative stem widths of *Narenga*, *Saccharum* and their F_1 hybrids (measurements after Barber).

Table 1. *Comparison of characters of* Saccharum officinarum (*Vellai*), Narenga porphyrocoma *and* F_1 *hybrids*

	Saccharum	F_1	*Narenga*
1. Habit	Perennial	Perennial	Perennial
2. Stem anatomy	Nodes and internodes present	←	Short rhizome Aerial stem develops during flowering only
3. Root eyes	Present	←	Absent
4. Bud	Ovate	Lanceolate	Elliptical
5. Ligular process	Present	←	Absent
6. Leaf blade	Non-fluted	←	Fluted
7. Upper epidermis	Non-hairy	→	Hairy
8. Main axis of inflorescence	Non-hairy	→	Hairy
9. Fourth glume	Absent	→	Present
10. Glumes	Membranous	→	Coriaceous
11. Callus hairs	Longer than glume	←	Equal to glume
12. Lodicules	Non-ciliate	→	Ciliate

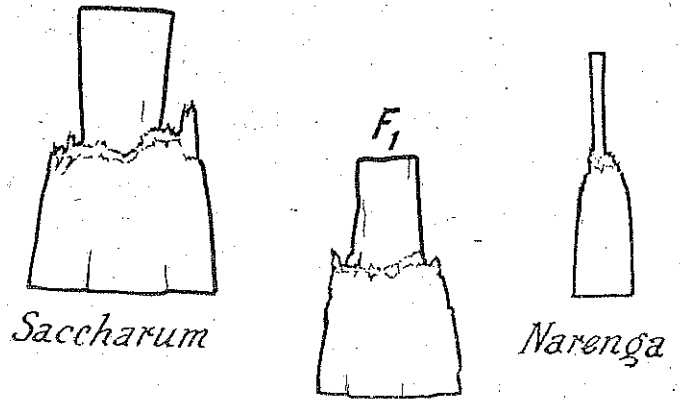

Fig. 4. The ligule in 'Vellai', *Narenga* and F_1 hybrid.

Saccharum-Narenga hybrids are similar to the *Zea* hybrid (Janaki-Ammal, 1941).

The generic character of coriaceous glumes in *Narenga* was modified to 'thinly coriaceous' in the hybrid.

4. CYTOLOGY OF PARENTS AND F_1 HYBRIDS

(*a*) *Somatic chromosomes*

The chromosome number of Vellai, $2n=80$ (Fig. 5*a*), has been recorded in the previous studies of this series. *Saccharum officinarum* is regarded as an octoploid. The 80 chromosomes of Vellai could be broadly classified into four types with regard to length. Secondary constrictions were found in the long chromosomes.

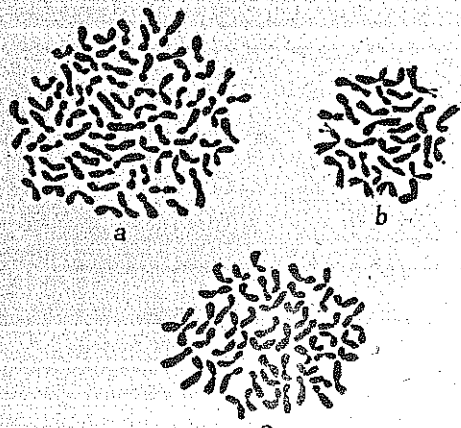

Fig. 5. Root-tip metaphase in (*a*) *Saccharum officinarum* (Vellai) ($2n=80$), (*b*) *Narenga porphyrocoma* ($2n=30$), (*c*) *Saccharum-Narenga* hybrid ($2n=55$). ×2000.

Root tips of all the clones of *Narenga porphyrocoma* examined had 30 chromosomes (Fig. 5*b*). This number verifies the count of Bremer (1925) from the 15 bivalents seen in pollen mother cells of this plant. It is the only genus in the Andropogoneae with this number. Its separation from *Saccharum* on morphological grounds by Bor (1940) can thus be supported cytologically.

Secondary constrictions were seen in the long chromosomes as in *Saccharum*. A single pair of chromosomes have trabants.

In root tips of sixteen of the hybrids I found 55 chromosomes (Fig. 5*c*). This number represents the sum of the haploid numbers of Vellai and *Narenga*. As the cane Vellai when crossed with *Sorghum Durra* produces both diploid and triploid hybrids ($2n=50$, 90; Janaki-Ammal, 1941), it

is to be presumed that only haploid egg cells of Vellai are fertilized by *Narenga*. In this respect the *Saccharum-Narenga* hybrids are similar to *Saccharum-Zea*. Such selective fertilization, or selective survival of fertilization types, seems to be characteristic of sugar-cane hybrids (Janaki-Ammal, 1941).

(*b*) *Meiosis and male sterility in* S. officinarum (*Vellai*)

Pollen mother cells of Vellai at diakinesis showed that the 80 chromosomes associate to form 40 bivalents (Fig. 6*a*). They form one or two chiasmata only. Occasionally two of the chromosomes are seen unpaired at metaphase. They then fail to congress on the metaphase plate, and

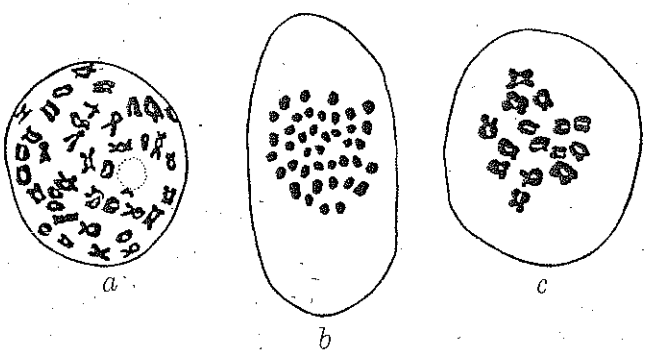

Fig. 6. (*a*) Pollen mother cells at diakinesis in Vellai. ×1800. (*b*) Polar view of metaphase II in pollen mother cells of Vellai. ×1800. (*c*) Prometaphase in pollen mother cells of *Narenga*. ×2000.

this may be responsible for the unequal numbers observed at metaphase II by Dutt & Subba Rao (1933). Except for this abnormality metaphase I is regular. As a rule 40 chromosomes were also counted at metaphase II (Fig. 6*b*). Tetrad formation is regular, though occasionally I came across triads in which one of the cells is binucleate. Anthers after tetrad formation showed a progressive deterioration in the pollen grains, generally beginning before the first division in the pollen grain. At this stage there is normally a change in the cytoplasm of the pollen grains, and starch grains are developed. In the degenerating pollen grains of Vellai starch is either poorly developed or totally absent. All such cells abort. About 99% of pollen grains in open spikelets were found to be aborted. As less than 2% of the anthers in an inflorescence burst, the pollen fertility is finally reduced to zero. That viability of the embryo-sac is not impaired is shown by the 200 seedlings obtained by Barber in this cross.

(c) *Meiosis in* Narenga porphyrocoma

The 30 chromosomes of *Narenga* form 15 bivalents (Fig. 6*c*), as recorded by Bremer (1925). Reduction division is regular, and pollen tetrads and grains are formed in the normal way. Pollen fertility is nearly 100%. On this evidence it is regarded as a hexaploid plant with a basic number of 5 instead of 10. Diploids of this basic number in the Andropogoneae are found only amongst the para-Sorghums.

(d) *Chromosome behaviour in* F_1 *hybrids*

Meiosis was studied in five seedlings. Pollen mother cells at diakinesis showed that the 55 chromosomes associate as bivalents, trivalents and quadrivalents (Fig. 7*a*). Table 2 gives the configurations noted in fifteen cells of three F_1 hybrids. My observations do not agree with those of

Table 2. *Degree of association in the three* F_1 *hybrids, A, B and C*

Configurations				No. of cells in			
IV	III	II	I	*A*	*B*	*C*	Total
2	2	19	3	5	1	1	7
2	1	20	4	5	5	3	13
2	0	21	5	2	1	4	7
1	2	21	3	3	2	0	5
1	1	22	4	0	4	2	6
1	0	24	3	0	0	2	2
1	0	23	5	0	2	3	5
							45 cells
Average	1·5	1	21	4			
% in *A*	6·7	5·3	74·6	13·4			
% in *B*	5·3	3·6	76·5	14·6			
% in *C*	5·5	1·6	77·7	15·2			

Singh (1934), who has recorded only bivalents and univalents in one plant he examined. The large number of bivalents present in the hybrid (19–23) shows that the chromosomes derived from the haploid complement of *Saccharum officinarum* are capable of pairing amongst themselves (by autosyndesis), like the *S. spontaneum* chromosomes in the cross with *Erianthus* (Janaki-Ammal, 1941). The percentage of configurations is fairly uniform for the three plants studied.

(e) *Behaviour of univalents*

The unpaired chromosomes in the hybrid, 3–5 in number, appear to be the largest of the complex. They probably belong to the *Narenga* parent. These chromosomes tend to be pushed towards the periphery of the nucleus even at the diakinesis stage. They are therefore at a positional disadvantage when the spindle is formed. They are always found on the edge of the spindle, where the forces of congression are apparently not so effective (Fig. 7*b*). The fate of the univalents during

meiosis depends on the degree of congression at metaphase I. Those univalents that are able to reach the plate in time divide at metaphase I (Fig. 7*c*) and generally lag at the second division. These lagging univalents move only through the agency of the stretching spindle, and not all of them reach the daughter nuclei of the tetrad stage.

Those univalents which are outside the effective sphere of action of the spindle at metaphase I either remain at the poles and become incorporated in the daughter nuclei, or may remain undivided at the plate.

In the first case they will divide normally at the second division, while in the second case they are seen to form a separate nucleus (Fig. 7*d*). Development is somewhat slower in these extra nuclei than in the main nucleus. They may form their own spindles at the second division (Fig. 7*e*), and then dyads with two micronuclei are seen (Fig. 7*f*).

There are usually five chromosomes—the maximum number of univalents observed—in these extra nuclei, indicating that they are formed when the general congression is weak.

The behaviour of the haploid chromosomes in *Narenga* in the hybrid is thus similar to that in a triploid plant with 5^{II} and 5^{I}.

Pollen sterility is very high, over 90%, in the hybrids. The few viable pollen grains seen in the anthers are not available as the anthers do not dehisce. The hybrids are also female-sterile.

The close resemblance between Barber's hybrids and the wild cane Hitam Rokhan (hitam meaning red) indicates the same origin. This cane, described by Bremer in 1925, was collected by J. B. Haga in 1916 on the banks of the river Rokhan in eastern Sumatra. It has the same chromosome number, $2n = 55$, as Barber's hybrids. It differs from them in the degree of autosyndesis of the chromosomes (Bremer, 1925). This is probably due to its being a hybrid of a different clone of *Saccharum officinarum*. The red colour of the cane points to one like Black Cheribon as the possible female parent.

Similarly the mosaic-resistant cane Kassoer, also found in the East Indies, is a natural hybrid between *S. officinarum* Black Cheribon and *S. spontaneum* (Bremer, 1923). Thus the two hybrids made by Barber in 1913 reproduce types occurring naturally. While the interspecific hybrids, both natural and artificial, proved of immense importance to the sugar-cane industry of Java and India, the intergeneric hybrids because of their sterility had to be discarded from all breeding programmes. It is to be hoped that the use of colchicine and other drugs for the production of amphidiploids will make even the sterile hybrids of some use.

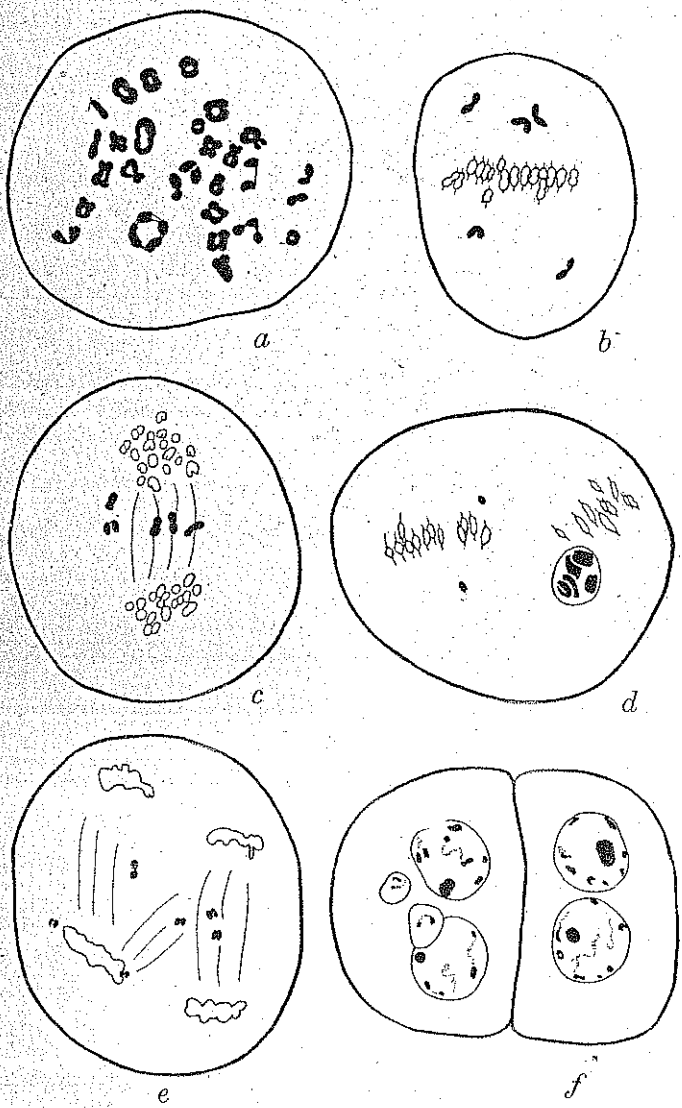

Fig. 7. Meiosis in *Saccharum-Narenga* hybrid. (*a*) Chromosome association at diakinesis in pollen mother cells. (*b*) Metaphase I showing position of univalents. (*c*) Telophase I with univalents at equator of plate. (*d*) Metaphase II with micronucleus of five univalents. (*e*) Telophase II with extra spindle formed by dividing micronucleus. (*f*) Dyad with two micronuclei. ×1800.

5. Summary

1. The hybrids made by C. A. Barber in 1913 between *Saccharum officinarum* (Vellai), $2n=80$, and *Narenga porphyrocoma*, $2n=30$, have 55 chromosomes.

2. They show detailed qualitative characters of each parent, but in general appearance are more like sugar canes.

3. In quantitative characters the hybrids are generally intermediate between the parents; only the minute cilia present on the lodicules of *Narenga* and absent in *Saccharum officinarum* were longer in the F_1 hybrids.

4. The 30 chromosomes of *Narenga porphyrocoma* form 15 bivalents and behave normally at meiosis.

5. The 80 chromosomes of *Saccharum officinarum* (Vellai) form 40 bivalents. Meiosis is generally regular. Male sterility in this sugar cane is due to defects in pollen-grain division.

6. The chromosomes in the *Saccharum-Narenga* hybrids show autosyndesis and associate as quadrivalents, trivalents and bivalents, while a few (3–5) remained as univalents.

7. Univalents, which are probably derived from *Narenga*, divided at metaphase I or II according to the degree of congression at metaphase I. Those outside the sphere of influence of the main spindle form extra nuclei which divide as separate units.

8. Male and female sterility in the hybrids is presumably due to autosyndesis of the chromosomes of both parents.

9. The wild cane Hitam Rokhan is evidently a natural hybrid between *Saccharum* and *Narenga*.

REFERENCES

Barber, C. A. (1916). Studies in Indian sugar canes. II. *Mem. Dep. Agric. India, Bot.* **8**, 103–99.

Barber, C. A. (1920). Sugar-cane seedling work in India. II. *Int. Sug. J.* **22**, 307–12.

Bor, N. L. (1940). Three new genera of Indian grasses. *Indian For.* **66**, 267–72.

Bremer, G. (1923). A cytological investigation of some species and species-hybrids within the genus *Saccharum*. *Genetica*, **5**, 97–148.

Bremer, G. (1925). The cytology of the sugar cane. III. The chromosomes of the primitive forms of the genus *Saccharum*. *Genetica*, **7**, 293–322.

Dutt, N. L. & Subba Rao, K. S. (1933). Observations on the cytology of the sugar cane. *Indian J. agric. Sci.* **3**, 37–56.

Janaki-Ammal, E. K. (1941). Intergeneric hybrids of *Saccharum*. I–III. *J. Genet.* **41**, 217–53.

Singh, T. S. N. (1934). Chromosome numbers in the genus *Saccharum* and its hybrids. *Indian J. agric. Sci.* **4**, 290–92.