INTERGENERIC HYBRIDS OF *SACCHARUM*

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(With Twenty-two Text-figures)

CONTENTS

PART I. *SACCHARUM-ERIANTHUS*

1. INTRODUCTION

IN the present series of papers I propose to deal with intergeneric hybrids between *Saccharum* and other grasses.

The genus *Saccharum* consists of some ten species (Bews, 1929) distributed throughout the warmer parts of the world. The species used in the present experiments are *S. spontaneum* and *S. officinarum*, both belonging to the section *Eu-Saccharum*, and hybrids between them.

S. spontaneum is a polymorphic species. I have collected clones in India with 48, 56, 64, 72 and 80 chromosomes (Janaki-Ammal, 1936, 1939). Others from Assam and Burma had 96 and from the East Indies 112, while Bremer (1929) found forms with 80 in Celebes and the Philippines. *S. officinarum*, the cultivated sugar cane in its common forms, is octoploid, $2n=80$. Like many important cultivated plants it is not known in the wild; its nearest wild relative is *S. robustum* ($2n=80$),[1] discovered by Brandes in New Guinea (1928).

The first successful cross between *S. officinarum* and *S. spontaneum* was made by Barber in 1914. Since that date a large number of hybrids and hybrid derivatives have been evolved both in India and Java (the so-called "nobilized" varieties). The first intergeneric hybrid of *Saccharum* was also made by Barber, in 1913 (Barber, 1916), when he crossed the clone "Vellai" of *S. officinarum* with the grass *Narenga narenga*. In 1927 Rumke (1934) crossed another clone "EK 28" with *Erianthus sara*. Since then a number of intergeneric hybrids of *S. officinarum* have been made (Venkatramam, 1938; Janaki-Ammal, 1938).

In 1934 I attempted a series of crosses between *S. spontaneum* and related grasses. Amongst the successful hybrids I described were those between two types of this species with 56 and 112 chromosomes and *Erianthus ravennae*, $2n=20$ (Janaki-Ammal, Report, 1936). The first of these did not flower; the present investigation is on the second, in which the Java clone of *S. spontaneum*, "Glagah", was used as the female parent. This clone was obtained from the Pasoeroean Experimental Station and has been propagated vegetatively at the Imperial Sugar Cane Station, Coimbatore, since its introduction in 1919.

The variety "purpurascens" of *Erianthus ravennae* was used as the pollen parent. It was collected from the Punjab and was designated "*Saccharum munja*, spiny" at Coimbatore until correctly identified by Mr Hubbard at Kew in 1935.

2. Methods

Spikelets of *S. spontaneum* selected for crossing were emasculated a day before their opening, and the rest of the spikelets removed from the "arrows" which were bagged both before and after pollination—a process which is usually thought detrimental to seed-setting in *Saccharum*. Five seedlings were obtained from this cross. The reciprocal cross set no seeds. All the F_1's were alike. My observations were made on one of the five F_1 plants—"SG 48·1" and its selfed seedlings.

[1] Brandes says $2n=84$; material examined by me had $2n=80$.

Root tips for chromosome counts were fixed in Allen's Bouin to contract the chromosomes and thus facilitate their counting. Root tips were immersed in crushed ice for several minutes before fixation, as this was found to give metaphase plates with the chromosomes well spaced. Pollen mother cells were fixed in 1 : 3 acetic alcohol. Acetocarmine smears were made both from fresh material and from pollen mother cells fixed in acetic alcohol and preserved in 70 % alcohol. Material thus preserved was rendered more suitable for staining in acetocarmine by immersion for a few minutes in acetic alcohol or Carnoy's fixative. Smears were made permanent by the method of McClintock (1929). Sections of root tips were cut at 10–12μ and of pollen mother cells at 16μ; all sections were stained in Heidenhain's iron-alum-haematoxylin.

3. GENERAL CHARACTERS OF PARENTS AND OFFSPRING

Hooker in his *Flora of British India* says of *Erianthus*: "Habit and character of *Saccharum* but glume 4-awned, rarely awnless." *Erianthus ravennae*, however, differs from *S. spontaneum* in a number of characters, of which the clearest is the absence of regular internodes.

Table 1 summarizes the general characters of taxonomic value noted in the two parents and the F_1. The F_1's resembled *S. spontaneum* more

Table 1. *Comparison of characters of* Saccharum spontaneum, Erianthus ravennae *and their* F_1

Characters	*S. spontaneum*	F_1 seedlings	*E. ravennae*
1. Stem: anatomy	Nodes and inter-nodes present	*S**	Short rhizomes. Aerial stem developed during flowering only
2. Stem: average thickness	0·93 cm.	1·3 cm.	1·1 cm.
3. Ligule	Ovate; zone of articulation present	*S*	Ciliate; zone of articulation absent
4. Leaf sheath	Hairy on side of ligule	*S*	Bearded at insertion of leaf
5. Leaf length	40 cm.	51 cm.	44 cm.
6. Leaf width	1·9 cm.	1·8 cm.	1·3 cm.
7. Inflorescence	Subsidiary branches simple	*S*	Subsidiary branches compound
8. Primary rach	Hairy	*S*	Glabrous
9. Callus hairs	4–5 times longer than glume	3·5–4 times longer	Equal or sub-equal
10. Glume I	Membranous with coriaceous base	*S*	Villous dorsally
11. Glume IV	(*a*) Minute	Long	Long
	(*b*) Linear	*S*	Ovate
	(*c*) Ciliate	*S*	Non-ciliate
	(*d*) Awnless	*S*	Awned
12. Lodicules	Ciliate	*S*	Glabrous

* *S* = character as in *Saccharum* parent.

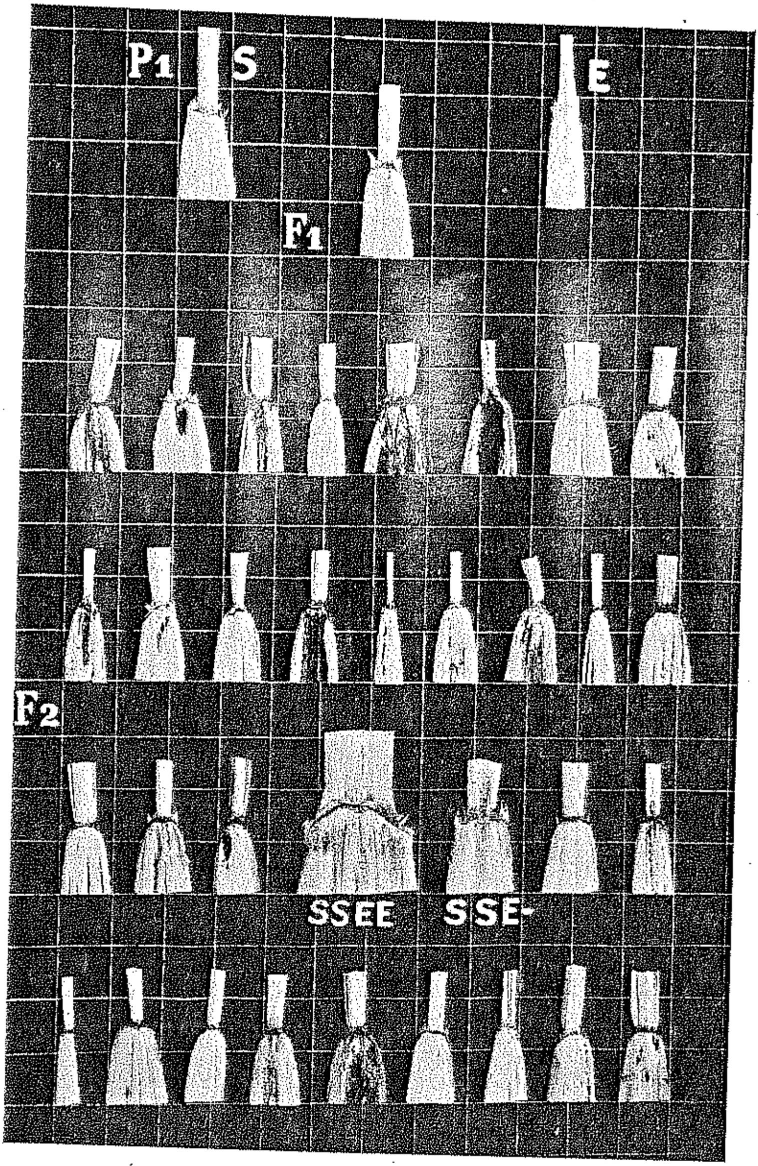


Fig. 1. Types of ligule in *S. spontaneum* and *Erianthus* P_1 and their F_1 and F_2 hybrids.

closely than *Erianthus*, but they had slightly thicker stems and longer leaves than the *Saccharum* parent and the inflorescence was on the average longer and denser. The hybrids produced abundant pollen. In the plant SG 48·1 selected for study the percentage of viable pollen was about 82 % as against 94 and 93 % noted in the *Saccharum* and *Erianthus* parents respectively. The plant set abundant seeds, even under bags.

4. MORPHOLOGY OF F_2 SEEDLINGS

Several hundred selfed seedlings were raised from bagged inflorescences of the *S. spontaneum* × *Erianthus ravennae* hybrid SG 48·1 in 1937; of these only fifty F_2 plants were grown for study. Owing to the drought conditions at Coimbatore in 1937 and 1938, and the salinity of the soil in which they were grown, several of the seedlings died. The remainder showed great variation in height and thickness of stem and width of leaves, some of them being much thicker and taller than any variety of *S. spontaneum*. The average width and length of the leaves was measured in the thirty-nine plants that survived. Fig. 1 shows the type of ligule in these. The frequency distribution of the seedlings in six class groups according to leaf width is recorded in Table 2. The modal class of pro-

Table 2. *Leaf width in parents and crosses*

	Leaf width in centimetres					
	1	1·5	2	2·5	3	3·5
Class of parents		*Erianthus*	*Saccharum*			
Class of F_1			F_1			
Frequency of F_2	11	19	3	2	3	1
Constitution of F_2 plants examined	$2x$, $SE+$				$3x$, $SSE-$	$4x$, $SSEE$

genies occurred in the 1·5 cm. group into which *Erianthus ravennae* also falls. Four plants stood out from the others by their great height. Their leaves, which exceeded 3 cm. in width, resembled those of sugar canes more than they did those of either parent or of the F_1. Fig. 2 illustrates the difference in the thickness of the stem, Fig. 3 the size of the inflorescence and spikelets in the parents F_1 and some of the F_2 seedlings. Class groups of stem diameter of forty seedlings are shown in Table 3. Plants with thicker stems had also larger inflorescences. In several individuals the subsidiary branches of the inflorescence were seen to be compound as in *Erianthus*. An awned glume was found to have segregated in the F_2. Where the awn was absent the fourth glume was generally longer than in *S. spontaneum*, as shown in Fig. 4. An important value in comparing these hybrids is the length proportion of callus hairs to glumes. The range of this value H/G is shown in Table 4. The F_2 distribution is unimodal and covers most of the range between the parental species.

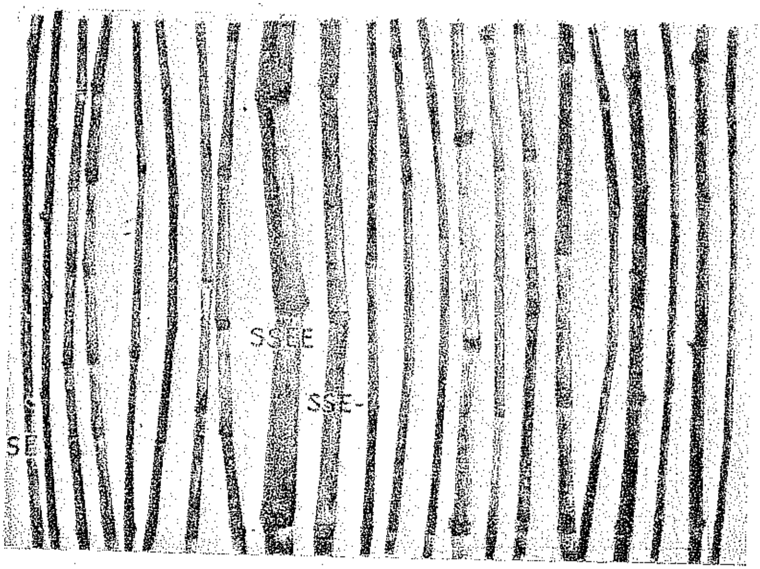


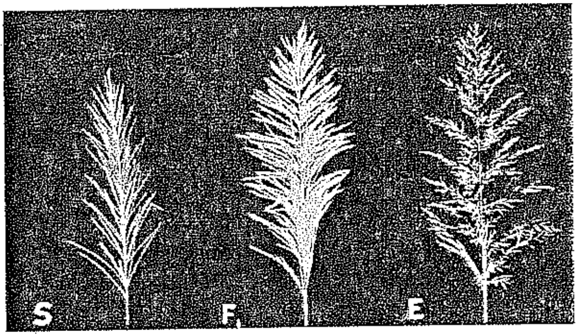
Fig. 2. Variation in stem thickness in the F_2 generation of *Saccharum-Erianthus* hybrids

Table 3. *Stem diameter in parents and crosses*

	Stem diameter in centimetres				
	0·5	1	1·5	2	2·5
Class of parents		*Erianthus*	*Saccharum*		
Class of F_1			F_1		
Frequency of F_2	2	31	3	3	1
Constitution of F_2 plants examined	$2x$, $SE+$			$3x$, $SSE-$	$4x$, $SSEE$

Table 4. *Distribution of* H/G *ratio in* S. spontaneum *and* E. ravennae *parents and hybrids*

Class	Class value	Class : parents and F_1	Frequency F_2
0·8–1·2	1	*E ravennae*	—
1·3–1·7	1·5	—	1
1·8–2·2	2	—	1
2·3–2·7	2·5	—	6
2·8–3·2	3	—	4
3·3–3·7	3·5	F_1	15
3·8–4·2	4	—	2
4·3–4·7	4·5	*S. spontaneum* "Glagah"	—



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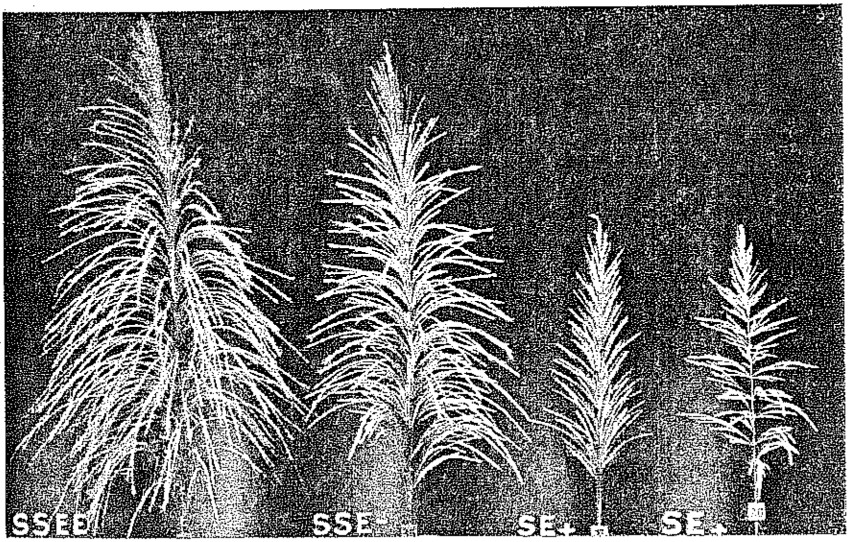

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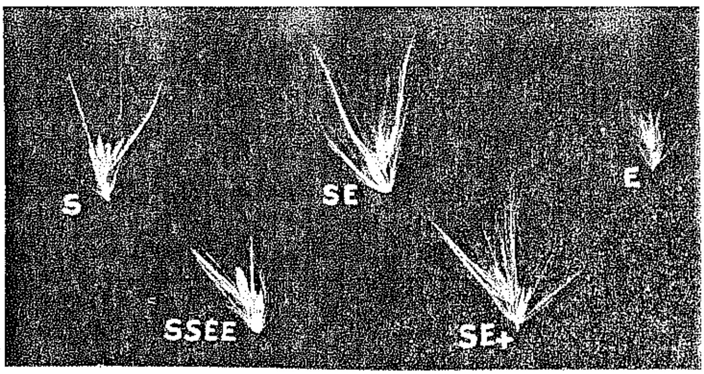


c

Fig. 3. *a*, the inflorescence of *S. spontaneum* (*S*), *Erianthus* (*E*) and their F_1 hybrid. *b*, relative size of arrows in F_2 of tetraploid (*SSEE*), triploid (*SSE*) and diploid (*SE*+). *c*, spikelets of *Saccharum* (*S*), *Erianthus* (*E*), their F_1 hybrid (*SE*) and two F_2 plants.

The F_2 seedlings varied a great deal in the degree of anthesis. In at least four of the seedlings that flowered there was total absence of anthesis. This was generally associated with low percentage of viable pollen.

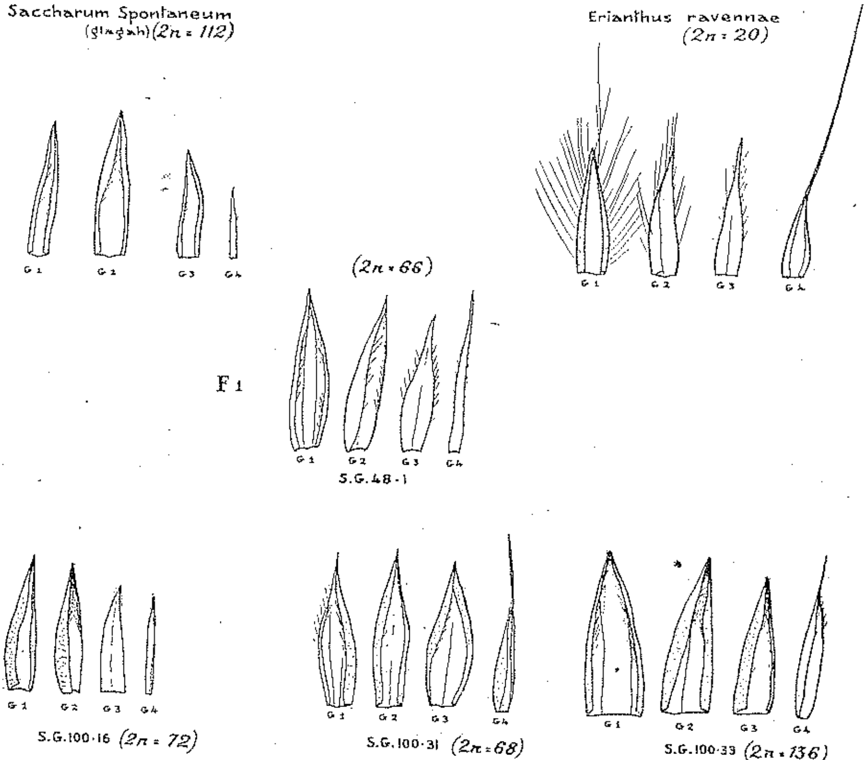


Fig. 4. Types of glume in spikelets of *Saccharum*, *Erianthus* and their F_1 and F_2 hybrids. The awned fourth glume, though not present in the F_1, appears in some of the F_2 seedlings.

5. Chromosome numbers in parents, F_1 and F_2

Root tips of *S. spontaneum* "Glagah" showed 112 chromosomes, as found by Bremer (1923). The somatic number of *Erianthus ravennae* is 20. In the variety "spiny" used in the present cross there was a small extra fragment. Four selfed seedlings of this plant showed the 20 chromosomes only. The basic number in the genus *Erianthus* is 10, and *E. ravennae* is therefore a diploid species.

All the five F_1 hybrids examined had $2n = 66$, the sum of the haploid numbers of the parents.

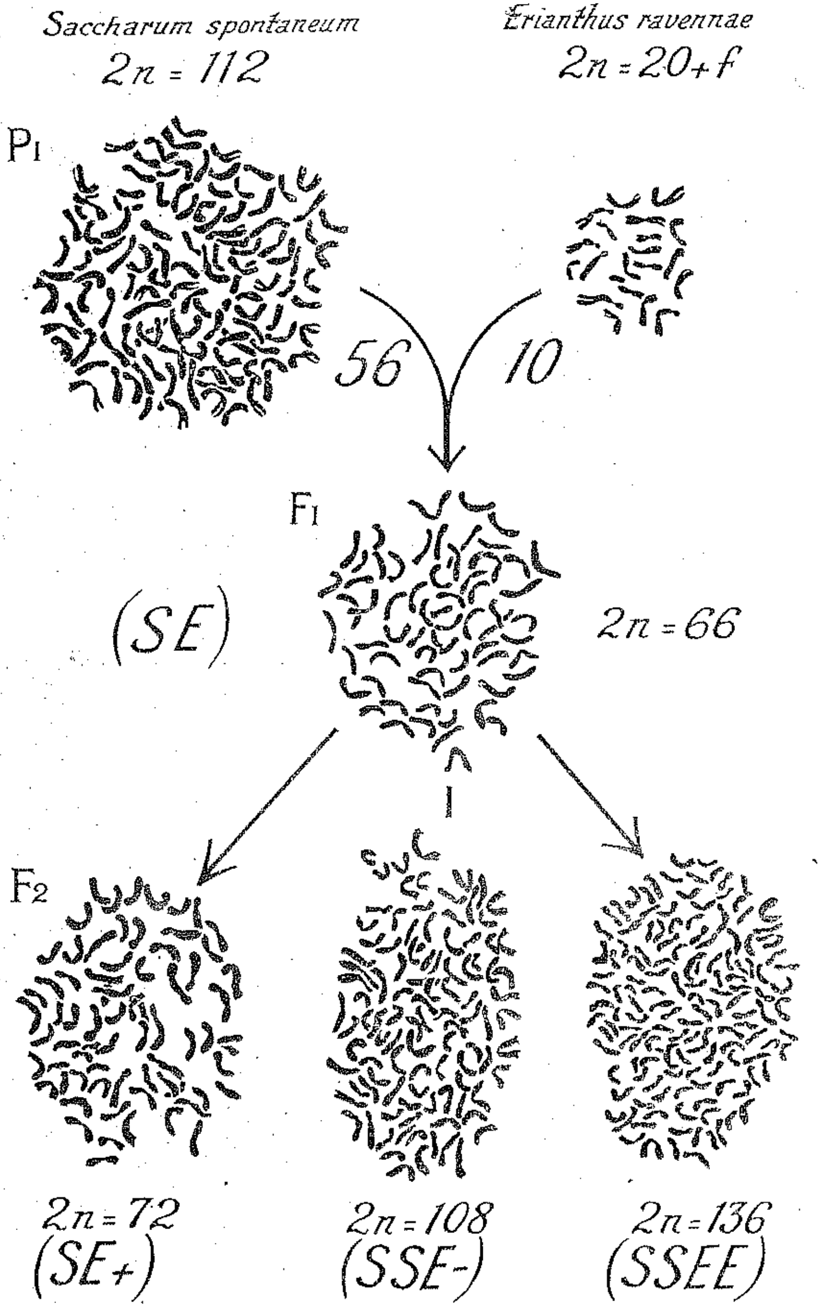


Fig. 5. Somatic metaphase in root tips of *S. spontaneum*, *Erianthus ravennae* and their F_1 and F_2 hybrids. ×2000.

The following are the chromosome numbers of the sixteen F_2 seedlings examined:

Chromosome no....	67	68	69	70	71	72	73	74	75	76	104	106	108	136
No. of plants	1	1	—	1	1	2	3	1	—	2	1	1	1	1
Presumed constitution	*SE* +										*SSE* −			*SSEE* +

Twelve of the sixteen seedlings examined had a chromosome number between 67 and 76, that is, close to that of the F_1 hybrids. Three plants had 104–108 chromosomes and are therefore "triploids", *SSE*, in relation to those in which the chromosome number ranged from 67 to 76. A single plant had $2n=136$ (Fig. 6) and would on the same evidence be considered a "tetraploid", *SSEE*. The higher chromosome numbers go with the larger size of stem, leaves and inflorescence.

The chromosomes of the parents, F_1 and three types of F_2 are shown in Fig. 5.

6. Meiosis in the parent species

The 112 chromosomes of *S. spontaneum* "Glagah" associate as 56 bivalents at diplotene (Fig. 6*a*). The number of chiasmata at this stage varies from one to two per bivalent. Fig. 6*b* represents the 56 bivalents

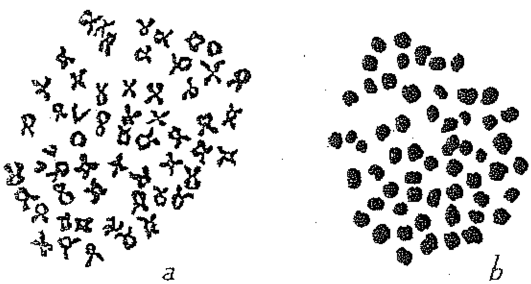


Fig. 6. Chromosome association in *S. spontaneum* "Glagah". *a*, diplotene; *b*, metaphase. ×1800.

at metaphase. Reduction division in this plant, which has also been dealt with by Bremer, shows regular distribution of the 56 bivalents during anaphase.

Fig. 7 *a–c* represents the stages of meiotic division in the male parent, *Erianthus ravennae*. The chromosomes associate as 10 bivalents, the number of chiasmata varying from two in the short chromosomes to three in the longer ones. The single centric fragment is not included in the metaphase plate and is lost in the cytoplasm. It is probably eliminated in gamete formation.

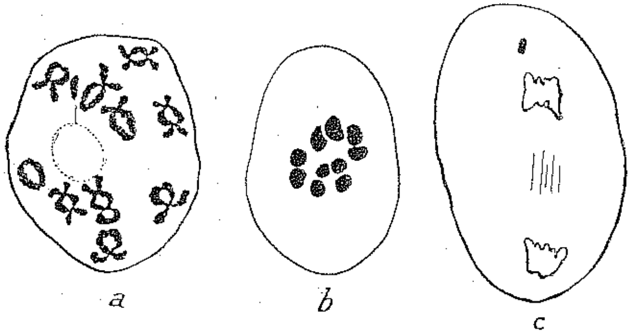


Fig. 7. Meiosis in *Erianthus ravennae*. *a*, diplotene; *b*, metaphase; *c*, telophase, with the fragment excluded. ×2000.

7. Meiosis in the F_1 hybrid

The F_1 hybrid flowered abundantly. Pollen mother cells at diakinesis showed that the 66 chromosomes associate into bivalents, trivalents and quadrivalents (Fig. 8*a*). Table 5 gives the configurations noted in ten

Table 5. *Degree of association of chromosomes in* F_1

Configurations				
IV	III	II	I	Cells
1	1	26	7	5
1	2	24	8	1
1	2	25	6	1
2	2	23	6	1
2	2	22	8	1
2	1	24	7	1

cells in which all the chromosomes were present. The large number of bivalents (22 to 26) present in the hybrid shows that the chromosomes derived from the haploid complement of the *S. spontaneum* are capable of pairing amongst themselves (autosyndesis) like the *Tripsacum* chromosomes in the cross between *Zea* and the tetraploid form of *Tripsacum dactyloides* (Mangelsdorf & Reeves, 1932).

The number of univalents varied from six to eight. These univalents probably represent the unpaired *Erianthus* chromosomes. The frequent presence of seven univalents associated with a single quadrivalent and a trivalent indicates that at least three of the chromosomes of *Erianthus* pair with those of *Saccharum*, forming the multiple associations noted. Not infrequently the number of these multiple associations is greater than one. An increase in their number is associated with a decrease in the number of bivalents rather than with any appreciable change in the number of univalents. We might infer from this that the gametic complement of *S. spontaneum* present in the hybrid is capable of forming higher

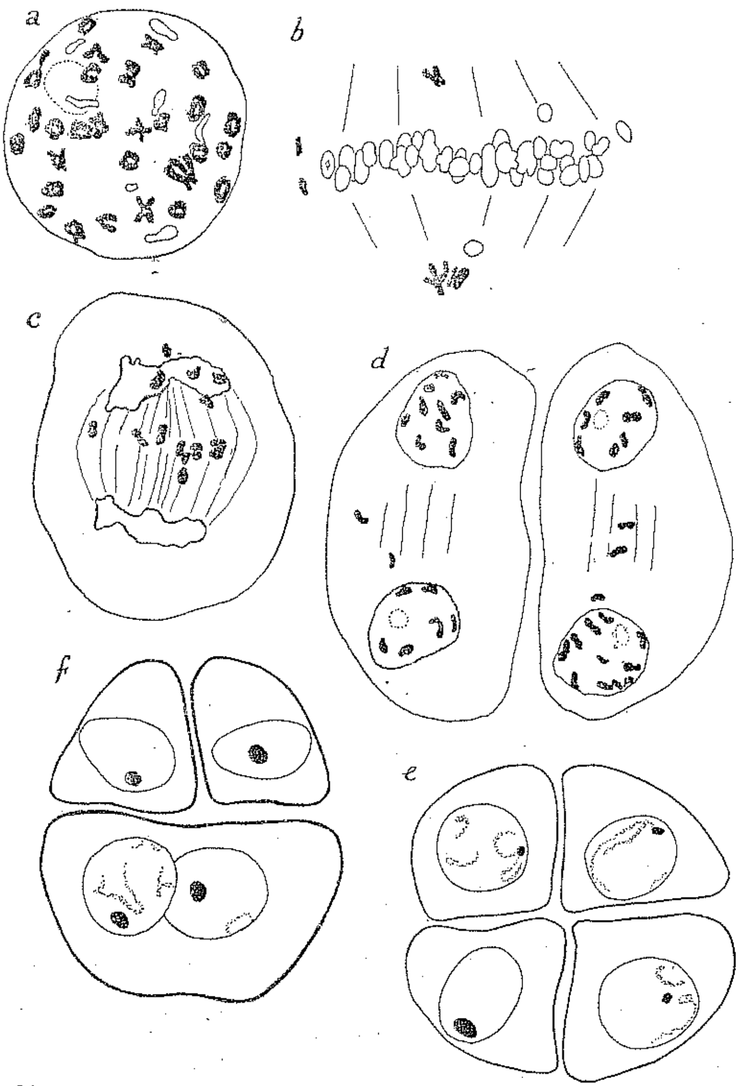


Fig. 8. Meiosis in *Saccharum-Erianthus* hybrid. *a*, diakinesis. *b*, first metaphase. *c*, telophase of first division, showing univalents dividing at the equator. *d*, telophase of second division, showing lagging chromosomes. *e*, normal tetrad. *f*, abnormal tetrad with binucleate dyad. (*a*, *b*, *c* and *d*, ×1800; *e* and *f*, ×1080.)

configurations by autosyndesis than it does in the polyploid parent. In this respect the *S. spontaneum* × *Erianthus* hybrid is similar to the diploid-hexaploid hybrid *Lolium perenne* × *Festuca arundinacea* in which Peto (1934) found trivalents, quadrivalents and quinquevalents.

Differential condensation of chromosomes was noticed in some of the cells. Fig. 8*b* shows a metaphase plate in which two bivalents seem to be at an earlier stage than the rest of the chromosomes. A variable number of univalents are seen to divide on the spindle, after the bivalents separate to opposite poles (Fig. 8*c*). At the second metaphase a number of daughter univalents are seen to lag and segregate at random without splitting. Some of these do not reach the poles before the nucleus is reconstructed (Fig. 8*d*). This variable segregation is responsible for the occurrence of F_2 seedlings with numbers ranging from 67 to 76. The development of the pollen grain was normal in a large percentage of the cells examined, but occasionally tetrads with two-nucleate cells and dyads were found (Fig. 8*f*). These would give rise to unreduced pollen grains. The occurrence of unreduced mother cells giving rise to unreduced embryo sacs is a common feature in *Saccharum* (Janaki-Ammal, 1939; S. Narayanaswamy, 1940). Fertilization of these diploid eggs by haploid and diploid pollen grains accounts for the occasional "triploids" and "tetraploids" found amongst the F_2 seedlings.

8. MEIOSIS IN F_2 SEEDLINGS

Fig. 9 *a*, *b*, *c*, represents the association of chromosomes in three of the "diploid" seedlings. The number of univalents was variable in all the plants studied. Chromosome association was chiefly in the form of bivalents with an occasional quadrivalent. In the "triploid" with 108 chromosomes I found occasional sexivalents besides bivalents (Fig. 9*d*).

Fig. 9 *e* and *f* illustrates the chromosome configuration in pollen mother cells of the single "tetraploid" plant SG 100·33 during diakinesis and metaphase. The chromosomes associate as bivalents, quadrivalents and occasionally sexivalents. The number of univalents was considerably less than in the diploid plants, and both first and second meiotic divisions were more regular than in these.

9. SUCROSE CONTENT OF HYBRIDS

Crossing *S. officinarum* with *Erianthus* results in hybrids with a reduced sugar content (Rumke, 1934). The F_1 hybrid between *S. spontaneum* "Glagah" and *Erianthus* also showed considerable reduction; the purity of the juice was also lowered. Table 6 gives the analysis of sugar in the cross as well as in five of the F_2 hybrids, two diploid, two triploid, and one tetraploid. It will be seen that, whereas the diploid hybrids were approximately as sweet as the F_1, the triploid and tetraploid plants showed a considerable increase in the percentage of sugar present. This is analogous to the findings in an autotriploid of *S. spontaneum* examined by the writer (Janaki-Ammal, 1939). It can be inferred from these

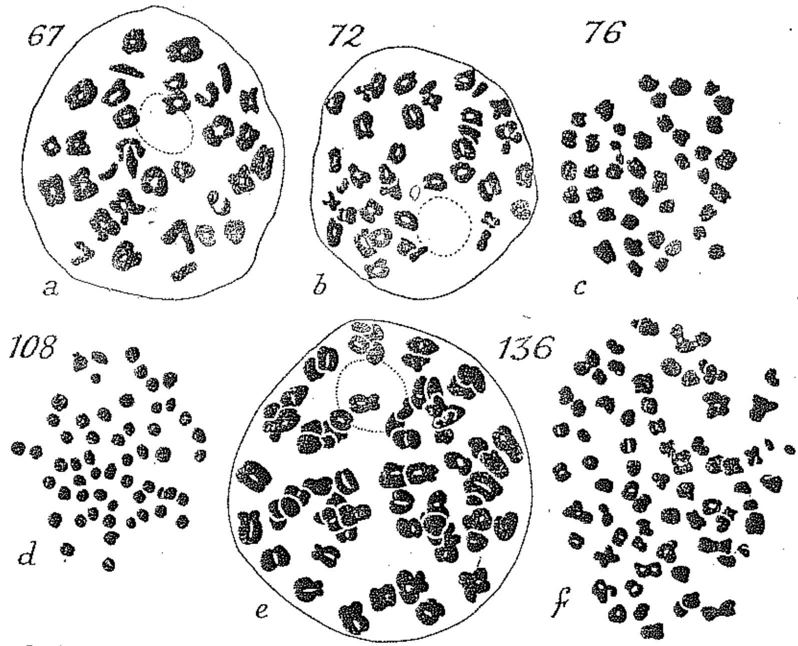


Fig. 9. Association of chromosomes in F_2 seedling. *a, b, c,* "diploids" with 67, 72 and 76 chromosomes. *d,* metaphase in triploid with 108 chromosomes. *e, f,* diakinesis and metaphase in tetraploid, $2n=136$, showing quadrivalents and sexivalents. ×1800.

Table 6. *Sugar analysis of* Saccharum × Erianthus *hybrids and* Saccharum *parent*

	Sucrose %	Purity %
S. spontaneum "Glagah"	7·93	60·5
S. spontaneum × Erianthus F_1	3·64	36·5
F_2's, diploid: S.G. 100·3	3·51	34·5
S.G. 100·16	2·33	27
F_2's, triploid: S.G. 100·5	5·45	42·4
S.G. 100·35	6·30	55·7
F_2, tetraploid: S.G. 100·33	6·51	58·3

observations that polyploidy results in an increase in sugar production in both *S. spontaneum* and its hybrids. The tetraploid F_2's, however, still lack the content of the *Saccharum* parent and can be used for commercial purposes only through further crossing with the cultivated sugar cane, *S. officinarum*.

10. SUMMARY

1. The Javanese variety "Glagah" of *Saccharum spontaneum*, $2n=112$, when crossed with *Erianthus ravennae*, $2n=20+f$, gave fertile hybrids with 66 chromosomes.

2. The F_1 hybrids resembled the two parents in proportion to their chromosome contributions (56 and 10).

3. The F_2 seedlings fell into three groups in regard to their chromosome numbers:

Diploids, $SE+$	68–76
Triploids, $SSE-$	104–108
Tetraploid, $SSEE+$	136

4. The diploid seedlings were the great majority. They showed segregation of the *Erianthus* characters—presence of awn and compound inflorescence—and a unimodal distribution of the length proportion of callus hairs to glumes. The triploid and tetraploid seedlings had thicker stems, wider leaves and a larger inflorescence than the diploids.

5. The sugar content of the *Saccharum* parent was greatly reduced in the diploid seedlings and slightly reduced in the triploids and tetraploid.

6. In the F_1 hybrid the gametic complement of *S. spontaneum* is capable of pairing by autosyndesis and may form higher configurations than in the parent. Some of the *Erianthus* chromosomes join with those of *Saccharum* to form trivalents and quadrivalents. The others are unpaired, and are lost or distributed at random in meiosis. Binucleate tetrads and dyads are formed by suppression of one division. Chromosomes condense differentially in some of the pollen mother cells.

7. At meiosis in the diploid F_2 hybrids, quadrivalents and many univalents are present and the division is irregular. In the tetraploids, though a few quadrivalents and even sexivalents are present, there are fewer univalents and the division is more regular.

PART II. *SACCHARUM-IMPERATA*

1. INTRODUCTION

The true octoploid species *S. officinarum*, when used as the female parent in intergeneric crosses, has given economically disappointing results. However, when Thomas and Venkatraman (1930) and Bourne (1935) crossed the hybrid cane of Java, "POJ 2725", $2n=106$, with *Sorghum* they obtained some seedlings of value, together with large numbers considered useless to the sugar cane breeder. Since then, "POJ 2725" and another Java cane, "POJ 213", $2n=124$, have been extensively used in breeding and spectacular results have been obtained with widely differing genera of grasses, including the bamboo (Venkatraman, 1937). In the present experiment I have used the cane "POJ 2725" in crosses with *Imperata*.

The grass *I. cylindrica* has a wide distribution in both the Old and the New World. It is a troublesome weed of cultivated land. There are several ecotypes, ranging from dwarfs of a few inches in height to swamp forms over 9 ft. tall with inflorescences up to 20 in. in length. The form I used for hybridization with the sugar cane was that known in Malay as "alang-alang", with a chromosome number of 20. It had a small inflorescence (Fig. 10). Bremer, who examined pollen mother cells (1925), found regular pairing and meiosis in the plant. Its gametic complement is therefore 10. The seed was supplied by the Department of Agriculture, Kuala Lumpur, Malaya.

In 1935 I pollinated an arrow of POJ 2725 with pollen of *I. cylindrica*, under a bag, and obtained thirty-five seedlings. All of these looked very much like sugar canes, though a few were inclined to dwarfness. From an unpollinated control arrow under a bag I obtained a single seedling in 1935 and four seedlings in 1936. The present investigation concerns some of the surviving seedlings. Only fourteen were examined cytologically.

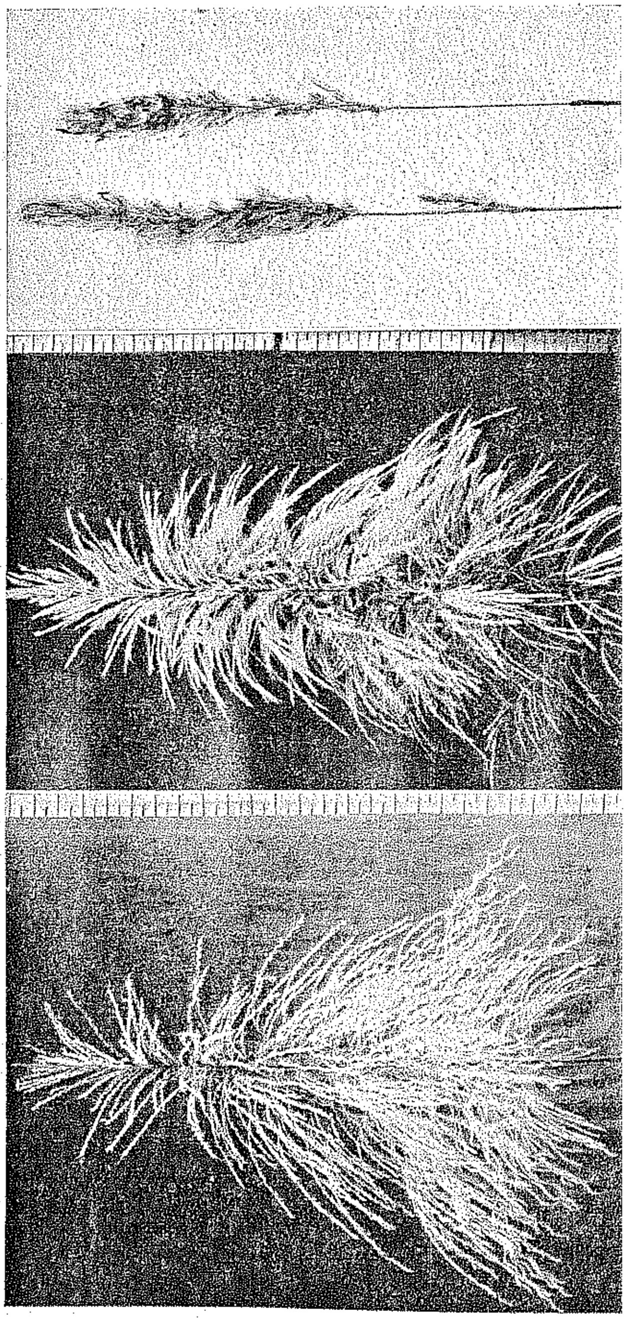
2. Methods

Canes of POJ 2725 were transported from the field to a pollinating shed when about to arrow. The arrows were bagged both before and after pollination. Root tips were fixed in Bouin's fixative and La Cour's 2BD after pre-treatment with ice. Pollen mother cells were fixed in acetic alcohol 1 : 3.

3. Cytology of the *Saccharum* parent, POJ 2725

The parentage of POJ 2725 is given in Table 7. It was produced at the Pasoeroean Station in Java by Jeswiet and has been described as "the product of the third nobilization of *S. spontaneum* Glagah".

When *S. officinarum* ♀ is crossed with *S. spontaneum* ♂, the F_1 in all cases examined is the result of the fertilization of an unreduced egg of the first species by a reduced pollen grain of the second (Bremer, 1929; Dutt & Subba Rao, 1933). It has the composition *OOS* where *O* and *S* stand for the complements of the two species. In back-crossing the hybrid "Kassoer", of this constitution, as male to *S. officinarum* ♀, fertilization was again confined to $2n$ eggs, so that POJ 2364, the female parent of the



POJ 2725 F_1 *Imperata cylindrica* (⅔ nat. size.)

Fig. 10. Inflorescence of POJ 2725, *Imperata cylindrica* and F_1.

Table 7. *Origin of the clone* POJ 2725

S. officinarum ♀ "Black Cheribon", $2n=80$ (OO) × *S. spontaneum* ♂ "Glagah", $2n=112$ (SS)

S. officinarum ♀ POJ 100, $2n=80$ (OO) × "Kassoer" ♂ $2n=136$ (OOS)

POJ 2364 ♀ $2n=148$ $(OOO+S/2)$ × *S. officinarum* ♂ EK 28, $2n=80$ (OO)

POJ 2725 $2n=106$

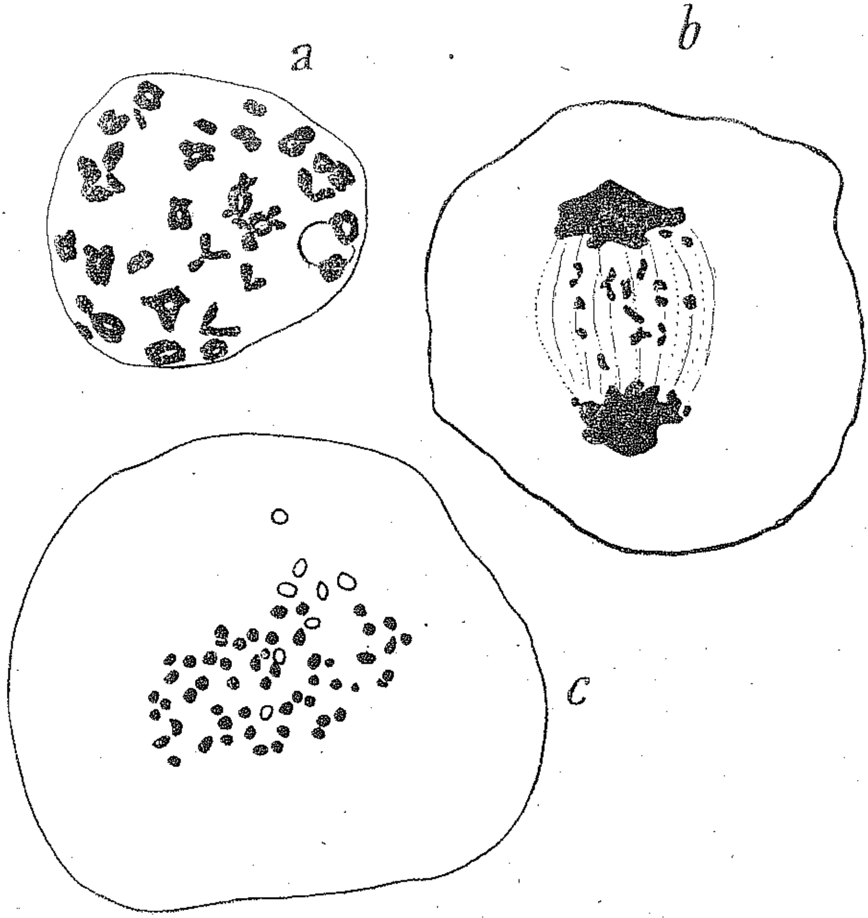


Fig. 11. Meiosis in POJ 2725. *a*, pollen mother cell at diakinesis showing multivalent and univalent association of chromosomes ×2400. *b*, anaphase of first division. *c*, metaphase in pollen grain with 62 chromosomes ×1800.

cane POJ 2725, may be considered as a triploid *S. officinarum* plus about half the gametic complement of *S. spontaneum*.

As would be expected, meiosis in POJ 2364 is very irregular. When backcrossed with *S. officinarum* it produced seedlings having a variable number of chromosomes, 106–120. According to Bremer (1928) POJ 2725 has 106–7 chromosomes; I was able to count 106 only. The chromosomes in pollen mother cells of POJ 2725 at diakinesis associate as bivalents, trivalents and quadrivalents (Fig. 11*a*). A number of univalents were also regularly observed, and many divided at first anaphase (Fig. 11*b*). Meiosis is consequently irregular. The irregular distribution of chromosomes gives gametes with variable numbers; 62 were found at metaphase in a pollen grain (Fig. 11*c*). Hence it seems unlikely that POJ 2725 will contribute its exact haploid number, 53, to any of its hybrids.

The percentage of viable pollen was about 21 %. Anthesis was poor and variable. In a sample from one arrow 2 % of the anthers had dehiscence pores. Selfing or parthenogenesis is therefore responsible for the few seedlings obtained under bag in 1936–8.

4. TRUE AND FALSE HYBRIDS

The following are the chromosome numbers of the fourteen seedlings:

	Chromosome no.	No. of plants	Origin	Presumed constitution	Pollen fertility
I	106	2	Vegetative embryony	*SS*	23–35 %
II	108 110 112	3 2 1	Sexual or diploid parthenogenesis	*SS* +	0·0–7 %
III	120 130 132 134	1 1 1 2	True F_1	*SSI*	50–80 %
IV	156	1	Triploid self	*SSS*	Non-flowering

It will be seen that they fall into four groups, of which representative types are shown in Fig. 12. First, there are those that have the same number as POJ 2725. These also resemble it closely in vegetative characters and have the same pollen fertility, 23–35 %. They are in all probability vegetatively apomictic plants and could be considered as clones of the mother plant (Fig. 13, type A).

To the second group belong plants in which the chromosome number is slightly greater than in POJ 2725. They show segregation of characters and are very highly sterile, the pollen fertility being 0–7 %. From the evidence of their chromosome number and morphology, they would appear to be either true selfs or parthenogenetic plants developed from unre-

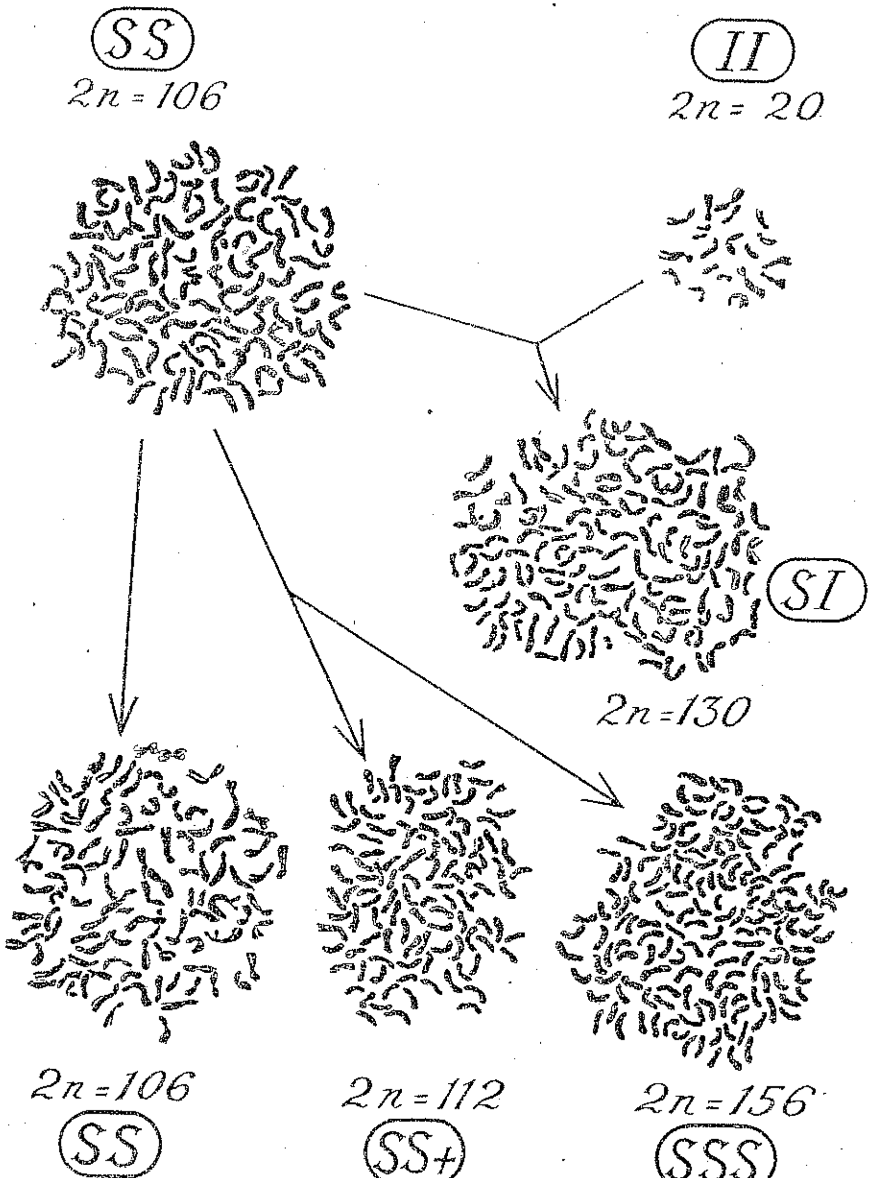


Fig. 12. Root tip metaphases in POJ 2725, (*SS*) *Imperata cylindrica* (II) and types of seedlings obtained in the cross. ×2000.

duced gametes (types B and D in Fig. 13). Bourne (1935) found that about 1 % of the seedlings produced by pollinating this cane with *Sorghum* are of a maternal type. Venkatraman & Thomas (1930) have made no reference to the existence of these types amongst their POJ 2725 *Sorghum* hybrids.

In the third group are plants with 120–134 chromosomes (type E, Fig. 13). These numbers represent the sum of the chromosomes from unreduced gametes of POJ 2725 and from the haploid gamete of *Imperata*.

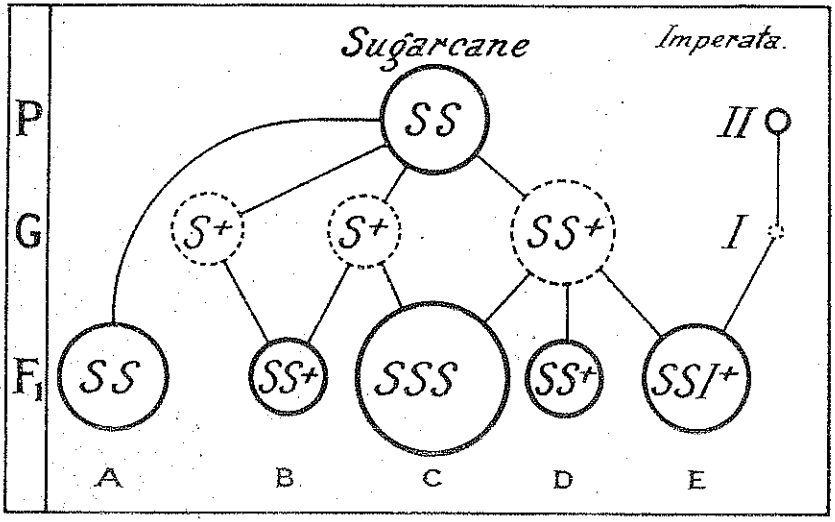


Fig. 13. Diagram of the genetic composition of seedlings from the cross POJ 2725 × *Imperata*.

They represent the only true hybrids of *Saccharum* and *Imperata*. These seedlings were highly fertile; they resembled sugar canes very closely, though they are more of the medium-cane type than the mother plant. A few showed multiple bud formation and the characteristic tillering of *Imperata*, but no importance was attached to these characters as they appear in hybrid sugar canes also.

The spikelets of *Imperata* differ from those of *Saccharum* in having fine hairs on the first and second glume. This character was found in some of the hybrids. The number of stamens also varied from three to four in odd spikelets of an inflorescence.

Meiosis in pollen mother cells of one hybrid with 120 chromosomes showed no configurations higher than bivalents (Fig. 14*a*). A variable number of univalents were present. These generally divided at the equator of the spindle after the bivalents had separated to the poles

(Fig. 14*b*), and were nearly always incorporated in the daughter nuclei. It is not possible to say whether they are unpaired chromosomes of *Imperata* or of *Saccharum*. Second division was fairly regular. The

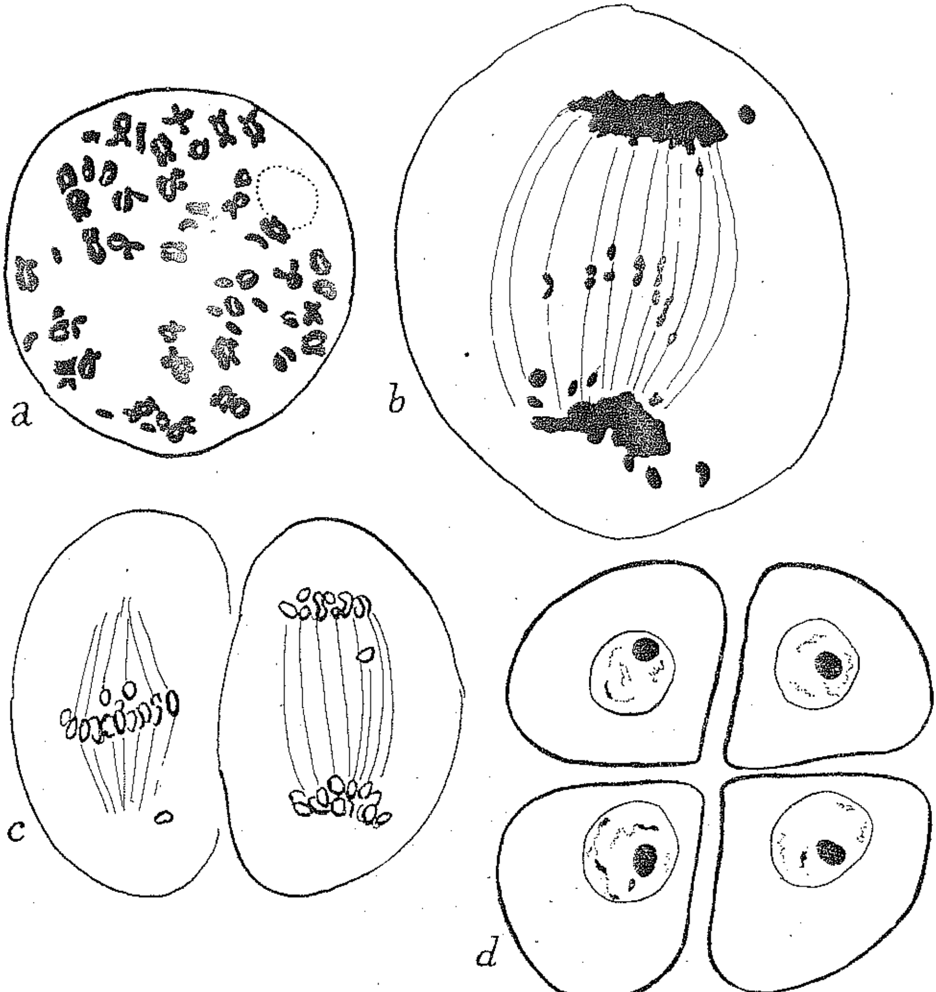


Fig. 14. Meiosis in a *Saccharum-Imperata* hybrid. *a*, diakinesis in pollen mother cell. *b*, first anaphase. *c*, second anaphase. *d*, tetrad formation. ×2000.

divided univalents were segregated at random to the poles (Fig. 14*c*), and normal tetrads formed (Fig. 14*d*).

All the hybrid F_1 plants were fertile, pollen fertility being as high as 80 % in some. An interesting series of F_2's was obtained from this plant,

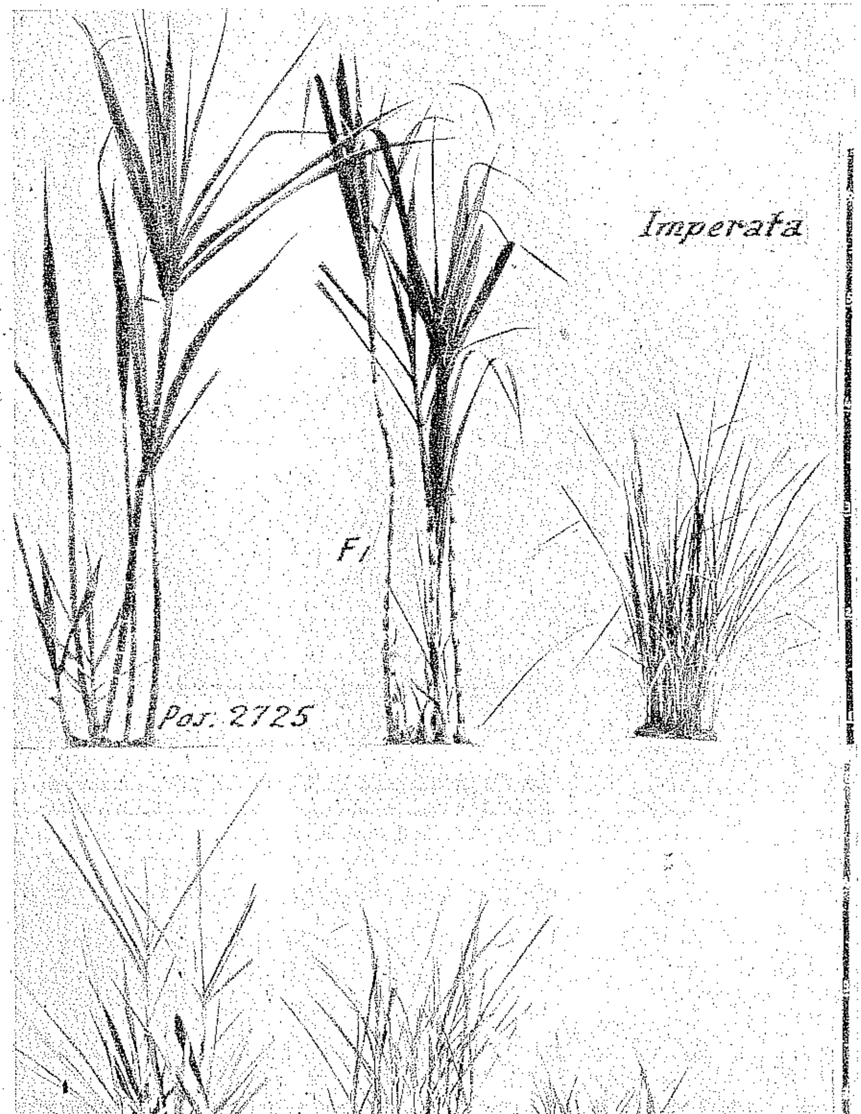


Fig. 15. General habit of POJ 2725, *Imperata cylindrica* and their F_1 and F_2 hybrids.

some of which were similar to *Imperata* in grass habit and size of leaf (Fig. 15). It will be seen then that when POJ 2725 is crossed with *Imperata*, hybrids are produced through the agency of unreduced embryo-sacs. This elimination of normally reduced eggs is responsible

(1) for the extremely few seedlings produced,
(2) for the sugar-cane like characters of the hybrids.

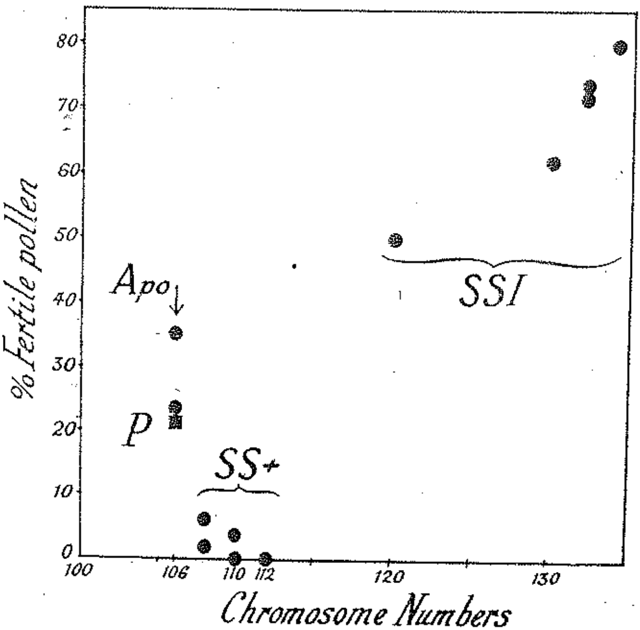


Fig. 16. Graph showing relative pollen fertility of POJ 2725 (*P*) and its apomictic (*Apo*), parthenogenetic or selfed (*SS* +), and hybrid (*SSI*) seedling.

The proportion of chromosomes of sugar cane to *Imperata* in the seedlings is 11 : 1 in the hybrids with the lowest number, and 12·4 : 1 in that with the highest number, 134.

The single plant with 156 chromosomes in the population may be considered as a "triploid" POJ 2725 (type C in Fig. 12). Unlike other triploids it is much smaller than the parent. It probably suffers from the disadvantage of having too many chromosomes for the size of the cell. So far it has not flowered.

Fig. 16 gives the pollen fertility of the three classes of seedlings.

5. SUCROSE CONTENT OF THE SEEDLINGS

Table 8 gives the sugar analysis of twenty-eight out of the thirty-five seedlings obtained by pollinating POJ 2725 with *Imperata*. The canes were analysed before they were fully mature, but the percentage of sugar present in the seedlings even at this stage is high enough to indicate the economic value of this cross.

Table 8. *Sugar analysis of twenty-eight seedlings produced by pollinating* POJ 2725 *with* Imperata

Seedling no.	Brix	Sucrose	Purity
63·1	15·98	13·27	83·00
63·2	19·99	17·21	86·10
63·3	16·88	13·68	81·00
63·4	17·38	13·67	78·00
63·5	19·19	16·08	83·80
63·6	18·29	14·80	80·90
63·7	21·40	16·14	75·40
63·8	19·04	15·82	83·10
63·9	20·49	17·79	87·00
63·10	15·21	11·95	78·60
63·11	16·14	13·49	83·60
63·12	15·64	12·18	79·50
63·13	15·67	12·14	77·50
63·15	18·07	15·19	84·10
63·21	17·27	14·29	82·80
63·23	19·20	17·12	89·20
63·24	17·53	15·20	86·70
63·25	21·25	18·60	89·60
63·26	19·75	17·63	89·20
63·27	18·14	15·80	87·10
63·28	17·63	14·79	83·70
63·29	19·04	17·28	90·70
63·30	17·23	14·08	81·70
63·31	15·93	12·77	80·20
63·32	19·44	16·87	86·80
63·33	17·73	15·34	86·50
63·34	19·24	17·56	91·30
63·35	20·25	18·19	89·80

Further analysis of some of the more promising seedlings showed that they were capable of still better performance. Thus the hybrid 63·32, $2n = 132$, which has an excellent erect habit and good tillering, had 19 % sugar when fully mature. The percentage was even higher in some others, but these were not so good from the agricultural point of view.

An interesting point is that the asexually produced seedlings *SS* with 106 chromosomes, whose genetic composition should be identical with that of POJ 2725, had a lower sucrose content than the sexually and subsexually produced *SS*+ type—probably because they are late in maturing.

The level of performance reached by the true hybrids *SSI* is as good as that of the selfed or diploid parthenogenetic seedlings, which is certainly due to the high proportion of sugar cane to *Imperata* chromosomes in this cross (12 : 1).

In Fig. 17 I have correlated the sucrose percentage with the genetic constitution of seedlings examined cytologically.

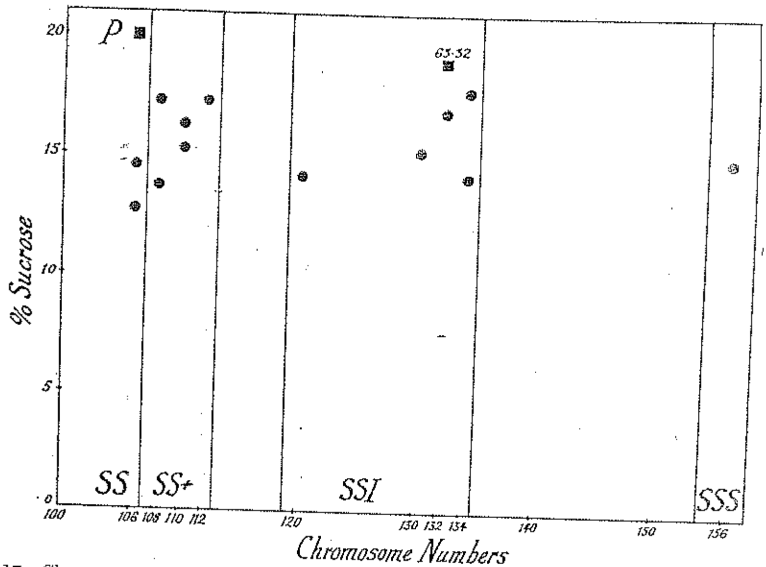


Fig. 17. Chromosome numbers and percentage sucrose in "true" and "false" hybrids of POJ 2725. The squares indicate maximum yield at full maturity, the circles yield of canes not yet fully matured.

6. Summary

1. By pollinating an arrow of the hybrid cane POJ 2725 with pollen of *Imperata cylindrica*, thirty-five seedlings of fairly high sucrose content were obtained.

2. Cytological analysis of the seedlings showed them to be of four types:

(i) Vegetative seedlings, *SS*, $2n = 106$.

(ii) Selfed or diploid parthenogenetic seedlings, *SS*+, $2n = 108$–112.

(iii) Triploid self, *SSS*, $2n = 156$.

(iv) True *Saccharum-Imperata* hybrids, *SSI*, $2n = 120$–134.

3. The vegetative seedlings resembled POJ 2725 in vegetative characters and degree of pollen sterility; parthenogenetic and selfed seedlings were completely sterile and the true hybrids highly fertile.

4. Elimination of all but unreduced eggs is responsible for the small number of F_1 seedlings produced. The high proportion of *Saccharum* chromosomes accounts for the predominance of *Saccharum* characters in the hybrids.

5. The "triploid" selfed seedling is much smaller than the parent, and has not flowered.

6. F_2 seedlings of the true *Saccharum-Imperata* hybrids show segregation of *Imperata* characters.

Part III. *SACCHARUM-ZEA*

1. Introduction

Crosses described in Parts I and II of this paper involved *S. spontaneum* or its cultivated derivatives. We now turn to a cross involving *S. officinarum* proper.

As mentioned above, Barber in 1913 produced a hybrid of *S. officinarum* "Vellai" with the grass *Narenga narenga*. The clone "Vellai" is male sterile owing to the suppression of anthesis, and this sterility is a genotypic character not due to irregular meiosis. The same character is found in other clones, including the Black Cheribon of Java. "Vellai" has been crossed with two species of *Sorghum* and with *Erianthus arundinaceus* at Coimbatore (Venkatraman, 1938).

These grasses are all within the group Andropogoneae according to the accepted classification of Bews (1929). A wider cross would be expected to succeed less readily. In 1936 I crossed several inflorescences of "Vellai" with pollen of *Zea Mays*, and obtained a single seedling, which proved to be a true hybrid (Janaki-Ammal, 1938*a*). I repeated the cross in 1938 and obtained another seedling, which, however, died early. Dr S. C. Harland tells me that, using a different type of sugar cane, he attempted a similar cross in Trinidad, but without success.

The variety of *Zea Mays* used as male parent was a sweet corn, "Golden Beauty". It was grown for several generations without chance of cross-pollination with other varieties, being the only kind of maize grown in the neighbourhood.

2. Methods

Canes of the male sterile *Saccharum officinarum* "Vellai" which was about to "arrow" were transported from the field and planted horizontally into a pollinating shed. The inflorescence, a large panicle, was supported by bamboo stilts to raise it above the ground. The arrows were bagged as soon as they emerged from the sheath. When stigmas started to appear they were dusted with pollen of *Zea Mays*. This was continued daily for about ten days. Two arrows under bag were allowed to remain in the same shed as control.

The seeds from the pollinated arrow were grown in the usual way (Barber, 1916) and a single seedling appeared. Seeds from unpollinated arrows failed to germinate.

The obstacle in crossing *Saccharum* with *Zea* seems to lie in the first stage of the operation, viz. in the widely different sugar concentrations required by the germinating pollen. Pollen of *Zea Mays* was tested for germination in different concentrations of sucrose in 1 % agar. *Zea* pollen germinates in concentrations ranging from 10 to 18 % sucrose. This is much below the concentration needed for sugar cane pollen, which demands the narrow range of 23–25 %, presumably the percentage of sugar present in the stigmas.

The seedling in its early stages was of very weak growth, and special treatment with nutrient solutions was used to keep it alive.

Root tips of the two parents and the hybrid were fixed in Allen's Bouin and in La Cour's 2BD. Roots of *S. officinarum* were pre-treated with ice.

3. Chromosome numbers in parents and hybrid

S. officinarum "Vellai" showed 80 chromosomes, as was found by Dutt & Subba Rao (1933). The length of the chromosomes varied from 3·6 to 1·6μ. Both primary and secondary constrictions were found, but in no case was I able to detect any chromosome with satellites (Fig. 18*a*).

Zea Mays "Golden Beauty" had besides the usual 20 chromosomes two *B* chromosomes, which could be distinguished by their deeper staining. The different chromosomes of the haploid complements of maize were recognizable by their sizes, the position of the centromere and the satellite of chromosome VI (Fig. 18*b*).

The hybrid *Saccharum-Zea* had 52 chromosomes, the sum of the haploid complements of *Saccharum* and *Zea*. The two *B* chromosomes seem to have been transmitted to the hybrid. It was not possible to distinguish all the *Zea* chromosomes in the hybrid, but a single satellite chromosome was seen (Fig. 18*c*) as well as one of the *B* chromosomes. The *Zea* chromosomes appear to have undergone considerable size reduction in the hybrid, indicating genotypic control of size (Darlington, 1937, p. 55).

4. MORPHOLOGY OF PARENTS AND HYBRID

The parent plants are too well known to need detailed description. The cane "Vellai"—the name means "white" in Tamil—is very probably the cane Lahaina of Hawaii. It was known as early as 1766 when

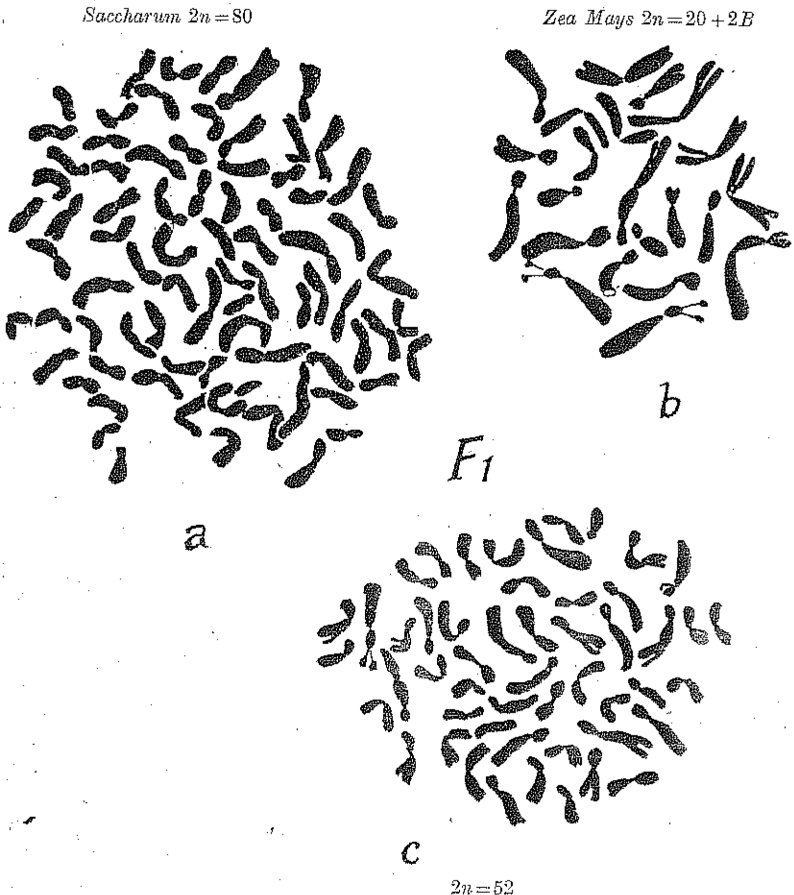


Fig. 18. Root tip metaphase in *a*, *Saccharum officinarum* Vellai; *b*, *Zea Mays* Golden Beauty; *c*, *Saccharum-Zea* hybrid. ×4000.

Bougainville introduced it from Otaheite to Mauritius and Bourbon, now Reunion (Deerr, 1921). In 1791 Captain Bligh brought it from Otaheite to Jamaica (Earle, 1928). It is known as the Otaheite cane, also as the Bourbon or Cana Blanca. It has a thick soft stem, and is still cultivated in many parts of the world as a chewing cane. A full description will be found in Barber (1916) and Earle (1928).

The *Zea* parent "Golden Beauty" is a sweet corn sold in Poona as a type suitable for cultivation in India. Like all maize it varies enormously in size according to soil and cultivation. In the loamy soil of Coimbatore it attained a height of about 5 ft. The ear was 6–7 in. long.

The hybrid *Saccharum-Zea* in the young seedling stage resembled a small *Saccharum*. After a year the plant was barely a foot high; during the second season it put out a number of tillers (Fig. 19). After four years it remains a dwarf bush, recalling in general appearance *Tripsacum dactyloides* as illustrated by Mangelsdorf & Reeves (1939). The growth of the main axis is very much retarded by the vigorous side branches. The

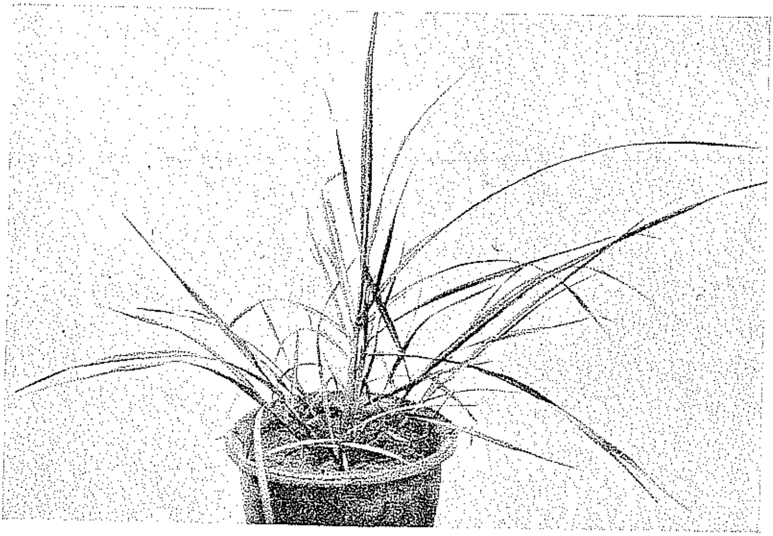


Fig. 19. *Saccharum-Zea* hybrid during second year of growth.

perennial nature of the hybrid has made it possible to propagate it vegetatively. Each tiller looks like a diminutive sugar cane with small *Saccharum*-like stem and leaves and has a ligular process, as found in Vellai but not in *Zea* (Fig. 20). The plant shows no sign of flowering.

The upper surface of the leaf is covered with long silky hairs, similar to but larger than those in *Zea* (Fig. 21). These silky hairs are a feature I have not found in any *Saccharum* except a freak cane "Troeboe" from Java. "Troeboe" is of unknown origin, and I am tempted to consider it as a possible hybrid between *Saccharum* and a member of the Maydeae. The possibility is strengthened by the fact that it does not bear perfect flowers; its inflorescence is aborted and forms a cauliflower-like mass in the sheath. Incidentally, this malformation seems to have led to the plant's survival in cultivation, as I am told the inflorescence is eaten as a salad by the peoples of the East Indies.

In Table 9 I have tabulated some anatomical and morphological characters of the two parents and the hybrid. The plants have been compared for twenty-four characters, fifteen qualitative and nine quantitative. It will be seen that the hybrid resembles its *Saccharum* parent in ten of the qualitative characters and *Zea* in three. One character is

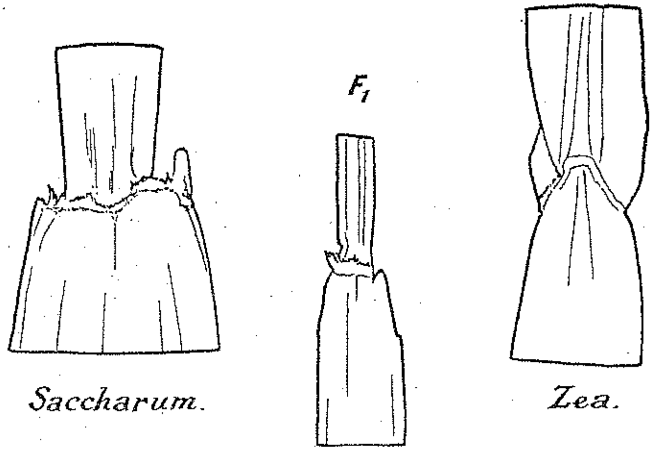


Fig. 20. The ligule in *Saccharum*, *Zea* and the F_1 hybrid.

intermediate and one is new in the hybrid—the depressed shape of the bulliform or motor cells.

In all measurements the hybrid is found to be smaller than either of the parents. This was noticed especially in the size of the cells. Exceptions are the length of the epidermal hairs and the number of vascular bundles in the stem of the hybrid plant. The concentration of vascular bundles (Fig. 22, F_1) can be explained as due to the extreme reduction of the stem and the consequent entry of large numbers of leaf traces into it.

The hybrid was not examined for its sugar content, but from the extreme woodiness of the stem it is unlikely that it will prove to have much sugar.

5. Summary

1. *Saccharum officinarum* "Vellai", $2n=80$, when crossed with *Zea Mays*, $2n=20+2B$, gave two seedlings, one of which survived and was found to have 52 chromosomes.

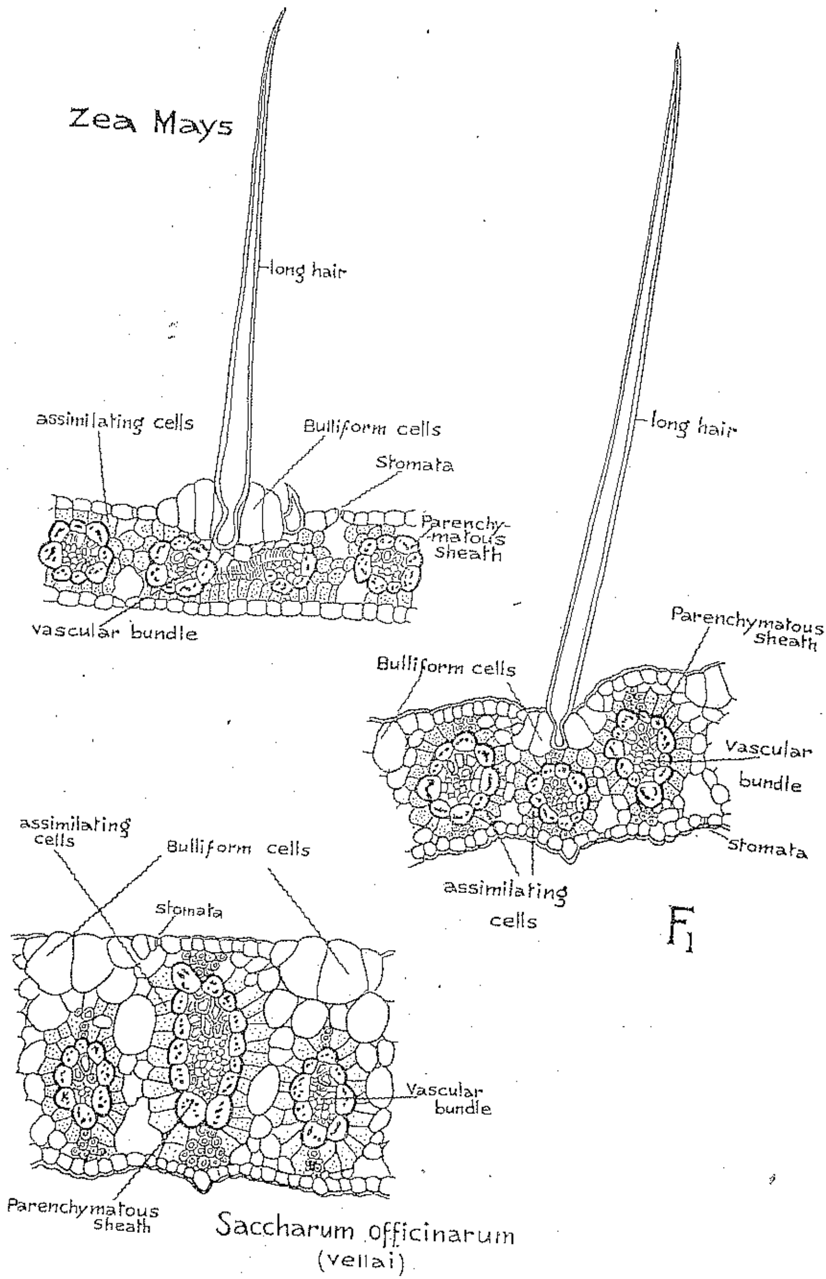


Fig. 21. Transverse section of leaf of *Saccharum*, *Zea* and the F_1 hybrid.

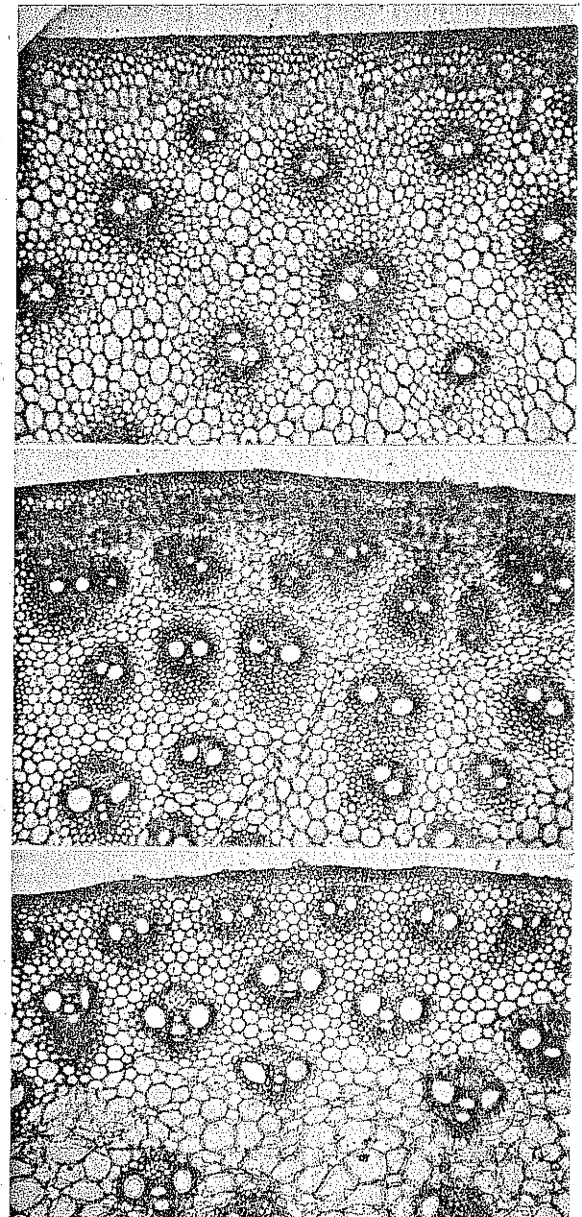


Fig. 22. Transverse section of internode of *Saccharum* (*S*), *Zea* (*Z*) and the F_1 hybrid.

2. The difficulty in making the cross seems to be that the concentration of sugar required by germinating maize pollen is much lower than that found in the *Saccharum* stigma.

3. The hybrid showed some of the detailed characters of each parent, but in general the growth was depressed. In four years the plant has

Table 9. *Comparison of characters of* Saccharum officinarum, Zea Mays *and* F_1 *hybrid*

	Saccharum	F_1	*Zea*
	Qualitative characters		
1. Habit	Perennial	←	Annual
2. Root stalks	Present	←	Absent
3. Root eyes	Present	←	Absent
4. Tillering	Many	←	Few
	Leaf		
5. Sheath	Hairy	→	Glabrous
6. Ligular process	Present	←	Absent
7. Upper epidermis	Non-hairy	→	Hairy
8. Lower epidermis	Asperites present	←	No asperites
9. Vascular bundle	Oval	Intermediate	Round
10. Sclerenchyma in bundle sheath	Present	←	Absent
11. Bulliform cells	In line with epidermis	Depressed	Raised
12. Shape of bulliform cells	Round	→	Linear
	Stem		
13. Cortex	Present	←	Absent
14. Distribution of vascular bundles	Diffuse	←	Peripheral
15. Direction of growth of bundle sheath	Towards stem centre	←	Equally around bundle
	Quantitative characters		
16. Average height (cm.)	600	75	180
17. Stem diameter (cm.)	4	1	2
18. Length of internode (cm.)	9	1·4	18
19. Leaf width (cm.)	6·5	1·5	4·5
20. Leaf length (cm.)	150	36	45
21. Number of bulliform cells	2–3	3–4	3–5
22. Number of vascular bundles per unit area	10	30	20
23. Average length of hairs (mm.)	—	0·75	0·5
24. Diameter of parenchyma cells (mm.)	0·15	0·075	0·125

failed to flower, although it has grown freely and has been propagated from tillers.

4. The vegetative abnormality of the hybrid is attributed to the remoteness of the parents.

5. The hybrid shows a hair character of the Maydeae which is also found in the highly aberrant and sterile cane "Troeboe". "Troeboe" may therefore be of similar hybrid origin.

Table 10. *Recorded intergeneric hybrids of* Saccharum

Clone		2n	Cross reported by	F_1 Chromosome	F_1 Fertility	Chromosome numbers determined by
			I. *S. officinarum*, $2n=80$			
1. Vellai	× *Narenga narenga*	30	Barber, 1916	55	S.*	E.K.J.‖ 1938 (R) ¶
2. EK 28	× *Erianthus sara*	60	Rumke, 1934	60–70	F.†	Rumke, 1934
3. Vellai	× *E. arundinaceus* (as *S. arundinaceum*)	60	Venkatraman, 1935	70	S. ?	E.K.J. 1938 (R)
4. Vellai	× *Sorghum durra*	20	Venkatraman, 1935	50, 90	N.Fl.‡	E.K.J. 1938 (R)
5. Vellai	× *S. halepense*	40	Venkatraman, 1938	60	P.S.§	E.K.J. 1938 (R)
6. Vellai	× *Zea Mays*	$20+2B$	E.K.J. 1938 *a*	52	N.Fl.	E.K.J. 1938
			II. *S. spontaneum*			
	2n					
7. Glagah	112 × *Er. ravennae*	20	E.K.J. 1936 (R)	66	F.	E.K.J. (unpublished)
8. Hole's no.1	56 × *Sorghum durra*	20	E.K.J. & Singh, 1936	38 66	S. F.	E.K.J. 1938 *b*
9. Coimbatore	64 × *Sorghum durra*	20	E.K.J. 1936 (R)	42	P.S.	E.K.J. (unpublished)
10. Gigas	124 × *Sorghum durra*	20	E.K.J. 1936 (R)	—	P.S.	—
11. Gigas	124 × *S. halepense*	40	E.K.J. 1936 (R)	—	P.S.	—
12. Coimbatore	64 × *Bambusa arundinacea*	72	E.K.J. 1939 (R)	68	P.S. 1 F_2 seedling	E.K.J. (unpublished)
			III. *S. officinarum* × *S. spontaneum* and derivatives			
13. POJ 2725	106 × *Sorghum durra*	20	Thomas and Venkatraman, 1930	63–4 116–118	P.S.	Singh, 1934
14. POJ 2725	106 × *Sorghum* sp. (as *Holcus Sorghum*)	20	Bourne, 1935	—	P.S.	—
15. POJ 2725	106 × *Imperata cylindrica*	20	E.K.J. 1938 (R)	120–134	F.	E.K.J. (unpublished)
16. POJ 2725	106 × *Bambusa arundinacea*	72	Venkatraman, 1937	90	F. ?	E.K.J. 1938 (R)
17. Kassoer	136 × *Sorghum durra*	20	E.K.J. 1936 (R)	—	—	—
			IV. *S. officinarum* × *S. Barberi*			
18. POJ 213	124 × *Bambusa arundinacea*	72	Venkatraman, 1937	96–100	F.	E.K.J. 1938 (R)

* S = sterile. † F = fertile. ‡ N.Fl. = non-flowering. § P.S. = pollen sterile. ‖ E.K.J. = E. K. Janaki-Ammal.
¶ R = *Report of work done under the Scheme for Research on the Genetics of Sugar Cane*, Government of India Press, Simla, 1936 *et seq.*

GENERAL DISCUSSION AND SUMMARY

Table 10 gives a list of the recorded intergeneric hybrids of *Saccharum*, including those described in the present paper.

It appears that in *Saccharum* high polyploidy has removed all obstacles to hybridization with other groups of Gramineae except those that depend upon the simply ascertainable conditions of pollen germination. The mode of action of high polyploidy is fully displayed in the versatile method of reproduction of the "nobilized" hybrids of *S. officinarum* and *S. spontaneum*, which in this respect resemble polyploids in *Poa* and *Rubus*. They are capable of producing from apparent hybridization with diploid species of other genera true diploid crosses, true triploid crosses and diploids and triploids which are not crosses at all. The fertility of the progeny depends not so much on the remoteness of the cross as on the internal pairability of the chromosomes derived from the polyploid parent, in other words on their capacity for autosyndesis. Systematic study of these properties will enable us in the future to recombine the materials of plant improvement on a scale that has not hitherto been realized.

ACKNOWLEDGEMENT

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