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E K Janaki Ammal made many pioneering contributions to the field of plant genetics. One of her major achievements was setting up intergeneric crosses between sugarcane and other related grasses. These studies were published in a classic series of papers in the *Journal of Genetics*. The first part of her paper published in 1941 is reprinted below. The paper documents her thorough and meticulous approach to science.

S Mahadevan

INTERGENERIC HYBRIDS OF *SACCHARUM*

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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.

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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 $2n=80$.



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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 10–12 μ and of pollen mother cells at 16 μ ; 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	<i>S. spontaneum</i>	F_1 seedlings	<i>E. ravennae</i>
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 (b) Linear (c) Ciliate (d) Awnless	Long S S S	Long Ovate Non-ciliate Awned
12. Lodicules	Ciliate	S	Glabrous

* S = character as in *Saccharum* parent.

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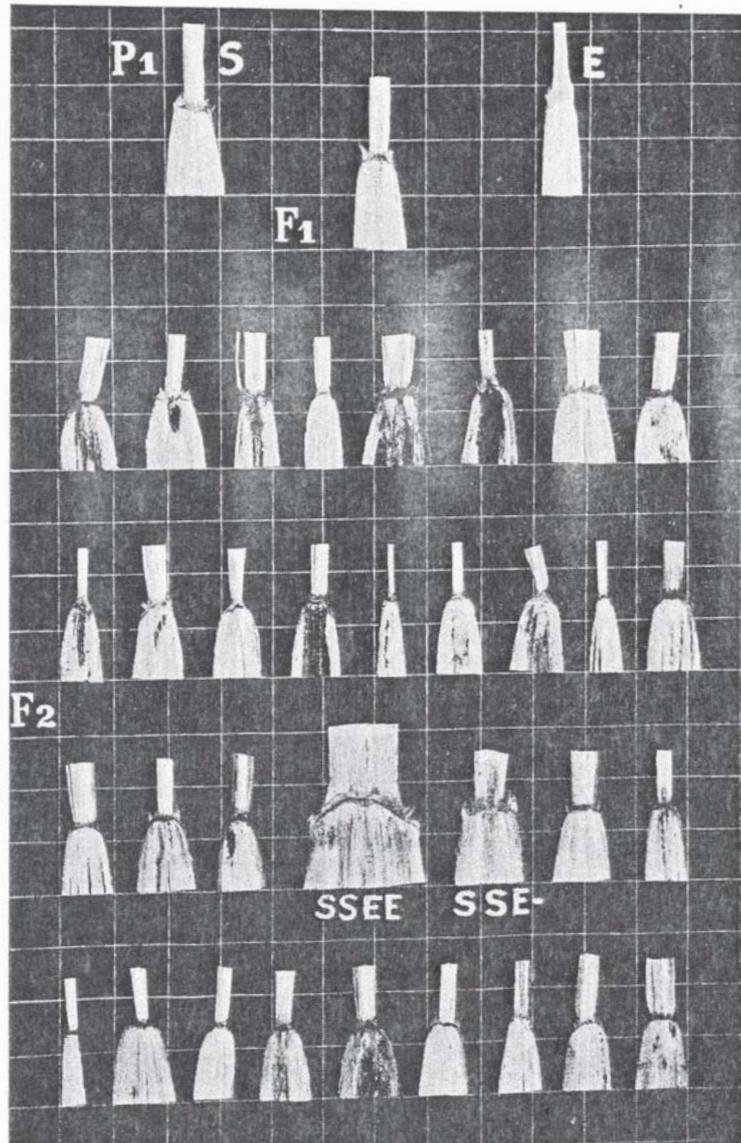


Fig. 1. Types of ligule in *S. spontaneum* and *Erianthus P₁* and their *F₁* and *F₂* hybrids.



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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

Class of parents	Leaf width in centimetres					
	1	1.5	2	2.5	3	3.5
Class of F_1	<i>Erianthus</i>		<i>Saccharum</i>		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

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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.

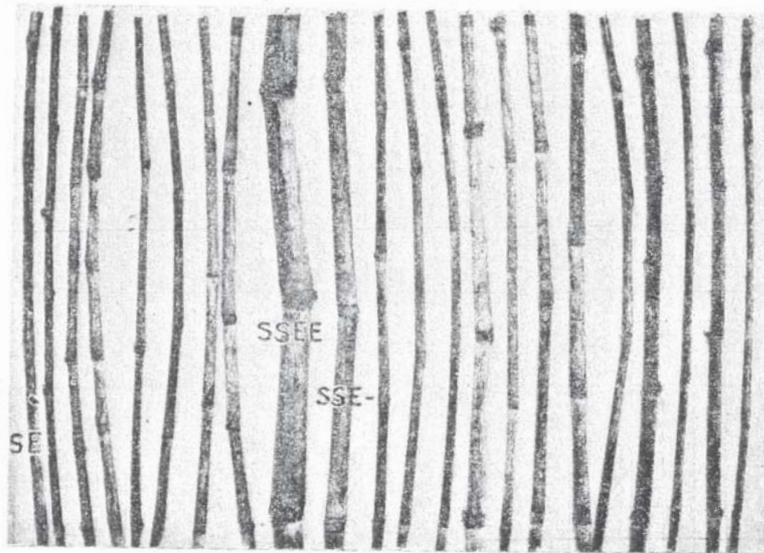


Fig. 2. Variation in stem thickness in the F_2 generation of *Saccharum-Erianthus* hybrids

Table 3. *Stem diameter in parents and crosses*

Class of parents Class of F_1 Frequency of F_2	Stem diameter in centimetres				
	0.5	1	1.5	2	2.5
		<i>Erianthus</i>	<i>Saccharum</i>		
	2	31	F_1 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	<i>E. ravennae</i>	—
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	<i>S. spontaneum</i> "Glagah"	—

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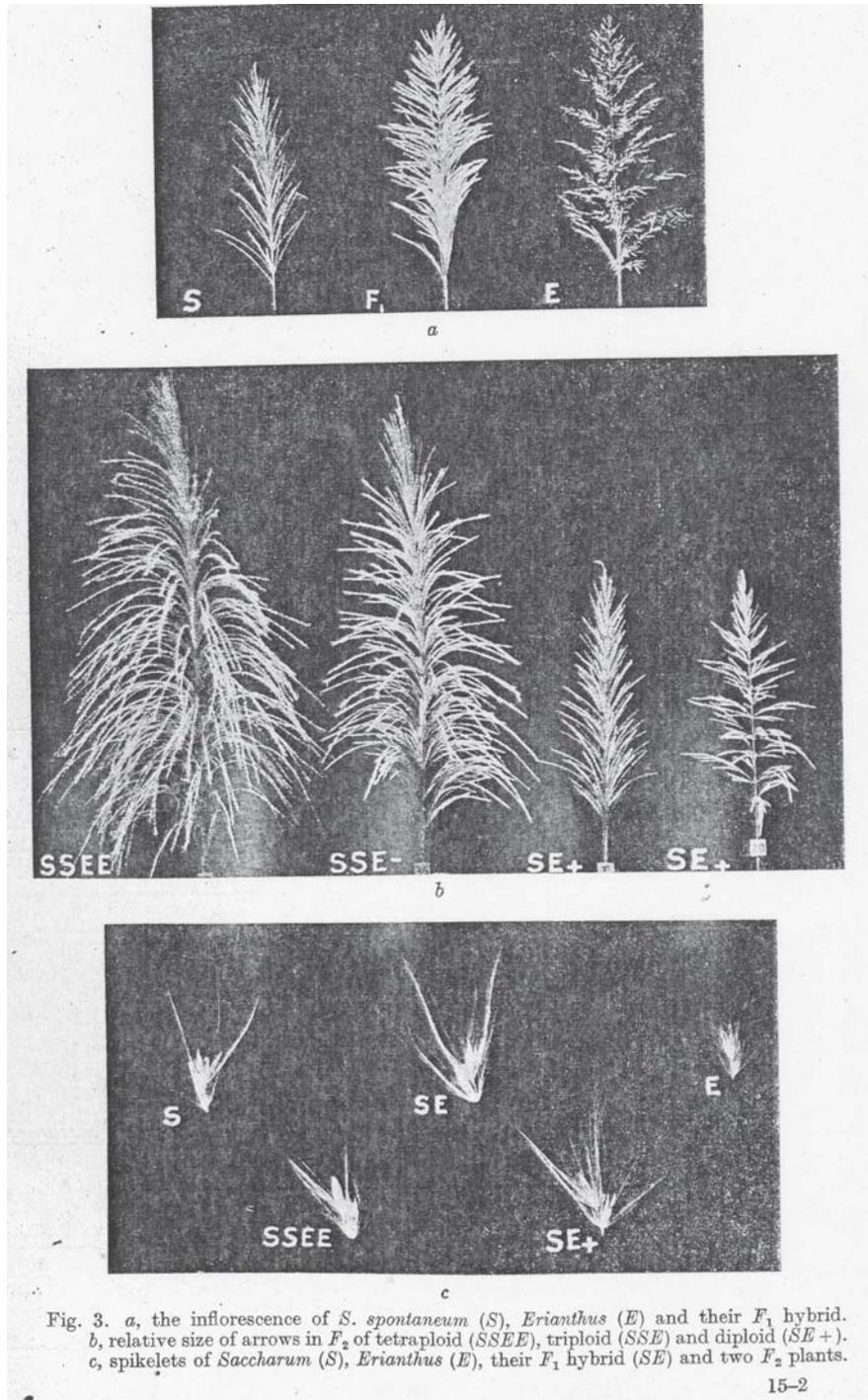


Fig. 3. a, the inflorescence of *S. spontaneum* (S), *Erianthus* (E) and their F_1 hybrid. b, relative size of arrows in F_2 of tetraploid (SSEE), triploid (SSE-) and diploid (SE+). c, spikelets of *Saccharum* (S), *Erianthus* (E), their F_1 hybrid (SE) and two F_2 plants.



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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.

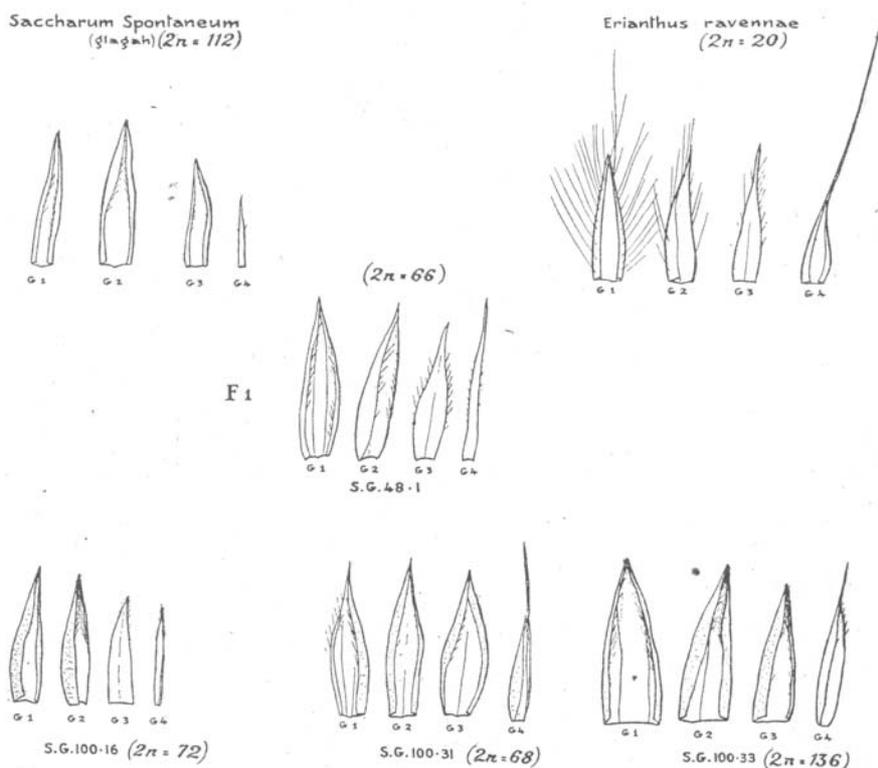


Fig. 4. Types of glume in spikelets of *Saccharum*, *Erianthus* and their F_1 and F_2 hybrids. The awned fourth glume, though not present in the F_1 , appears in some of the F_2 seedlings.

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.

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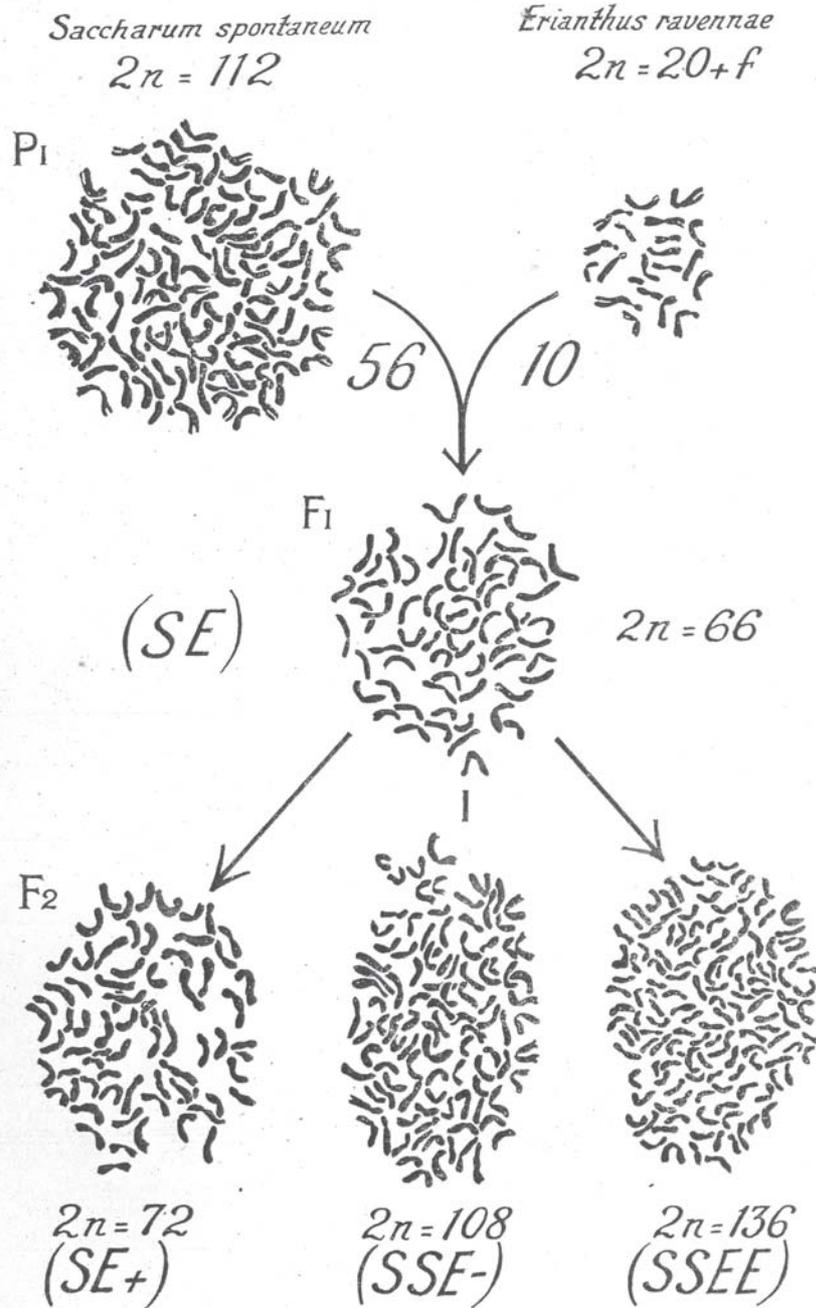


Fig. 5. Somatic metaphase in root tips of *S. spontaneum*, *Erianthus ravennae* and their F_1 and F_2 hybrids. $\times 2000$.

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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. 6a). The number of chiasmata at this stage varies from one to two per bivalent. Fig. 6b represents the 56 bivalents

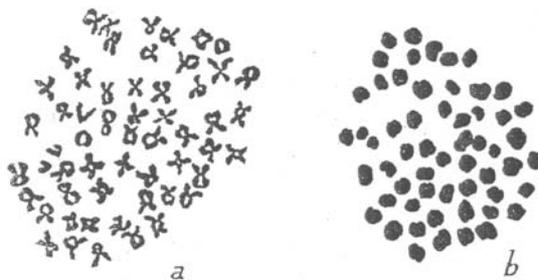


Fig. 6. Chromosome association in *S. spontaneum* “Glagah”. a, diplotene; b, metaphase. $\times 1800$.

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.

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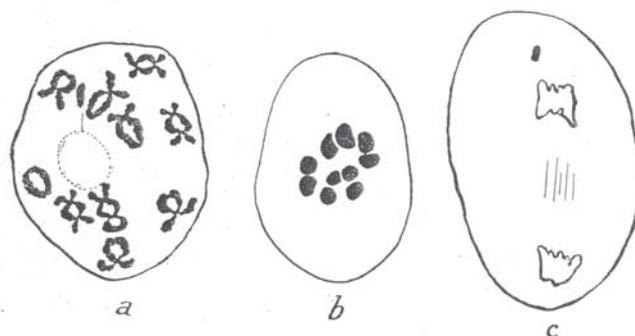


Fig. 7. Meiosis in *Erianthus ravennae*. a, diplotene; b, metaphase; c, telophase, with the fragment excluded. $\times 2000$.

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. 8a). Table 5 gives the configurations noted in ten

Table 5. Degree of association of chromosomes in F_1

Configurations				Cells
IV	III	II	I	
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 (autosomesyndesis) 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

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number of univalents. We might infer from this that the gametic complement of *S. spontaneum* present in the hybrid is capable of forming higher

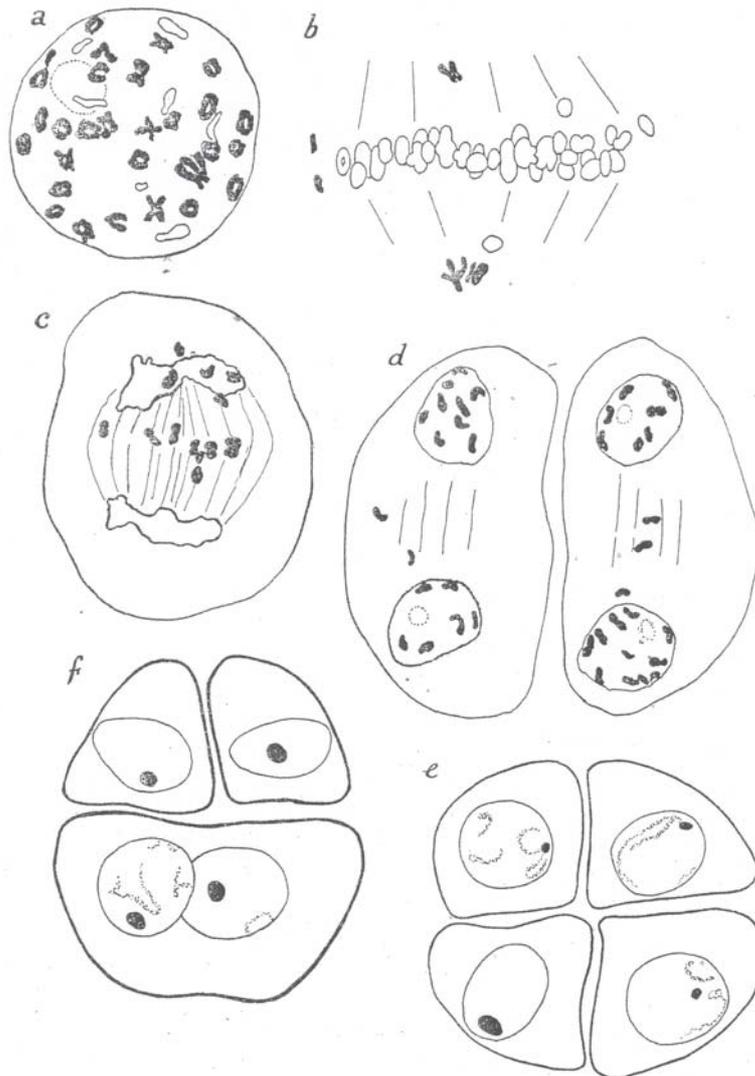


Fig. 8. Meiosis in *Saccharum-Erianthus* hybrid. *a*, diakinesis. *b*, first metaphase. *c*, telophase of first division, showing univalents dividing at the equator. *d*, telophase of second division, showing lagging chromosomes. *e*, normal tetrad. *f*, abnormal tetrad with binucleate dyad. (*a*, *b*,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

configurations by autosome synthesis than it does in the polyploid parent. In this respect the *S. spontaneum* \times *Erianthus* hybrid is similar to the



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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



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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

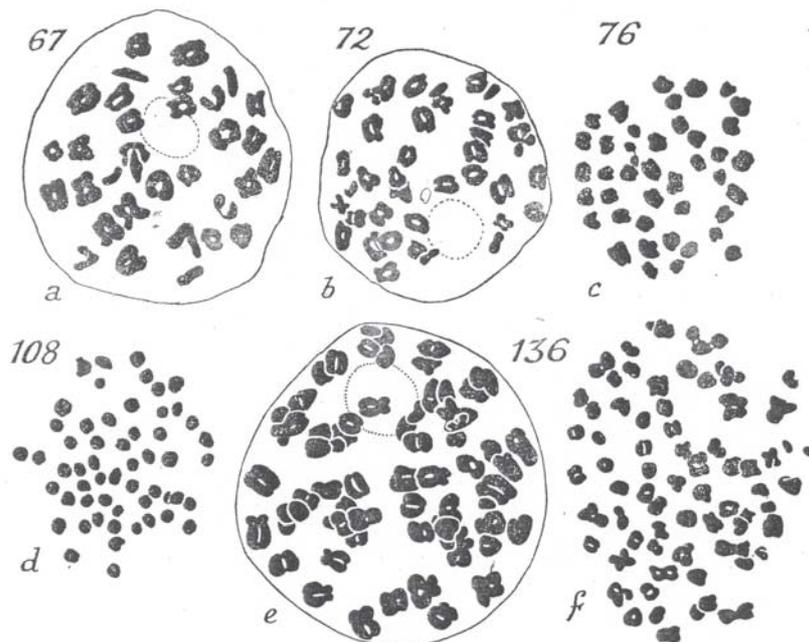


Fig. 9. Association of chromosomes in F_2 seedling. *a, b, c*, "diploids" with 67, 72 and 76 chromosomes. *d*, metaphase in triploid with 108 chromosomes. *e, f*, diakinesis and metaphase in tetraploid, $2n=136$, showing quadrivalents and sexivalents. $\times 1800$.

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

	Sucrose %	Purity %
<i>S. spontaneum</i> "Glagah"	7.93	60.5
<i>S. spontaneum</i> \times <i>Erianthus</i> 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*.



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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.

