CLASSIC PAPERS
E.K. JANAKI AMMAL

HUNDRED YEARS OF EXCELLENCE IN SUGARCANE RESEARCH

SBI 100 YEARS

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Sugarcane Breeding Institute
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CLASSIC PAPERS ON SUGARCANE
E.K. JANAKI AMMAL

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CONTENTS

Dr. E K Janaki Ammal – A Brief Biography

Dr. E K Janaki Ammal, a renowned botanist and plant cytologist who made significant contributions to genetics, evolution, phytogeography and ethnobotany was born in Tellichery, Kerala, in a cultured middle class family on 4th November 1897. Ammal's father was a sub-judge in what was then the Madras Presidency. She had six brothers and five sisters. After schooling in Tellichery, she moved to Madras where she obtained the Bachelor's degree from Queen Mary's College and her Honours degree in Botany from the Presidency College in 1921. She then taught at the Women's Christian College (WCC), Madras (now Chennai), with a sojourn as a Barbour Scholar at the University of Michigan in USA where she obtained her Master's degree in 1925. Returning to India, she continued to teach at the WCC, but went to Michigan again as the first Oriental Barbour Fellow where she obtained her D.Sc in 1931. On her return, she became Professor of Botany at the Maharaja's College of Science, Trivandrum and taught there during 1932–34.

From 1934–1939 she worked as Geneticist at the Sugarcane Breeding Institute at Coimbatore. During that period Sir T S Venkataraman developed the internationally famous Coimbatore canes such as Co 419 with qualities of drought- and disease resistance. The Co varieties were grown in all parts of India and were also preferred for cultivation in other countries where sugarcane was an important crop. It was in this scenario that Ammal quit her teaching position in Trivandrum and joined the Institute at Coimbatore. Ammal's pioneering work at the Institute on the cytogenetics of *Saccharum officinarum* (sugarcane) and interspecific and intergeneric hybrids involving sugarcane and related grass species and genera such as Bambusa (bamboo) is epochal. She made extensive studies on several intergeneric

hybrids notably *Saccharum* x *Zea*, *Saccharum* x *Erianthus*, *Saccharum* x *Imperata* and *Saccharum* x Sorghum.

Ammal then left for England and during 1940–45 and worked as Assistant Cytologist at the John Innes Horticultural Institution in London, and as Cytologist at the Royal Horticultural Society at Wisley during 1945–51. During the years (1939-1950) she spent in England, she did chromosome studies of a wide range of garden plants. Her studies on chromosome numbers and ploidy in many cases threw light on the evolution of species and varieties. The Chromosome Atlas of Cultivated Plants which she wrote jointly with C D Darlington in 1945 was a compilation that incorporated much of her own work on the many species on which she worked. The focus on polyploidy and evolution of plants which effervesced then, continued on her return to India and Ammal worked on some of the most important genera: *Solanum*, *Datura*, Mentha, *Cymbopogon,*and *Dioscorea*, besides a range of medicinal and other plants too many for mention here. Ammal was an original thinker and she attributed the higher rate of plant speciation in the cold and humid northeast Himalayas as compared to the cold and dry northwest Himalayas to polyploidy. Also, according to her, the confluence of Chinese and Malayan elements in the flora of northeast India led to natural hybridization between these and the native flora in this region, contributing further to plant diversification.

Comprehending her innate scientific acumen, the then Prime Minister of India, Jawaharlal Nehru, invited her to accept an assignment as Special Officer to reorganize the Botanical Survey of India (BSI), she returned to India in 1951. From then on, besides the reorganization of the BSI, Ammal continued to be in the service of the Government of India in various capacities including heading the Central Botanical Laboratory at

Allahabad and Officer on Special duty at the Regional Research Laboratory in Jammu and Kashmir. She worked for a brief spell at the Bhabha Atomic Research Centre at Trombay and then settled down in Madras in November 1970, working as an Emeritus Scientist at the Centre for Advanced Study in Botany, University of Madras. Following her retirement, Ammal continued to work unabated, focusing special attention on medicinal plants and ethnobatany and published original findings of her research. In the Centre for Advanced Study Field Laboratory where she lived and worked she developed a garden of medicinal plants with great zeal and dedication. Though cytology was her forte all through, her work embraced genetics, evolution, phytogeography and ethnobotany. She lived and worked in the Centre's Field Laboratory at Maduravoyal near Madras until her demise in February 1984.

Ammal was elected as Fellow of the Indian Academy of Sciences in 1935 (the year it was founded by Sir C V Raman) and of the Indian National Science Academy in 1957. The University of Michigan conferred an honorary LLD on her in 1956. The Government of India conferred the Padma Sri on her in 1957 and the Ministry of Environment and Forestry instituted the National Award of Taxonomy in her name in 2000.

From a young age, she was endowed with the courage to make choices and the versatility to change course and adapt where and when required. With her passion for plants, she defined for herself her goals and purpose, and her mission in life. Having done that, she kept her mission above everything else and stuck to it till the end. Crop plants, garden plants, plantation crops, medicinal plants, plants in the wild and plants of the tribals – all species were interesting to her. She just worked on what was on hand and within reach. And, there was much that was on hand and within reach.

Her familiarity with British plants was matched by her familiarity with tropical species.

She led a simple life of total dedication to her mission, remaining single. Her physical needs were few and she was unostentatious and modest to the core. Ammal was thoroughly Indian in attire and habits, and Gandhian in her lifestyle. She was too selfless to seek favours or the limelight and yet honours came to her unsought, something that is true of many great women and men. The honorary LLD which the University of Michigan conferred on Ammal in 1956 in recognition of her contributions to botany and cytogenetics said: Blessed with the ability to make painstaking and accurate observations, she and her patient endeavours stand as a model for serious and dedicated scientific workers. When required, she did not shirk fighting for a cause or for a right. Her integrity and professional ethics are beyond doubt. She lived up to her own definition of greatness which combined virtue in life and passion in the pursuit of her science. There is thus much for us to emulate in her life and work.

Only Ammal can endow the above 'cradle to cremation' recount with flesh and blood. However, one can look at the course of her life and work in the context of her times, of her nature and upbringing, of challenges and opportunities before her, and of her view of life and work reflected in her own life and work. She will be ever and remembered by the scientific fraternity for her immense contribution to cytogenetics and ethnobotany

Indian Journal of Agricultural Science VI:1105-1106 (1936)

A PRELIMINARY NOTE ON A NEW SACCHARUM × SORGHUM HYBRID

BY

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(Received for publication on 17th June 1936)

(With plates LXII and LXIII)

In sugarcane-breeding it has been the custom to use as female parents canes that are pollen-sterile. Such plants are also generally complex hybrids. Thus in the successful intergeneric hybrids evolved at Coimbatore [Venkatraman and Thomas, 1930] the female parent used, P. O. J. 2725, was a noble cane from Java with a complex heredity. It has further about 106 chromosomes.

With a view to understanding the genetic relationship between the genus *Saccharum* and *Sorghum*, a number of crosses were made in 1934-1935 in which the female parents were simpler forms of *Saccharum*. Amongst such parents employed was the wild species, *Saccharum spontaneum* L. which is being increasingly used in Coimbatore for the breeding of disease and drought-resisting sugarcanes. A number of chromosomal forms are found in this species [Janaki Ammal, 1936] and the hybrid reported is a cross between a form from Dehra Dun ($2n=56$) and *Sorghum durra* (*Periamanjal* of Coimbatore), the same parent used in the intergeneric cross P. O. J. 2725×*Sorghum*. In *Sorghum durra* the chromosome number $2n=20$.

As the *S. spontaneum* used was nearly 100 per cent pollen-fertile, pollination with *Sorghum* was effected after emasculation of spikelets. The arrows were bagged after dusting with *Sorghum* pollen. Thirteen seedlings were produced, all of which are now in field. In appearance they resemble *S. spontaneum* more than *Sorghum* (Plate LXII). The plants arrowed five months after germination, *i.e.* four months before the usual time of flowering in *Saccharum*. This early flowering habit is apparently inherited from *Sorghum*. It was however found to be completely pollen-sterile.

Plate LXIII shows the nature of the spikelet of the parents and hybrid.

S. spontaneum differs from *S. officinarum* in having a rudimentary IV glume. This IV glume, which is also present in *Sorghum*, is awned in this genus. The presence of this awned IV glume in the hybrid as well as the callus hairs inherited from *Saccharum* makes the validity of this new intergeneric cross unquestionable. The number of chromosomes in root tip of the hybrid is thirty-eight which is the sum of the haploid numbers of the Dehra Dun *S. spontaneum* and *Sorghum*. A more detailed study of its cytology will appear elsewhere.

REFERENCES

Janaki Ammal, E. K. (1936). *Ind. J. Agric. Sci.* Vol. 6, 1-8.

Thomas, R. and Venkataraman, T. S. (1930). *A. J. L.*, 25, 2, P. 164.

S. spontaneum (Dehra Dun) × *Sorghum Durra.*

FIG. 1. Spikelets of the intergeneric hybrid *S. spontaneum* (Dehra Dun) × *Sorghum Durra*.

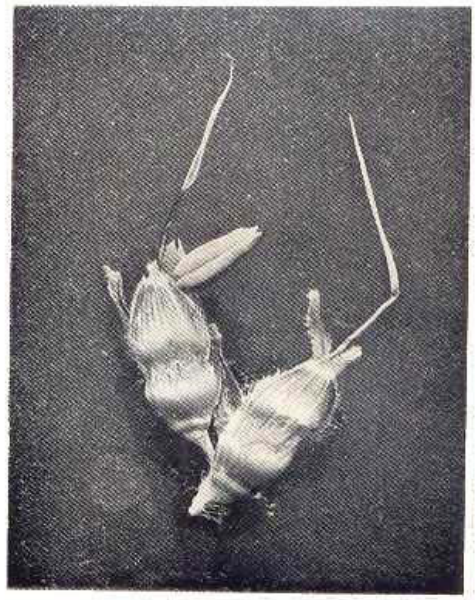


FIG. 2. Spikelets of *Sorghum Durra*.

FIG. 3. Spikelets of *S. spontaneum* (Dehra Dun).

Indian Journal of Agricultural Science VI:1-8 (1936)

ORIGINAL ARTICLES

CYTO-GENETIC ANALYSIS OF *SACCHARUM SPONTANEUM* L.

1.—CHROMOSOME STUDIES IN SOME INDIAN FORMS

BY

E. K. JANAKI-AMMAL,

Sugarcane Geneticist, Imperial Sugarcane Station, Coimbatore

(Received for publication on 15th August 1935)

(With Plates I-V)

CONTENTS

I. INTRODUCTION

The use of *Saccharum spontaneum* L. in sugarcane breeding has been fully justified by the results obtained both in India and Java. Beginning with Co. 205, which is an inter-specific hybrid between the thick cane "Vellai" and the local form of *S. spontaneum*, most of the canes produced at Coimbatore have in them traces of *spontaneum* 'blood'.

S. spontaneum is widely distributed in the Old World. It extends from Africa through Southern Asia to Australia and is represented in India by a number of distinct forms. √Hackel classified the Indian varieties into,

Sub-species (*a*) *Indicum* with very narrow lamina and slender racemes ;
Sub-species (*b*) *Oegyptiacum* with broader lamina and dense racemes.

He mentions the prevalence of "intermediates" between these two sub-species. Hole [1911], while recognizing these different forms, considers them as purely ecological types.

The method of approaching taxonomic problems has changed considerably within the last decade and additional evidence of cytology and genetics is now brought to bear on the purely morphological studies on which species were hitherto differentiated. Our concept of species has, therefore, been very much widened.

In the present study an attempt is made to bring together observations on the chromosome behaviour of some of the Indian forms of *S. spontaneum* in the hope that the cytological facts presented will help to throw light on the phylogeny of this widely-distributed Linnean species.

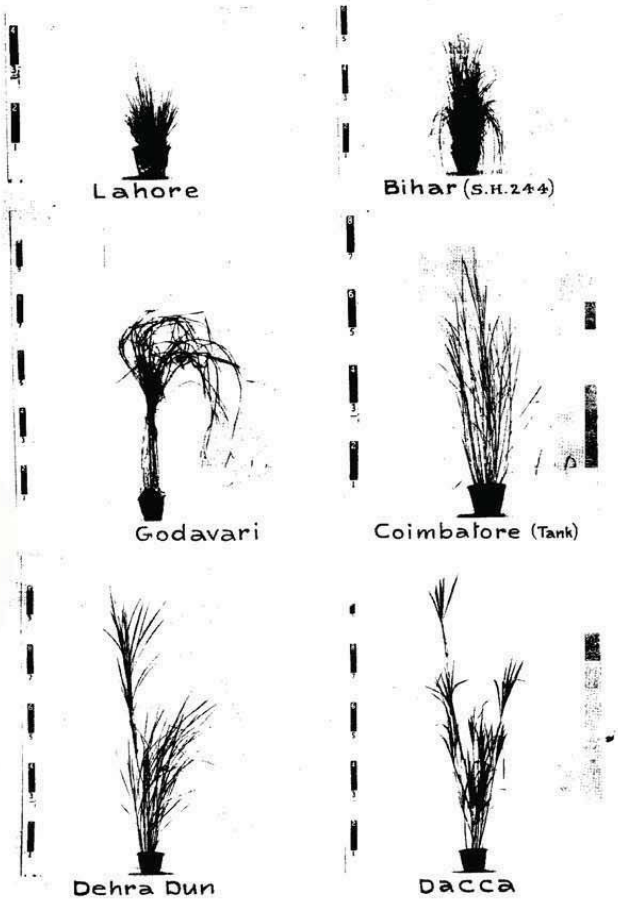
II. Material and methods

The material studied was grown at the Imperial Sugarcane Breeding Station, Coimbatore. The different types are named after the places from which they were collected. The following types were examined :—

Lahore,
Dehra Dun.
Coimbatore (Tank).
Godavari (Rellagaddi).
Dacca.
Bihar (S. H. 244 and S. H. 248).
Cochin.

Morphological descriptions of the first five forms are included in Panje's "Studies in *Saccharum spontaneum*" [1933]. Except in the case of Lahore, which does not arrow at Coimbatore, both root tips and pollen mother-cells were studied. Root tips were fixed in Allen's modification of Bouin's fixative, PFA 15b [McClung, 1929] and in La Cour's 2 BD [La Cour, 1931]. Of these two fixatives, Bouin's was found more satisfactory for the study of metaphase plates. Freezing the root tips for about two to three minutes just before fixing, by surrounding them with crushed ice, had the effect of spacing the chromosomes evenly, and facilitated their counting. This is very important in a genus like *Saccharum* where the chromosomes are many.

Spikelets at the right stage were selected after preliminary examination of anthers in aceto-carmine [Belling, 1923]. They were then fixed in Allen's modification of Bouin's fixative (PFA 15b), and acetic alcohol in the proportion of one part acetic acid to three parts absolute alcohol. Materials fixed in acetic alcohol and preserved in 70 per cent alcohol proved excellent for aceto-carmine smears, even after a period of several months. An exhaust pump was used to hasten penetration.



Some Indian types of *Saccharum spontaneum*.

SOMATIC METAPHASE IN SOME INDIAN TYPES OF *SACCHARUM SPONTANEUM* L.

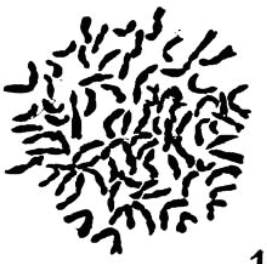


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FIG. 1. Dacca $2n=80$.

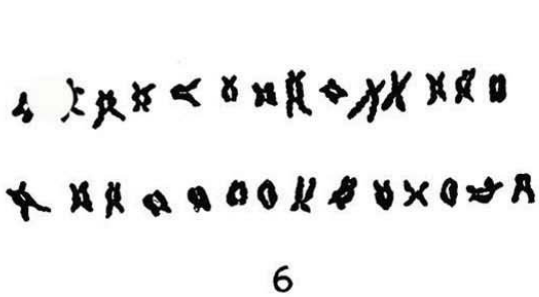
FIG. 2. Bihar $2n=64$.

FIG. 3. Rellagaddi $2n=64$.

FIG. 4. Dehra Dun $2n=56$.

FIG. 5. Lahore $2n=48$.

CHROMOSOME ASSOCIATION IN THE DEHRA DUN AND GODAVARI FORMS



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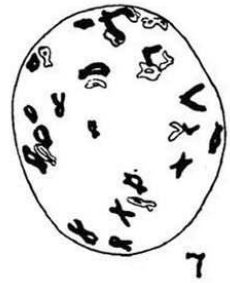

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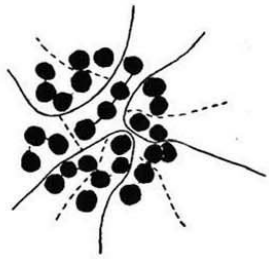

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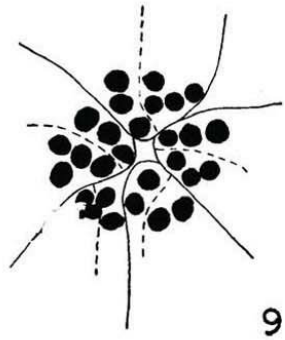

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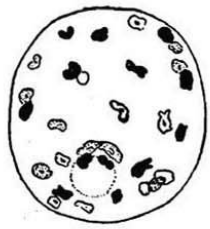

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FIG. 6. Bivalents from one P.M.C. of the Dehra Dun form $n=28$ (aceto-carmine smear).

FIG. 7. Diakinesis in the Dehra Dun form.

FIGS. 8 & 9. Prometaphase & metaphase stages in the Dehra Dun form showing "Secondary association."

FIG. 10. Early anaphase in Rellagaddi ($n=32$).

FIG. 11. Diakinesis in Rellagaddi.

FIG. 12. Prometaphase in Rellagaddi.

Sections were cut, 10μ in the case of root tips and 12 to 15μ in the case of anthers. Iron alum haematoxylin with picric acid as a de-stainer gave clearer differentiation than Iodine gentian violet.

Drawings were made at bench level with a Spencer Abbe camera lucida, a Leitz objective, 1·92 (N.A. 1·32) and a x25 eye piece, to give a magnification of 4000. For the study of aceto-carmine smears a water immersion objective (N. A. 1·2) was used.

III. The chromosome complement in *Saccharum spontaneum*

Cytological studies in *S. spontaneum* were first made by Bremer [1925] who gives the chromosome number of the Java form (Glagah) $n = 56$ and of the Celebes form " Glagah Tabongo " $n = 40$.

Chromosome counts of some of the types examined have already been published. Dutt and Rao [1932] were the first to point out that the Coimbatore form of *S. spontaneum* differs from the Java and the Celebes forms in having sixty-four chromosomes in its somatic cells. Singh [1934] reported thirty-two bivalents in the Godavari form, twenty-seven bivalents in the Dehra Dun and thirty-nine in Dacca. I find the following numbers in the types examined by me :—

	Somatic chromosomes
Lahore	48
Dehra Dun (Holes I)	56
Coimbatore (Tank)	64
Rellagaddi	64
Bihar (S. H. 244)	64
Bihar (S. H. 248)	64
Cochin	64
	80
ling from the Dacca form	80

The above observations suggest that the different types of *Saccharum spontaneum* represent a polyploid series with a basic number eight.

Plate II, figs. 1 to 5 give the somatic metaphase plates of the ' chromosal ' types studied. The chromosomes are found to vary both in size and morphology—at least three types can be distinguished and possibly five in some of the varieties. Both medium and secondary constrictions are found. There is indication of the occurrence of trabants in a few chromosomes of some of the varieties examined.

IV. Pollen mother-cell division

The general facts regarding meiosis are the same for all the types studied and to avoid repetition the cytology of the Dehra Dun form is given in some detail, as the one having the smallest number of chromosomes next to Lahore. This

form from Lahore—which has little or no lamina—has not so far produced any arrows in Coimbatore. It was, therefore, not possible to include in this study, observations on its meiosis.

Early prophase studies have not been attempted. Observations herein recorded have been mainly on the stages from early diakinesis or diplotene to metaphase.

(*a*) *The Dehra Dun form*

At zygotene, single threads are seen lying side by side in various grades of proximity and at diplotene loops are formed with several points of contact which are found to be ' chiasmata ' or exchange of partners between the four chromatids. An examination of early diakinesis shows the fifty-six chromosomes associated into twenty-eight bivalents by means of chiasmata (Plate III, figs. 6 and 7).

Bivalents are of two kinds, some with one chiasmata and others with two. In the case of bivalents with single chiasmata, the chiasma are localized and medium. Terminalization seldom occurs in these. They have the appearance of X's even in late diakinesis. In those with two chiasmata, terminalization is complete in the smaller chromosomes, which have the appearance of O's. Chiasmata observed at late diakinesis are never completely terminalized. Contraction of chromosomes is very intense in diakinesis. This may have been intensified by the reagent used.

With the termination of diakinetic repulsion there is a sudden convergence of the bivalents to the centre of the nucleus. From a study of large numbers of pollen mother-cells at this (pro-metaphase) stage the existence of "secondary association" of bivalents is very clear. The bivalents arrange themselves into ten groups (Plate III, fig. 8). Due partly to their close association and partly as an artefact of fixation there is generally a thin strand of linen connecting the bivalents of a group. These bivalents orient themselves to form the characteristic metaphase plate (Plate III, fig. 9). In a polar view this "secondary association" can be clearly observed. The chromosomes are very much condensed at this stage and look like spherical bodies. Inter-kinesis is short and at the second metaphase, the chromosomes are found to be still associated. Tetrad formation is normal.

(*b*) *The Godavari form* (*Rellagaddi*) 2n=*64*

This is the form of *S. spontaneum* growing on the banks of the Godavari river. Its chromosome number reported by Singh [1934] is $n=32$, which I have also observed (Plate III, fig. 10). Plate III, fig. 11 shows a pollen mother-cell at late diakinesis. Thirty-two bivalents are generally observed, but in a number of nuclei examined at this stage, a single tetravalent could be observed. The number of bivalents with single chiasmata are slightly less than those in the Dehra Dun form. As pairing takes place not between chromosomes but blocks of chromosomes

CHROMOSOME ASSOCIATION IN THE BIHAR AND DACCA FORMS

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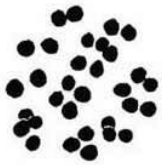

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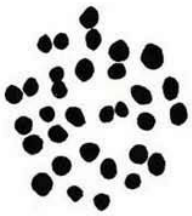

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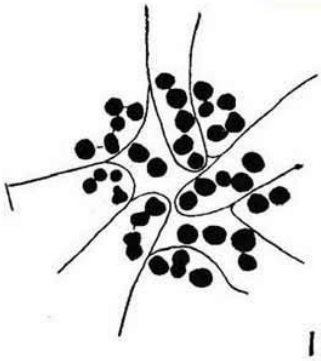

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FIG. 13. P.M.C. at diakinesis stage in the Bihar form showing 28 bivalents (aceto-carmine smear).

FIG. 14. Metaphase in the Bihar form showing chromosome association.

FIG. 15. Metaphase plate in the Coimbatore form.

FIG. 16. Diakinesis in the Dacca form.

FIG. 17. Metaphase in the Dacca form showing chromosome association.

PLATE V.

DIAGRAMATIC REPRESENTATION OF THE DIFFERENT TYPES OF *SACCHARUM SPONTANEUM* IN INDIA

[Darlington, 1932], the increase in the number of chiasmata observed may be an indication of structural changes in the two chromosomes of a bivalent. The presence of a tetravalent is also noteworthy. Plate III, fig. 12 shows a pollen mother-cell at prometaphase stage. The chromosomes associate in groups of two, three and four which seems to be the prevailing type of secondary association in this variety. The later stages of meiosis are normal.

(c) *The Bihar form* (*S. H. 244*), $2x = 64$

This is another form in which there are thirty-two bivalents in the pollen mother-cell (Plate IV, fig. 13). No tetravalents were observed. Secondary association is more lax than in the Dehra Dun form. Ten groups were differentiated in which at least five were associations of two bivalents (Plate IV, fig. 14).

(d) *The Coimbatore form* (*Tank*), $2n = 64$

Thirty-two bivalents are observed at metaphase (Plate IV, fig. 15). Secondary association of bivalents is of frequent occurrence. They are associated in groups similar to the Bihar form (S. H. 244) and about ten groups could be traced. A number of bivalents with single chiasma, which remain unterminalized, was observed at diakinesis giving a configuration like X.

(e) *The Dacca form* $2n = 80$

Plate IV, fig. 16 shows a reconstructed nuclei from two sections of a pollen mother-cell at diakinesis. Variation in length of chromosomes is very marked in this variety. In particular a number of very small chromosomes are found to associate in groups at pro-metaphase. "Secondary association" is very marked (Plate IV, fig. 17) and the groups are composed of two, four and five bivalents. An analysis of the groups shows that in a number of pollen mother-cells the forty bivalents arrange themselves into four groups of five, four groups of four and two groups of two, making in all ten groups.

V. EVIDENCE OF HYBRIDITY WITHIN THE SPECIES

Secondary polyploidy.—It would appear from the chromosome counts of the various clones examined that the species *S. spontaneum* represents a polyploid series. The association of more than two chromosomes at metaphase of meiosis was first observed by Kuwada [1910] in tetraploid *Oryza sativa*. This phenomenon has since been observed in many polyploids. Except for the single tetravalent observed in Rellagaddi, no multivalents have been observed so far in the Indian species. Its absence goes to prove that polyploidy in this species must have occurred through hybridization and not through somatic duplication of chromosome sets, as in auto-polyploids like *Tulipa*.

Besides the formation of multivalents, which is the result of prophase pairing, a secondary or post-synaptic association of bivalents, arising from a general affinity of relatively homologous bivalents, was first observed by Darlington [1926] in Prunus. It is generally observed in plants with small chromosomes. Lawrence [1932] has shown that "secondary association" is of wide occurrence in many species and provides "valuable criterion for homology in hybrid polyploids". We have in *S. spontaneum* strong evidence of this post-synaptic association. The bivalents generally associate into groups of five or ten, which is the basic number of other Andropogonae like *Sorghum*, *Erianthus*, *Imperata* and *S. officinarum*.

In *S. spontaneum* (Dehra Dun) the ~~eighteen~~ twenty-eight bivalents arrange themselves into ten groups as follows :—

Five groups of two bivalents
Two groups of three bivalents
Three groups of four bivalents

There is also an indication of a closer affinity between two of these ten groups so that in some nuclei a more compact association into five groups may be observed. If *A*, *B*, *C*, *D* and *E* represent the basic chromosomes, we can consider the gametes in the Dehra Dun form as being made up approximately of the following chromosome complement :—

1. *A.A.A* + *A.A.A.A.*
2. *B.B.B* + *B.B.B.B.*
3. *C.C* + *C.C.C.C.*
4. *D.D* + *D.D.*
5. *E.E* + *E.E.*

We thus see that the twenty-eight bivalents are composed of two groups of sixteen and twelve bivalents respectively. Since these numbers correspond to the haploid number of the Peninsular forms with thirty-two chromosomes and the Lahore form with forty-eight chromosomes, the Dehra Dun form may be a hybrid between these two chromosome types.

The presence of five pairs of bivalents in the Peninsular form, which stand apart from the rest, seems to indicate that this variety is also a product of hybridization. It is highly probable that the thirty-two chromosome form have arisen as natural crosses between a type like Dacca ($n = 40$) and a type like Lahore ($n = 24$). In its morphological characters the Peninsular form is found to be intermediate between the wire-leafed "Lahore" and the broad-leafed "Dacca". The range of variation found in this group is also very wide which may be an additional evidence for its being considered a hybrid.

Chiasma behaviour.—The chromosomes of *S. spontaneum* examined fall into two types from the point of chiasma behaviour.

Type I with a single chiasma which is localized.

Type II with one or two chiasmata which are formed at random.

The latter have a fairly high frequency of terminalization.

The presence of bivalents with greater frequency of chiasmata in proportion to their length is noteworthy (Plate III, fig. 6). This is in accordance with the "chiasma theory of pairing" of Darlington. If the frequency of pairing is proportional to length, some of the shorter bivalents would be unpaired at metaphase which would then lead to irregularity in germ formation and thus interfere with sexual reproduction of the species. This property may thus be considered as a genetic factor influencing the behaviour of chromosomes [Darlington, 1932].

This differential behaviour in the chiasma formation of the *S. spontaneum* may be taken as another evidence of its hybridity. This will be dealt with more fully in a separate paper.

VI. Conclusions

Barber, in his "Studies of *Saccharum*" [1918], found the broad-leafed form from Dacca to resemble cultivated canes more than any of the other Indian forms examined by him. This form is now found to have the same number of chromosomes as *Saccharum officinarum*. The discovery of other types with $n = 40$ chromosomes, like *S. robustum* from New Guinea and Glagah Tabongo, from Celebes points to this form having a wide distribution in the tropics. From a study of its morphological characters, the Dacca form comes nearest to Hackel's subspecies, *ægyptiacum*.

It seems that the wire-leafed form from Lahore with $2n = 48$ represents a form of *Saccharum spontaneum* having a basic number six or twelve instead of the usual five or ten found in other Andropogonae. There is strong indication that the forms with thirty-two and twenty-eight chromosomes, which represent the "intermediate" forms of Hackel, are natural hybrids or their back-crosses, between the two types ($n = 40$) and ($n = 24$). These intermediate forms, because of their wide distribution, rightly represent the sub-species '*indicum*', variety "*genuinum*" of Hackel. They show great variation in vegetative characters and are best suited for the study of the differentiation of the species from an ecological point of view. The wire-leafed form, which Hackel describes as subspecies *indicum*, variety *juncifolium*, deserves to be classed as a separate species perhaps "*Saccharum juncifolium*", because of its distinctive morphological characters and its unique chromosome complement.

It is highly probable that the more primitive types of *S. spontaneum* are those with lesser number of chromosomes. These studies on Indian forms

indicate that the primitive forms occur in India. It is likely that a more comprehensive study of the species will lead to the discovery of more "ecotypes" with chromosome number forty-eight and fifty-six than those hitherto examined.

VII. Summary

The Indian types of *S. spontaneum* form a polyploid series with the following chromosome numbers :—

$2n = 48$
$2n = 56$
$2n = 64$
$2n = 80$

2. The somatic chromosomes show primary and secondary constrictions. They also vary in length.

3. Pairing of chromosomes at meiosis is by means of chiasmata. Chromosomes are always associated as bivalents except in Rellagaddi in which a single tetravalent was observed.

4. The number of chiasmata observed is not always proportional to length of chromosomes.

5. "Secondary association" of bivalents is observed. The chromosomes arrange themselves into five or ten groups which is the basic number of the Andropogonae.

6. There is evidence of hybridity within the species. Of the Indian forms examined, the type with forty-eight chromosomes represents Hackel's variety "*juncifolium*" and the type with eighty the variety "*ægyptiacum*". Those with intermediate numbers seem to be natural hybrids between these two forms.

REFERENCES

Barber, C. A. (1918). *Mem. Dept. Agric. Ind., Bot. Ser.* **9** No. 4, 140.
Belling, J. (1923). *Amer. Nat.* **57**, 92-96.
Bremer, G. (1925). *Genetica* **7**, 293.
Darlington, C. D. (1928). *J. Gen.* **18**, 249.
———————— (1932). 'Recent Advances in Cytology'.
Dutt, N. L. and Subba Rao, K. S. (1933). *Ind. J. Agric. Sci.* **3**, 41-42.
Hole, R. S. (1911). *For. Bot. Ser.* **1**, 1-126.
Kuwada, Y. (1910). *Bot. Mag., Tokyo* **24**, 267-281.
La Cour, L. (1931). *J. Roy. Micr. Sci.* **51**, 119-126.
Lawrence, W. J. C. (1931). *Cytologia* **2**, 252-384.
McClung, C. E. (1929). 'Hand book of microscopical technique', 424.
Panje, Rama Rao (1933). *Ind. J. Agric. Sci.* **3**, 1017.
Singh, T. S. N. (1934). *Ind. J. Agric. Sci.* **4**, 290-94.

Indian Journal of Agricultural Science VI:9-10 (1936)

CYTO-GENETIC ANALYSIS OF *SACCHARUM SPONTANEUM* L.

2.—A TYPE FROM BURMA

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(Received for publication on 15th August 1935)

(With Plates VI-VIII)

According to Barber [1918] *Saccharum spontaneum* in Burma represents a series of forms ranging from a thick "Land form" to an aquatic type like the Dacca form of India. The only Burma variety of *S. spontaneum* in the collection at the Imperial Sugarcane Station is from a clone from Mandalay collected in 1929. This variety is the most outstanding of the *S. spontaneums* grown at Coimbatore. It attains a height of fifteen to eighteen feet, propagates very profusely by means of underground stems and has a splendid erect habit (Plate VI). The stem has a much longer internode than any of the other *S. spontaneums*. Panje [1932] notes a number of other morphological characters in which this form differs from the Indian and the East Indies types studied by him. The plant flowers very rarely in Coimbatore and only a single inflorescence was observed in 1933.

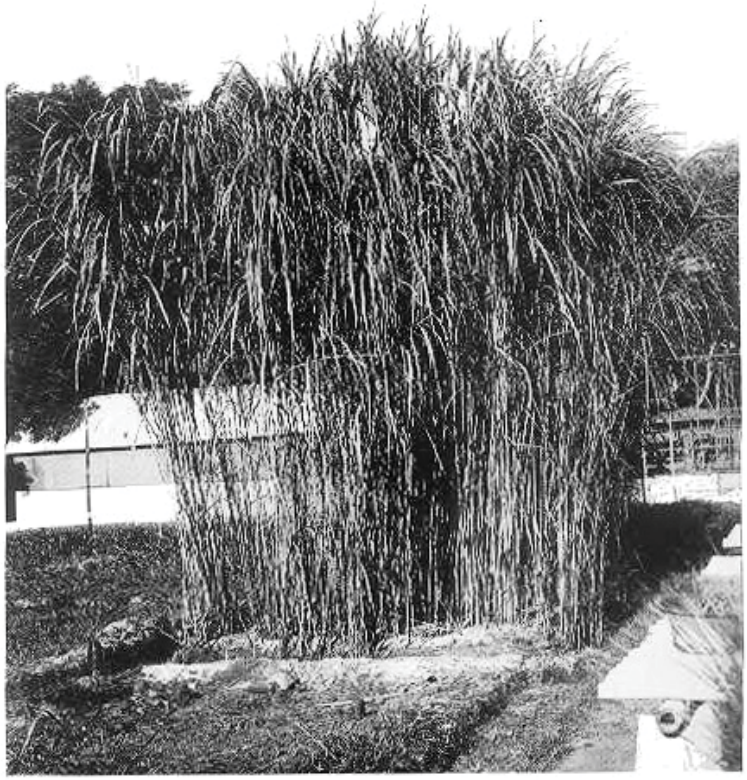
Cytological observation.—As the plant did not arrow in 1934-35, only root tips were available. These were fixed in Allen's modification of Bouin's PFA-15b after freezing, sections cut at 10μ and stained with Iron Alum haematoxylin with picric acid as de-stainer. The metaphase plate of somatic division showed ninety-six chromosomes (Plate VII, fig. 1). This is the first record of this number for any type of *S. spontaneum* studied. The chromosomes appeared similar to those of the Indian *S. spontaneum* studied, in having both primary and secondary constrictions. The prevalence in India of forms of *S. spontaneum* with chromosome $2n=64$ [Dutt and Rao, 1933 and Singh, 1934] and the occurrence in Sumatra of a "gigas" form with $2n=124$ [Singh, 1934] points to the Burma form being a triploid with basic chromosome number $x=32$.

Triploids occur in nature either as the result of a cross between diploids and tetraploids of the same species or as the result of the failure of reduction in the archesporium of one of two diploid parents and the consequent fusion of an x with an unreduced $2x$ germ cell. According to Bremer [1929] doubling of chromosomes in the female parent is a common phenomenon in *Saccharum*. The regionally intermediate Burma form might be either a natural cross between an Indian form with $n=32$ and a tetraploid form like the " gigas " form of Sumatra (Plate VIII) or it may represent the product of fertilization of an abnormal diploid gamete with a haploid one as noted by Bremer in many other *Saccharum* species. Cytological evidence from pollen mother-cell studies is necessary to throw further light on the origin of this interesting Burma form. Its possible position amongst other *S. spontaneum* is shown in the diagram.

Progeney of the Burma form.—The single arrow produced in 1933—and left unbagged—yielded a number of seedlings which showed marked differences in morphological characters. Many of these were thicker than the parent and looked like very medium sugarcanes. Root tips of some of these were examined and they showed 96 to 124 chromosomes at somatic metaphase (Plate VII, figs. 2-6). As the arrow was unbagged, and consequent possibility of cross-pollination, much value cannot be placed on the variation observed in their chromosome numbers. However the numbers found fall within the expected frequency of chromosome distribution in a triploid with $2n=96$.

REFERENCES

Bremer, G. (1929). *Facts about Sugar* **24**, 926.
Dutt, N. L. and Subba Rao, K. S. (1933). *Ind. J. Agric. Sci.* **3**, 41-42.
Panje, Rama Rao (1933). *Ind. J. Agric. Sci.* **3**, 1017.
Singh, T. S. N. (1934). *Ind. J. Agric. Sci.* **4**, 292.



Burma variety of *Saccharum spontaneum*.

CHROMOSOME NUMBERS IN A BURMA *SACCHARUM SPONTANEUM* L. & ITS SEEDLINGS

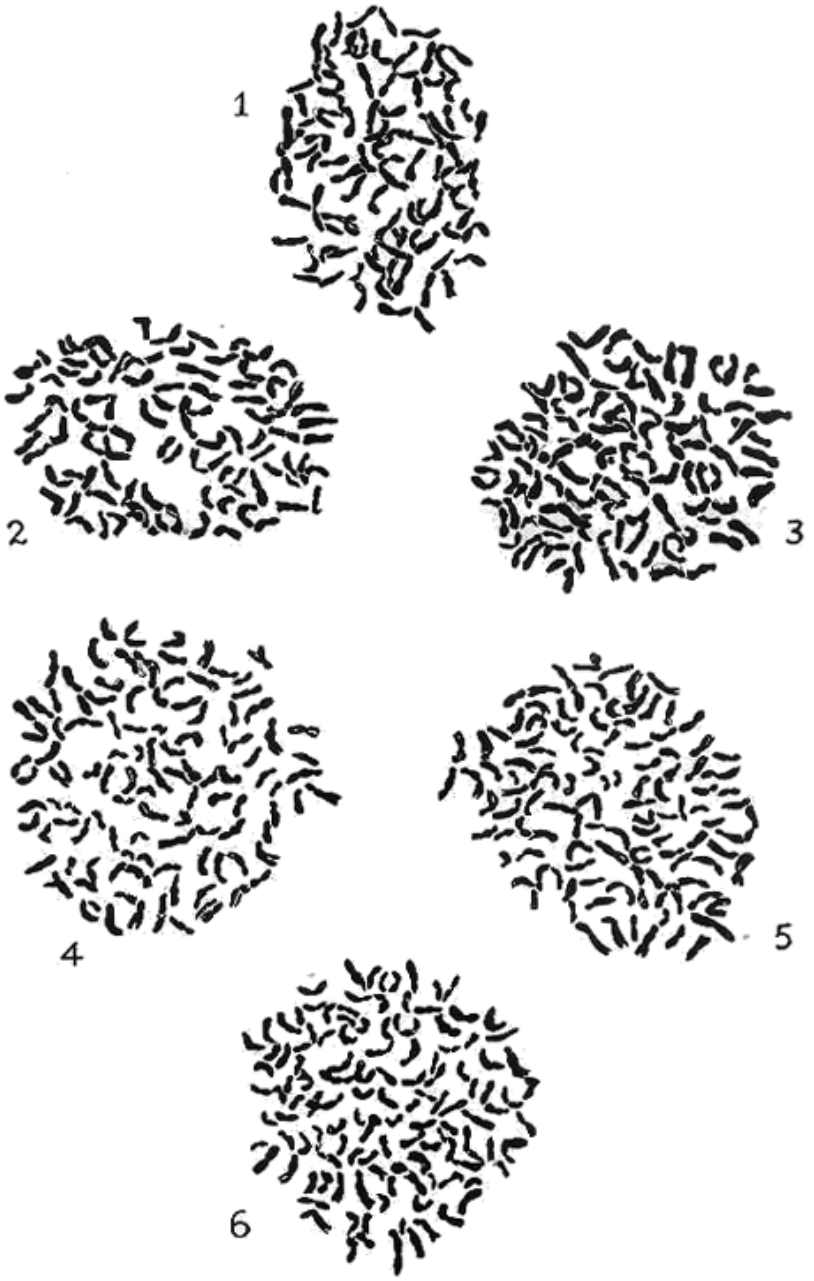


FIG. 1. Somatic metaphase from the root tip of the parent Burma *S. spontaneum* $2n=96$.

FIGS. 2–6. Somatic metaphases from the root tip of some seedlings of the Burma form. $2n=96$; $2n=108$–110; $2n=112$–114; $2n=114$; $2n=124$; respectively.

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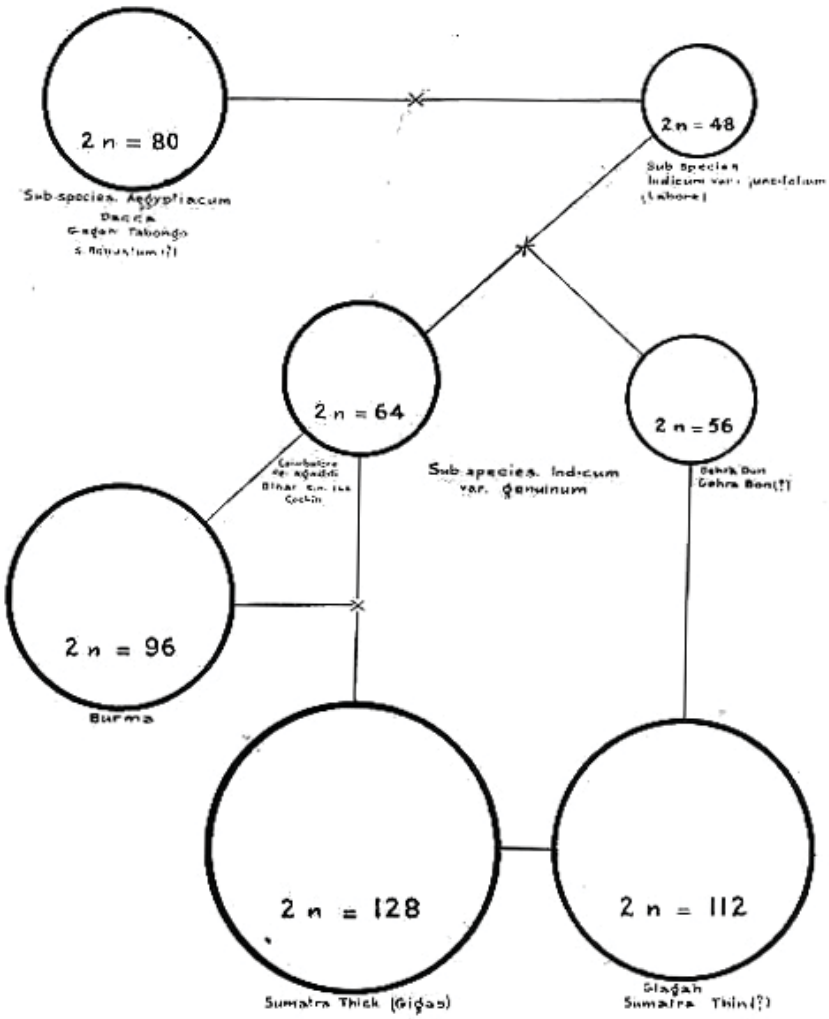
DIAGRAMATIC REPRESENTATION OF THE DIFFERENT TYPES OF *SACCHARUM SPONTANEUM*



smaller molecule than that in the deproteinated serum.

To the discussion of the view of Waldschmidt-Leitz that the polarographically active substance in carcinomatic deproteinated serum may be a sulphur-free mucoid[4], I would point out that this assumption is not in accord with my experimental facts; the hydrolysates of the deproteinated serum solutions in question show clearly the presence of cystine and, moreover, in the same relative content as found in the various non-hydrolysed deproteinated pathological or normal sera; the cystine content in the deproteinated serum is of the order of 10^{-4} molar, and is always higher in the carcinomatic case; against the necessity for a mucoid theory is also the fact that an identical polarographic effect is evoked by a deproteinated solution of pure crystalline albumin, if the albumin is first degradated with the alkali or pepsin[5].

The experimental evidence thus shows convincingly that the changes in pathological sera polarographically detected consist in a proteolytic degradation of serum proteins by which cystine containing high molecular products, bearing the character of albumose, are split off. The origin of this proteolysis taking place in the blood must be sought in the increase of some products of the pathological metabolism, of the type of Abderhalden's proteolytic reactions.

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[1] Brdička, R., *Acta Unio Internationalis contra Cancrum*, **3**, 13 (1938).
[2] Bergh, F., Henriques, O. M., and Wolffbrandt, C. G., NATURE, **142**, 212 (1938).
[3] Rosenthal, H. G., *Mikrochemie*, **22**, 233 (1937).
[4] Waldschmidt-Leitz, E., *Angew. Chem.*, **51**, 324 (1938).
[5] Brdička, R., to be published elsewhere.

Photo-ammonification of Organic Nitrogenous Compounds in the Soil

IN recent years Gopala Rao and Dhar[1], Gopala Rao[2], Dhar and co-workers[3] and Corbet[4] have shown that nitrification in soils occurs partly as a photochemical reaction under the influence of sunlight. Dhar and co-workers[5] have also brought forward considerable evidence to show that fixation of atmospheric nitrogen is favoured by sunlight.

We have now found that the decomposition of various nitrogenous compounds, the so-called ammonification, occurs as a purely photochemical reaction in the presence of photocatalysts like heated soil or ignited ferric oxide. Aqueous solutions of various nitrogenous compounds were exposed to sunlight (for 30 hours) in 'Pyrex' glass flasks under sterile conditions. The amount of ammonia liberated in the decomposition process is estimated by Folin's method. The results are as given below.

	Milligrams of ammoniacal nitrogen per litre	
	Ferric oxide as photocatalyst	Heated red soil as photocatalyst
M/20 glycine ..	43·75	13·85
,, alanine ..	61·25	14·00
,, aspartic acid	65·65	17·30
,, glutamic acid	8·75	7·00
,, urea ..	28·00	12·72

It thus appears that many important chemical reactions in the soil can be brought about by the photochemical action of sunlight, independently of bacteria.

Further work is in progress.

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[1] *Soil Science*, **31**, 379 (1931).
[2] *Soil Science*, **38**, 143 (1934).
[3] NATURE, **133**, 213 (1934); **137**, 462 (1936).
[4] *Biochem. J.*, **28**, 1575 (1934); **29**, 1086 (1935).
[5] NATURE, **137**, 629, 1000 (1936); **133**, 648, 1060 (1936).

Effect of Pyridine Compounds on the Nutrition of *Staphylococcus aureus*

RECENT investigations have established the necessity of nicotinic acid (or amide) for the growth of *Staphylococcus aureus*[1]. In a previous report, it was shown that the ability of this organism to utilize compounds related to nicotinic acid is limited. We have since prepared several compounds of interest in this connexion, and the determination of their biological activity is herewith reported.

The synthetic amino acid - glucose medium of Fildes *et al.*[2] was employed in testing the activity of the series of compounds. The compounds were tested in the presence of an excess of thiamine (0·05 gamma per 10 c.c. of medium) using an 18-hour culture of *S. aureus*.

Nicotinyl glycine exhibited growth-promoting activity in the same order of concentration as nicotinic acid. Trigonelline, pyridine betaine β-carboxylic acid, α-amino pyridine, and α-amino pyridine β-carboxylic acid, were completely inactive as growth factors for *S. aureus*. It may be of interest to recall that Ackermann[3] isolated nicotinyl glycine and trigonelline from urine following the administration of nicotinic acid.

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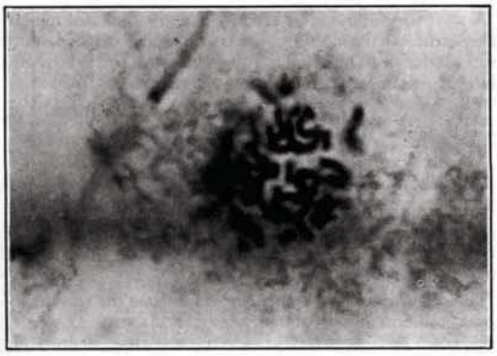
[1] Knight, B. C. J. G., *Biochem. J.*, **31**, 731 and 966 (1937). Koser, S. A., Finkle, R. D., Dorfman, A., and Saunders, F., *J. Bact.*, **35**, 6 (1938). Landy, M., *Proc. Soc. Exp. Biol. Med.*, **38**, 504 (1938).
[2] Fildes, P., Richardson, G. M., Knight, B. C. J. G., and Gladstone, G. P., *Brit. J. Exp. Path.*, **17**, 481 (1936).
[3] Ackermann, D., *Z. Biol.*, **59**, 17 (1912).

A *Saccharum* - *Zea* Cross

BOTH *Saccharum* and *Zea* are distinguished by the readiness with which they cross with related genera. For example, while Mangelsdorf and Reeves[1] have crossed *Zea Mays* with *Euchlæna* and *Tripsacum*, Venkatraman and Thomas[2] have crossed *S. officinarum* with a species of *Sorghum* and even the remotely related *Bambusa*[3]. I have also crossed *S. officinarum* with *Imperata Cylindrica* Beaew. and *S. spontaneum* L. with *Sorghum Durra* and *Sorghum halepense*. In spite of *Zea* and *Saccharum* being in two different sections of the Gramineæ—Andropogoneae and Maydeae (Bews)—I thought it worth while to cross them, and after several attempts using many thousands of flowers of a male sterile variety (Vellai) of *S. officinarum* $2n = 80 = 8x$ as the female parent, and variety

Golden Beauty of *Zea Mays* $2n = 20$, $2B$ as the male parent, I obtained a single seedling. This plant has received the expected 40 chromosomes from the *Saccharum* parent and 12 chromosomes from the male parent *Zea*. Amongst these the VI nucleolar chromosome of *Zea Mays* is recognizable.



PHOTOMICROGRAPH OF THE CHROMOSOME COMPLEX OF THE HYBRID BETWEEN *S. officinarum* (VELLAI) AND *Zea Mays* (GOLDEN BEAUTY) SHOWING THE SINGLE VI NUCLEOLAR CHROMOSOME AND THE *B* CHROMOSOMES RECEIVED FROM THE MAIZE PARENT.

The hybrid resembles the *Saccharum* parent more closely as we should expect from these chromosome contributions, but it has the characteristic epidermal hair found on the upper side of the leaf in *Zea Mays* and related genera. The cross, however, is dwarf in habit and although it has tillered freely, has not produced flowering canes after twenty-two months. It lacks the vigour and early maturity found in *Saccharum* - *Sorghum* hybrids.

This cannot be due simply to the remoteness of the cross since the *Saccharum-Bambusa* hybrids are very vigorous. It must rather be due to the inequality of the contribution of the polyploid and diploid parents. The same consideration is likely to vitiate the fertility of the hybrid. The occurrence of these remote crosses in experiments indicates that the degree of anastomosis in the ancestry of polyploid species may be much greater than is commonly suspected.

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[1] Mangelsdorf, P. C., and Reeves, R. G., "Hybridization of Maize, *Tripsacum* and *Euchlæna*", *J. Hered.*, **22**, 327–343 (1932).
[2] Thomas, R., and Venkatraman, T. S., "Sugarcane—Sorghum Hybrids, *Agri. J. India*, **25**, 164 (1930).
[3] Venkatraman, T. S., "Sugarcane—Bamboo Hybrids", *Ind. J., Agri. Sci.*, **7**, Pt. III, 513 (1937).

Vowel Vibrations and Vowel Production

THE dark band in Fig. 1 is the reproduction of the speech track of a vowel (*a* in *hatch*) on a sound film. The serrated upper edge is the registration of the vibratory movement of the particles of air, that is, it is the curve of vibration. It is seen to consist of a series of portions—vibratory 'bits'—each of which begins strong and fades away to zero. Such a curve is the registration of a free vibration aroused by an impulse that is not a vibration. The glottal action consists of the repeated opening and shutting of the glottal slit. A puff of air is sent into the vocal cavity at each opening movement; each puff sets the air in the cavity into vibration.

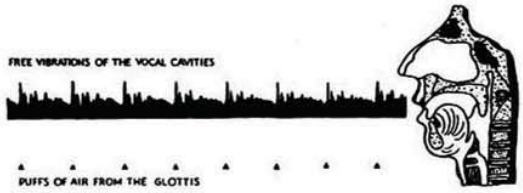

Fig. 1.

The 'profiles' in the vibratory bits are different for the different vowels (Fig. 2). The vocal cavity, therefore, has a different form in each case. The progressive change in the profiles of a vowel indicates that the vocal cavity changes its form constantly.

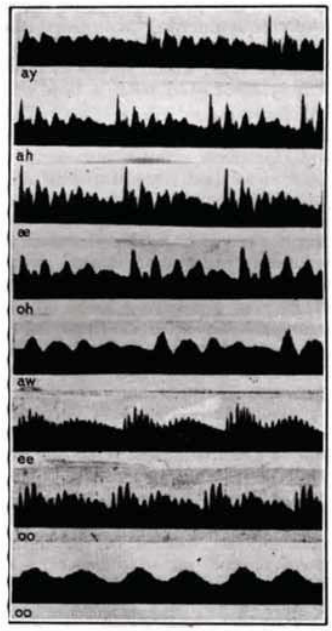

Fig. 2.

Every element in a vibratory bit has its characteristic rate of fading (logarithmic decrement). This is always large and never zero. Forced—or resonance—vibrations do not fade; their logarithmic decrement is zero. The vowel vibrations are, as the tracks show, not forced vibrations; they cannot have been produced by resonance.

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Chromosome Numbers in Sugarcane × Bamboo Hybrids

THE sugarcane × bamboo hybrids recently produced at this station (Venkatraman, 1937), provide material for the study of the phylogenetic relationship of the genus *Saccharum* with other grasses. The gap covered by this cross is considerably wider than in the case of the *Saccharum* × *Sorghum* hybrids[1,2], or the *Saccharum* × *Erianthus* hybrids effected in Java[3].

The female parents employed were the two Java canes: *P.O.J.* 213—an interspecific hybrid between *S. officinarum* (Black Cheribon, $2n = 80$) and *S. barberi* (Chunnee, $2n = 82$); and *P.O.J.* 2725—a rather complicated hybrid cane between a number of 'noble' types and the *S. spontaneum* (*Glagah*) of Java. The male parent was *Bambusa arundinacea* Willd., a species which is common in South India.

Root tips of *P.O.J.* 213 showed 124 chromosomes at metaphase, somatic pairing of homologous chromosomes being very apparent. Bremer has reported 62 bivalents at the reduction division of pollen mother cell of this plant. He gives $2n = 106$–107 as the chromosome number of *P.O.J.* 2725[4]. Reduction division is somewhat irregular in this cane, and like a number of other hybrid sugarcanes, it has been known to produce both diploid and haploid gametes. Pollen sterility is very high in both the canes. As the ovules, however, are fertile, these canes have been used a great deal as female parents in the breeding programme at Coimbatore. 72 chromosomes were counted in the root tips of *Bambusa arundinacea*. Root tips of five of the hybrids between *P.O.J.* 213 and *Bambusa arundinacea* showed 96–100 chromosomes and one plant of the cross *P.O.J* 2725 × *Bambusa arundinacea* examined had 90 chromosome. These numbers represent approximately the sum of the haploid numbers of the two parents.

All the *Saccharum* × bamboo hybrids so far examined differ from the *Saccharum* × *Sorghum* hybrids in the fact that whereas the chromosome numbers of the latter indicate that viable embryos are formed from fertilization of both haploid and diploid gamates[5], in the former only those derived from fertilization of haploid gametes were found to be viable. They also differ from the *Saccharum* × *Imperata* hybrids (Janaki Ammal, unpublished), where viability is limited to such embryos as are derived from the fertilization of diploid gametes only.

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[1] Thomas, R., and Venkatraman, T. S., "Sugarcane × Sorghum Hybrids", *Agri. J. Ind.*, **25**, 164 (1930); Venkatraman, T. S., "Sugarcane × Bamboo Hybrids", *Ind. J. Agri. Sci.*, **7** (1937).

[2] Janaki Ammal, E. K., and Singh, T. S. N., "A Preliminary Note on a New Saccharum × Sorghum Hybrid", *Ind. J. Agric. Sci.*, **6**, 1105–1106 (1936).

[3] Rumke, jun., C. L., "Saccharum-Erianthus Bastaarden", *Archief voor de Suikerindustrie in Nederlandsche-Indie*, Deel II (1934). Mededeelingen van het Proefstation voor de Java suikerindustrie, Jaargang 1934, No. 7.

[4] Bremer, "Remarks on the Cytology of Saccharum", *Facts about Sugar*, **24**, 926–7 (1929).

[5] Singh, T. S. N., "Chromosome Numbers in Sugarcane × Sorghum Hybrids", *Ind. J. Agri. Sci.*, **4**, 1050 (1934).

Pedocalic Tendencies in Soils of Southern England

THE late Dr. C. F. Marbut proposed the division of the soils of the world into two primary groups, namely, pedocals and pedalfers. Pedocals are soils developed under climates which are too dry to maintain a continuous downward movement of water to the water table. They therefore contain a horizon of accumulation of calcium carbonate (and sometimes other salts also) in the soil profile, usually in the *B* horizon. Pedalfers are soils developed under more humid climates. They are completely leached and contain no horizon of calcium carbonate accumulation. Thus the steppe and desert soils of the semi-arid and arid regions are distinguished from the podsolic and lateritic soils of the humid temperate and humid tropical climates. It has been generally accepted that the climate of Great Britain is too wet for pedocals to develop. Under free drainage conditions, therefore, horizons of secondary calcium carbonate accumulation should not occur.

Observations on the brown forest soils of Rumania, developed from calcareous loess, were made by me, through the courtesy of Dr. Cernescu of Bucharest, in the spring of 1937. These showed calcium carbonate concretions at the base of the *B* horizon, although brown forest soils should belong to the pedalfer group. This anomalous position led me to make detailed observations of the soils in Berkshire developed from porous calcareous parent materials, since it was thought that the deposition of secondary calcium carbonate might be a function of the calcareous nature of the parent material.

Consequently, pits were dug on the Malmstone (Upper Greensand) and Calcareous Grit formations. The Malmstone soil, developed under mixed broad-leafed woodland and free drainage conditions, revealed small soft calcium carbonate concretions at a depth of 3 ft. 9 in. at the base of the *B* horizon and in the top of the *C* horizon. The soil on the Calcareous Grit, developed under very free drainage conditions, under a stand of Scots pine at least a hundred years old, revealed secondary deposition of calcium carbonate on the upper surface and in the cracks of bands of shattered rock. These occurred at a depth of 15 in. and again at 37 in.

Analytical data showed that the Malmstone profile was weakly podsolized, since some alumina had been lost from the surface horizons. Iron oxide was apparently stable, although exceptionally low in amount. The Calcareous Grit profile showed lateritic tendencies, since there was considerable loss of silica from the soil horizons. The silica-sesquioxide ratio is about 2·0 in the soil horizons and about 3·0 in the parent material. It is suggested in this connexion that all red-brown soils developed from calcareous parent material may show loss of silica from the soil horizons.

Observations on shallow downland soils near Wantage, developed from Upper Chalk, revealed secondary deposition of calcium carbonate at a depth of 6–8 in., the horizon being about 3 in. deep. The profile was calcareous to the surface, high in organic matter, with a good crumb structure, and showing the typical profile of an English rendzina. Chalk rock fragments occurred throughout the profile, but whereas these are normally humus-stained and therefore dark brown in colour in the soil, the secondary calcium carbonate, which was structureless, showed up as a white layer. This layer was very intermittent, frequently entirely absent, and in places only represented by isolated spots. I consider, however, that this profile strongly resembles the Continental *tshernosem* (steppe soil). Its shallowness is, of course, the result of erosion. In some localities the surface horizon may be completely leached of calcium carbonate and may be slightly acid in reaction, but it does not lose its steppe-like character.

refractive index, etc., and $(1-x)$ and (x) the mol. fractions of the solvent and solute respectively. When P_{solute} is plotted against concentration a remarkable similarity is observed in the behaviour of all the curves (Figs. 1 and 2), which show breaks at definite points. *viz.*,

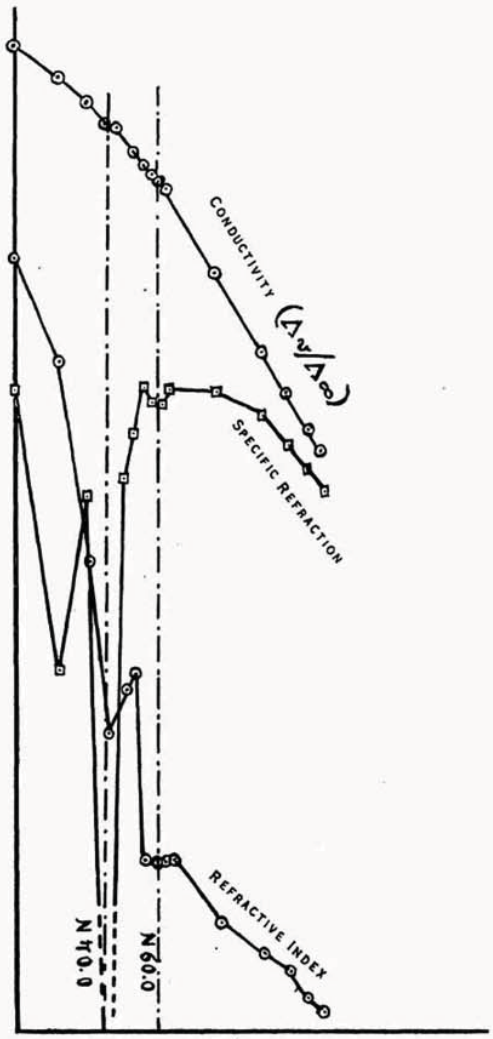

FIG. 2

0·1N. and 0·04N, indicating thereby that the phenomenon observed is a genuine one.

Evidences from Raman spectra[8] have also been obtained, which rule out the possible explanation of the formation of hydrates.

The following reactions are suggested to explain the various observations made:—

$$\underset{0\cdot10N}{\tfrac{1}{3}(HIO_3)_3} \rightleftharpoons \underset{0\cdot04N}{\tfrac{1}{2}(HIO_3)_2} \rightleftharpoons HIO_3 \rightleftharpoons H^{\cdot} + IO_3'$$

The detailed paper will be published in *Zeitschrift fur anorganische und allgemeine Chemie.*

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[1] Thomson, J., *Ber.*, 1874, **7**, 112.

[2] Ostwald, W., *J. prakt. Chem.*, 1885, **32** (2), 310.

[3] Walden, P., *Z. phys. Chem.*, 1888, **2**, 64.

[4] Rosenheim and Liebknecht, *Lieb. Ann.*, 1899, **308**, 40.

[5] Groschuff, E., *Z. anorg. Chem.*, 1905, **47**, 331.

[6] Dhar and coworkers, *J. Ind. Chem. Soc.*, 1928, **5**, 585; 1929, **6**, 784; 1932, **9**, 322.

[7] Nayar, M. R., and Gairola, T. R., *Z. anorg. Chem.*, 1934, **220**, 163–68.

[8] Nayar, M. R., and Sharma, P. N., *ibid.*, pp. 169–72; Venkateswaran, C. S., *Proc. Ind. Acad. Sci.*, 1935, **2**, 119; Shen, Ya and Wu, *Phys. Rev.*, 1937, **51**, 235.

Triplo-Polyploidy in *Saccharum spontaneum* L.

AN examination of a large number of clones of *Saccharum spontaneum* collected from various parts of India, Burma, and the East Indies have shown that this species includes a polyploid series with $2n = 48$, $2n = 56$, $2n = 64$, $2n = 72$, and $2n = 80$ in India and $2n = 80$, $2n = 96$, $2n = 112$ in further India and the East Indies. One would be led to conclude from these chromosome numbers that 8 is the basic number in this species and forms with $2n = 56$, $2n = 64$ and $2n = 72$ are $7x$, $8x$ and $9x$ forms respectively. The presence of an odd set of chromosomes in the $7x$ and $9x$ forms would naturally lead one to expect univalents or multivalents at meiosis in these types. A study of the chromosome behaviour of a number of clones collected in India have shown, however, that 28, 32 and 36 bivalents are formed regularly at meiosis in these types. Consideration of the above has led me to conclude

that these forms of the species are dibasic, having arisen by hybridization of a form with ($x = 10$) and one with ($x = 6$).

Of these two types $2n = 80$, $8(x = 10)$ which is a wide-leafed form (variety *aegyptiacum* of Hackel) is found distributed in the more tropical parts of South-Eastern Asia and East Indies, while the $2n = 48$, $8(x = 6)$ which is a thin-leafed form (variety *juncifolium* of Hackel, is confined to the subtropical region of North-Western Asia and Southern Russia. The form very widely distributed in Peninsular India is one with $2n = 64$, which I have considered as a natural hybrid between the $2n = 48$ and $2n = 80$ types (Janaki Ammal, 1936).[1] Its chromosome complement can be represented as $4(x = 6) + 4(x = 10)$.

Plants with 56 and 72 chromosomes have a more restricted distribution being found in mixed populations of the $2n = 64$ chromosome type and the two primary forms (varieties *juncifolium* and *aegyptiacum*) with 48 and 80 chromosomes respectively. I have also been

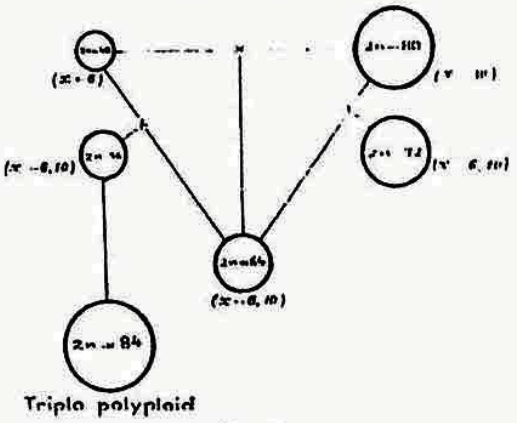

FIG. 1

Diagrammatic representation of the type of *S. spontaneum* found in India and the relation of the "triplo polyploid" to the other forms

able to synthesize the $2n = 72$ type by crossing the wide-leafed Dacca *S. spontaneum* ($2n = 80$)

FIG. 2

A field of *S. spontaneum* showing the "triploid" giant amongst the diploids

with the local Coimbatore form ($2n = 64$) (Janaki Ammal, 1936 b).[2] On this basis they may be considered as true back crosses (Fig. 1). Their chromosome complexes are, therefore, as follows:

$$2n = 56 = 6(x = 6) + 2(x = 10)$$
$$2n = 72 = 2(x = 6) + 6(x = 10).$$

In studying a population of 100 selfed seedlings of a form with $2n = 56$ collected at Dehra Dun, I noticed two giant plants which stood out amongst the rest by their greater height, thickness of stem, width of leaves, size of inflorescence and increased sugar content (Figs. 2, 3 & 4). Chromosome counts in root tips of these plants showed 84 chromosomes which is thrice the haploid complement of the parent (Figs. 5 & 6). The plants are therefore "triploids", having arisen by the fertilization of an unreduced ($2n = 56$) gamete with a reduced one ($n = 28$). They are semi-sterile, but have yielded a number of seedlings on selfing. A study of meiosis in the "triploid" revealed the presence of univalents and multivalents besides a large number of bivalents. As the plant from which this "triploid" has arisen is already a complex polyploid, I have used the term "triplo-polyploid" to designate this type of derivation. In vegetative characters these "triplo-polyploid" plants stand intermediate between the *S. spontaneum* of India and the indigenous cultivated sugarcanes which they resemble. It is therefore not unlikely that

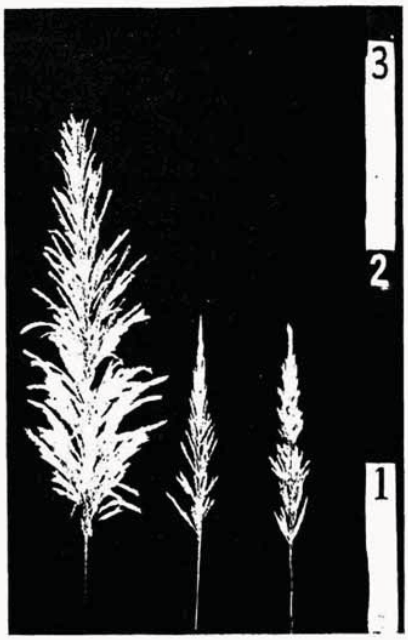

FIG. 4

Inflorescence of a "triploid" and two "diploid" *S. spontaneum* from the culture of selfed seedlings of the Dehra Dun form

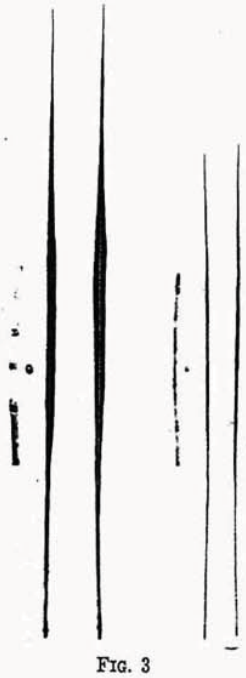

FIG. 3

Stem and leaves of "triploid" seedling of *S. spontaneum* (Dehra Dun) by the side of its "diploid" parent

FIG. 5

Somatic metaphase in *S. spontaneum*, Dehra Dun ($2n = 56$)

FIG. 6

Somatic metaphase in "triploid" seedling of the Dehra Dun *S. spontaneum* ($2n = 84$)

some of the sugarcanes of India have arisen from *S. spontaneum* as "triplo-polyploids". If it is so, there exists an interesting parallelism between the chromosome history of a cultivated plant like the sugarcane and the triploid mutants whose propagation as clones has likewise provided the best varieties of apples, pears, tulips and hyacinths.

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Imperial Sugarcane Station,
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Coimbatore,
January 19, 1939.

[1] Janaki Ammal, E. K., *Ind. Jour. Agri. Sci.*, 1936, 5, 1.

[2] — *Report of the Sugarcane Geneticist*, from July 1935 to March 1936.

Separation of Lac into Sclerolac and Soft Lac Resin

PREVIOUS work[1] has shown that lac could be separated into sclerolac, the hard lac resin, and the soft lac resin. The superiority of sclero- to ordinary lac[2] which consists in its higher melting point, low water absorption value, low acid value, better adhesive strength and its capacity for thermo-hardening, has not only extended the applications of lac but to a considerable extent stabilised its position in the varnish and electrical industries. Further, this has provided the necessary impetus for the manufacture of hard lac resin on a commercial scale.

There are three methods in vogue for the preparation of sclerolac from lac: (i) direct extraction with organic solvents which extract the soft lac resin,[3] (ii) 'cold polymerisation process'[4] involving the addition of urea to an acetone solution of lac whereby, the polymerised hard lac is precipitated, and (iii) extraction of lac with dilute alkali solutions or fractional precipitation from dilute alkali solutions.[2,5]

The first two methods entail the employment of rather expensive solvents and the extractions are accompanied by heat treatment which is not very desirable in the case of a thermo-hardening material like the hard lac resin. The third method, though it does not yield pure hard lac resin, is an economical process. The small amounts of buffer salts employed for the process, will have to be thoroughly removed and the product dried before use in varnish manufacture.

We carried out experiments to find out the nature of the products yielded on precipitating lac from an alcoholic solution with water. This is analogous to the precipitation of lac from an alcoholic solution with ether, although the separation is not quite so distinct and complete. By this method, the less acidic ingredients of lac separated out as a viscous mass from the solution, the more acidic ones remaining in the supernatant. A very important factor in favour of this method is that it does not involve much extra cost when adopted by manufacturers producing machine-made shellac. Moreover, there is no likelihood

TABLE I

Treatment	Soluble fraction		Insoluble fraction	
	Acid value	Wt. in gm.	Acid value	Wt. in gm.
*25 c.c. alcoholic solution of lac + 700 c.c. ethyl ether ..	100·8	4·04	56·0	2·8
* ,, ,, ,, + 8 c.c. distilled water ..	88	3·34	71·8	3·68
* ,, ,, ,, + 10 ,, ,, ..	104·6	1·86	75·0	5·05
* ,, ,, ,, + 12 ,, ,, ..	125·7	1·09	75·8	5·68
* ,, ,, ,, + 14 ,, ,, ..	131·1	0·82	76·7	6·10
25 c.c. alcoholic solution of bleached lac *plus* 12 c.c. distilled water	107·6	1·39	77·0	3·35

* The original alcoholic solution of lac had an acid value of 81·8 and a solid content of 27·65%.

Vitamin C in Pulmonary Tuberculosis

PHYSIOLOGICAL properties of Vitamin C such, among others, as its action on vascular permeability, its role in intoxication and in tissue respiration and its action on formative cells, suggest its application in Pulmonary Tuberculosis therapy. Some useful work has already been done in this field. A comprehensive study on the relation between Vitamin C and pulmonary tuberculosis is being carried on in this Department by Dr. S. K. Roy and the results already obtained are encouraging.

A study of the urinary excretion of Vitamin C by the method of Harris and Ray[1] as later modified,[2] proved that the system is highly unsaturated with Vitamin C in pulmonary tuberculosis cases. Fig. 1 gives a picture of

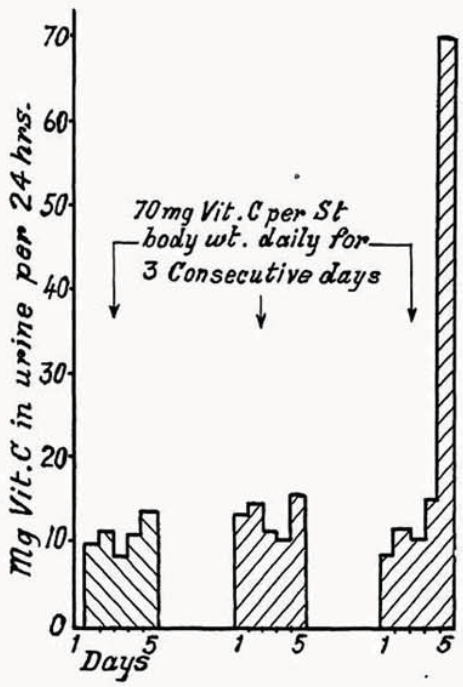

FIG. 1

this state of unsaturation. Administration of 70 mg. of Vitamin C per kg. of body weight for three consecutive days failed to saturate the body in most cases of pulmonary tuberculosis. In hæmoptysis, Vitamin C excretion falls rapidly and rises with the stoppage of hæmoptysis.

Administration of 350 mg. of Vitamin C daily to pulmonary tuberculosis patients, makes a decided improvement in the general blood picture, sedimentation rate, von Bonsdorff's count and Houghton's index.

The investigation is being continued.

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Patna,
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[1] *Lancet*, 1935, **1**, 71.
[2] *Ibid.*, 1935, **2**, 1399.

Supernumerary Chromosomes in Para-Sorghum

A CYTOLOGICAL examination of a number of plants of *Sorghum purpuero-sericeum* Ashers et Schwenf, grown at the Imperial Sugarcane Breeding Station, Coimbatore, from seeds received from Kew as originally collected from the Sudan, showed that the chromosome number in this species of Sorghum varies from $2n = 10$ to $2n = 14$. The haploid set of 5 which was also found in the two other para-Sorghums examined, *S. versicolor* Anderss and *S. dimidiatum* Stapf. could be easily identified in plants of *S. purpuero-sericeum* with 10 chromosomes, by their different lengths and the nature of their attachment constrictions (Fig. 1). A pair

FIG. 1
Mitotic metaphase in *S. purpuero-sericeum*
$2n = 10$ × 2700

of nucleolar chromosomes possessing the characteristic satellite could be easily distinguished. The extra chromosomes in plants having more than 10 chromosomes are found to be identical with the smallest or fifth chromosome in length

and in having a sub-median attachment constriction (Fig. 2). At meiosis these extra

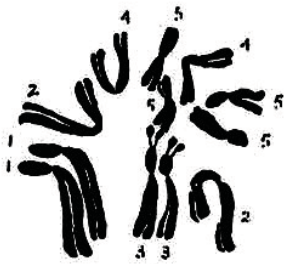

FIG. 2

Mitotic metaphase in *S. purpureo-sericeum*
$2n = 10 + 2$ × 2700

chromosomes may be seen as univalents or they may pair amongst themselves or with the fifth chromosome to form bivalents, trivalents or tetravalents.

The plants in which these extra chromosomes occur are found to be in no way different from those in which they are absent. It is highly probable that these supernumerary chromosomes like those first observed in Maize (Longley, 1927) and in Paspalum (Avdulov and Titova, 1933) are impoverished of genes. They, however, differ from the "B" chromosomes of maize in having a well-defined attachment constriction and in being homologous with one of the chromosomes of the normal haploid set.

Ten has been commonly reported as the basic number in the Andropogoneæ and the discovery of the five-chromosome species *S. nersicolor* (Karper, 1930), has been considered as a direct evidence for this. However, multivalent associations are found in nearly all diploid species of Sorghum, and associations higher than quadrivalents reported in the tetraploid *S. halepense* by Huskins and Smith (1934). These workers have not found fewer than 7 units of association in the Sorghum material examined by them. This, and the frequency with which the chromosome number 7 and its multiples occur in Gramineæ, raise the possibilities of this number rather than 5 being the basic number in Sorghum. The discovery of types amongst *S. purpureo-sericeum* with chromosomes ranging from $2n = 10$ to $2n = 14$ seems to indicate that this species of Sorghum probably represents one of the stages in the process whereby chromosomes are gradually eliminated in the evolutionary fall in the basic chromosome number from 7 to 5.

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Coimbatore,
May 1, 1939.

Avdulov, N., and Titova, N., "Additional Chromosomes in *Paspalum stoloniferum* Bosen." *Bull. Appl. Bot.*, Leningrad, 1933, Ser. 2 (2), 165–72.

Huskins, C. L., and Smith, S. G., "A Cytological Study of the Genus Sorghum," *Jour. Gen.*, 1934, 28.

Longley, A. E., "Supernumerary Chromosomes in *Zea Mays*," *Jour. Agri. Res.*, 1927, 35, 769–84.

Karper, R. E., "Inheritance in Grain Sorghums," *Texas. Agri. Exp. Sta. Records*, 43rd Ann. Report, 1930.

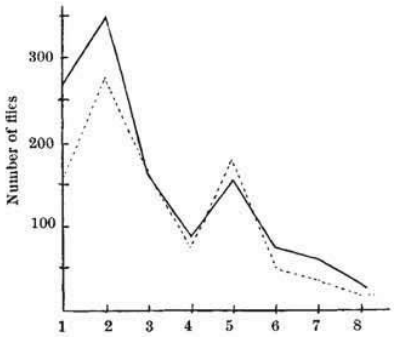
How Mid-Rib Hardness affords Resistance to the Sugarcane Top-borer *Scirpophaga nivella* F., in India

THE sugarcane top-borer, *Scirpophaga nivella* F., is found almost all over India where at present about 4,500,000 acres are under sugarcane owing to the recent rapid development of the sugar industry.

In some of the sugarcane tracts about 70% of the sugarcanes at harvest time are found attacked by the top-borer. Attacked canes exhibit a drying shoot and become stunted and often have a bunchy top owing to the upper side buds developing into branches. Besides this damage, the attack by this pest kills off many young shoots and prevents the growth of many shoots into millable canes. At harvest time millable canes bored by this pest show an average loss of 20% in weight. During some years the loss is much more.

It has been found as a result of field observations carried out during 1937 and 1938 that some varieties of sugarcanes are definitely more resistant to this pest than others. All these resistant varieties have in common very strong hard mid-ribs in their leaves. The varieties that are badly attacked have rather weak mid-ribs often with drooping leaves.

6·2; 5·0; 2·5; etc., and 16·8; 28·8; 17·2; 7·7; 18·6; 5·0; 3·7; 1·7; etc. Here the female curve also showed a high peak on the second day and another but slightly lower peak on the fifth day of emerging. This fifth day female peak was much more pronounced than that of the criss-cross generation mentioned above. The difference between the number of females and males for the fifth day was consequently not so large as in the case of the criss-cross generation.



Data treated on similar lines and obtained from observations on *Drosophila melanogaster* (wild) and *Drosophila simulans* (wild) showed a pronounced second day peak for both sexes but no fifth day peak. Hereafter there was an unarrested decline. The females were in the majority, more or less to the same degree for every day of emerging. The daily percentages of the total of females and males respectively for *Drosophila melanogaster* were: 28·7; 40·9; 19·5; 9·2; 1·1; 0·27; 0·27; and 22·8; 35·7; 26·0; 12·5; 0·6; 0·3; etc. The corresponding percentages for *Drosophila simulans* were: 18·6; 32·3; 15·7; 13·2; 8·4; 5·7; 2·8; 1·9; etc., and 11·4; 35·8; 20·0; 14·3; 8·6; 3·9; 2·6; 2·6; etc.

It is important to note that especially for the criss-cross generations involving the sex-linked genes *y* and *w*, and probably also other sex-linked genes, the sex-ratio may be inherently different from the first to the last day of emerging.

The percentage of females for the consecutive days of emerging of the criss-cross generation were: 68; 58; 51·5; 49·4; 36·1; 46·9; 18·7; 55·1; etc., the average being approximately 51·6.

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Chromosome Numbers in *Sclerostachya fusca*

Sclerostachya fusca A. Camus (*Saccharum fuscum* Roxb.) is a grass closely related to *Saccharum spontaneum* and similar to it in habit. It is distinguished from Saccharum by its rachis being tough instead of fragile and by its pedicelled spikelets being female instead of hermaphrodite. (The sessile spikelets in both are hermaphrodite.)

This grass grows in association with *Saccharum spontaneum* in Assam and Orissa. I collected several clones from both these localities in 1937. The peasants use it extensively for roofing and making framework for their mud houses, as well as for fencing. It goes by the names of *yekkada* and *ikra* in these provinces.

The Orissa form proved to have 48 chromosomes, and the Assam form, which was of larger habit, had 96 chromosomes.

Most of the Andropogoneæ, including *Saccharum officinarum*, have 10 as their basic number. Exceptions to this are Miscanthus with 36 chromosomes and the dibasic *Saccharum spontaneum* in which forms with $x = 6$ and $x = 10$ have been found.

The doubling within the species is analogous to the condition of *Saccharum spontaneum* with its west-to-east transition from 48 to 112 chromosomes[1]. The discovery of another genus, more closely related to Saccharum than Miscanthus, with the basic number of 6, makes it easier to understand the origin of the dibasic cultivated sugar canes.

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[1] Janaki-Ammal, E. K., "Triplo-polyploidy in *Saccharum spontaneum* L.", *Current Sci.*, **8**, 74–76 (1939).

Amœba lescheri (= *Chaos lescheri*): a New Species of Amœba

EARLY in 1938 I discovered a new species of Chaos (better known as Amœba in Great Britain) which I propose to name *Chaos lescheri* = *Amœba lescheri*, to honour the memory of Mary Adela Lescher, founder of Notre Dame College, Glasgow. A full account of the life-history of this amœba will appear in the *Quarterly Journal of Microscopical Science*. The amœbæ of the genus Chaos are large and form pseudopodia that are sub-cylindrical, blunt and filled with granular endoplasm throughout. Conspicuous longitudinal ridges and grooves are characteristic of the ectoplasm. *Amœba lescheri* is readily distinguished from the other species of Chaos by its crystals, which are square prisms (maximum size 2 μ). Outsized spherical individuals attain a diameter of 525 μ, creeping individuals a length of 600 μ. The average for the former is 350 μ and for the latter 400–500 μ.

Translucency in amœbæ is a factor of age and physiological condition, depending to a large extent on the cytoplasmic inclusions. Having regard to these considerations, *Amœba lescheri* is more translucent, as it is also slightly smaller than *A. proteus* Y[1]. The shapes assumed by the contrasting amœbæ also differ, more of the bulk of the cytoplasm being concentrated in the pseudopodia of *A. lescheri*. Normally there is present one nucleus, discoid in shape. Alternate aspects of the nucleus, 'plan and elevation', are presented to the observer as the nucleus is subjected to the streaming movements of the endoplasm. Frequently the nucleus has the appearance of a biconcave lens. Since it normally lies in the peripheral endoplasm it is quickly found in a microscopical preparation of the living animal. The chromatin is distributed on an achromatic network and takes the form of regularly arranged blocks under the nuclear membrane distinct from, and slightly separated from,

TABLE 1.

GROWTH FACTORS FOR *Cl. acetobutylicum* AND RELATED SUBSTANCES.

Substance	Amount of substance per ml. of basal synthetic medium					
	2×10^{-5} μ gm.	2×10^{-4} μ gm.	2×10^{-3} μ gm.	2×10^{-2} μ gm.	2×10^{-1} μ gm.	2 μ gm.
1. Yeast concentrate	−	−	+	+	+	+
2. Yeast concentrate (after ether extraction)	−	−	−	−	−	−
3. *p*-benzoyl amino benzoic acid (from yeast concentrate)	−	+	+	+	+	−
4. *p*-amino benzoic acid	+	+	+	+	+	−
5. *m*-amino benzoic acid	−	−	−	−	−	+
6. *o*-amino benzoic acid	−	−	−	−	−	+
7. Novocaine	−	+	+	+	+	−
8. Sulphanilamide	−	−	−	−	−	−

Growth +, no growth −

A comparison of these results with the anti-sulphanilamide tests of Woods reveals a remarkable correlation. In this work the growth factor activity is: *p*-amino benzoic acid $6\cdot8\times10^{-8}$ *M.*, novocaine $1\cdot2\times10^{-8}$ *M.*, *ortho* and *meta* amino benzoic acid probably inactive. In regard to Weizmann's statement, it is presumed that his biotin, which stimulated growth, contained *p*-amino benzoic acid.

The bacteriostatic action of sulphanilamide on growth was determined by subculturing the organisms in the basal medium containing varying quantities of 'p.a.b.' and constant amounts of sulphanilamide. The results recorded in Table 2 confirm Woods's findings[6] on the anti-sulphanilamide action of 'p.a.b.' They also illustrate how it is possible to titrate the two antagonists using growth as the end point.

TABLE 2.

TITRATION OF *p*-AMINO BENZOIC ACID AGAINST SULPHANILAMIDE.

Concentration of sulphanilamide	Amount of *p*-amino benzoic acid per ml. of basal medium					Molecular ratio 'p.a.b.' : sulphanilamide
	5×10^{-3} μ gm.	1×10^{-3} μ gm.	2×10^{-1} μ gm.	3×10^{-1} μ gm.	4×10^{-3} μ gm.	
$\frac{M}{1,650}$	−	−	−	−	−	23,000
$\frac{M}{3,300}$	−	−	+	+	+	23,000
$\frac{M}{6,600}$	−	+	+	+	+	23,000

Growth +, no growth −

On the basis of the above figures one molecule of 'p.a.b.' antagonizes 23,000 molecules of sulphanilamide. This tremendous disproportion between these two antagonistic reagents makes it difficult to conceive how the growth activator 'p.a.b.' can overcome the effect of the growth inhibitor (sulphanilamide) if the two molecules are destined towards the same receptor site on the organism. The chances of the activator making first contact seem fairly remote when considered from a physico-chemical point of view.

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Oct. 22.

[1] Fildes, *Lancet*, **1**, 955 (1940).
[2] Stamp, *Lancet*, **11**, 10 (1939).
[3] Green, *Brit. J. Exp. Path.*, **21**, 38 (1940).
[4] Brown, Wood and Werkman, *J. Bact.*, **38**, 631 (1939).
[5] Weizmann, *Biochem. J.*, **33**, 1376 (1939).
[6] Woods, *Brit. J. Exp. Path.*, **21**, 74 (1940).

Chromosome Diminution in a Plant

THE genus Sorghum consists of two groups. Eu-Sorghum includes the tetraploid millet species and an octoploid fodder species[1]. Para-Sorghum includes only diploid species ($n = 5$) which are not cultivated. One of these is *S. purpureo-sericeum*, from the Sudan, in which I have described plants with varying numbers of extra chromosomes. These chromosomes I supposed to be inert[2].

I now find from meiosis in the pollen mother cell that, amongst a wild 1931 collection of seeds given me by Mr. C. E. Hubbard of Kew, 40 out of 100 had these extra chromosomes (Table 1).

TABLE 1.

PLANTS WITH *B* CHROMOSOMES IN *Sorghum purpureo-sericeum*.

No. of *B*'s	0	1	2	3	4	5	6	Total
Total plants	60	12	20	5	1	1	1	100
Tested in roots	—	8	7	2	1	1	1	20

Thus there appears to be the same kind of distribution of extra *B* chromosomes in the natural population of this species as occurs in certain cultivated varieties of maize[3]. Again as in maize, these *B* chromosomes do not pair at meiosis with the five ordinary members of the complement. They have, therefore, lost all effective relationship with the active chromosomes, alongside which they must have maintained a separate existence for a great time.

That these chromosomes are in some respects active, and disadvantageously active, is shown by the proportion of healthy pollen (from 500 grains each) in plants having different numbers of *B*'s (Table 2). Their long maintenance in the species therefore demands that they have certain compensating advantages for the plant.

TABLE 2.

EFFECT OF *B*'S ON AVERAGE POLLEN FERTILITY.

No. of *B*'s	0	1	2	3
No. of Plants	6	3	5	2
Good pollen	93·6%	83·6%	63%	11·5%

From mitosis in the root-tips, however, a new and remarkable property of these *B* chromosomes appears. Twenty plants having *B*'s in the pollen mother cells were examined. None had *B*'s in the roots. All had the ordinary chromosome complement of 10 (second line, Table 1).

Re-examination of the flower-tissues of these plants showed the presence of *B*'s at metaphase of mitosis. The resting cells also frequently contained small extra nuclei, which had doubtless arisen from lagging *B*'s. Since these chromosomes are maintained in the tissue, it seems that the extra nuclei must rejoin their companions during mitosis in the flower parts. In the early growth of the roots, on the other hand, they must be lost.

Now irregularities (attributed to a deficient centromere) have often been found in the mitotic movements of extra chromosomes, but never before has a regular loss of such chromosomes been recorded in a particular tissue of a plant. The case at once recalls the well-known *diminution* of chromosomes in Ascaris and, still more forcibly, the exclusion of certain 'sex-limited' chromosomes from the somatic line in Sciara[4].

The further study of these highly controlled chromosomes is therefore likely to throw light on

the physiology and mechanics of the more elaborate and more abstruse systems of chromosome diminution found in animals.

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Nov. 26.

[1] Hunter, A. W. S., *Canad. J. Res.*, **11**, 213–241 (1934).
[2] Janaki-Ammal, E. K., *Curr. Sci.*, (1939).
[3] Darlington, C. D., and Upcott, M.B. (1940). *J. Genet.* (in the Press).
[4] Metz, C. W., *Amer. Nat.*, **72**, 485–520 (1938).

Compression of Cylinders of Soft Materials

SOME time ago, we proposed an equation[1] to describe the behaviour of soft bodies under compression, with special reference to the compression of cylinders. Whereas for a true fluid we have $\eta = s\sigma^{-1}t^1$, and for an elastic solid $n = s\sigma^{-1}t^0$; we proposed for 'intermediate' materials

$$\psi = s\sigma^{-1}t^k$$

where s is shearing stress, σ is shearing strain calculated by the logarithmic formula, t is time of compression, η is viscosity, n is shear modulus, and ψ was described as the "firmness" and k as "a measure of elasticity", though we should no longer care to use the latter expression, since such properties as work-hardening and dilatancy would reduce k without increasing the elastic recovery.

This equation obviates the difficulties, both practical and theoretical, involved in attempting to divide σ into two parts, recoverable and non-recoverable, in order to calculate η and n for such materials.

In a later paper[2], psychological experiments were described which we believe to justify the use of ψ as a criterion of firmness, in spite of, or rather because of, its peculiar physical dimensions. In neither paper was it possible to give any data to test the equation, nor was it claimed that ψ and k would be expected to be constants for all materials independent of stress and strain conditions. It was, however, hoped that the new treatment would prove simpler than the classical analysis, in which very complex variations in η and n with varying stress and strain conditions and histories are to be found[3].

A direct test of the equation has now been made possible as a result of the design and construction by Dr. P. White and Mr. J. Cotton of an apparatus, to be described shortly, in which cylinders can be loaded in such a way that the load increases proportionally to the change in cross-section of the cylinder, the value of s thus remaining constant throughout the compression.

Under these conditions, the equation may be written :

$$\log \psi = k \log t - \log \sigma + \text{const.};$$

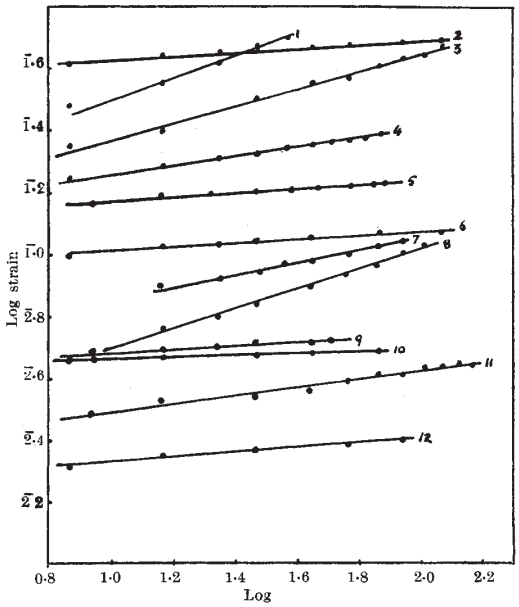
or, in the case where the $\log \sigma/\log t$ curves are linear :

$$\log \psi = k - \log \sigma_{10} + \text{const.},$$

where σ_{10} is the strain produced in 10 sec.

In view of the principle underlying Fechner's law, it seems not unlikely that 'firmness' as judged subjectively may be related directly to $\log \psi$, and for this reason as well as because the logarithmic values are easier to handle, we prefer to keep the data in the logarithmic form.

The new instrument was designed for experiments with cheese and butter, so that the stress range available is somewhat limited, but it has been possible



No.	Material	Radius	Shearing stress	Log ψ	k
1.	Worked butter	1·5 cm.	58,260 dyne cm.$^{-2}$	5·61	0·35
2.	Wet clay soil	1·0	131,100	5·53	0·035
3.	Rested butter	1·5	58,260	5·67	0·28
4.	Cake	1·0	102,500	5·89	0·14
5.	Acrylic acid polymer	1·0	192,600	6·18	0·06
6.	Mod. wet clay soil	1·0	131,100	6·17	0·055
7.	Cheddar cheese	1·0	131,100	6·47	0·20
8.	Stale bread	1·5	58,260	6·40	0·325
9.	Apple (flesh)	1·0	192,600	6·66	0·055
10.	Potato	1·0	192,600	6·66	0·035
11.	Plasticine-rubber Vaseline mixture	1·0	192,600	6·93	0·14
12.	Dry clay soil	1·0	131,100	6·86	0·07

to test, at a constant temperature of 60° F., a number of very varied materials, curves for some of which are shown in the accompanying graph. The $\log \sigma/\log t$ curves are remarkably linear, except perhaps at very small strains, where measurements are, in any event, decidedly inaccurate.

Experiments with cylinders of Californian bitumen, which approximates very closely to truly fluid behaviour, indicate that the instrument is compensating correctly for the change in cross-section, unless exceptionally small loads are used. The only case where this error is likely to be just significant is for No. 4, but the curve is shown because of its intrinsic interest.

Our views as to the significance of k have altered in the light of experience with the apparatus. This will be discussed when the experiments are described more fully elsewhere.

G. W. SCOTT BLAIR
F. M. VALDA COPPEN.
National Institute for Research in Dairying,
University of Reading. Nov. 25.

[1] Scott Blair, G. W., and Coppen, F. M. V., *Proc. Roy. Soc.*, B, **128**, 109 (1939).
[2] Scott Blair, G. W., and Coppen, F. M. V., *Brit. J. Psychol.*, **31**, 61 (1940).
[3] Schofield, R. K., and Scott Blair, G. W., *Proc. Roy. Soc.*, A, **138** 707, (1932); **139**, 557 (1933); **141**, 72 (1933); **160**, 87 (1937.)

Annals of Botany V: 83-87 (1941)

The Breakdown of Meiosis in a Male-Sterile Saccharum

BY

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With twenty-one Figures in the Text

INTRODUCTION

IN many hermaphrodite plants individuals occur with defective anthers. This defect arises in many ways, but it is always genetically determined (cf. Lewis, 1940), and may sometimes play an important part in the evolution of the genetic system (Frankel, 1940). Studying the precise means of breakdown, however, is of importance from an entirely different point of view. Beadle (1933) has shown that in maize there are some dozen genetically distinct determinants of male sterility, and these determinants act in many different ways. Some of them, such as the polymitotic gene, are already of great physiological interest and others await more exact interpretation.

Types of male sterility may be conveniently subdivided into those which act through the intermediacy of the chromosome-development and those which are seen directly to affect the spindle. Of the first the asynaptic gene in *Crepis capillaris* (Richardson, 1935) is an example; of the second, the breakdown of tetrad formation in Kniphofia (Moffett, 1932). Physiologically, a more obvious means of classification is that depending on the onset of breakdown. Abnormalities which in Chrysanthemum affect premeiotic divisions (Shimotomai, 1931) are at the one end of the scale; at the other extreme might be placed those types of pollen in hybrid species of Oenothera which although externally normal fail to germinate.

The types of male sterility which affect the development of the spindle are of particular interest for comparison with the results of experimental treatment and of non-pairing of chromosomes, which has always of course a secondary effect on spindle development. In a recent account Darlington and Thomas (1937) have been able to show in a Festuca-Lolium derivative that a failure in the combination of the separate spindles developed by the centromeres of each chromosome leads to a splitting of the spindle at anaphase and the formation at telophase of three nuclei instead of two. They also found that at the second division spindles could be developed which ran round the apparently quiescent nucleus and were developed, therefore, without any

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relationship to the centromeres, from which the first division spindles were most palpably derived.

In the present study I am concerned with another grass, a derivative of *Saccharum officinarum* with 110 chromosomes. The individual concerned was pollen-sterile. Its ovule fertility has not been tested by cross-pollination. It arose by diploid parthenogenesis from the Java cane POJ 2725 ($2n = 106$) after pollination with *Imperata cylindrica*, as I have recorded elsewhere (Janaki-Ammal, 1940).

FIG. 1. Somatic metaphase in root-tip of spindle defective plant. $2n = 110$. (×3,000.)

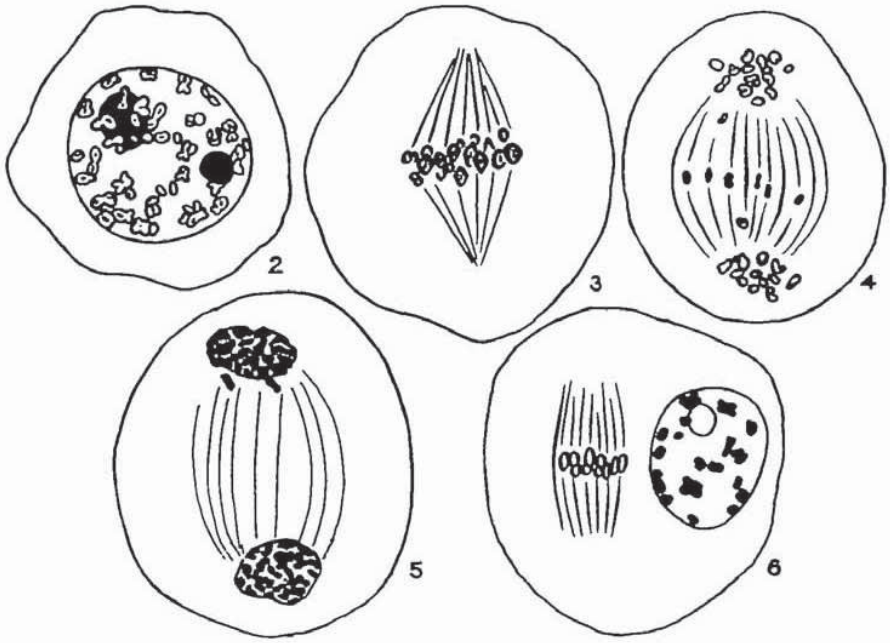
METHODS

Root-tips were fixed in Allen's Bouin after immersion in ice, and stained in Heidenhein's iron-alum-haematoxylin (Fig. 1). Pollen mother-cells were fixed in 1:3 acetic alcohol and transferred to 70 per cent. alcohol, in which they were left for several months. Material thus preserved was stained in aceto-carmine after immersion for a few minutes in acetic alcohol. Smears were made permanent according to McClintock's method.

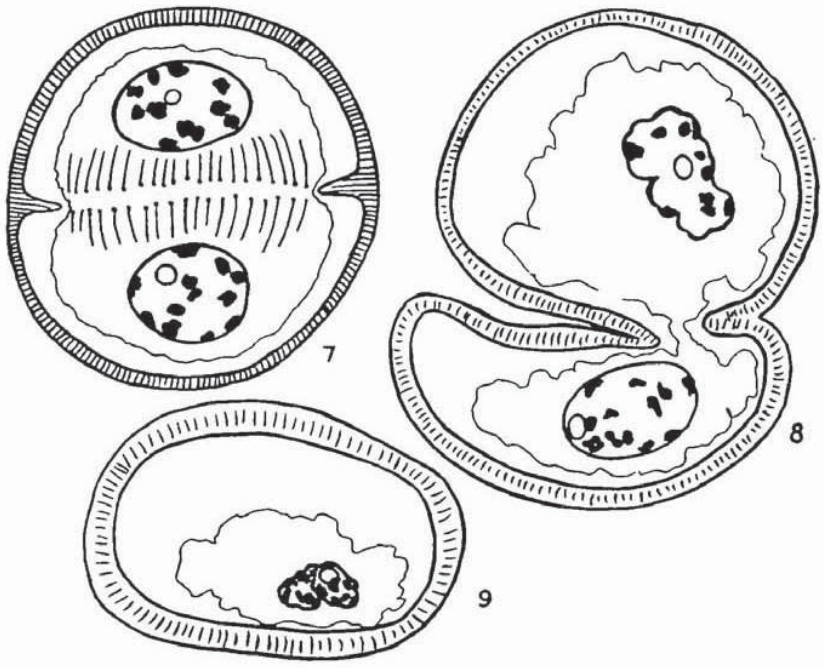
OBSERVATIONS

Meiosis follows a uniform course until the first anaphase. There are a variable number of unpaired chromosomes and multivalents (Figs. 2–5), but these do not seem to hinder spindle development seriously. Even after this stage, in rare instances, telophase, second division (Fig. 6), and tetrad formation may succeed one another to give separate pollen grains with separate nuclei. These, however, when formed rapidly degenerate. Alternatively, the second division may be omitted, and irregular dyads formed which afterwards degenerate (Figs. 7–9).

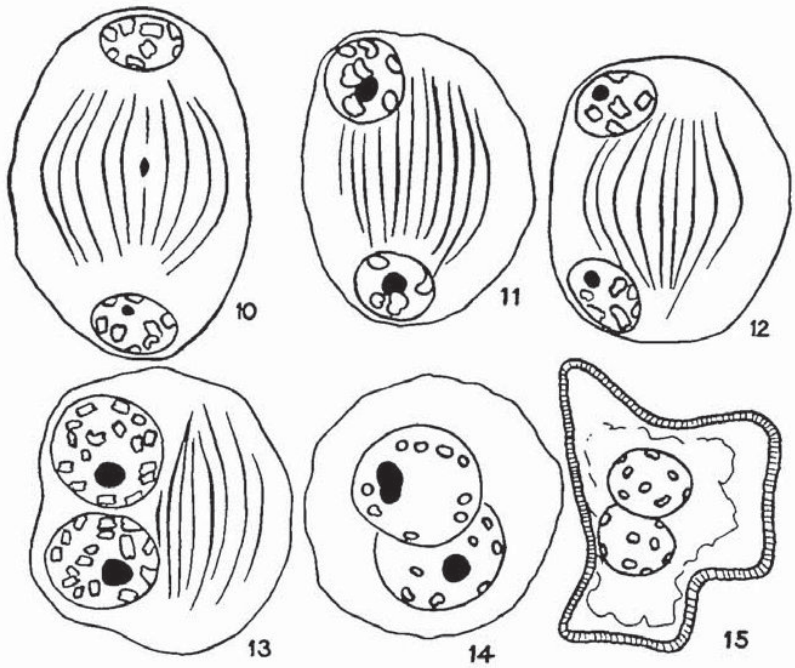
The great majority of cells show abnormalities, which fall sharply into two classes. In both the first telophase nuclei lose contact with the spindle before a cell plate can be formed. The loss of contact is not merely a visible relationship in the fixed material. It is correlated with a loss of relationship in movement. The two courses that may follow are quite simple. In the one case, as in Figs. 11–15, the two nuclei leave the ends of the spindle and move towards one another, one on each side. They fail, however, to meet, and produce, when the spindle has disappeared, a single, ill-formed, binucleate pollen grain. In the second case, which is even more surprising (Figs. 16–21), the two nuclei move towards one another through the spindle. They meet inside the spindle, and having met fuse to form a single nucleus from which a single pollen grain develops.



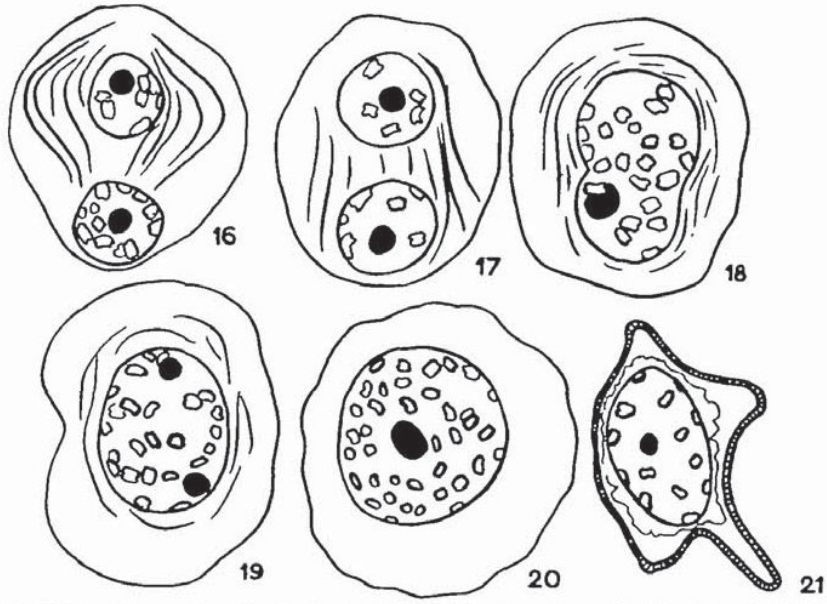
FIGS. 2–5. Diakinesis to first telophase in pollen mother cell.
FIG. 6. A rare case of second division (see text). (×1,200.)



FIGS. 7–9. Formation of irregular dyad pollen grains through omission of second division. (×1,200.)



FIGS. 10–15. First telophase to formation of binucleate pollen grains. (×1,200.)



FIGS. 16–21. Formation of uninucleate pollen grains by fusion of the first telophase nuclei. (×1,200.)

What is significant about this behaviour is that nuclei, which are distinguished by the accidents of their position and movement, in one course of development fuse and in another fail to do so. They fuse when they are within the spindle; they fail to fuse when they are within the cytoplasm. A second point of interest is the loss of contact of nucleus and spindle, which recalls the absence of contact of the second division in the Lolium derivative. This loss of contact is of course associated with the loss of function in the deposition of the cell plate. We should therefore describe the spindles, for all their apparent structure and strength, as dead spindles.

Summary

Sterility in a parthenogenetically derived seedling of the sugar-cane POJ 2725 is due to inactivity of the spindle after the first telophase.

The two nuclei formed either move down the sides of the spindle or pass through it. In the latter case a restitution nucleus results.

In rare cases tetrad and dyad grains are formed; these degenerate.

Literature Cited

Beadle, G. W., 1933: Further Studies of Asynaptic Maize. Cytologia, iv. 269–85.

Darlington, C. D., and Thomas, P. T., 1937: The Breakdown of Cell Division in a Festuca-Lolium Hybrid. Ann. Bot., N.S., i. 747–61.

Frankel, O. H., 1940: Studies in *Hebe*, II. The Significance of Male Sterility. Journ. Genet., xl. 171–84.

Janaki-Ammal, E. K., 1940: Intergeneric Hybrids of *Saccharum*, I–III. Journ. Genet. (in the press).

Lewis, D., 1940: Male Sterility in Natural Populations. I. The Equilibrium between Females and Hermaphrodites to be expected with Different Types of Inheritance. New Phytol. (in the press).

Moffett, A. A., 1932: Studies in the Formation of Multinuclear Pollen Grains in *Kniphofia*. Journ. Genet., xxv. 315–37.

Richardson, M. M., 1935: Meiosis in *Crepis*, II. Failure of Pairing in *Crepis capillaris L.* Journ. Genet., xxxi. 119–43.

Shimotomai, N., 1931: Über die abnorme Reduktionsteilung in Pollenmutterzellen die einen riesigen oder überzähligen Zwergkern enthalten. Bot. Mag. Tokyo, xlv. 356–63.

Journal of Genetics 41:217-254 (1941)

INTERGENERIC HYBRIDS OF *SACCHARUM*

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John Innes Horticultural Institution, Merton

(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.

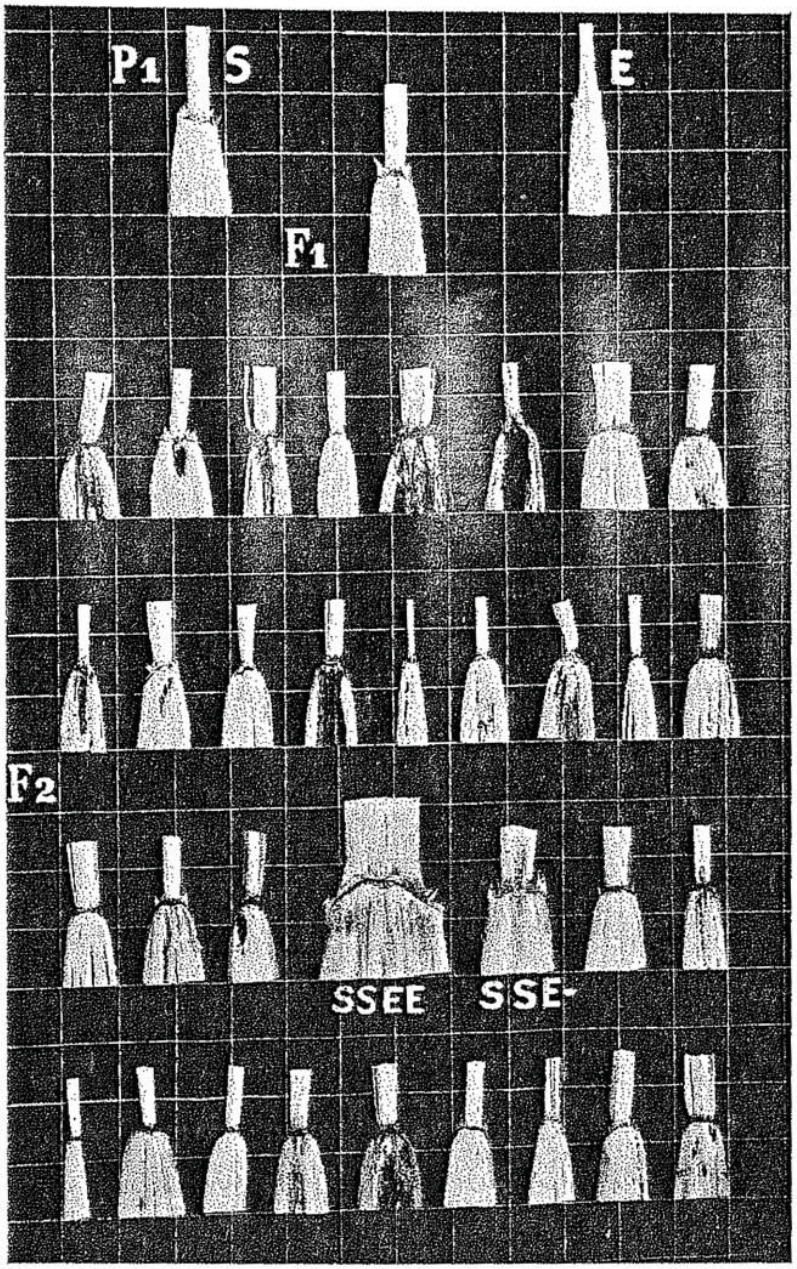


Fig. 1. Types of ligule in *S. spontaneum* and *Erianthus* P_1 and their F_1 and F_2 hybrids.

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.

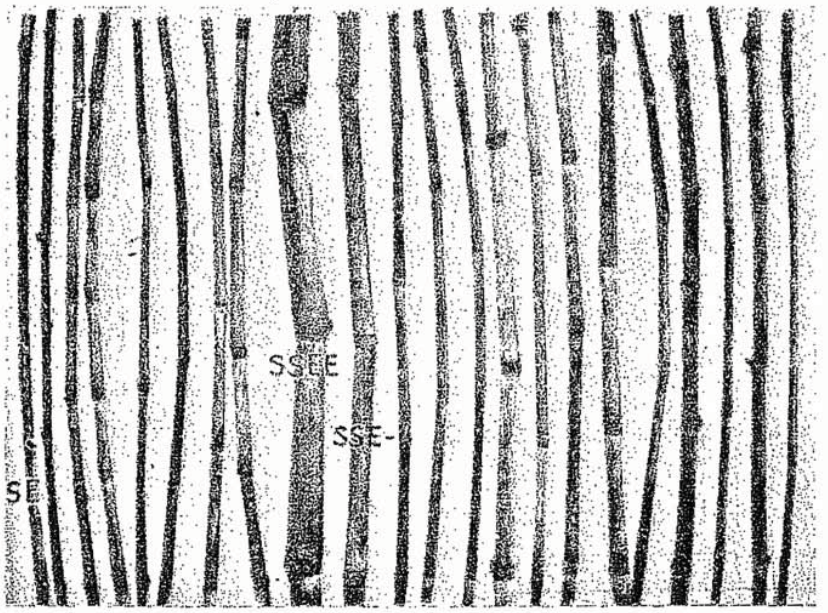


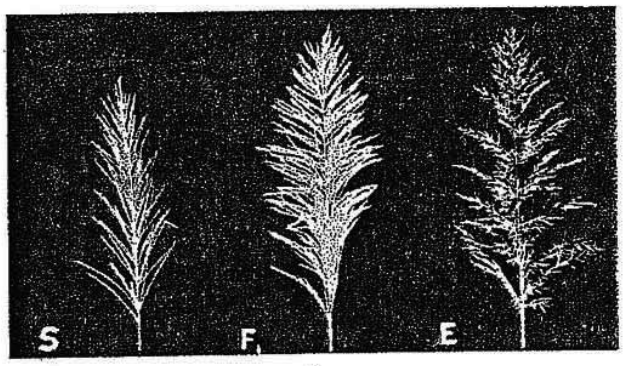
Fig. 2. Variation in stem thickness in the F_2 generation of *Saccharum-Erianthus* hybrids

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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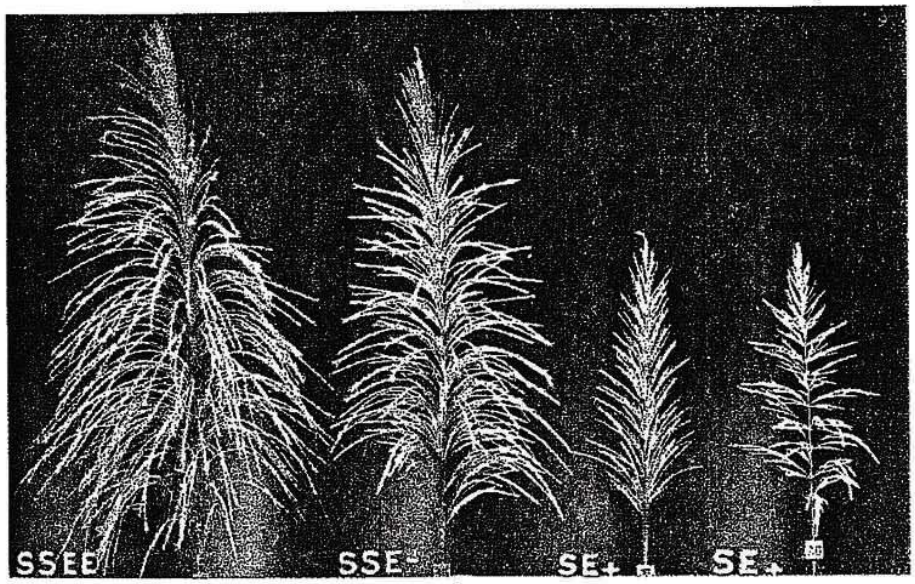

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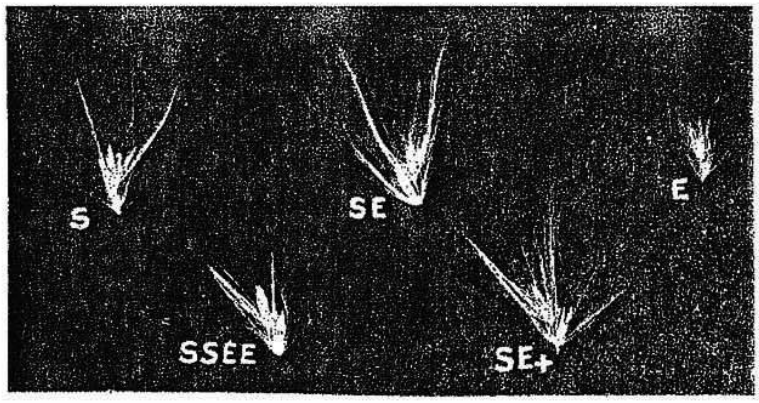


c

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.

15–2

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.

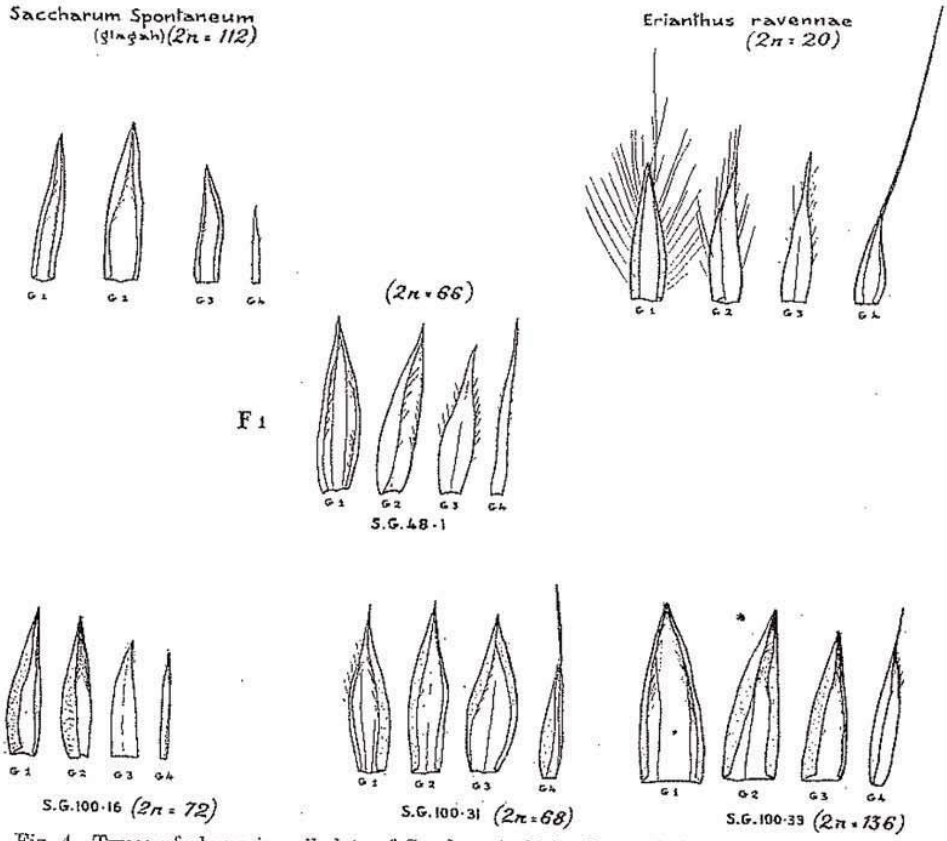


Fig. 4. Types of glume in spikelets of *Saccharum*, *Erianthus* and their F_1 and F_2 hybrids. The awned fourth glume, though not present in the F_1, appears in some of the F_2 seedlings.

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.

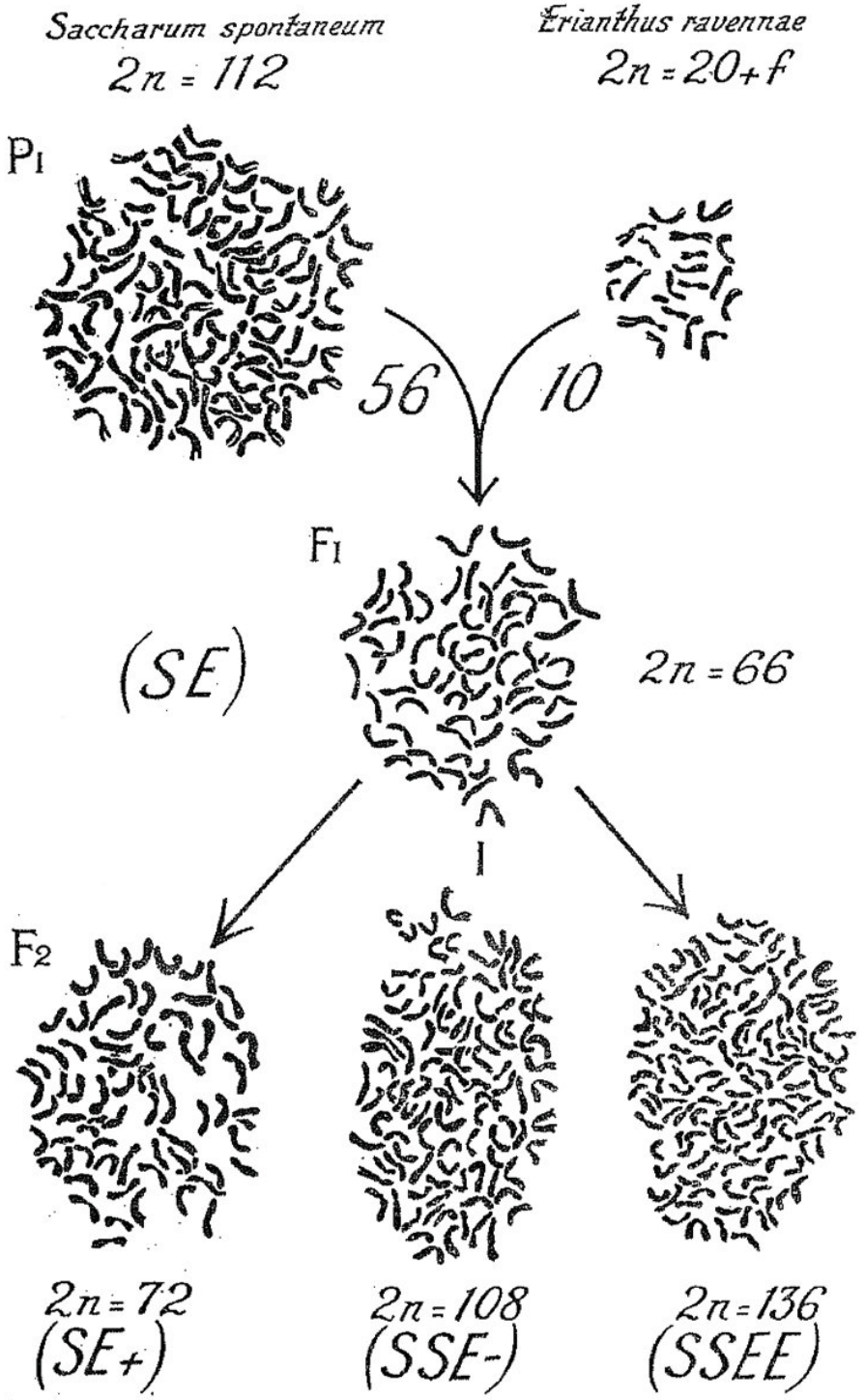


Fig. 5. Somatic metaphase in root tips of *S. spontaneum*, *Erianthus ravennae* and their F_1 and F_2 hybrids. ×2000.

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

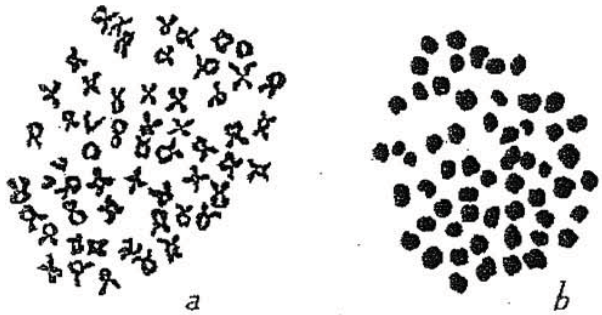


Fig. 6. Chromosome association in *S. spontaneum* "Glagah". *a*, diplotene; *b*, metaphase. ×1800.

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.

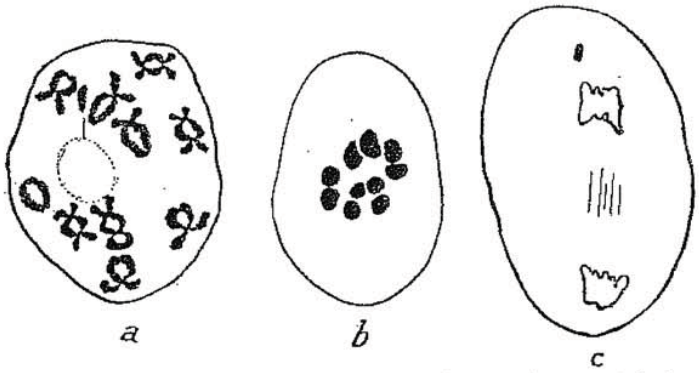


Fig. 7. Meiosis in *Erianthus ravennae*. *a*, diplotene; *b*, metaphase; *c*, telophase, with the fragment excluded. ×2000.

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

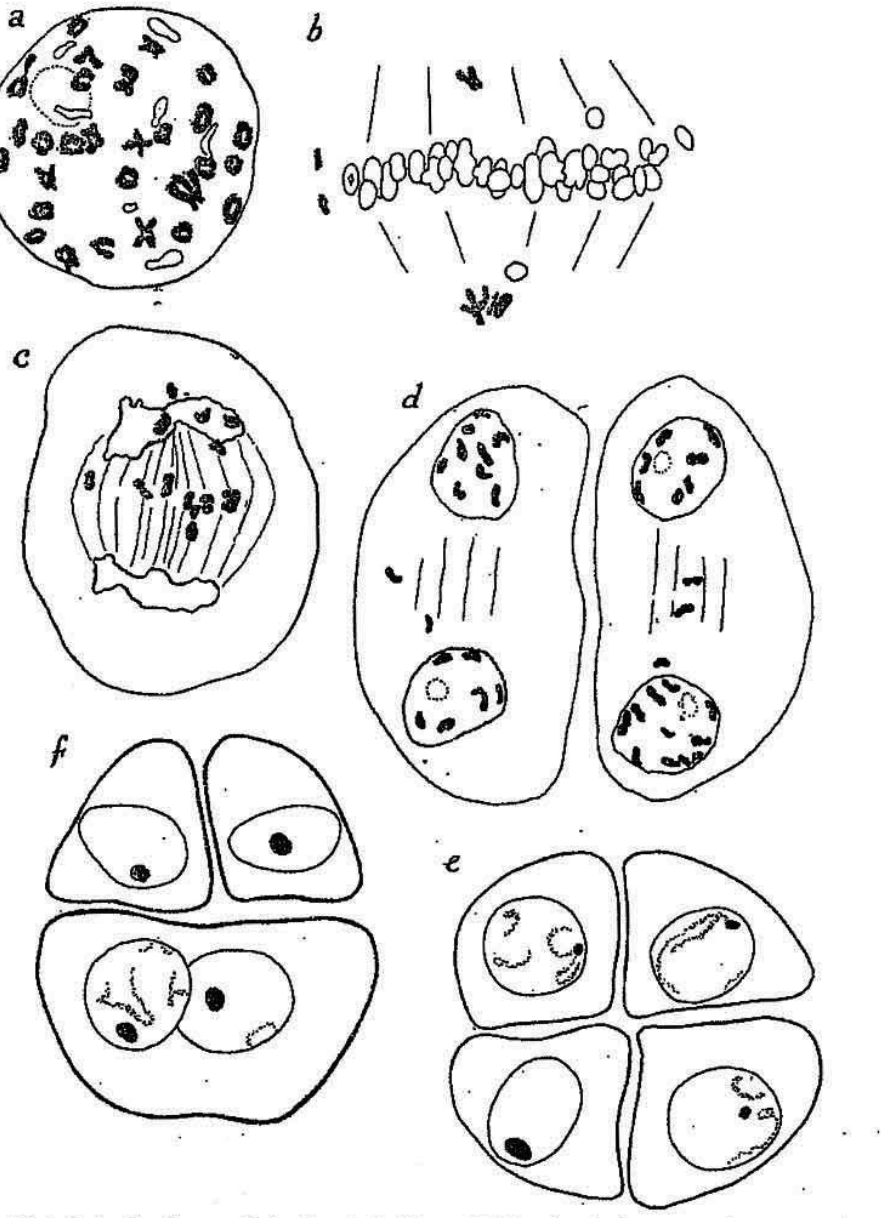


Fig. 8. Meiosis in *Saccharum-Erianthus* hybrid. *a*, diakinesis. *b*, first metaphase. *c*, telophase of first division, showing univalents dividing at the equator. *d*, telophase of second division, showing lagging chromosomes. *e*, normal tetrad. *f*, abnormal tetrad with binucleate dyad. (*a*, *b*,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

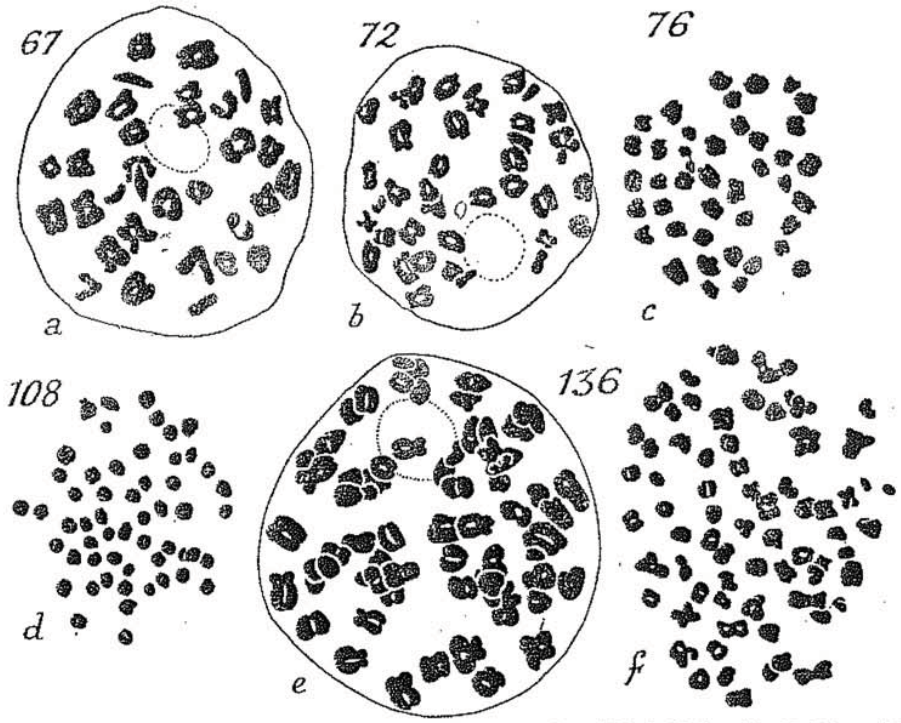


Fig. 9. Association of chromosomes in F_2 seedling. *a*, *b*, *c*, "diploids" with 67, 72 and 76 chromosomes. *d*, metaphase in triploid with 108 chromosomes. *e*, *f*, diakinesis and metaphase in tetraploid, $2n=136$, showing quadrivalents and sexivalents. ×1800.

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.

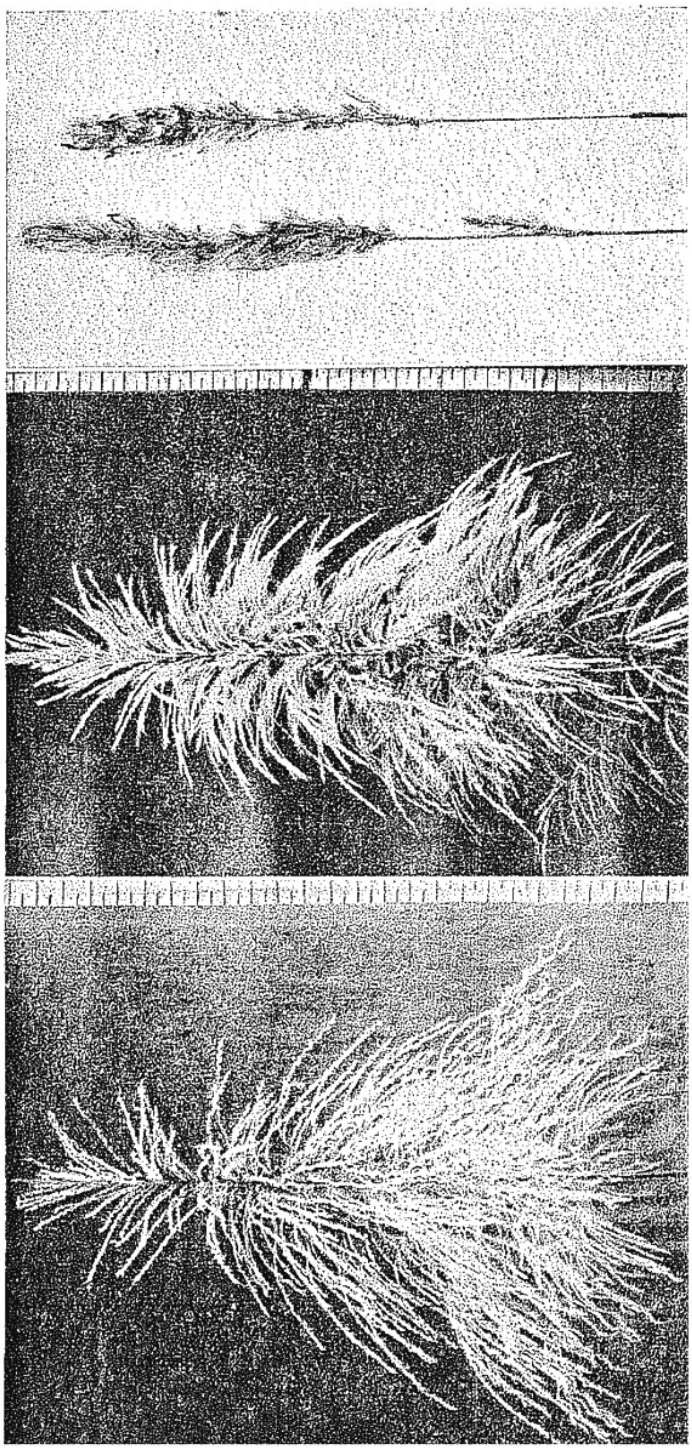
2. Methods

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



Imperata cylindrica (§ nat. size.)

F_1

POJ 2725

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*)

"Kassoer" ♂ $2n=136$ (*OOS*)

S. officinarum ♀ POJ 100, $2n=80$ (*OO*) ×

POJ 2364 ♀ $2n=148$ (*OOO* + *S*/2) ×

S. officinarum ♂ EK 28, $2n=80$ (*OO*)

POJ 2725 $2n=106$

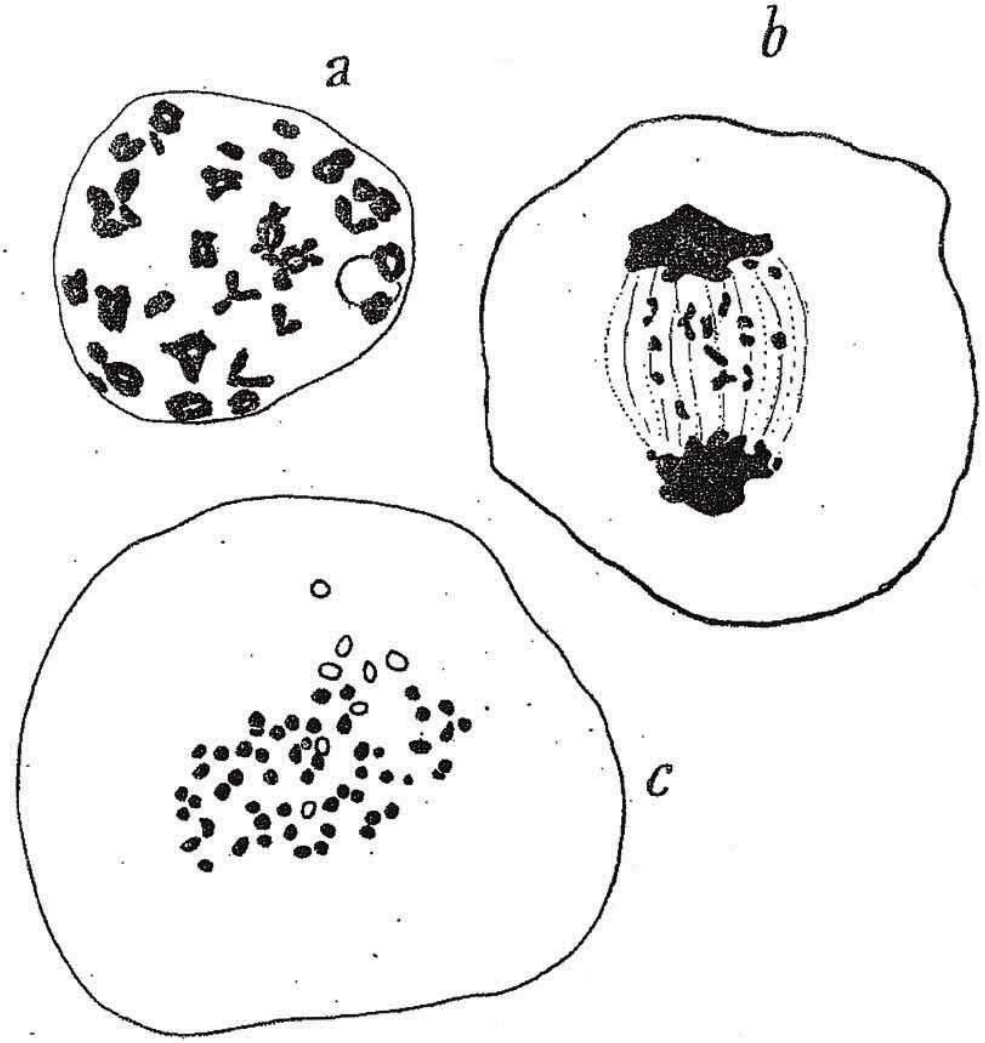


Fig. 11. Meiosis in POJ 2725. *a*, pollen mother cell at diakinesis showing multivalent and univalent association of chromosomes × 2400. *b*, anaphase of first division. *c*, metaphase in pollen grain with 62 chromosomes × 1800.

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	3	Sexual or diploid parthenogenesis	*SS*+	0·0–7 %
	110	2			
	112	1			
III	120	1	True F_1	*SSI*	50–80 %
	130	1			
	132	1			
	134	2			
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-

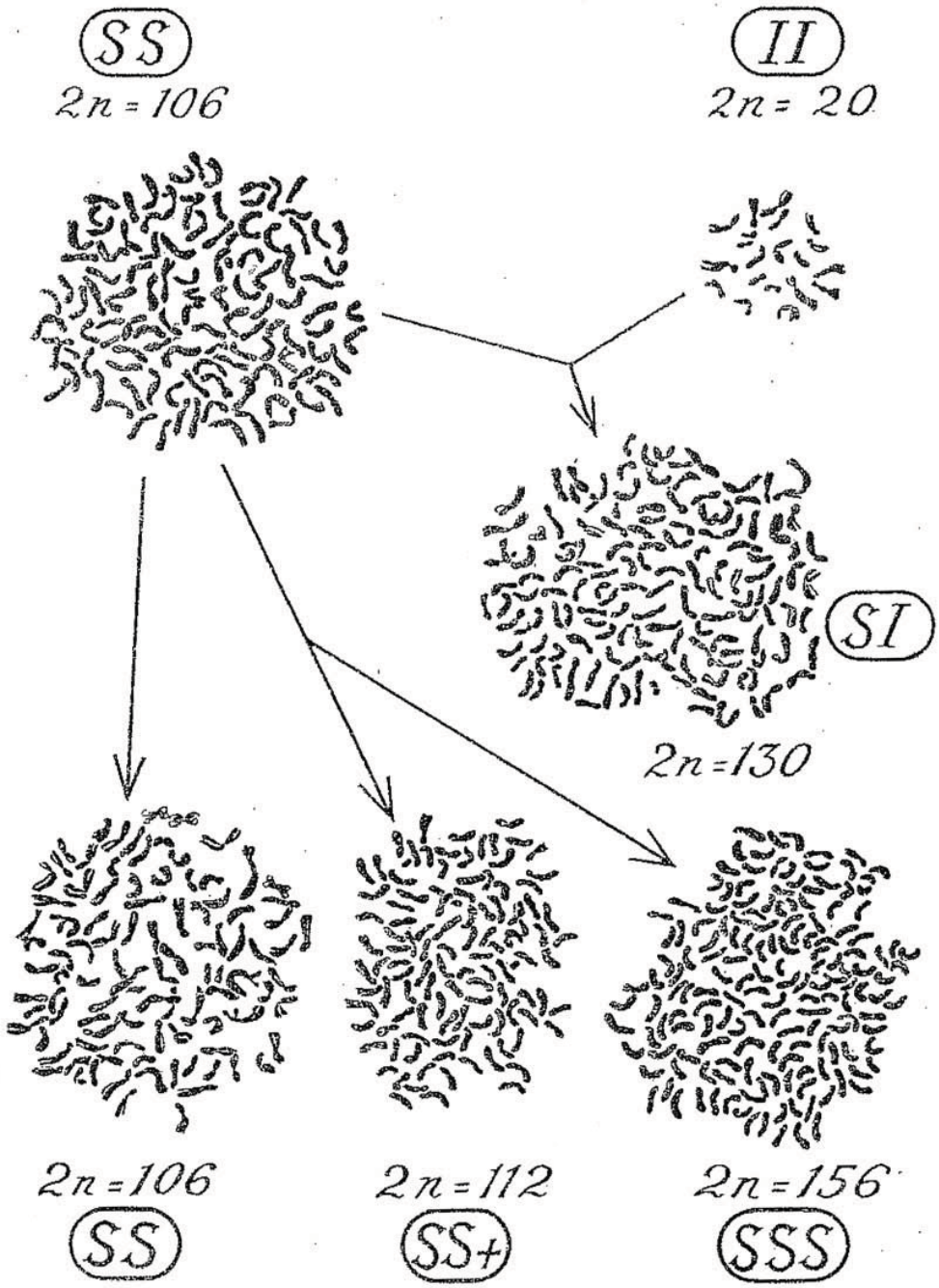


Fig. 12. Root tip metaphases in POJ 2725. (*SS*) *Imperata cylindrica* (II) and types of seedlings obtained in the cross. ×2000.

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*.

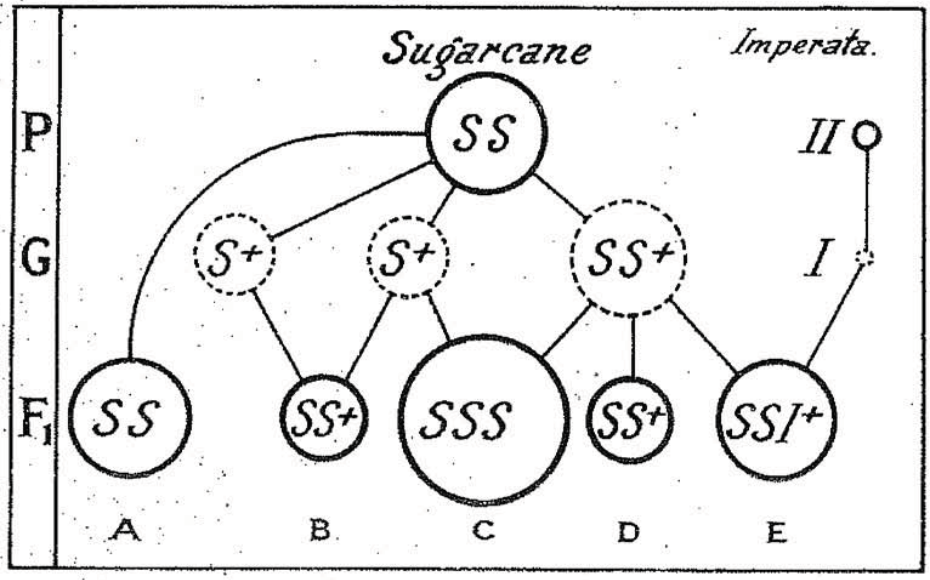


Fig. 13. Diagram of the genetic composition of seedlings from the cross POJ 2725 × *Imperata*.

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

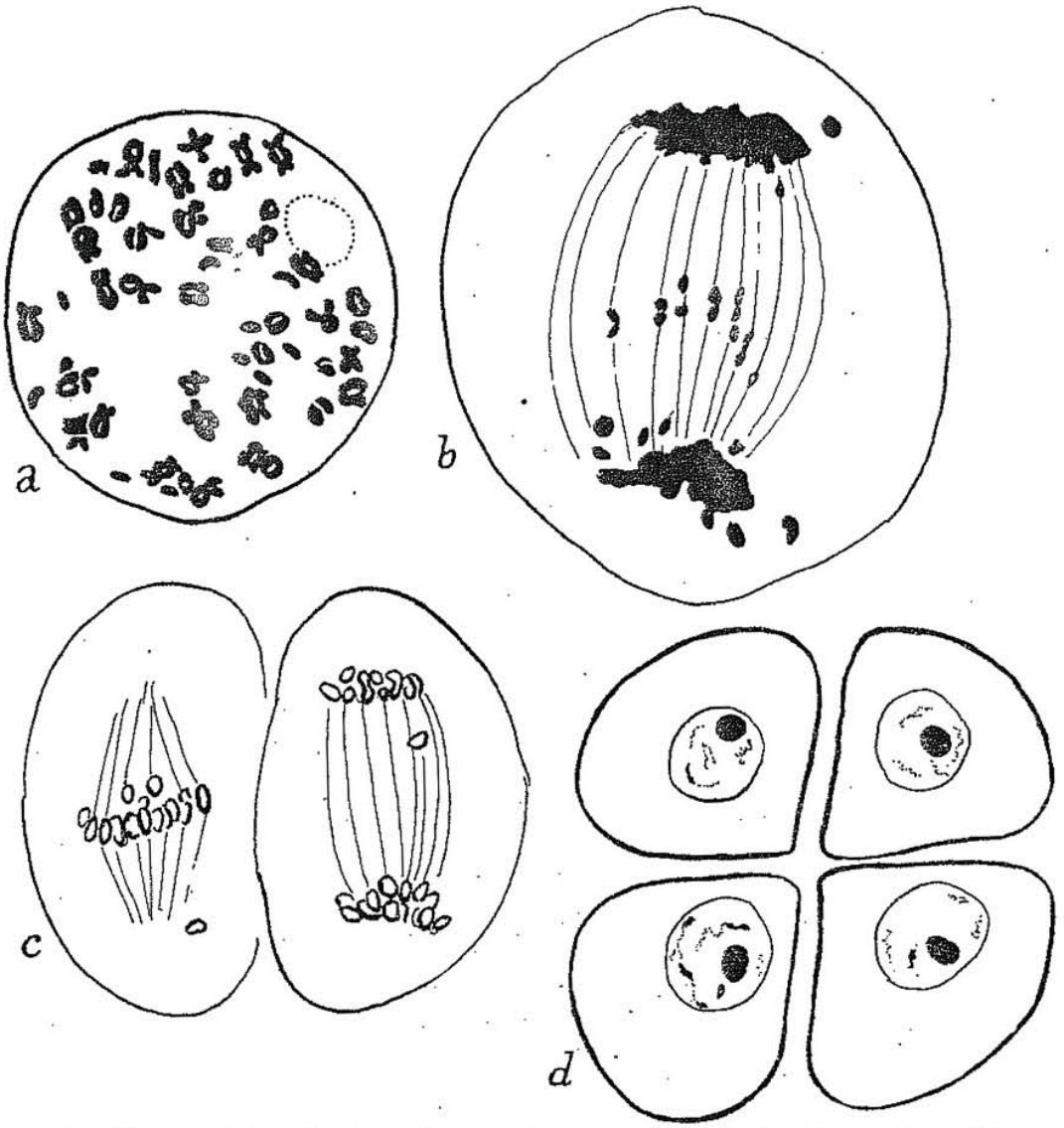


Fig. 14. Meiosis in a *Saccharum-Imperata* hybrid. *a*, diakinesis in pollen mother cell. *b*, first anaphase. *c*, second anaphase. *d*, tetrad formation. ×2000.

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,

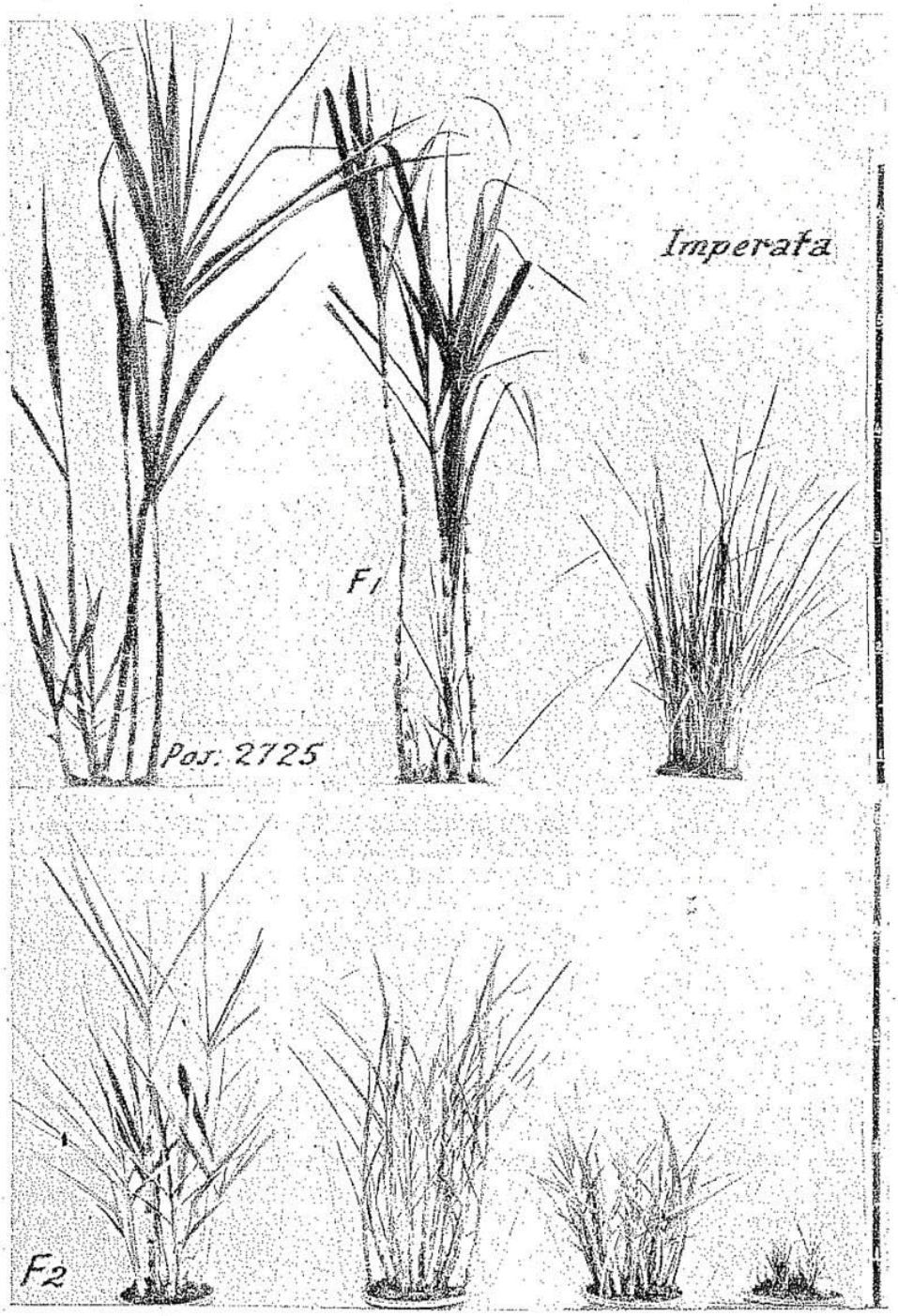


Fig. 15. General habit of POJ 2725, *Imperata cylindrica* and their F_1 and F_2 hybrids.

16-2

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.

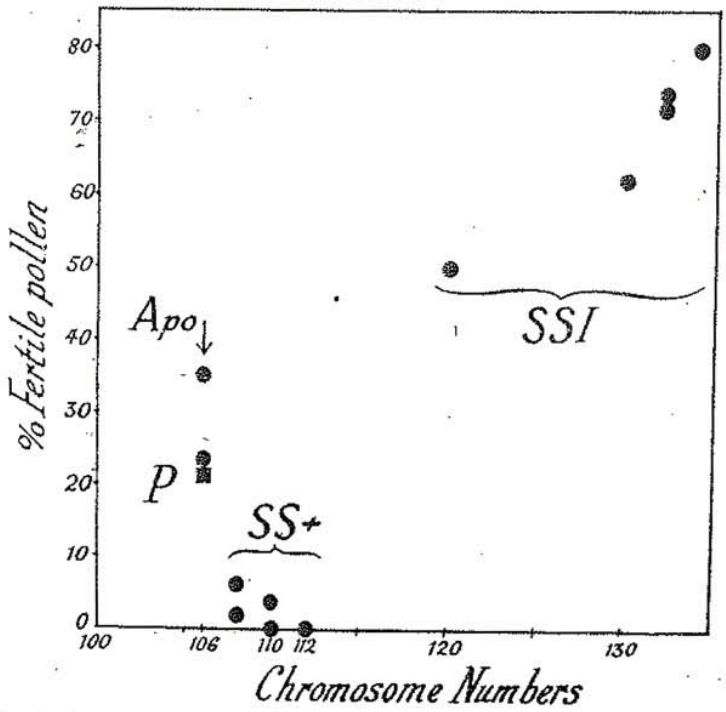


Fig. 16. Graph showing relative pollen fertility of POJ 2725 (*P*) and its apomictic (*Apo*), parthenogenetic or selfed (*SS+*), and hybrid (*SSI*) seedling.

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	17·6[illegible]	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.

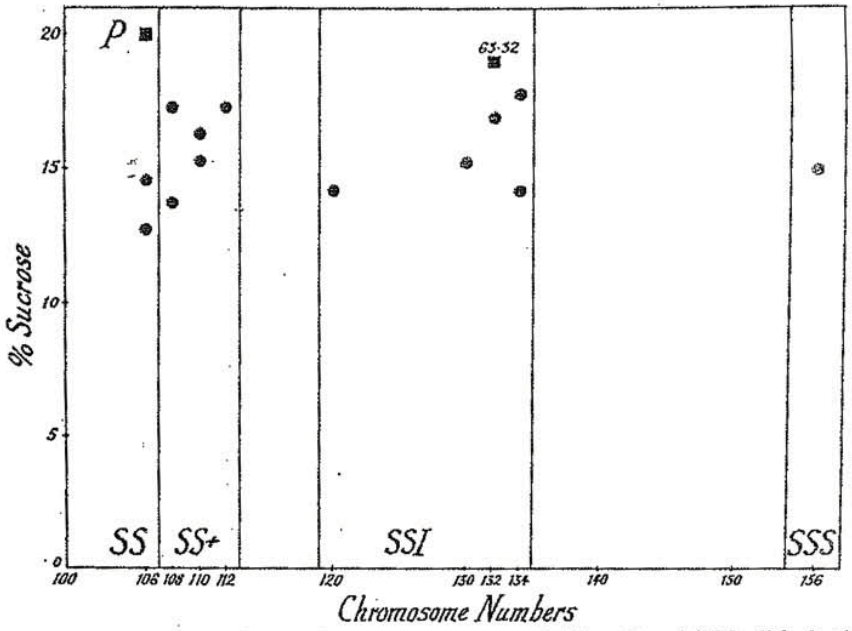


Fig. 17. Chromosome numbers and percentage sucrose in "true" and "false" hybrids of POJ 2725. The squares indicate maximum yield at full maturity, the circles yield of canes not yet fully matured.

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

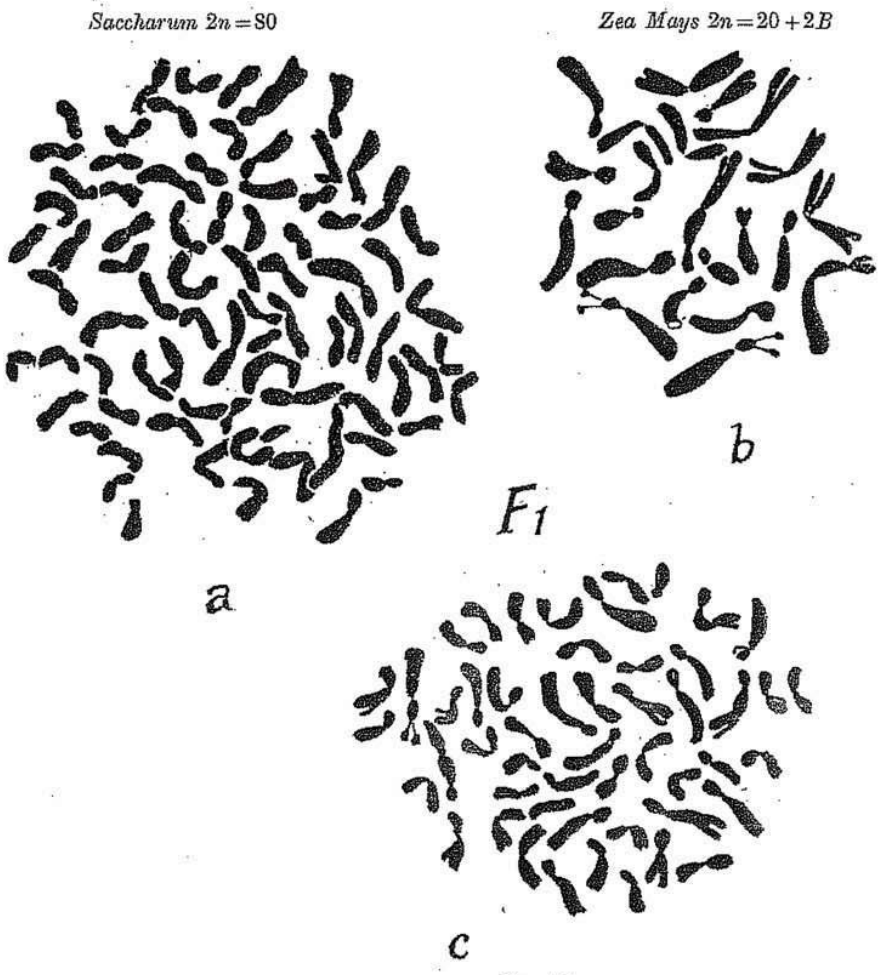


Fig. 18. Root tip metaphase in *a*, *Saccharum officinarum* Vellai; *b*, *Zea Mays* Golden Beauty; *c*, *Saccharum-Zea* hybrid. ×4000.

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

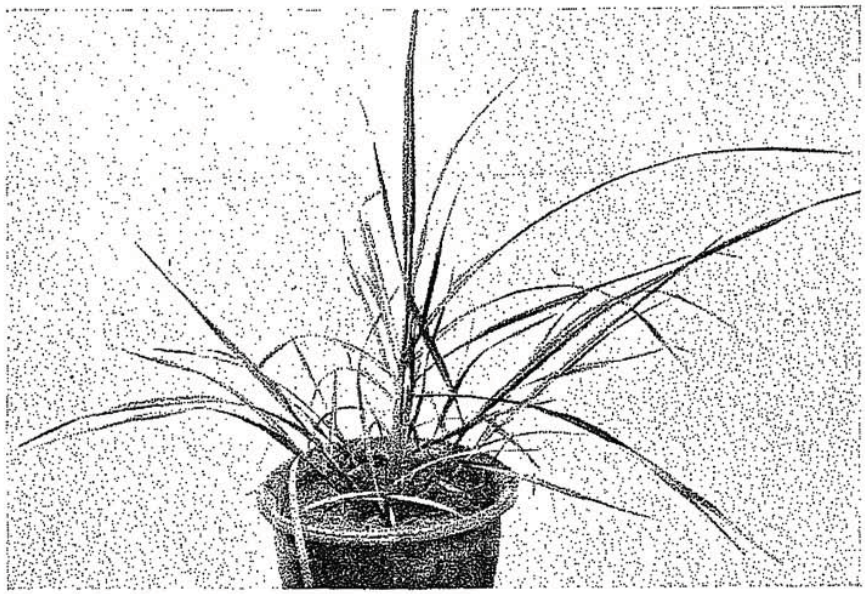


Fig. 19. *Saccharum-Zea* hybrid during second year of growth.

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

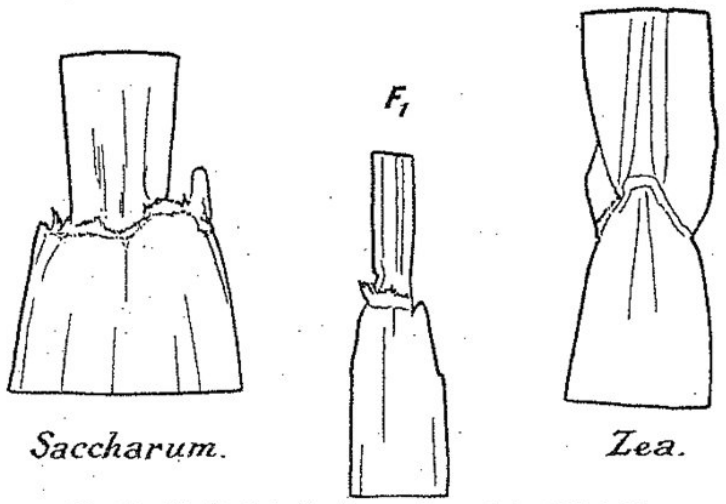


Fig. 20. The ligule in *Saccharum*, *Zea* and the F_1 hybrid.

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.

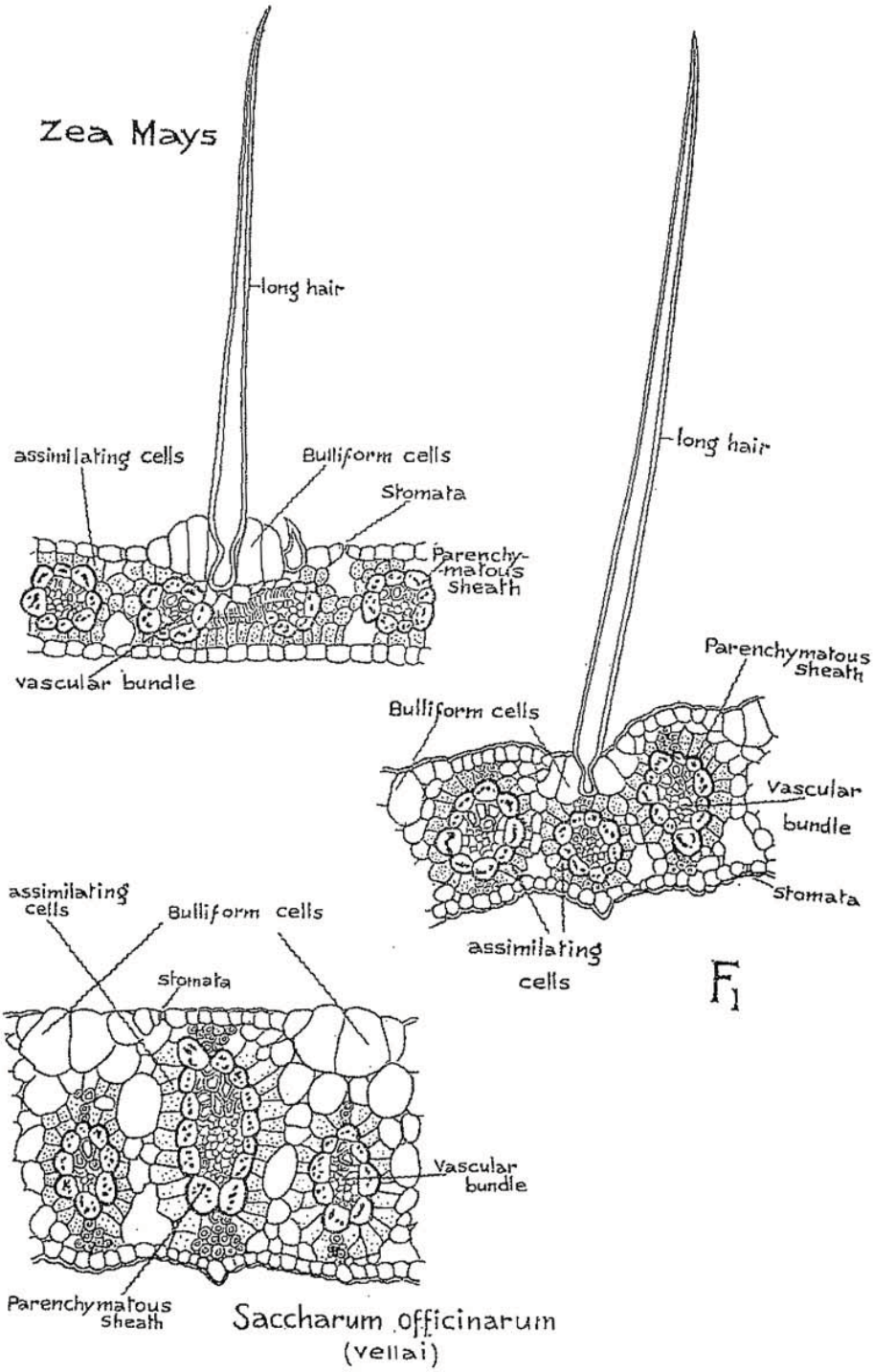


Fig. 21. Transverse section of leaf of *Saccharum*, *Zea* and the F_1 hybrid.

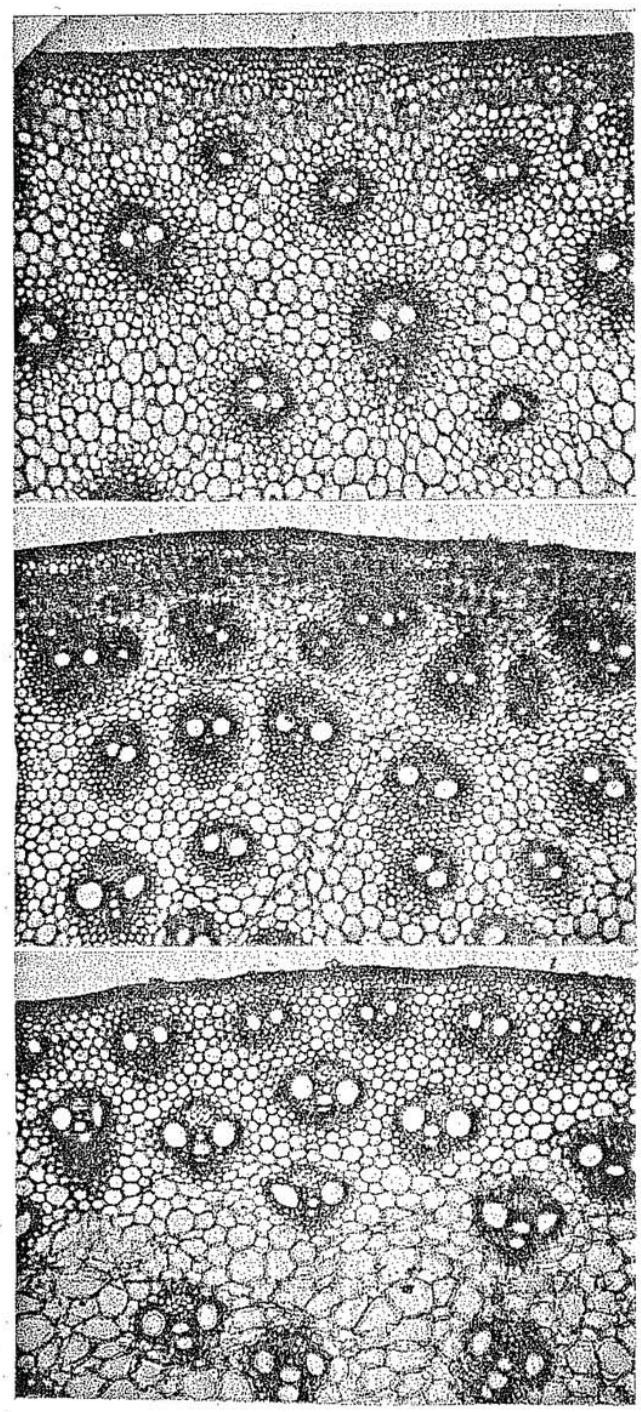


Fig. 22. Transverse section of internode of *Saccharum* (*S*), *Zea* (*Z*) and the F_1 hybrid.

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		2*n*	Cross reported by	F_1 Chromosome	F_1 Fertility	Chromosome numbers determined by
			I. *S. officinarum*, 2*n* = 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 + 2*B*	E.K.J. 1938 *a*	52	N.Fl.	E.K.J. 1938
			II. *S. spontaneum*			
7. Glagah	2*n* 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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REFERENCES

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

BEWS, J. W. (1929). *The World's Grasses*. London: Longmans.

BOURNE, B. A. (1935). "A comparative study of certain morphological characters of sugar cane × Sorgho hybrids." *J. agric. Res.* **50**, 538–51.

BRANDES, E. W. (1936). "Sugar cane, its origin and improvement." *U.S. Dep. Agric. Yearb.* 1936, pp. 561–611.

BREMER, G. (1923). "A cytological investigation of some species and species hybrids within the genus *Saccharum*." *Genetica*, **5**, 97–148, 273–326.

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

—— (1928). "De cytologie van het suikerriet. IV. Een cytologisch onderzoek der bastaarden tusschen Saccharum officinarum en Saccharum spontaneum." *Arch. Suikerind. Ned.-Ind.* pp. 565–696.

—— (1929). "The cytology of *Saccharum*." *Proc. 3rd Cong. Internat. Soc. Sugar Cane Technol.* (Sourabaya), pp. 408–15.

DARLINGTON, C. D. (1937). *Recent Advances in Cytology*. London: Churchill.

DEERR, N. (1921). *Cane Sugar*. London: Rodger.

DUTT, N. L. & SUBBA RAO, K. S. (1933). "Observations on the cytology of the sugar cane." *Ind. J. agric. Sci.* **3**, 37–56.

EARLE, F. S. (1928). *Sugar Cane and its Culture*. New York: Wiley.

JANAKI-AMMAL, E. K. (1936). "Cytogenetic analysis of *Saccharum spontaneum* chromosome studies in some Indian forms." *Ind. J. agric. Sci.* **6**, 1–8.

—— (1938*a*). "A *Saccharum-Zea* cross." *Nature, Lond.*, **142**, 618–19.

—— (1938*b*). "Chromosome behaviour in *S. spontaneum* × *Sorghum durra* hybrids." *Ind. Sci. Cong.* 1938 (Abstract).

—— (1938*c*). "Chromosome numbers in sugar cane × bamboo hybrids." *Nature, Lond.*, **141**, 925.

—— (1939). "Triplopolyploidy in *Saccharum spontaneum* L." *Curr. Sci.* **8**, 74–6.

—— (1936–9). *Reports on the work done under the scheme for research on the genetics of sugar cane*. Simla: Govt. India Press.

JANAKI-AMMAL, E. K. & SINGH, T. S. N. (1936). "A preliminary note on a new *Saccharum* × *Sorghum* hybrid." *Ind. J. agric. Sci.* **6**, 1105–6.

MCCLINTOCK, B. (1929). "A method of making acetocarmine smears permanent." *Stain Techn.* **4**, 53–6.

MANGELSDORF, P. C. & REEVES, R. G. (1932). "*Tripsacum-Zea* hybrids." *Proc. 6th Int. Genet. Cong., Ithaca*, **2**, 351.

—— —— (1939). "The origin of Indian Corn and its relatives." *Bull. Texas agric. Exp. Sta.* no. 574.

NARAYANASWAMY, S. (1940). "Megasporogenesis and the origin of triploidy in *Saccharum*." *Ind. J. agric. Sci.* (in the Press).

PETO, F. H. (1934). "The cytology of certain intergeneric hybrids between *Festuca* and *Lolium*." *J. Genet.* **28**, 113–53.

RUMKE, C. L. (1934). "*Saccharum-Erianthus* Bastaarden." *Arch. Suikerind. Ned.-Ind.* pp. 211–63.

SINGH, T. S. N. (1934). "Chromosome number in sugar cane × *Sorghum* hybrids." *Ind. J. agric. Sci.* **4**, 1050.

THOMAS, R. & VENKATRAMAN, T. S. (1930). "Sugar-*Sorghum* hybrids." *Agric. J. Ind.* **25**, 164.

VENKATRAMAN, T. S. (1937). "Sugar cane × bamboo hybrids." *Ind. J. agric. Sci.* **7**, 513.

—— (1938). "Hybridization in and with the genus *Saccharum* (its scientific and economic aspects)." *Pres. Address, 25th Ind. Sci. Cong., Calcutta*, pp. 1–18.

VENKATRAMAN, T. S. & THOMAS, R. (1930). "Brief note on sugar cane-*Sorghum* hybrids." *4th Cong. Internat. Soc. Sugar Cane Technol.*

VENKATRAMAN, T. S. (1935). "Report of the government sugarcane expert." *Sci. Rep. Imp. Inst. agric. Res. Pusa*, 1934–5.

Journal of Genetics 44:23-32 (1942)

INTERGENERIC HYBRIDS OF *SACCHARUM*

IV. *SACCHARUM-NARENGA*

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(With Seven Text-figures)

CONTENTS

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

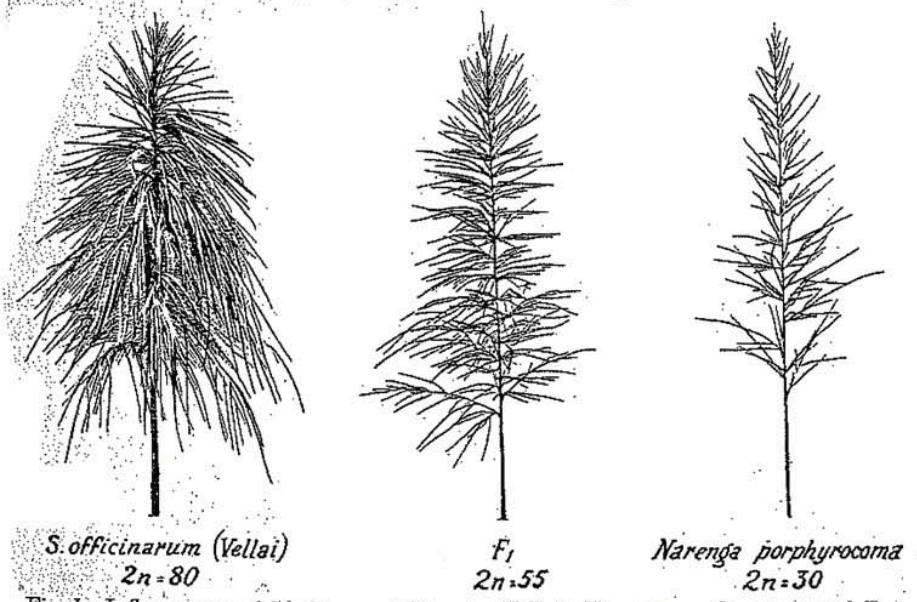

Fig. 1. Inflorescence of *Saccharum officinarum* (Vellai), *Narenga porphyrocoma* and F_1.

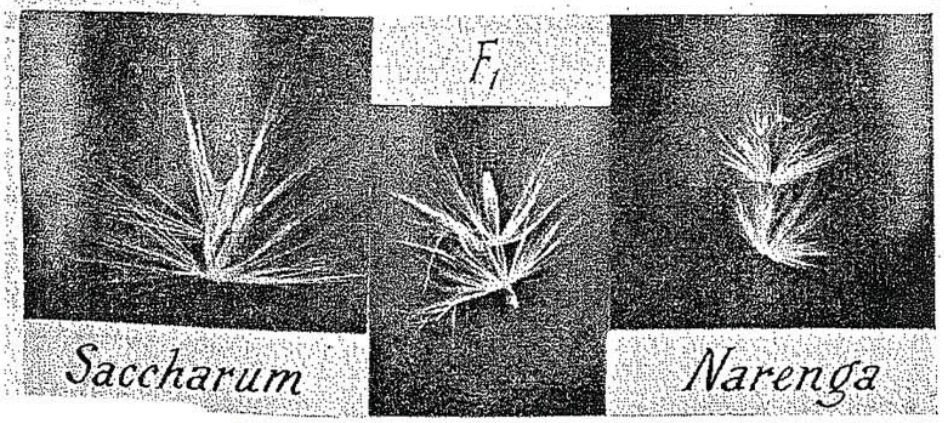

Fig. 2. Spikelets of *Saccharum*, *Narenga* and F_1.

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

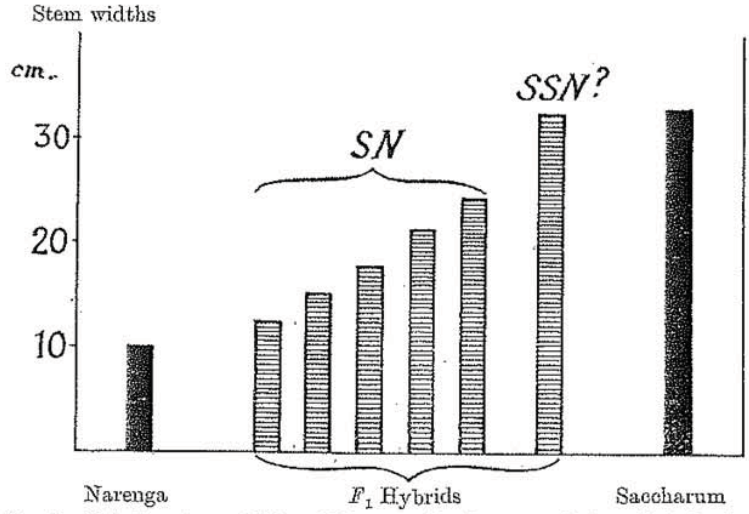

Fig. 3. Relative stem widths of *Narenga*, *Saccharum* and their F_1 hybrids (measurements after Barber).

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

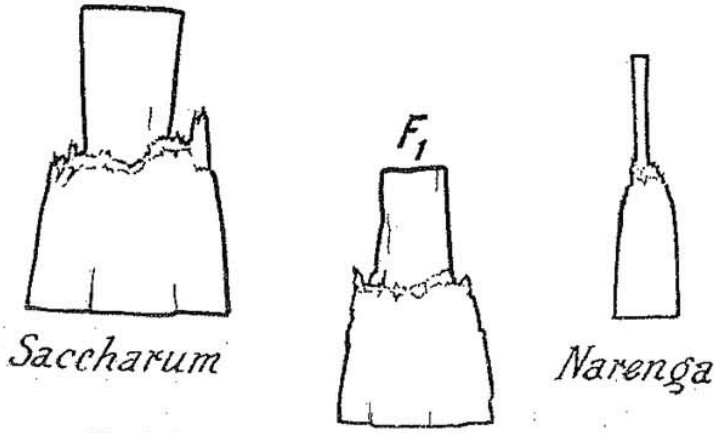

Fig. 4. The ligule in 'Vellai', *Narenga* and F_1 hybrid.

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.

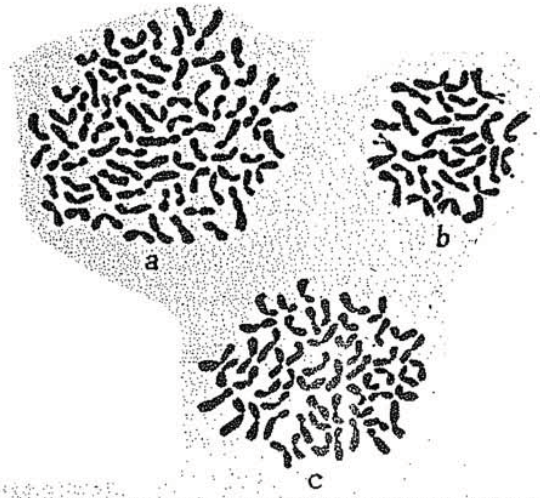

Fig. 5. Root-tip metaphase in (*a*) *Saccharum officinarum* (Vellai) ($2n=80$), (*b*) *Narenga porphyrocoma* ($2n=30$), (*c*) *Saccharum-Narenga* hybrid ($2n=55$). ×2000.

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

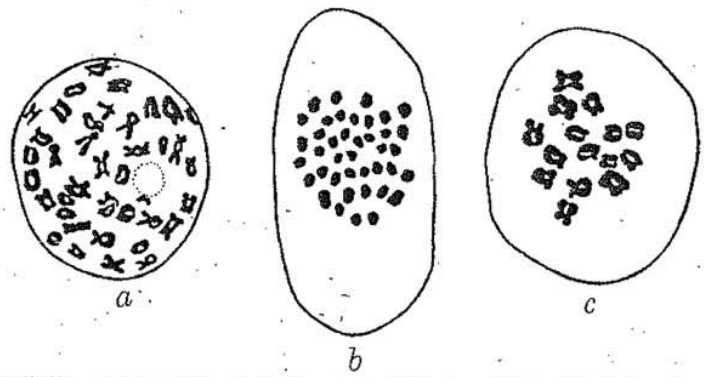

Fig. 6. (*a*) Pollen mother cells at diakinesis in Vellai. ×1800. (*b*) Polar view of metaphase II in pollen mother cells of Vellai. ×1800. (*c*) Prometaphase in pollen mother cells of *Narenga*. ×2000.

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.

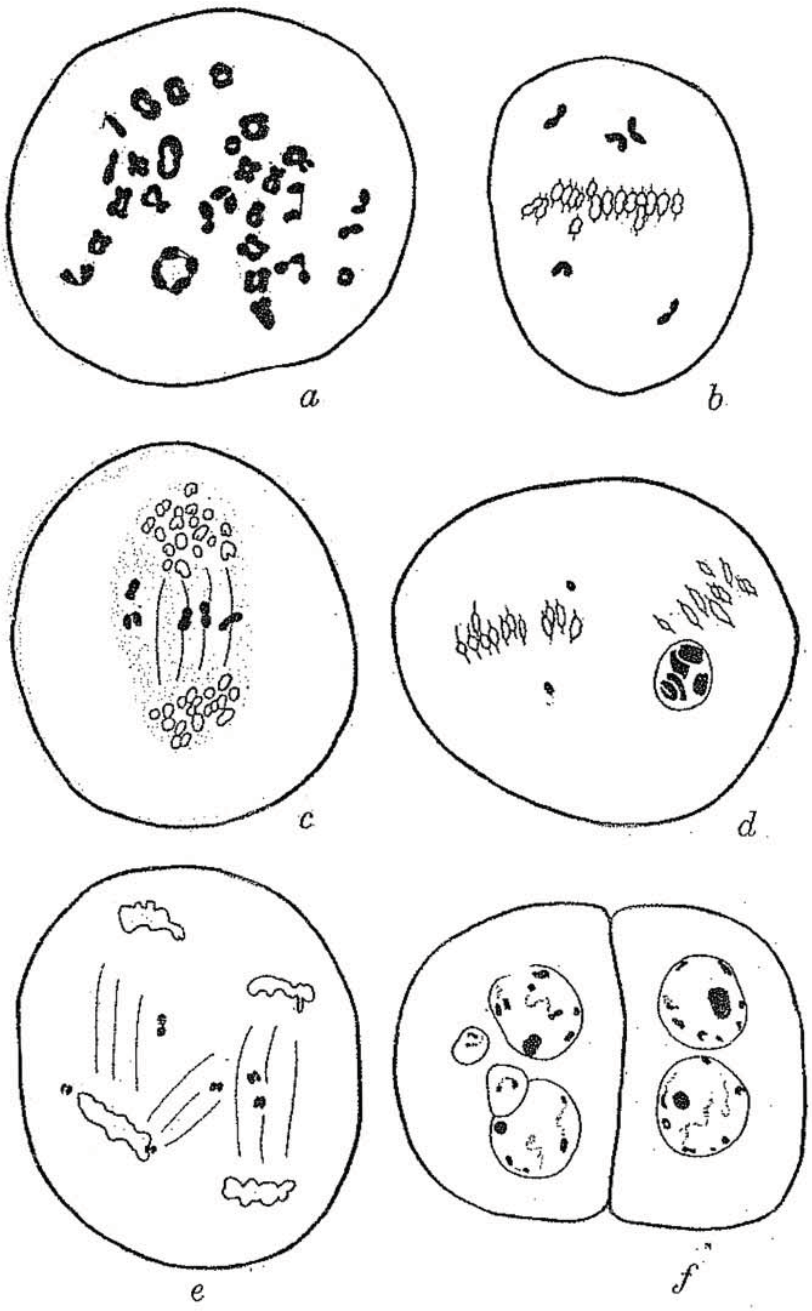

Fig. 7. Meiosis in *Saccharum-Narenga* hybrid. (*a*) Chromosome association at diakinesis in pollen mother cells. (*b*) Metaphase I showing position of univalents. (*c*) Telophase I with univalents at equator of plate. (*d*) Metaphase II with micronucleus of five univalents. (*e*) Telophase II with extra 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.

NOTES AND COMMENTS

FURTHER STUDIES IN SACCHARUM-ZEA HYBRID
I. MITOTIC STUDIES*

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1. Introduction

The first intergeneric hybrid in *Saccharum* was made in 1913 by C. S. Barber at Coimbatore. He crossed *S. officinarum* var. Vellai ($2n = 80$) with *Narenga porphyrocoma* ($2n = 30$) and obtained two types of hybrids. One ($2n = 95$) had a diploid gamete of *S. officinarum* ($2n = 80$). The other ($2n = 55$) had only the haploid gamete ($n = 40$).

The same variety of *S. officinarum* was pollinated with *Zea mays* var. Golden Beauty which had two additional B chromosomes, by E. K. Janaki Ammal in 1938. A single seedling was the outcome. It had 52 chromosomes, being the sum of the haploid complements of *Saccharum* ($n = 40$) and of Zea ($n = 10$) with the two B chromosomes of maize (Janaki Ammal, 1941). Tillering was most abundant and the plant which was clonally propagated survived 30 years but without producing any inflorescence. This failure to produce "arrows" was found to be due to the non-emergence of the inflorescence out of the boot leaf which when dissected was found to be lying within the flag leaf. Naidu and Ramakrishnan (1970) induced the inflorescence to emerge by the application of gibberelic acid and it was found to be similar to that of *Saccharum* (figs. 1 and 2). A fourth glume which does not occur in either parent was present in the hybrid, though some of the *S. officinarum* clones do have one. There were three stamens as in the parents.

2. Materials and methods

For the study of somatic chromosomes, root tips were treated with a saturated solution of α-bromonaphthalene for 2 hours at 10° C. They were washed in running water for 30 minutes and fixed in a fixative containing 60 ml. methanol, 30 ml. chloroform, 20 ml. distilled water, 1 g. mercuric chloride, and 1 g. picric acid for 18 to 24 hours. They were then hydrolysed in 1 NHCl for 13 minutes at 60° C., stained with leuco-basic fuchsin and squashed in 45 per cent. acetic acid. For anaphase studies root tips were fixed without pretreatment.

3. Somatic chromosomes

S. officinarum var. Vellai has 80 somatic chromosomes with median centromeres (fig. 3). There is one pair of satellited chromosomes. The

* Memoir No. 100 from the Centre for Advanced studies in Botany.

longest chromosome is about 3·20 microns in length (Jagathesan and Ratnambal, 1967).

Maize has eight chromosomes with sub-median and two (chromosomes 1 and 5) with median centromeres. They can be distinguished by the presence of knobs at pachytene (Rhoades, 1955).

In the hybrid we find the number of chromosomes varies in root tip cells even of one cutting. In 23 metaphase plates, the number varied from 52 to 58 (figs. 4 and 5). The frequency of distribution of these extra chromosomes is shown in table 1. By length we find that out of 56 chromosomes, 40 belong to *S. officinarum* and 16 (10A+6B) to *Zea mays*. All the B chromosomes of *Zea* could be identified in the somatic plate (fig. 5 indicated by arrow). Two types could be distinguished by length.

TABLE 1

Frequency of B chromosomes in 23 root tip cells of hybrid

No. of B chromosomes	2	3	4	5	6	7	8
No. of cells	1	2	4	4	8	—	4

Anaphase was seen in 133 cells and out of these 85 were normal. Others showed abnormalities such as bridges in 40 cells, fragments and lagging chromosomes. These abnormalities had disappeared by telophase, in 80 cells which were studied.

4. Discussion

The increase in chromosome number over the early observation is due to the accumulation of B chromosomes in the course of 30 years of purely vegetative propagation. The abnormalities observed at anaphase are presumably in the A chromosomes. Since the B chromosomes are of two kinds, misdivision seems also likely to be occurring.

5. Summary and conclusions

1. A hybrid between sugarcane and maize, perhaps the widest plant cross ever made, was raised in 1938. It had the expected 40+10 chromosomes and in addition two B chromosomes.

2. The hybrid was induced to open its inflorescence by gibberellin treatment in 1969 and 1970. The anthers, however, were sterile.

3. Growth was probably restricted by irregular mitosis. But the restriction may have favoured the accumulation of B chromosomes which rose in number to six or more in the course of the 30 years of vegetative propagation.

6. References

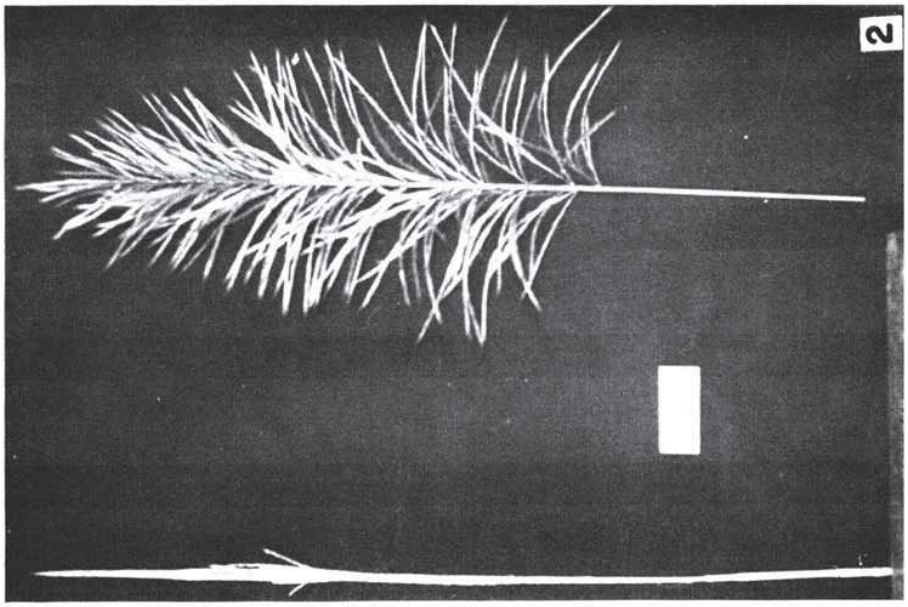
JANAKI AMMAL, E. K. 1941. Intergeneric hybrids of *Saccharum*. *J. Genetics*, *41*, 217-253.

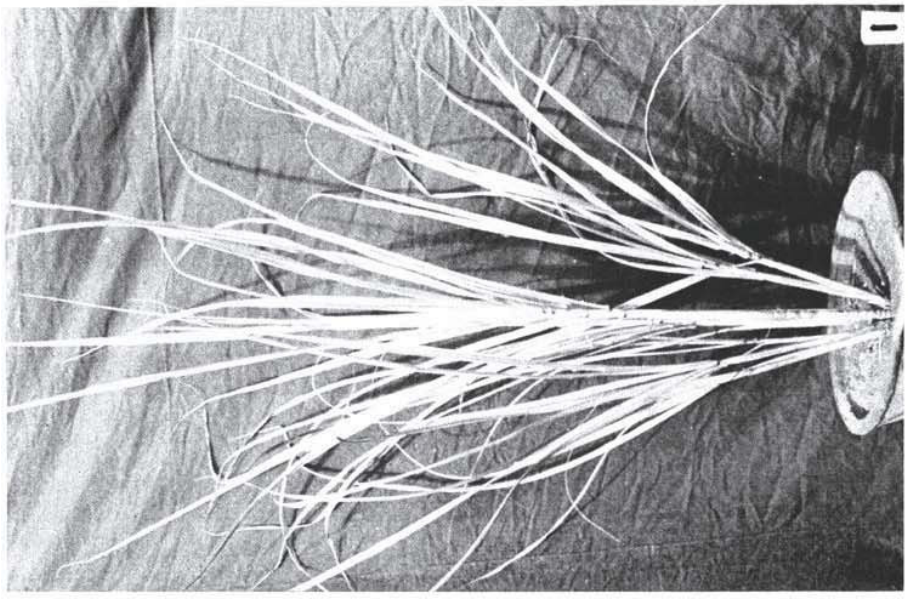
JAGATHESAN, D., AND RATNAMBAL, M. J. 1967. Karyotype analysis in *Saccharum officinarum*. *Nucleus*, *10*, 159-167.

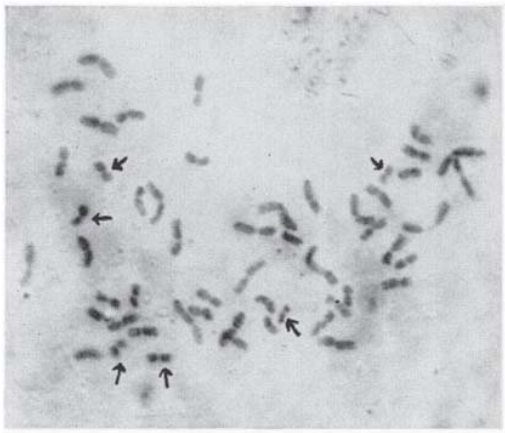
NAIDU, K. M., AND RAMAKRISHNAN, S. 1970. Flowering behaviour of sugarcane × maize hybrid and forcing its tassel emergence with gibberelic acid. *Indian J. Agri. Sci.*, *41*.

RHOADES, M. M. 1955. The cytogenetics of maize. *In Corn and Corn Improvement*, Ed. G. Sprague. Academic Press, New York.

Plate I







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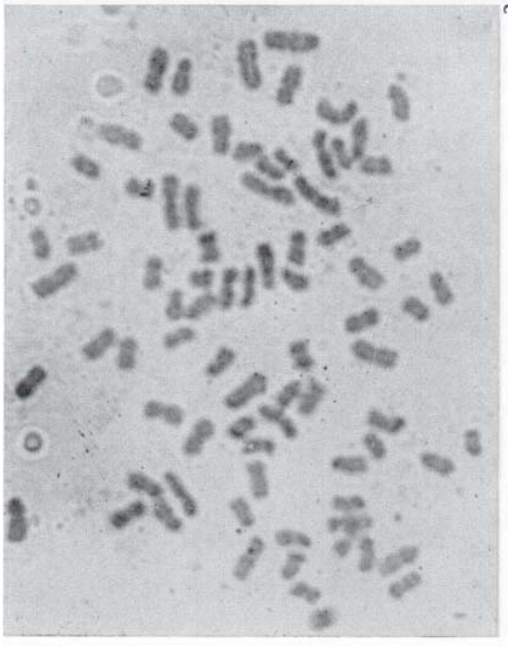

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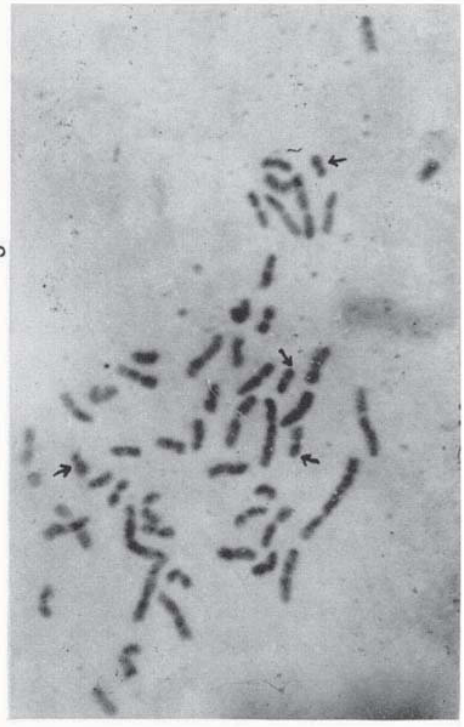

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FIG. 1.—*Saccharum* x *Zea* hybrid.

FIG. 2.—Inflorescences. *Left*: Enclosed in leaf sheath. *Right*: After emergence by gibberellic acid treatment. (Courtesy, Naidu and Ramakrishnan, 1970).

FIG. 3.—Root-tip squash of *S. officinarum* showing 80 chromosomes. ×4000.

FIG. 4.—Root-tip squash of hybrid showing 56 chromosomes (six B chromosomes arrow-marked). ×3000.

FIG. 5.—Root-tip squash of hybrid showing 54 chromosomes (four B chromosomes arrow-marked). ×4000.