Microsporogenesis of *Saccharum spontaneum* with Special Reference to Its Chromosome Number ¹⁾

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The cytological investigation reported in the present paper was undertaken with the main purpose of working out the details of meiosis in *Saccharum spontaneum* Linnaeus sub-species *indicum* Häckel, with special attention to its chromosome number. It was thought that a knowledge of its chromosome number might be useful in the solution of some of the practical problems arising in the experimental studies on the local sugarcane varieties.

Saccharum spontaneum subsp. *indicum* Häckel is widely distributed throughout the Philippines, and is commonly known as *talahib* in Tagalog, *bugang* in Visaya and *lidda* in Ilocano. It possesses many interesting and important features. It is a hardy and more or less tufted or gregarious type of grass and is very resistant to some common destructive diseases that usually attack the sugarcane. Apparently it can stand drought and flood.

The *Saccharum spontaneum* in Java, known as *glagah*, has played an important role during the last four decades in the extensive investigations of sugarcane in the Experimental Station at Pasoeroean. Several experiments have been conducted by various workers with the chief purpose to obtain a hybrid which in addition to high sugar content of the parents combine the dimension of the one and the resistance to common sugarcane diseases of the other. Among these prominent researchers on sugarcane may be mentioned H. Wakker, former Director of the Experimental Station at Pasoeroean, G. Wildbrink, J. Jeswiet and G. Bremer.

Numerous and repeated crossings have been made between the *glagah* in Java and *Saccharum officinarum*, and also their hybrids. From these crossings very complicated combinations have been obtained. In 1924, Jeswiet (5) by crossing 2364 POJ × EK28 obtained 2878 POJ and 2883 POJ. From these two generations, 2878 POJ has been considered the best. Bremer (5) states that this cane has strong, well developed roots and is not susceptible to yellow

1) Preliminary results read before the First Philippine Science Convention, March 15, 1932. Abstract Nat. and Applied Sci. Bull. Univ. of the Phil. 2. (1932) 211.

stripes-diseases, and is until now immune to sereh-diseases, and at the same time the weight of the cane and its yield are very high. The following diagram indicates the names of the ancestors of 2878 POJ and the outline of the different crossings made by Jeswiet:—

The above diagram was based upon the exhibit of the original preserved specimens of the *glagah* and different sugarcane varieties used by Jeswiet in the crossings and who arranged them in the form of a diagram on the laboratory table in the Arboretum and Institution for Plant Taxonomy at the University College of Agriculture at Wageningen, Holland, during the 6th International Botanical Congress at Amsterdam, September 2–7, 1935.

The chromosome number of *Saccharum spontaneum* was first reported in 1915 by Kuwada (7, 8). He indicated that both *S. spontaneum* and *S. officinarum* have 68 chromosomes in the diploid phase. Bremer (2, 3, 4) in his extensive investigation on *S. spontaneum* and *S. officinarum* published in 1924 and 1925, states that Kuwada has estimated the diploid number a little too low. He claims that *S. spontaneum* variety in Java always show 56 chromosomes in the haploid phase and a variety of *S. spontaneum* in Celebes has 40 chromosomes, and in *S. officinarum* he also found 40 chromosomes in the haploid phase and 80 in the diploid phase. Working on the chromosomes of the primitive forms and some species and species-hybrids of the genus *Saccharum* Bremer (2, 3, 4 and 5) obtained a great variation of their chromosome number. The list below shows the chromosome numbers of some species and species hybrids under the genus *Saccharum* determined by Bremer (2, 3, 4, & 5).

<i>Saccharum spontaneum</i>	<i>n</i>		<i>n</i>
Glagah alas Soekapoera	56	Glagah alas Soemberpoetih ...	56
Glagah alas Troeno.....	56	Glagah alas Sempol	56
Glagah alas Djatiroto	56	Glagah alas Soembawa	56
Glagah alas Kloet	56	Glagah alas Kepandjen	56
		Glagah Tabongo (Celebes)	40

<i>Saccharum officinarum</i>	<i>n</i>		<i>n</i>
Green German New Guinea ..	40	I 1072	68
Ardjoeno	40	I 1078	68
Batjan	40	I 1079	68
Black Borneo	40	I 1080	68
Black Cheribon	40	I 1086	68
Fidji	40	K 1527	68
Bandjarmassin hitam	40	2775 POJ	68
Sampang A	40	2722 POJ	54
247 B	40		
EK 28	40	<i>S. officinarum</i> × Kassoer	
SW 3	40	1807 POJ	74
		2191 POJ	74
		2222 POJ	74
<i>Saccharum arundinaceum</i>			
Tananggé	30	100 POJ × Kassoer	
<i>S. munja</i>	30	2323	75
<i>S. ciliare</i>	20	2354 POJ	75
<i>S. narenga</i>	15	2364 POJ	75
Hitam Rokan	55/2		
Labaina	40	Bandjarmassin hitam × Loethers	
Loethers	99/2	100 POJ	45
British-Indian cane varieties		<i>S. officinarum</i> × Chunnee	
{ Chunnee	± 46	36 POJ	62
{ Ruckree	± 46	213 POJ	62
{ Katha	± 46		
<i>Saccharum spontaneum</i> × <i>S. officinarum</i>		2722 POJ × EK 28	
Kossoer	68	2940 POJ	48
G 101	68		
G 107	68	2875 POJ × EK 28	
I 1052	68	2944 POJ	60
I 1056	68		
I 1060	68	2364 POJ × EK 28	
I 1070	68	2878 POJ	60
		2883 POJ	57

It is quite interesting, therefore, to find the chromosome number of the Philippine variety of *Saccharum spontaneum*.

Material and Methods

Three sets of material collected in different years and from different places were used in the study. The first set was collected at the pregnant stage during the later part of August, 1928, in the neighborhood of Manila. This material upon sectioning and microscopic examination showed mostly premeiotic stages including synzesis and open spireme. The second set consists of three portions, one of which was gathered at Del Carmen, Pampanga Province at about the end of August, 1929; another portion was collected at Canlubang, Laguna province, about the beginning of the first week of September of the same year; and the rest was gathered at Manila during the middle part of the same week in which the second portion was col-

lected. All three portions were collected during the "flag" stage. Of this material more advanced stages were obtained, including the open spireme, second contraction, diakinetic stages and some beautiful phases of the metaphase. The third set was collected at Manila about the end of August, 1931, two days after the beginning of the "flag" stage. This material yielded not only various premeiotic, diakinetic, and metaphasic stages, but also different phases of the second division of the pollen mother cells.

The material was fixed between 9 and 10 o'clock in the morning and between 2 and 3 o'clock in the afternoon. The material collected from the provinces of Pampanga and Laguna was fixed in the field; while part of the material collected at Manila was fixed in the field and part was fixed in the laboratory.

The method employed in the field fixation consists of removing the protecting leaf sheath of the inflorescence and taking some lateral axes at various heights from it. These lateral axes are put into the mouth and after partially sucking the air from between the glumes and a more or less thoroughly wetting them with saliva, they are cut into segments and placed into the various vials containing the fixing fluids. Similar lateral axes were taken at various heights of the same inflorescence, but into segments and put directly into the vials containing the fixing fluid. In the laboratory this untreated material was subjected to the vacuum pump in order to remove the air from the glumes and from the tissue. The material fixed in the laboratory was fixed in the same manner as that of the field, the only difference being that the portion of the plants with the inflorescence were cut off and placed in a bottle containing water and brought into the laboratory. Furthermore, the material placed directly into the vials containing the fixative were immediately subjected to the vacuum pump in order to remove the air from the glumes and from the tissue.

The fixatives used were (a) strong Flemming's fluid; (b) 1 per cent chrom-acetic acid, with about ten drops of 1 per cent osmic acid to 50 c.c. of the solution; (c) weak Flemming's fluid, and (d) Carnoy's fluid. The first two gave the best results, while the last two gave fair results. The material fixed in Carnoy's fluid showed marked contraction or shrinkage.

The material was allowed to stay in the different fixatives for about 24 hours, and then washed in the usual way, dehydrated, and infiltrated, and embedded in paraffin with a melting point of 65°C. However, some of the spikelets were dissected under the dissecting microscope and deprived of their glumes before they were dehydrated and embedded, while the others were embedded whole. The flowers treated with saliva came out as good as those subjected to the vacuum

pump. The sections were made from 6 to 15 μ in thickness and stained with Heidenhain's iron-alum haematoxylin, in some cases contrasted with Bismark brown.

The chromosome counts as well as the drawings, were all made with the aid of the camera lucida, under the Spencer 1.5 mm. N.A. 1.30 apochromate objective in combination with Carl Zeiss Orthoscop-ocular 28 $\times$, giving a magnification of 5,500 diameters. The critical examination of the spireme and chromosomes was made with Carl Zeiss K20, which gives a clearer definition, although it has a slightly smaller magnification than the Orthoscop-ocular 28 $\times$. The drawings were reduced to one-half in reproduction.

Observation

Archisporial phase. The very young anthers of *Saccharum spontaneum* var. *indicum* in cross section appear somewhat closely arranged in triangular form surrounded by three glumes and two paleae. At the stage following the last somatic division in the sporogenous tissue, each anther shows four distinct lobes consisting of two pairs of young microsporangia. The wall of the microsporangium consists of four layers of cells; namely, (a) the *epidermis* or the outermost layer; (b) the *endothecium* or the outer parietal layer; (c) the inner parietal layer, which becomes obliterated during the later stages; (d) the *tapetum*, or the jacket of nourishing cells which are in immediate contact with the sporogenous tissue. Within the tapetum the pollen mother cells are located. The pollen mother cells during the early stages are wedge shaped or polygonal in outline and are arranged in a very compact form with distinct large nuclei and extremely dense protoplasm, and they vary in number from 3 to 7. Their outer wall or sides are in contact with the tapetal cell. In the longitudinal section the sporogenous tissue appears as two columns closely fitted into one another and bordered by the tapetal cells. As the microsporangium grows larger and the pollen mother cells increase in size and advance to spireme stages, a central lacuna is developed. The angular edges or polygonal outline of the pollen mother cells gradually assume a rounded or elliptic form, but they remain in the peripheral side in the form of a ring with their outer side touching the tapetal cells.

The tapetal cells are present during all the stages of the reduction division as a ring of tangentially elongated cells, which line the locules of the center. During the early synizetic stages of the mother cells, the nuclei of the tapetal cells divide and each cell becomes binucleate. These tapetal binucleate cells remain in this condition until

the mother cell wall is ruptured, when they disintegrate and disappear.

The anther opposite the outer glume matures slightly ahead of the anthers at the two lateral sides and the pollen mother cells in the ventral locules or microsporangia apparently mature slightly ahead of those in the dorsal locules, but the pollen mother cells in a locule are in the same stage of meiosis. The difference is notable during the later stages of the post synizesis. This interesting feature makes possible critical observation and more accurate interpretation of the nature and behavior of the chromatic structure of the nucleus during the pre- and post-synizetic stages.

Pre-synizesis. The microsporocytes of *Saccharum spontaneum*, like those of *Elodea* (12, 13) and *Cocos* (14), just before they pass into prophase, are more or less uniform in general appearance and structure. They are fitted closely into one another and their protoplasts are angular. The cytoplasm is very dense, granular in structure, and takes a deeper stain uniformly. The nucleus is large, rounded, measures from 0.0075 to 0.0108 millimeter in diameter, and has a definite nuclear membrane. In the nuclear cavity are extended irregularly very fine delicate granular chromatin threads. These chromatin threads are connected with each other by numerous extremely fine fibers, making the whole chromatic structure appear as a network spread all over the nuclear cavity. At the point of attachment of the connecting fibers the chromatin granules are usually larger than those between the intersections. Generally there is only one large rounded nucleolus measuring from 0.0018 to 0.0028 millimeter in diameter and it lies at one side or at the center of the nuclear cavity; but in some cases there are two or sometimes even three. When there are two or three nucleoli present in a nucleus, their sizes are proportionately reduced. During this stage which is usually of short duration, the reticulum is somewhat evenly distributed in the nuclear cavity. The beaded chromatic threads run or radiate in various directions with no trace of regular parallelism. Fig. 1 represents a nucleus at the stage just before the manifestation of synizesis, and fig. 2 shows a tangential section of a nucleus at the same stage.

Synizesis. This interesting feature of the microsporocyte during the first stage of meiotic division, as usual, is manifested by the gradual rearrangement, condensation, and separation of the chromatin threads from the nuclear membrane and the growth of the nucleus. The rearrangement of the chromatin threads apparently is due either to the dissolution of the fine connecting fibers or to their contraction

bringing closer some portions of the threads more or less in a parallel arrangement. This phase is illustrated in fig. 3. As the nuclear changes proceed, the chromatin threads become irregularly thickened. The granules come closer together by contraction of the threads, and they increase in size either by fusion with the granules from the other or same threads and they take a deeper stain. Some of the meshes become bigger and others become smaller and disappear, so that the reticulum acquires a ragged appearance. This is followed by the gradual withdrawal or separation of the reticulum from the nuclear wall; this generally takes place at one side of the nucleus, but in some cases it starts almost all around the nuclear membrane as represented in fig. 4. As the contraction advances steadily, the nucleus grows in size from 0.014 to 0.017 millimeter in diameter (figs. 5 and 6). The condensation proceeds until all the strands of the reticulum are drawn into a compact and characteristic mass or knot at one side of the nucleus (fig. 7). The chromatin structure at this stage is highly complicated. A critical examination of a tangential section of the synizetic knot and the few small loops that surround it reveals that the chromatin granules are brought close together by condensation. As in *Cocos nucifera*, described by the writer (14), the granules are arranged in single rows in some places and in two rows running side by side in other places. This observation is confirmed by the later stage as shown in fig. 8, in which the thread or spireme appears somewhat distinctly as a single and continuous thread. The nucleolus is either entirely or partially entangled with the contracted chromatin reticulum, but usually is only partly covered and located at one side of the synizetic knot toward the center of the nuclear cavity. Up to this stage there is no material change in the structure of the nucleolus except that it has grown from 0.004 to 0.0065 millimeter in diameter.

Post-synizesis. Subsequent to the long and critical period of the synizetic condition in which a certain complicated rearrangements of the chromatin threads has taken place, the nucleus passes slowly into another interesting phase. The first significant indication that this important event is about to take place is the apparent reduction of the number of the chromatin threads and the formation of small loops at the sides of the synizetic knot. A closer examination of the chromatin mass at this stage, which previously was in a very compact form, reveals that as a whole there is a general loosening movement, or expanding of its entire structure, and that a more definite, coarser, and continuous filament or spireme is developed. Apparently there is a general movement throughout the entire length of its structure,

Figs. 1-10. *Saccharum spontaneum* subsp. *indicum* Häckel. 1. A typical resting nucleus of the pollen mother cell, showing the more or less uniform reticulum in a single nucleolus. 2. A tangential section of a resting nucleus, showing the characteristic indicated in fig. 1. 3. An early stages of a heterotypic prophase in which the chromatin threads become irregularly thickened and in some cases slight parallelism is observed. 4. An early stage of the synizetic contraction, showing the withdrawal or separation of the reticulum from the nuclear membrane and thickening of the chromatin threads. 5. A more definite stage of an early synizesis, showing the contraction of the reticulum in which the two nucleoli are inclosed. 6. A more advanced synizetic contraction indicating the massing or aggregation of the reticulum and the growth of the nuclear cavity. 7. A typical synizesis. The threads and the masses are collected into a close mass. 8. The loosening of the spireme from the synizetic knot. 9. A more advanced stage of the loosening of the spireme, showing the loops extending from the synizetic knot to the nuclear membrane. 10. spireme. $\times 2700$.

resulting in the formation of beautiful loops at different sides of the knot. A careful observation of the spireme shows that there is a marked shortening and condensation, in which the chromatin granules were brought very close together and fused. It is more or less uniform in diameter and in some portions exhibits doubleness. This double nature of the spireme has been found to be constant among the various microsporocytes at the same stage, but unlike that of *Elodea* (12, 13), in each portion it can be traced for a short distance only. Free ends are seldom observed, as in the case of some tangential sections of the nucleus in which part of the chromatin mass is included. The free ends are evidently cut ends. Fig. 8 is a typical representation while the formation of the loops or loosening of the spireme is in progress.

As the loops increase in number and slowly spread themselves throughout the nuclear cavity, the nucleolus usually becomes gradually released from the entangled thread system, as shown in fig. 9. Fig. 10 illustrates a more-advanced stage known as open spireme. Even at this stage there is no material or visible change observed in the structure of the nucleolus. As usual it is rounded and takes deep stain uniformly. The spireme is rearranged in a series of irregular loops extending throughout the nucleus. It appears more uniform in diameter, although in some places there are nodal enlargements. It is distinctly thicker and shorter. It does not show any splitting, except in some portions where there is slight indication of doubleness. Similar to the condition found by the writer (14) in *Cocos nucifera* all the evidence indicates that the chromatin granules from a thin thread reticulum are brought closer and closer until they form a thick spireme by synizesis. This consists largely in the contraction of certain threads and absorption of their contents into the body of other threads, a process which involves occasional parallelism.

Considering the frequency of the above stages, from the time the spireme comes out from the synizetic stage, it is quite obvious that the loosening of the spireme is of a long duration. Melbourn (9) states that the loosening of the spireme in wheat occupies a much longer period of time than does the contraction of the reticulum to form the synizetic knot.

Second contraction. Early stages preparatory to this next interesting phase of the meiosis are shown in figs. 11 and 12. A marked condensation, shortening, and thickening of the spireme can be observed. The loops are beginning to rearrange themselves in a very irregular manner, and the sides of some of them are brought close to one another. The spireme, which was previously more or less

uniform in structure, now shows notable variations in diameter. It shows conspicuous attenuation at various alternating regions.

As the general condensation and shortening of the spireme filament advance slowly and steadily, some of the loops are gradually detached by partial segmentation and these become entangled with the other portions of the spireme and collected usually at the sides of the nucleolus. Figs. 13 and 14, represent the initial stages in which the loops are becoming entangled with each other and gradually collected either at one, two, or three sides of the nucleolus.

The two arms or sides of the loops become crooked and sometimes the sides of a given loop twist around each other. A critical study of the chromatin segments at this stage revealed that they are unsplit, and that occasionally there is a constriction at the distal end of the loop. This characteristic is one of the indications that each side of each loop represents a univalent chromosome. While the collecting of the filaments is in progress, the transverse segmentation is completed. Figs. 15 and 16 represent the character of the second contraction in *Saccharum spontaneum*. After the examination of a considerable number of sections the writer failed to find a typical second contraction. In most cases each segment is partly entangled only with two or more loops, and the arms or sides of each loop either twist around each other or lie closely side by side. The individual segments cannot be counted accurately, but there is a distinct reduction in the number of the filaments. This type of second contraction is not unusual. Grégoire (6) states that it may or may not occur in *Lilium speciosum*. According to Overton (11), it may occur in plants which possess long chromosomes and it seems not to occur in plants with short chromosomes. Recently Beal (1) failed also to find a typical second contraction in cotton and he agrees with Overton (11) that it may occur in plants with long chromosomes. Grégoire (6), Overton (11), Beal (1) and some other followers of the parasynapsis theory believe that second contraction has neither special significance nor any importance in this phase. However, since there is no strong evidence as to the double nature or splitting of the spireme up to the formation of the loop segments in *Saccharum spontaneum*, it seems that the only plausible explanation is that the univalent chromosomes have conjugated end to end and that the two members of each pair are brought to a side-by-side position by the second contraction as clearly indicated in fig. 16. It was observed that a large proportion of the folded members of each pair remained closely connected at the original point of conjugation. It is evident therefore, if the true significance of synapsis is the conjugation of homologous elements, as suggested by Moore (10), then the second contraction in *Sac-*

Figs. 11-16. *Saccharum spontaneum* subsp. *indicum* Häckel. 11. An early stage of the second contraction or synapsis, showing the irregular distribution of the loops. 12. An older stage leading to the second contraction, showing the segmentation of the spireme or separation of individual loops. 13. A more definite stage of the beginning of the second contraction. The loops are beginning to be entangled with one another. 14. An advance stage of the second contraction or synapsis. The individual segments or loops are aggregating irregularly, at least in the four sides in the nuclear cavity of the mother cells. 15. A typical second contraction. 16. Another stage of a second contraction showing the pairing of the two members of each loop. $\times 2700$.

charum spontaneum must be a case of synapsis, while the first contraction is synizesis. In fact, according to Sharp (15), it is now customary to refer to chromosome conjugation as "*synapsis*" and to contraction, natural or otherwise, as "*synizesis*."

Diakinesis. Following the brief period of aggregation of the chromatin material and the completion of the segmentation of the spireme, the folded segments are gradually dispersed throughout the nuclear vacuole. Fig. 17 exhibits the characteristic redistribution of the segments in the nuclear cavity. The chromatin segments are shown in varying stages of evolution, some are still entangled with one another, while the others are more or less separated. The original loops, although reduced in size, can be traced. Their sides are still slender and irregularly thickened, some are crooked and crossed over each other or twisted around each other, and some are lying either intimately or loosely side-by-side. Fig. 18 illustrates a more advanced stage, in which the chromatin segments are distinctly shorter, denser, and somewhat thicker in diameter. However, they still exhibit a ragged appearance due to a marked attenuation at certain alternating regions, which results in lumped, heavy-staining node-like masses with alternating thinner and more slightly staining regions in each segment. The members of each folded segment are now mostly observed lying side-by-side more intimately. The writer failed to observe a phenomenon similar to that in *Cocos nucifera* (14) where the sides of most of the folded segments untwist far apart, resulting in the formation of various configuration, namely, J's, O's, X's, Y's, U's and V's. A careful examination shows that the sides of the folded segments, which represent the sides of the original loops, are unsplit and remain closely attached, parallel with each other until further condensations and shortening has taken place. This clearly indicates that each side of each loop represents a univalent chromosome, which has been joined end to end to its mate during the formation of the spireme.

Soon after the young bivalent chromosomes are more or less evenly distributed in the nuclear vacuole, they undergo a more rapid condensation, shortening and thickening and they assume a position close to the nuclear membrane. This stage is represented in fig. 19a, a median section and 19b, a tangential section, both of which are from the same nucleus. Most of the bivalent chromosomes clearly show their double origin and exhibit various stages of evolution. In many instances the chromosomes, which have formed the bivalent elements of each pair, are closely appressed to one another and in some cases they are still somewhat loosely arranged side-by-side, connected partly

near their middle portion, and still, in a few cases, are V-shaped. Generally the paired chromosomes appear more uniform in structure, with deeper stain, similar to two short and straight or bent closely

Figs. 17-21. *Saccharum spontaneum* subsp. *indicum* Häckel. 17. An early diakenesis stage showing the bivalent segments dispersed in the nuclear cavity after the second contraction. 18. A slightly more advanced diakenesis stage showing the young bivalent chromosomes. 19a. A diakenesis stage showing the peripheral distribution of highly condensed bivalent chromosomes. 19b. A superficial view of a diakinesis stage, a portion of the mother cell shown in fig. 19a. 20. A side view of an early metaphase showing the migration of the bivalent chromosomes toward the equatorial region. 21. polar view of a metaphase stage with 40 chromosomes. $\times 2700$.

appressed rods, but, in some cases, the univalent members are still attenuated and unevenly stained and have ragged characteristics. Through further condensation they become nearly ellipsoidal or globular and the univalent halves of each pair become so intimately applied to each other that their double nature is almost indistinguishable.

From the early to the later stages of diakinesis the chromosome number was carefully considered. During the early stages while the bivalent chromosomes are still slender, it is almost impossible to determine their number, because of too much overlapping. Very often two or more of the young bivalent chromosomes closely adhere to the nucleolus. Moreover, they cannot all be seen in one plane or focus. In the later stages several attempts were made, but since the chromosomes are mostly in the peripheral sides of the nuclear vacuole, similar difficulty was encountered. The number obtained in these countings varies from 37 to 41 and sometimes even 44 or 45. This may be accounted for by the loose arrangement of some of the chromosome elements at this stage, presumably some of them were counted twice or taken as two overlapping chromosomes. A satisfactory result was obtained when the chromosomes were collecting in a heap preparatory to the heterotypic metaphase, in which they are more or less far-apart and have acquired definite form. Furthermore, they can almost all be placed in one focus with a little manipulation only. Numerous counts varying from 40 to 50, were made separately from the different sections and from the preparations of the material collected from Del Carmen, Canlubang and Manila. The number obtained from the different preparations was always 40. This number is exactly the same as with the haploid chromosome number of *Saccharum spontaneum* of Celebes and *officinarum*, both reported by Bremer (2 to 4).

During the later diakinetik stages there is a notable decrease in the size of the nucleolus as well as of the nuclear cavity. The nucleolar content seems to have been greatly reduced and its affinity to stains is considerably lessened. The nuclear vacuole becomes somewhat ovoid, and the nuclear membrane changes from a definite and smooth structure to an irregular and granular one. The cytoplasm around the nuclear membrane becomes denser, which is followed by the simultaneous disappearance of the nucleolus and the nuclear membrane. Subsequent to the dissolution of the nuclear membrane very fine granulated fibers begin to emerge from three or more sides of the nucleus and form multipolar spindles. These spindles become attached to definite parts of the bivalent chromosomes, which at this stage are arranging themselves in a heap.

Heterotypic mitosis. The transitional period from the late diakinesis to the multipolar condition and from this to the bipolar stage is of a very short duration only; for while these stages are frequently met in some preparations, they are not found in great number. Immediately after the formation of the multipolar spindle fibers, these become rearranged into bipolar spindle. Fig. 20 shows the spindle fibers stretching from one side to the other of the microsporocyte. They have lost their granular characters, and toward the upper part, their ends have already concentrated into one pole, while on the lower side they are still grouped into two. The bivalent chromosomes, which have now reached the maximum condensation, appear as globular or diamond-shaped masses almost uniform in size, and their double nature is not conspicuous. They are migrating to the equatorial region preparatory to the metaphase stage. While the chromosomes seem not to reach the region at the same time, the heterotypic division does not take place until they are all arranged in one plane and in a somewhat symmetrical form.

Fig. 21 represents a polar view just at the time when the bivalent chromosomes have reached the equatorial region. Similar stages are indicated on figs. 23 and 24. With but a little manipulation almost all the chromosomes can be seen simultaneously. They are either rounded or ovoid and sometimes more or less angular. Their double nature is very difficult to detect. The two univalent chromosome elements forming them are so appressed together that they cannot be traced, except in a comparatively few instances in which a very faint sign of splitting is noted. This is the most favorable stage for the accurate determination of the chromosome number in *Saccharum spontaneum*. for at this stage the bivalent chromosomes, aside from having a definite shape or outline, are still far apart and there is only a little overlapping. The several counts made at this stage from the different collections all gave 40 chromosomes.

The chromosomes viewed from the sides at metaphase appear in a straight line. At this stage it is absolutely impossible to establish any count, because the inner chromosomes are almost completely covered by those of the upper portion. Fig. 22, represents a typical metaphase stage of *Saccharum spontaneum*. All the visible bivalent chromosomes show that they are splitting simultaneously. The two members that form each of the bivalent chromosomes are either attached at, or close to, their ends by small bundles of spindle fibers. Their free posterior ends, which are still united, appear as a thickening with darker stain.

Shortly after the separation of the daughter chromosomes, they move away from the equatorial region and migrate toward the poles.

During this stage they acquire either a J or a V shape and become partially condensed and contracted. As soon as the chromosomes

Fig. 22-29. *Saccharum spontaneum* subsp. *indicum* Häckel. 22, a side view of a definite metaphase stages; 23 and 24, two polar views of metaphase stages each of which showing 40 chromosomes; 25, late anaphase or early telophase of heterotypic mitosis showing the aggregation of the daughter chromosomes at the two poles; 26, a late telophase showing the formation of the nuclear membrane; 27 and 28, two successive stages of a daughter nuclei showing the distribution of the daughter chromosomes; and 29, a polar view of the daughter chromosomes at the metaphase stage of the homoeotypic mitosis. $\times 2700$.

reach the corresponding poles, they form a compact structure, which becomes surrounded by a thin membrane and by cytoplasm. They are somewhat homogeneous in structure and exhibit no sign of splitting. Fig. 25, shows how the daughter chromosomes are assembled as they reach the poles. The fig. 26 represents a more-advanced stage, showing that after the massing of the chromosomes at the poles they become surrounded by a thin membrane and cytoplasm followed by the formation of a clear space around them.

Upon the development of the nuclear membrane and eventually the formation of the nuclear vacuole, the daughter chromosomes loosen themselves and become longer and thinner. The end of one chromosome becomes connected with the end of another chromosome by means of fine strands until they are all connected with one another. As the nucleus continues to grow in size the daughter chromosomes, which now have a ragged appearance, are evenly distributed throughout the nuclear cavity (fig. 27). A small nucleolus suddenly appears between the chromatin masses (fig. 28).

During the interkinesis no distinct cell wall is developed between the daughter nuclei as observed in *Saccharum spontaneum* (Glagah alas Djatiroto) by Bremer (2). The cell wall separating the two daughter nuclei becomes conspicuous only after the homoeotypic mitosis has taken place, in which the granddaughter nuclei are fully developed (fig. 33).

Homoeotypic mitosis. As usual the daughter nuclei following the hetero-typic division do not pass into a typical resting condition. The daughter chromosomes, which are connected by strands, become somewhat drawn out into two or three parts. Immediately after the chromatin material is evenly distributed in the nuclear vacuole and the daughter nucleus has grown in size, they become reorganized into chromosomes, while the nucleolus and the nuclear membrane disappear simultaneously. The young chromosomes pass into a rapid condensation until they become more or less ellipsoidal or globular in shape. Subsequently these condensed chromosomes are attached by the spindle fibers and arranged in one plane at the equatorial region as pictured in polar view on fig. 29. Although the univalent chromosomes are comparatively smaller in size at this stage and assembled almost in compact form, in several counts made from different sections, always gave the number 40. Evidently the haploid number of the chromosomes, of *Saccharum spontaneum* var. *indicum* of the Philippines is 40. The spindle fibers of the two sets of chromosomes as usual may lie parallel or at right angles to each other, or in few cases in a V-shaped position. Fig. 30 shows a case in which the

axes of the two daughter nuclear spindles cross one another at right angles and, as a result of this crossing, one plate is observed in side view, while the other plate shows the polar view. The latter shows distinctly 40 chromosomes. On the same fig. 31 represents a case with the axes of the nuclear spindles more or less in V-shaped arrangement. The axes of these two nuclear spindles, however, might have been lying parallel to one another, but because of the splitting of one side of the cytoplasm, they become V-shaped.

The chromosomes are mostly attached close to the end and divide

Figs. 30-34. *Saccharum spontaneum* subsp. *indicum* Häckel. 30, the two daughter nuclei at the metaphase stage, one in the side view and the other in the polar view; 31, side views of the daughter nuclei at metaphase; 32, a typical homoeotypic anaphase of a daughter nucleus; 33, two young granddaughter nuclei, showing the disintegrating granddaughter chromosomes; and 34, a resting nucleus of a young pollen grain. $\times 2700$.

simultaneously. A typical anaphase of one of the daughter nuclei is pictured on fig. 32. The two sets of granddaughter chromosomes are seen in perfectly straight lines at right angles to the axes of the spindle fibers. The individual chromosomes are, as a whole, V-shaped. As in the telophase in the first division the chromosomes at the corresponding poles draw together in a mass. Subsequent to this massing they become surrounded by a dense cytoplasm and the nuclear membrane is developed. Upon the formation of the nuclear membrane the chromosomes, which are now connected to each other by strands, loosen themselves and are distributed throughout the nuclear cavity (fig. 33). While the chromosomes undergo the process of alveolization, the cell walls between the four granddaughter nuclei are formed. Fig. 34 shows a detail picture of the nucleus of a young pollen grain.

Discussion of Results

The reduction division of *Saccharum spontaneum* Linnaeus subsp. *indicum* Häckel from the Philippines is quite normal. At the diakinesis stage all the gemini are distinctly doubled or paired in character and are mostly crowded in the peripheral part of the nuclear cavity. This makes it very difficult to determine accurately their number at this stage. Several chromosome counts made at early diakinesis stage always gave variable results, ranging from 37 to 41, and in some instances even 44. This may be accounted for by the greater overlapping and besides since the members of each bivalent chromosome are not yet intimately joined, each of some of them may have been counted as two. A constant number was obtained during the later stages of diakinesis from the polar view, at early metaphase in which the bivalent chromosomes have already attained a more definite form and they are arranged in a single plane at the equatorial region preparatory to the heterotypic division. At this stage as shown in fig. 21 and figs. 23 and 24, the chromosomes are somewhat far apart and are seen practically in the same focus. They appear more or less globular or diamond-shaped and have almost the same size. The several counts made at this stage from different microsporocytes of the material collected from Del Carmen, Canlubang, and in Manila, have always given 40 chromosomes.

The 40 gemini, in the side view, appear in a straight line across the spindle fibers. The heterotypic division proceeds in a perfectly normal manner. There is no lagging or other irregularity. The daughter chromosomes are practically all uniform in size and shape. They move away from the equatorial region and reach the corresponding poles simultaneously. The same chromosome number was ob-

tained in the different counts made at the polar views of the different metaphase stages examined just before the homoeotypic division takes place.

The chromosome number of the Philippine variety of *Saccharum spontaneum*, therefore, can be safely put as 40 in the haploid phase, a chromosome number exactly the same as that of *Saccharum spontaneum* or *Glagah Tabongo* in Celebes and also the same as the chromosome number of *S. officinarum*, both of which are reported by Bremer (4). In fact the results obtained from this investigation are perfectly in accord with the conclusion arrived at by Bremer (4), who, after determining the chromosome number of the glagah-variety of Celebes writes as follows:

"The fact that a glagah-variety occurs in Celebes with 40 chromosomes in the haploid phase, which moreover has a normal reduction division, in connection with the fact that Kuwada's investigation makes it very probable that in Japan also glagah with 40 chromosomes exists, which possibly, are distributed over islands to the north of Java, such as Celebes, the Philippines, Formosa and Japan"

In view of these facts, the writer is of the same opinion as Bremer (4), that Kuwada (7), who reported 68 chromosomes as the diploid number of the Japanese variety of *Saccharum spontaneum*, based upon a somatic count, has estimated the diploid number rather too low. Furthermore, the univalent chromosomes during the somatic division are not only greatly elongated but generally closely arranged, and therefore there is a greater degree of overlapping among the chromosomes, and the counts made at this phase are usually unreliable. Thus, the determination of the chromosome number of the Philippine variety makes it more evident that the Japanese *Saccharum* variety must have 40 chromosomes in the haploid phase and 80 instead of 68 in the diploid phase.

Summary

1. The chromatin reticulum is granular in character and is more or less evenly spread out in the nuclear vacuole.
2. The chromatin threads during the heterotypic prophase radiate in various directions and there is no regular parallelism.
3. The synizesis is due to the gradual contraction of the reticulum and growth of the nuclear cavity.
4. The spireme formed during the synizetic contraction is doubled in some places only and single in the other portions.
5. During the loosening of the spireme from the synizetic condition beautiful loops, more or less equal to the gametic number, are formed.

6. Following the distribution of the spireme in the form of loops in the nuclear cavity and the separation of the individual loop by segmentation, the second contraction or synapsis takes place.

7. The pairing of the two arms of the loops, representing the two univalent chromosomes is evident.

8. The nuclear membrane and the nucleolus disappear simultaneously, while the multipolar spindles suddenly appear.

9. The number of the chromosomes in the haploid phase is 40.

10. The bivalent chromosomes are more or less the same in shape and size.

11. The heterotypic division takes place in a normal manner, no lagging or other irregularity was observed.

12. The daughter nuclei do not pass into resting stage as usual but pass directly into a normal homoeotypic division, forming the four daughter nuclei of the pollen grains.

Literature Cited

1. Beal, S. M. A study of the heterotypic prophases in the microsporogenesis of cotton. *La Cellule* 38, (1928) 247-263.
2. Bremer, G. A cytological investigation of some species and species hybrids within the genus *Saccharum*. *Genetica* 5 (1923) 97-148 and 273-326.
3. — The cytology of the sugarcane. *Genetica* 6, (1924) 497-525.
4. — The cytology of the sugarcane. *Genetica* 7, (1925) 293-322.
5. — De cytologie van het suikerriet 4^e bijdrage. *Archief voor de suikerindustrie in Nederlandsch-Indië*. 36^{ste}, 3^e deel Mededeeling. van het Proefstat. voor de Java-suikerindust. No. 11, (1928) 565-696.
6. Grégoire, V. La formation des gemini hétérotypiques dans les végétaux. *La Cellule*, 24, (1907) 369-420.
7. Kuwada, Yoshinari. Über die Chromosomenszahl von *Zea Mays* L. *Bot. Mag. (Tokyo)*, 29 (1915) 87.
8. — Die chromosomenzahl von *Zea Mays* L. *Journ. Coll. Sci., Imp. Univ. Tokyo* 39, Art. 10 (1919) 35.
9. Melbourn, M. C. Heterotypic prophases in the absence of chromosome pairing. *Canadian Journ. Res.* 1, (1929) 512-527.
10. Moore, J. E. S. *Quar. Jour. Micr. Sci.* 38, (1896) 275-314.
11. Overton, J. B. On the organization of the nuclei in the pollen mother cells of certain plants, with especial reference to the permanence of the chromosomes. *Ann. of Bot.* 23, (1909) 51-52.
12. Santos, J. K. Differentiation among chromosomes in *Elodea*. *Bot. Gaz.* 75, (1923) 42-47.
13. — Determination of sex in *Elodea*. *Bot. Gaz.* 77, (1924) 353-371.
14. — A cytological study of *Cocos nucifera* Linnaeus. *Phil. Journ. Sci.* 37, (1928) 417-433.
15. Sharp, L. W. *An Introduction to Cytology*, 2nd edition (1926) 277.