

CYTOLOGICAL STUDIES OF SUGARCANE AND ITS RELATIVES:

XVII. Trigeneric hybrids of *Saccharum officinarum* L., *Sclerostachya fusca* A. Camus, and *Miscanthus japonicus* Anderss.⁽¹⁾

H. W. LI, T. S. WENG, K. C. SHANG and P. C. YANG⁽²⁾

In our previous paper (Li *et al.*, 1959) clone SG 310/6 which was a cross of G 3662×*Sclerostachya fusca*, was found to have 72 chromosomes. G 3662 in turn was a cross of *S. officinarum* var. Vellai×*S. fusca*. This was found to have 55 chromosomes, of which 40 came from the female parent and 5 from the male (Shang *et al.*, 1960). By back-crossing SG 310/6 to the recurrent parent *Sclerostachya*, SG 363/4 was obtained which was found to have 50 chromosomes.

Sugarcane can be crossed with *Miscanthus japonicus* (Li *et al.*, 1948) and it can be crossed also with *Sclerostachya*. However, crossing involving *Miscanthus* and *Sclerostachya* has never been made. It would be interesting to find out whether or not such a cross is feasible by using sugarcane as an intermediary. If successful, the relationship of these genera would be revealed by their chromosomal behavior in the hybrid. Fortunately, two hybrid plants were obtained by crossing SG 310/6 with *M. japonicus*. The detailed cytological studies are reported in this paper.

Material and Method

There were 18 seedlings obtained from the cross SG 310/6×*M. japonicus*. Thirteen of them were fixed and studied. All but two, 58-1 and 58-2, were found to be selfs of SG 310/6 which had about 70% pollen fertility. These selfed plants had about 70 chromosomes and were further identified by the absence of awns on their fertile lemmas. Whereas, the two true hybrids, in addition to having a different chromosome number as will be described later, had awned fertile lemma.

Thirteen seedlings were obtained from the cross SG 363/4×*M. japonicus*, but all of them were found to be selfs.

Acetocarmine method was used throughout this experiment.

(1) Contribution from Institute of Botany, Academia Sinica, Taiwan, China.

(2) Director of Institute of Botany, Research Assistant of Institute of Botany, Senior Plant Breeder of Taiwan Sugar Experiment Station, and Assistant.

Experimental Results

Hybrid 58-2

Hybrid 58-2 was a cross of SG 310/6 ($2n=72, 42(O)+30(S)$) \times *M. japonicus* ($2n=38(M)$) and was found to have 55 chromosomes, 21 of which derived from *Saccharum*, 15 from *Sclerostachya* and 19 from *Miscanthus*. As was mentioned before, the fertile lemma of hybrid 58-2 was awned (Plate II). This character was shared by the male parent *M. japonicus* in effecting this cross as well as the other hybrid 58-1 (Plate II). It is apparent therefore that the awned lemma character is dominant. It was also true that both paired spikelets were pediceled, the same for *M. japonicus* and 58-1 (Plate II). Whereas in *Saccharum*, *Sclerostachya*, and BC_1 hybrids of them, one spikelet was pediceled and the other sessile. Again, as in the case of awned lemma, pediceled spikelet is dominant.

The chromosome association at MI of this hybrid plant is shown in Table 1.

Table 1. Chromosome association at MI of hybrid 58-2.

Chromosome association							Frequency	Chromosome association							Frequency
VII	VI	V	IV	III	II	I		VII	VI	V	IV	III	II	I	
			1	1	20	8	1				2	1	13	18	1
			1	1	22	8	2				1	1	14	18	1
				3	18	10	1			1	1	1	15	18	5
			1	1	19	10	4				1	1	17	18	8
				1	21	10	1	1			2	2	12	18	1
			1	2	17	11	1				2	2	11	19	1
			1		20	11	3			1	1	1	14	19	1
					22	11	7			1		2	15	19	2
			1	1	18	12	3				1		16	19	5
				1	20	12	5				1		18	19	16
			1	4	13	13	1				1	1	14	20	5
			1	2	16	13	2				1	1	16	20	9
				2	18	13	1				3		11	21	1
			1		19	13	1				2		14	21	1
					21	13	7				1		15	21	1
			1	3	14	14	1						17	21	12
			2	1	15	14	1					1	15	22	4
			1	1	17	14	1					2	13	23	1
				1	19	14	8				1		14	23	1
			1	2	15	15	2						16	23	10
				2	17	15	4				2	1	10	24	1
			1		18	15	9				1	1	12	24	1
					20	15	13					1	14	24	2
			1	3	13	16	1						15	25	3
				3	15	16	1					1	13	26	2
			1	1	16	16	3						14	27	1
				1	18	16	15				1	1	10	28	1
			1	2	14	17	1				1		12	28	1
			2		15	17	4						13	29	1
			1		17	17	5								
					19	17	21								
			1	3	12	18	1								
Total													230		

There were altogether 230 cells studied. The chromosome association was mostly tetra-, tri-, bi-, and univalents. Very infrequently did pentavalents occur. Only once was a heptavalent observed. The frequency of each type of association was found to be very much varied and diversified. There was no way of

determining which would be the most predominant type. The average per cell of tetra-, tri-, bi-, and univalents were 0.34, 0.60, 17.28, and 17.19 respectively. It went without saying that $19^{II}+17^I$ was the modal class. In one cell, the highest number of bivalents found was 22, and the lowest 13 (Table 1). The highest number of tetravalents found in one cell was 3, and that of trivalents 4. For the univalents, the highest number in one cell found was 29, and the lowest 8. The significance of all these will be discussed later.

Types of Bivalents:

As can be seen from Plate III, Figs. 1 and 2, and Plate IV, Figs. 4 and 5, there were two kinds of bivalents found, i. e., closed and open. Of the open type, there were unequal ones, dissimilar either in size mostly, or form or both. The average of these types from the study of 140 sporocytes is shown in Table 2.

Table 2. Frequency of different types of bivalents found in 140 sporocytes (multivalents are not included)

		Average number of bivalents	
		17.53	
		Closed	Open
Average number		12.87	4.66
%		73.40	26.60
		26.60	
		Equal	Unequal
Average number		2.83	1.83
%		60.80	39.20

The average number of bivalents in these 140 cells studied was 17.53. Of these, roughly 3/4 belonged to the closed type, and the remaining 1/4 was of open type.

The open type bivalents as a rule divided precociously at MI ahead of the closed ones. This was particularly true with the unequal bivalents (Plate IV, Figs. 1, 2, and 3). It was found that in the early prophase stages the unequal bivalents behaved differently from the equal bivalents. At leptonema (Plate IV, Fig. 6) the end with unequal length did not even come close to one another. At pachynema, singleness was evident at the unequal end. Otherwise, close synapsis and doubleness were observed for the other end (Plate IV, Fig. 6b). The number of chiasmata seemed to be greatly decreased in the case of the unequal bivalent at diplonema (Plate III, Fig. 6c). However, there was no actual count of the number of chiasmata per chromosome belonging to different types. At diakinesis (Plate IV, Fig. 6e), terminalization of these unequal bivalents seemed to be rather complete as compared with the closed chromosome on the right. As a result, precocity in division was readily explainable.

Multivalents:

Trivalents and tetravalents found in different cells were drawn as shown in

Plate IV, Figs. 2 and 3. It was evident that some of these multivalents were composed of unlike partners. In the case of trivalents, the partnership might have been constituted by 2 *Saccharum* chromosomes and either a *Sclerostachya* chromosome or a *Miscanthus* chromosome. Sometimes, the three partners might consist of chromosomes of all three genera.

In the case of tetravalents, the partners might have come from two genera, or else, chromosomes of all three genera might be involved in constituting the tetravalent.

Univalents:

The univalents behaved typically as in other hybrids. At MI they were scattered all over the cell. Either they grouped themselves at the nearest pole after the separation of the bivalents and multivalents, or they congressed at the equator to divide themselves or to pass to the poles at random. In a few cases observed, however, the univalents shown in outlines (Plate IV, Fig. 3) were at the polar regions ahead of the bivalents and multivalents which congressed at the equator yet to be divided. Whether or not these univalents would come back to the equator to divide or to be redistributed, nothing has been known definitely about this.

Hybrid 58-1

Hybrid 58-1 was the sister plant of the cross SG 310/5 × *M. japonicus*. It was found to have 106 somatic chromosomes. Morphologically it behaved exactly like its sister hybrid plant 58-2 with awned lemma and pediceled spikelets (Plate II, Fig. 5). Undoubtedly, 58-2 was made up of reduced gametes from both parents and presumably 58-1 was made up of a zygote whose chromosomes were being doubled right after fertilization and before the onset of embryogenesis. Being so, 38 chromosomes would come from *Saccharum*, 30 from *Sclerostachya* and 38 from *Miscanthus*. Had there been two more chromosomes from *Saccharum*, this tri-generic hybrid would have been perfect as far as having all the chromosome complement from *Sclerostachya* and *Miscanthus* and half the complement from *Saccharum*. Li *et al* (1959) found that the chromosomes of *Saccharum* in the reduced numbers (80 → 40 → 20) would form bivalents almost exclusively. Theoretically, therefore, the pairing of the chromosomes would be perfect. However, this was not found to be true. The chromosome association at MI and diakinesis is shown in Table 3.

The average for the bivalents was 48.82, and univalents, 7.90. There were also a few multivalents found in the course of our study (Plate III, Figs. 6 and 7). The tetravalents were in chains as well as in rings.

As many as 8 univalents were found as an average in a cell. This would merit some consideration. It is true that non-pairing of some of the chromosomes

Table 3. Chromosome association at MI and diakinesis of 58-1.

Chromosome association				MI	Frequency	
IV	III	II	I		Diakinesis	Total
		52	2	2		2
1		49	4	1	1	2
		51	4	2	1	3
	1	49	5	1		1
1		48	6	2		2
		50	6	11	1	12
		49	8	10	1	11
		48	10	9	1	10
		47	12	2		2
		47	12	2		2
1		44	14	1		1
		46	14	4		4
Total				47	5	52

did exist while almost perfect pairing would be expected. It can be explained simply by physiological disturbance. This physiological disturbance may be conjectured to be due to the non-orientation of the chromosomes from the male gamete in a foreign cytoplasm of the egg. Unless experimental proof could be carried out, this may serve as a starting hypothesis. Other explanations, however, are not precluded.

Pollen Fertility

Pollen was studied for all the parents and the hybrids. Since Vellai does not flower in Taiwan, 28 NG 264 was used in its stead.

Table 4. Pollen fertility and size for all the clones involved.

Clone	28 NG 264	SG 310/6	S. fusca	M. japonicus	58-1	58-2
% good pollen	60	70	86	78	0	0
Number counted	660	1,000	609	900		
Size in μ :						
Average	33.93	42.28	32.36	36.37	36.89	30.28
Range	43.5-26.1	60.9-26.1	43.5-26.1	52.2-26.1	52.2-26.1	34.8-26.1

The representative photo-micrographs of the pollen grains are shown in Plate III, Figs. 8-13 inclusive. 58-1, with whole chromosome complements of *Miscanthus* and *Sclerostachya* and half of *Saccharum*, was expected to have good fertility so that a new species would thus be created. Unfortunately, it

was completely male sterile. That 58-2 was completely male sterile was within the realm of expectation. It was interesting to notice that the size of the empty pollen grains of 58-1 was almost twice as large as those of 58-2. This was in conformity with the chromosome number of these two plants.

Discussion

SG 310/6, the female parent used in effecting this cross with *Miscanthus*, had 72 chromosomes. Its chromosome constitution, as was studied earlier (Li *et al.*, 1959), was 42(O)+30(S) while (O) representing chromosomes of *Saccharum* origin and (S) those of *Sclerostachya* origin. When this was crossed to *M. japonicus* ($2n=38$), two true hybrids were obtained:

1. 58-1 ($2n=106$) had chromosome constitution of 38(O)+30(S)+38(M) while (M) representing chromosomes of *Miscanthus* origin. With whole chromosome complements of both *Sclerostachya* and *Miscanthus* present plus almost half the complement of *Saccharum*, almost perfect pairing was expected because each chromosome would have its own homologue.

2. 58-2 ($2n=55$) had chromosome constitution of 21(O)+15(S)+19(M). The 21 chromosomes of *Saccharum* would naturally form 10 pairs+1 univalent as a rule (Li *et al.*, 1959). The chromosomes of both *Sclerostachya* and *Miscanthus* are in reduced state. That is to say, each chromosome would not have any homologue of its own. This would mean further that in this hybrid plant, there are roughly 10 bivalents +1 univalent of *Saccharum* origin plus 2 haploid sets of *Sclerostachya* and *Miscanthus*.

Katayama (1935) made the distinction of monohaploid and polyhaploid according to whether the haploid is derived from diploid species or from allopolyploids. Tometorp (1939) found only 5.6% of PMC while one bivalent was observed in *Hordeum distichum* haploid. Olsson and Hagberg (1955) observed 2-8 or 9 bivalents at MI in haploids of *Brassica napus* L. Since there was practically no pairing between the chromosomes of the haploid sets of either *campestris*-genome or *oleracea*-genome, both of which constituted the amphidiploid *B. napus*, the bivalents seen at meiosis of amphihaploid rape were composed of one *compestris*- and one *oleracea*-chromosome each.

Shang *et al.* (in press) found less than 1 bivalent per cell in *Sclerostachya* haploid set. No study was ever being made of the pairing condition of *Miscanthus* haploid. If it should behave likewise as *Sclerostachya*, then the pairing in 58-2 in addition to the 10 pairs which originate from *Saccharum* can be easily explained. In our study, we found 17.28 bivalents per cell besides tetravalents and trivalents. The highest number of bivalents found in a single cell was 22. This would mean that the chromosomes of the haploid sets of both *Sclerostachya*

and *Miscanthus* would pair with each other besides having very little pairing between the chromosomes of one haploid set of one species.

Were this to be true, it can easily explain the existence of the unequal bivalents found in this hybrid. These unequal bivalents have only one segment in common. From the very beginning of meiosis, each bivalent of this type does not have very close association between the chromosomes. Chiasma formation is greatly decreased later in diplotema and there is a complete terminalization at diakinesis. Perhaps, some of the bivalents belonging to the open type (Table 2) may belong to this category even though the inequality is not distinctly visible microscopically.

Unequal bivalents were found in *Secale vavilovii* (Jain 1959), in hybrids of *Lycopersicum esculentum* and *Solanum pennellii* (Rick, 1960), in haploid rape (Olsson and Hagberg, 1955), and in other plants. However, the classical work would be the study of the haploid wheat by Sears and Okamoto (1957). From pairing and crossing-over in the haploid, translocation rings and chains of 4 were found in the back cross generation. Eleven translocations were fully examined and seven were found to reveal genetically related chromosomes. These involved pairing of homoeologous chromosomes. However, the other four cases involved pairing of non-homoeologous chromosomes.

Summing up, from 7 to as many as 12 chromosomes of *Sclerostachya* and *Miscanthus* would pair with each other in 58-2. This would mean to indicate that roughly 10 chromosomes from each genus are partially homologous. Since some of the chromosomes of *Sclerostachya* and *Miscanthus* are partially homologous with *Saccharum* (Li *et al.*, 1959) and with each other, consequently, multivalents are observed.

In the study of the phylogenetical relationship of the relatives of sugarcane, should direct crossings between two different genera fail, the method by using sugarcane as an intermediary in order to effect the bringing together of two different genera can be applied, so that their mutual relationships can be studied in the hybrid.

Crossing of SG 363/4 with chromosome constitution of 20(O)+30(S) and *Miscanthus* failed to obtain any hybrid. Chances are that the seedlings obtained were too few in number.

Summary

Crossing SG 310/6 ($2n=72$) with *Miscanthus japonicus* ($2n=38$), two true hybrids were obtained. Both were identified by having awned lemma and pediceled spikelets whose characters were contributed by the male parent.

SG 310/6 had the chromosome constitution 42(O)+30(S), while (O)=*Saccharum officinarum* ($2n=80$) and (S)=*Sclerostachya fusca* ($2n=30$).

One of the hybrids, 58-1, has 106 somatic chromosomes. Presumably, the chromosomes in the zygote were doubled after fertilization of the gametes with reduced number of chromosomes before the onset of embryogenesis.

The chromosome constitution might be:

$$38(O)+30(S)+38(M) \quad (M)=M. japonicus.$$

Normal pairing of the chromosomes within each set derived from different parents was expected and realized. However, a few univalents were found in each cell as well as occasional occurrence of multivalents.

This plant was male sterile. However, it was not fully tested about its female fertility.

The other hybrid 58-2 had only 55 chromosomes. It was the result of fertilization of gametes of reduced number of chromosomes from both parents. Its chromosome constitution was therefore $21(O)+15(S)+19(M)$.

Pairing was observed to exist between the chromosomes of *Sclerostachya* and *Miscanthus*, 7-12 pairs besides the bivalents formed by the sugarcane chromosomes.

These bivalents were mostly of open type and some of these were unequal either in size or in form or both.

Conclusion could be drawn therefore that *Sclerostachya* and *Miscanthus* were closely related to each other. Perhaps they shared a common ancestor in the course of evolution.

As multivalents were frequently found indicating that not only *Sclerostachya* was related to *Miscanthus* but they in turn were related to *Saccharum* as both could be crossed with *Saccharum*.

58-2 was also male sterile. This was to be expected. Its female fertility was yet to be fully tested.

甘蔗及其近親植物的細胞學研究

李先聞 翁登山 項公傳 楊必卿

以 SG 310% ($2n=72$) 與 *Miscanthus japonicus* ($2n=38$) 雜交後，獲得了兩株雜種植物，58-1 及 58-2。兩者的小花都有柄，其外穎尖端伸長為芒。這些特性都是來自 *M. japonicus*，所以易於識別。

SG 310% 的染色體組結構為 $42(O)+30(S)$ ，其中(O)表示來自 *Saccharum officinarum* ($2n=80$) 的染色體，(S)則表示來自 *Sclerostachya fusca* ($2n=30$) 的染色體。

雜種 58-1 含有 106 個體細胞染色體。這似乎是由於兩個減數的雌雄配偶子接合後，在接合子未開始分化以前，染色體數發生倍數化所致。它的染色體組結構可能為： $38(O)+30(S)+38(M)$ ，其中(M)表示來自 *M. japonicus* 的染色體。在 58-1 的花粉母細胞中，來自不同親本的各組染色體均能自行配對，但有的時候亦可見有若干單價體及多價體。這株雜種，為雄不稔性。其雌稔性的程度如何，因未作進一步的試驗，故無法判定。

雜種58-2含有55個體細胞染色體。它的由來顯然是起於兩個減數的雌雄配偶子的接合，因此它的染色體組結構為：21(O)+15(S)+19(M)。在58-2的花粉母細胞中，來自 *Sclerostachya* 的染色體能與來自 *Miscanthus* 者相互配對，其頻度約為7-12對不等。這些二價體多於開展式 (Open type)，其中有的形狀與大小都不相等 (所謂 Unequal type)。這株雜種亦是雄不稔性——這是可以預料的。不過的雌稔性尚待測驗。

根據上面的觀察可以下一結論：即 *Sclerostachya* 與 *Miscanthus* 的親緣相當近。它們在進化的過程中可能來自同一祖先。再者，多價體的時常出現，更可證明 *Sclerostachya* 與 *Miscanthus* 又分別與甘蔗有親緣關係。這是顯而易見的，因為無論 *Sclerostachya* 或 *Miscanthus* 都能與甘蔗雜交。(摘要)

Literature Cited

- JAIN, S. K. Über das Vorkommen einer heteromorphischen Bivalenten in *Secale vavilovii* ($2n=14$). *Cytologia* 24: 326-329, 1959.
- KATAYAMA, Y. Karyological comparisons of haploid plants from octoploid *Aegilotriticum* and diploid wheat. *Jap. Jour. of Botany* 2: 349-380, 1935.
- LI, H. W., C. S. LOH, and C. L. LEE. Cytological studies of sugarcane and its relatives. I. Hybrids between *Saccharum officinarum* and *Miscanthus japonicus*. *Bot. Bull. Acad. Sinica* 2: 147-160, 1948.
- LI, H. W., K. C. SHANG, YVONNE Y. HSIAO, and P. C. YANG. Cytological studies of sugarcane and its relatives. XV. Basic chromosome number of *Saccharum officinarum* L. *Cytologia* 24: 220-226, 1959.
- OLSSON, GÖSTA and HAGBERG, ARNE. Investigation on haploid rape. *Hereditas* 41: 227-237, 1955.
- PARTHASARATHY, N. Origin of noble cane (*S. officinarum* L.). *Nature* 161: 608-611, 1948.
- RICK, C. M. Hybridization between *Lycopersicum esculentum* and *Solanum pennellii*: Phylogenetic and cytogenetic significance. *Proc. Nat. Acad. Science* 46: 78-82, 1960.
- SEARS, E. R. and OKAMOTO M. Genetic and structural relationships of non-homologous chromosomes in wheat. *Proc. Int. Gen. Symposium* 1956, Tokyo & Kyoto: 332-335, 1957.
- SHANG, K. C., S. S. CHU, T. S. WENG, and H. W. LI. Cytological studies of sugarcane and its relatives. XVI. Further studies of basic chromosome number of *Saccharum officinarum* L.: F₁ hybrid of Vellai and *Sclerostachya fusca* A. Camus. *Bot. Bull. Acad. Sinica*, n. s. 1: 23-28, 1960.
- TOMETORP, G. Cytological studies on haploid *Hordeum distinchum*. *Hereditas* 25: 241-254, 1939.