Cytogenetical Studies On The Sterile Wild Senna (Cassia Tora L.) Produced By The Atomic Bomb Explosion : Ⅳ. On the gigas mutants segregated from the asynaptic wild senna (Cassia Tora L.)

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CYTOGENETICAL STUDIES ON THE STERILE WILD SENNA (*CASSIA TORA* L.) PRODUCED BY THE ATOMIC BOMB EXPLOSION

IV. On the gigas mutants segregated from the asynaptic wild senna (*Cassia Tora* L.)

TAIRA KATAYAMA

I. INTRODUCTION

In 1949 Nagamatsu[10] reported briefly on a sterile wild senna, *Cassia Tora* L., produced after the atomic bomb explosion in Nagasaki in 1945. Nagamatsu maintained that the sterility had been caused by an asynaptic characteristic which was inherited as simple Mendelian recessive. This fact was confirmed later by the author through more detailed cytological and genetical observations.[7,8]

Due to the failure of bivalent formation, the chromosome behaviour of the asynaptic plant during meiosis was very irregular. This phenomenon resulted in a high percentage of abortive pollen grains. Such irregular meiotic chromosome behaviour, which usually causes high sterility, may enhance the probability of chromosomal mutations, such as, haploid, triploid, heteroploid, etc.

In 1949 the author discovered two gigas type plants (No. 21-8 and No. 65-1) among the offspring of asynaptic wild senna. These two gigas plants were very similar morphologically, and had one extra chromosome each. The morphological aspects and chromosome behaviour in the pollen mother cells of these gigas plants and their offspring (No. 21-8-1 and No. 21-8-2) will be described in this paper.

II. MATERIAL

The materials used in this study were as follows: two gigas type plants (No. 21-8 and No. 65-1), and two progenies (No. 21-8-1 and No. 21-8-2). The former were discovered among the offspring of wild senna which have already been reported as asynaptic plants,[8,10,12] while the latter were produced by the self-pollination of plant No. 21-8.

The pedigrees of these materials are as follows:

1946	1947	1948	1949
Culture No. 18	→ E. 18-23 Pollen normal. →	E. 17-10 Pollen normal. Percentage of asynaptic plants in Line No. 17 was 9.09. →	E. 21-8 Pollen Abortive. All other plants in the Line E. 21 were normal.
	→ E. 18-82 Pollen normal. →	E. 22-4 Percentage of abortive pollen was 99.15. Percentage of asynaptic plants in Line No. 22 was 29.03. →	E. 65-1 Pollen abortive. Line E. 65 contained only one plant.

III. MORPHOLOGICAL CHARACTERISTICS (No. 21-8 and No. 65-1)

As stated elsewhere,[7] the asynaptic sterile plant of the wild senna is not distinguishable from the normal one morphologically. They can be discriminated only by the high sterility of the asynaptic plant at their fruit-bearing time. The two gigas mutants described here are distinctly different from both the normal and the asynaptic wild senna in such characteristics as gigas type, delayed flowering time, and high sterility (Table 1 and Figs. 1-4).

As shown in Table 1, the normal plants started to flower about July 25, while the gigas mutants produced buds about September 10 and their first flowers on September 20. The normal adult plant was approximately 105 cm. high, while the gigas

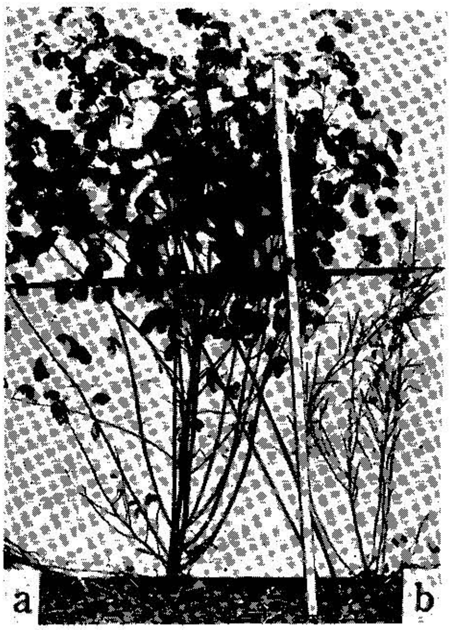


Fig. 1. Normal and gigas plants (trisomic plant) of *Cassia Tora* L.
a. Gigas plant (No. 21-8). b. Normal plant.

Table 1. Measurements on the normal and the gigas plants and their offspring.

	Normal plant	Gigas mutants		Offspring of No. 21-8	
		21-8	65-1	21-8-1	21-8-2
Date of the 1st flower	July 25	Sept. 20	Sept. 20	July 21	July 4
Height (cm)	104,9	180,0	178,0	160,0	120,0
No. of branches per plant	15,51	99,0	96,0	50,0	101,0
Stem diameter (cm)	1,10	2,27	2,07	—	—
No. of placentas per plant	30,47	30,60	31,50	—	30,39
No. of seeds per pod	10,08	2,67	0	2,03	11,02
Length of rachis (cm)	5,79	8,35	7,68	7,5	6,8
Length of 1st leaflet (cm)	3,22	4,72	4,63	4,5	4,3
Width of 1st leaflet (cm)	2,14	3,15	3,15	2,7	3,2
Length of 2nd leaflet (cm)	4,14	6,18	6,08	5,8	5,5
Width of 2nd leaflet (cm)	2,61	3,78	3,71	3,5	3,8
Length of 3rd leaflet (cm)	4,50	7,03	6,95	6,5	6,8
Width of 3rd leaflet (cm)	2,71	4,08	3,84	3,4	4,2
Length of guard cells	7,03	6,84	6,94	7,01	7,04
% of sterile pollen grains	23,75	89,35	86,42	90,13	44,21

mutants were nearly as high as 180 cm., which is almost twice as high as the former. The diameter of the main stem of the mutant was also nearly twice as large as that of the normal. The total number of branches on the normal plant was 15.5, while those of the gigas mutants numbered about 100. The rachis of the gigas mutants were longer than those of the normal by about 2.0–2.5 cm. The length and width of each leaflet were also greater

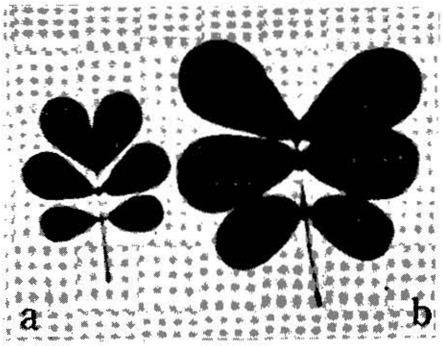


Fig. 2. Compound leaf.
a. Normal plant. b. Gigas plant.

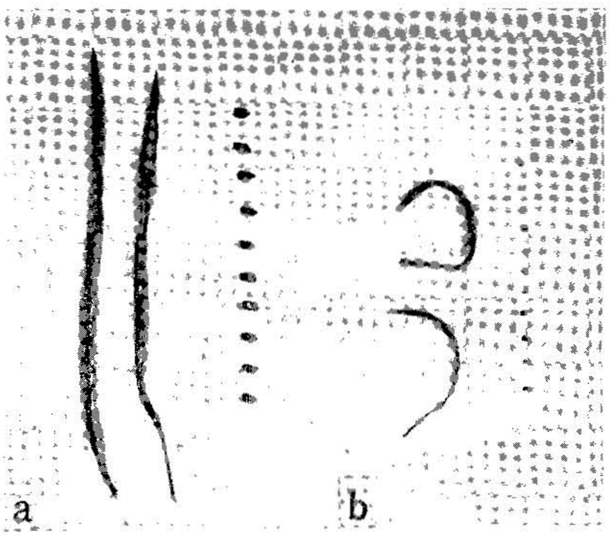


Fig. 3. Pods and seeds.
a. Normal plant. b. Gigas plant.

by about 1–2 cm., respectively. Another distinct difference was also observed in the size of the flower-vase. However, no difference was noticed in the length of the guard cell and in the number of placentae per legume between the two types.

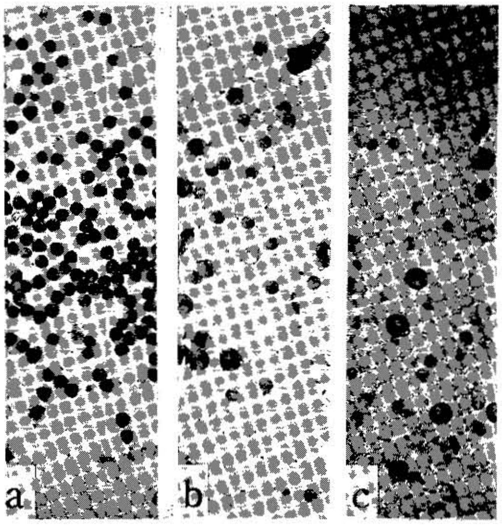


Fig. 4. Pollen grains (× 54).
a. Normal plant.
b. Gigas plant (No. 21-8).
c. „ „ (No. 65-1).

IV. METHODS

The root tips were fixed with Navashin's solution and stained with Heidenhain's iron-alum-haematoxylin.

The pollen mother cells were fixed with acetic alcohol (alcohol 3 : glacial acetic acid 1) for about one hour, and then stained with Heidenhain's iron-alum-haematoxylin and Newton's gentian violet. A good result was obtained from the latter.

Sections of the root tips were sliced as thick as 7 to 8μ, and those of the pollen mother cells from 18 to 20μ.

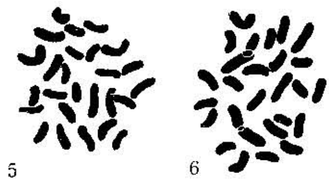
Pollen tetrads and pollen sterility were investigated by the smear method with iron acto-carmine.

V. Cytological Observations
(No. 21-8 and No. 65-1)

Since the behaviour of the chromosomes in the two gigas mutants, discovered in 1949 were similar to each other, the author will describe only the result of the cytological observations on plant No. 21-8.

Root Tips

As stated elsewhere,[8] the root tip cells of the normal wild senna contained 26 chromosomes (Fig. 5). On the other hand, 27 chromosomes (2n+1) were observed in the root tip of the gigas



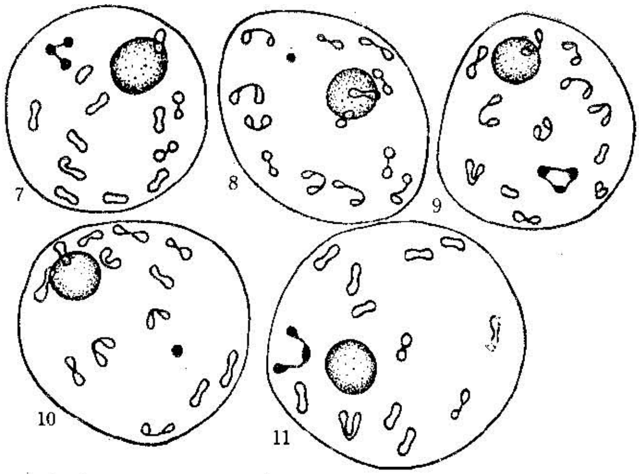
Figs. 5-6. Somatic chromosomes (ca. ×2250).
5. Normal plant (2n=26). 6. Gigas plant (2n=27).

mutants (Fig. 6). This fact indicated that the gigas mutants were so-called "trisomics", with one extra chromosome. It was impossible, however to distinguish the extra chromosome morphologically.

Pollen Mother Cells

(Heterotypic division)

In the diakinetic nucleus of the pollen mother cells, chromosome conjugations of $1_{III}+12_{II}$ were counted in about 59% (Fig. 7), and $13_{II}+1_{I}$ in about 41% (Fig. 8; Table 2). At the first metaphase, $1_{III}+12_{II}$ were observed in half of the pollen mother cells (Figs. 14 and 19). $13_{II}+1_{I}$ were next to the $1_{III}+12_{II}$ chromosomes in number (Figs. 13 and 20). However, the $1_{IV}+1_{III}+10_{II}$ (Fig. 12) were rarely found (Table 2). In these cases, univalents, bivalents, and trivalents could be distinguished from one another



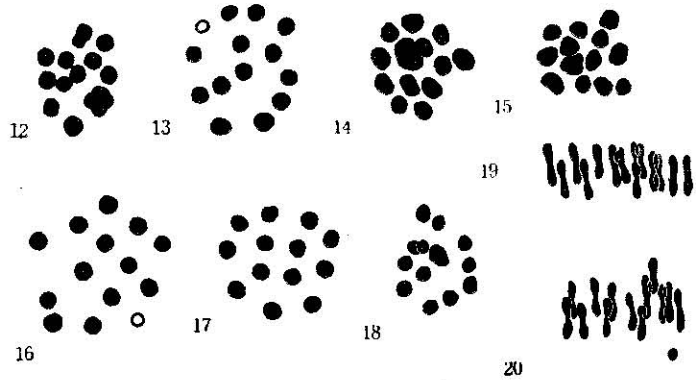
Figs. 7-11. Diakinesis. 7-8. No. 21-8, showing $1_{III}+12_{II}$ and $13_{II}+1_{I}$ respectively. 9-10. No. 65-1, showing $1_{III}+12_{II}$ and $13_{II}+1_{I}$ respectively. 11. No. 21-8-1, showing $1_{III}+12_{II}$ (ca. ×2250).

Table 2. Frequencies of PMCs with various degrees of chromosome association at diakinesis and M-I.

Culture No.	Stage	Frequencies of chromosome association			
		$1_{IV}+1_{III}+10_{II}$	$1_{III}+12_{II}$	$13_{II}+1_{I}$	13_{II}
Normal plant	Diakinesis	—	—	—	20
	M-I	—	—	—	23
21-8	Diakinesis	—	26	18	—
	M-I	1	22	18	—
65-1	Diakinesis	—	9	8	—
	M-I	—	20	17	—
21-8-1	Diakinesis	—	22	15	—
	M-I	—	13	11	—
21-8-2	Diakinesis	—	—	—	10
	M-I	—	—	—	13

by shape, location, and degree of stain, while the tetravalents were determined by the shape and size.

At the first anaphase, the bivalents were normally disjoint, the halves moving toward each pole. At the first telophase, they were included in the daughter nuclei. Trivalents are usually disjoint; two chromosomes moving to one pole and the other moving toward the opposite pole. In most cases, it was observed that the univalents moved toward one pole without splitting, or without dividing after splitting (Fig. 21). However, the split halves rarely passed toward opposite poles (Figs. 21 and 23). The univalents were often delayed in moving, as compared with the



Figs. 12–18. Polar views of M-I. 12–14. No. 21-8, showing $1_{IV}+1_{III}+10_{II}$, $13_{II}+1_{I}$ and $1_{III}+12_{II}$ respectively. 15-16. No. 65-1, showing $1_{III}+12_{II}$ and $13_{II}+1_{I}$ respectively. 17. No. 21-8-2, showing 13_{II}. 18. No. 21-8-1, showing $1_{III}+12_{II}$ (ca. ×2250).

Figs. 19-20. Side views of M-I. 19. No. 21-8, showing $1_{III}+12_{II}$. 20. No. 65-1, showing $13_{II}+1_{I}$ (ca. ×2250).

bivalents (Figs. 21 and 23), and consequently at interkinesis, some micronuclei besides the two daughter nuclei were frequently formed (Fig. 23). The polyvalent chromosomes, such as the tetravalent, were rarely found at M-I (Fig. 12).

(Homeotypic division)

Usually 13 (Fig. 27) or 14 (Fig. 28), and occasionaly 12 chromosomes (Fig. 26) could be counted during the second metaphase. Some lagging chromosomes were also observed at the second anaphase. Occasionally, at cytokinesis, these laggards formed the micronuclei in addition to four daughter nuclei.

(Tetrads and pollens)

At the stage, corresponding to the pollen tetrads, the irregular sporads consisted of monads to hexads. In No. 21–8 and No. 65–1, the number of tetrads was equivalent to 70 and 77%, respectively, of all sporads; 67 and 47%, respectively, of the tetrads were distinctly abnormal in size or shape (Table 3).

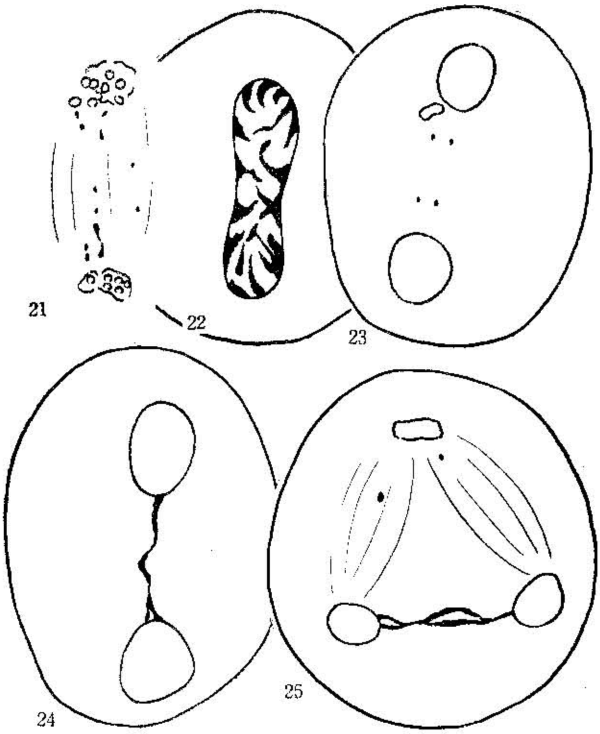
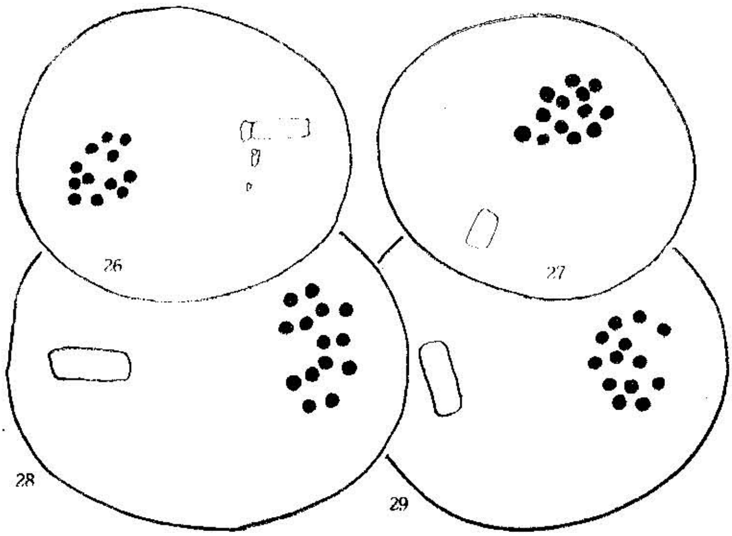


Fig. 21. A-I of No. 21-8, showing lagging chromosomes.
Fig. 22. Regression in No. 21-8.
Fig. 23. No. 21-8, showing a micronucleus and some laggards.
Fig. 24. No. 21-8, showing a chromosomal bridge at interkinetic stage.
Fig. 25. No. 21-8, showing tripolarity (ca. ×2250).

According to the experiment conducted at the end of October in 1949, if the pollen, not stained with acetocarmine, was presumed as the abortive pollen, than these gigas mutants showed sterility of pollen as high as 89 and 86% respectively, while the pollen sterilities of the normal plants showed about 24% (Fig. 4a, b, and c; Table 1).

In the normal plant the size of the pollen grains was comparatively equal, ranging from 3.0 to 4.5, (where the figures refer to the graduations on the micrometer scale), with the mode at 3.75. On the other hand, in the gigas mutants the size varied, and ranged from 3.0 to 6.5, with the mode between 4.25 and 5.25 (Fig. 4a, b, c; Table 4).



Figs. 26-28. M-II of No. 21-8, showing 12, 13 and 14 chromosomes respectively.
Fig. 29. M-II of No. 21-8-1, showing 13 chromosomes (ca. ×2250).

Table 3. Frequencies of various sporads in tetrad stage.

Culture No.	Monads	Diads	Triads	Tetrads Normal	Tetrads Ab-normal	Pentads	Hexads	Total
Normal plant	—	—	—	99	—	1	—	100
21-8	4	12	47	118	148	24	2	355
65-1	3	32	41	240	77	19	2	414
21-1-1	5	16	55	132	161	33	4	406
21-1-2	—	—	5	78	28	9	—	120

In spite of numerous self-pollinations, the gigas mutants always showed high sterility. Thus, the assumption was made that most of their pollen was abortive.

(Other Irregularities)

Other types of irregularities such as chromosome bridges (Figs. 24 and 25), tripolarity (Fig. 25), and regression (Fig. 22), were rarely observed during the first anaphase and telophase.

Table 4. Size distributions of pollen grains of the normal and the trisomic plants and their offspring.

1 unit=3.3	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	Total	$\bar{x}$	s^2
Normal plant		—	109	73	—	—	—	—	—	—	182	3.95	0.0604
21-8		8	21	54	35	95	14	2	—	—	229	4.77	0.4152
65-1		18	30	58	58	60	4	1	—	1	230	4.55	0.4690
21-1-1		7	26	51	48	89	9	1	—	—	231	4.72	0.3817
21-1-2		71	170	58	—	—	—	—	—	—	299	3.73	0.1077

VI. THE OFFSPRING OF No. 21-8.

In 1950 11 seeds, obtained from the self-pollination of plant No. 21-8 in 1949, were sowed in Wagner's pot (1/20.000 Tan; one acre equals 4.0806 Tan). Only three of these seeds germinated, and the two plants grown were put into a sun-glass room until flowering time. The pots were then moved from the room.

Morphological Observations

(No. 21-8-1)

Unfortunately, this plant died at the beginning of September. This plant was very similar to its parent (No. 21-8), except for the discrepancy in the beginning of flowering time and in number of branches (Table 1). The flowering time of this plant was earlier than that of the parent. This phenomenon may have resulted because of its environment in the sun-glass room during the earlier stage. In addition, death in the early stage may have resulted in a shorter stem and fewer branches.

(No. 21-8-2)

The morphological characteristics of this plant were very similar to those of the normal one, although the former showed more abortive pellen grains (Table 1). The reason for the premature flowering of this plant also seems to be the same as that stated in the preceding paragraph; and the multiplicity in the number of branches may be due to the fact that it was provided with sufficient space for growth.

Cytological Observations

(No. 21-8-1)

The somatic chromosome number of No. 21-8-1 was counted as 27 (2n+1).

At diakinesis $1_{III}+12_{II}$ and $13_{II}+1_{I}$ were often perceived (Fig. 11). Although the number of cases observed were not so many during the first metaphase, $1_{III}+12_{II}$ (Fig. 18) and $13_{II}+1_{I}$ were shown as a result. In most cases, 13 chromosomes were counted in one plate during the second metaphase (Fig. 29), although 12 and 14 chromosomes were observed. The behaviour of the chromosomes during the first anaphase, interkinesis, and second metaphase, as well as the general appearance of the tetrads and pollen were similar to those of the parent (No. 21-8).

(No. 21-8-2)

In the root tips of plant No. 21-8-2, 26 chromosomes were counted. At diakinesis and the first metaphase 13_{II} were observed (Fig. 17); at the second metaphase 13 chromosomes were counted. The behaviour of the chromosomes appeared to be similar to that of the normal ones.

VII. Discussion

In *Datura*,[3] *Nicotiana*,[5,14] *Oryzae*,[8,11] and *Oenothera*,[2] trisomic plants were artificially produced by exposure to X-rays or radium.

In wild senna, it seems that the gigas plants, having one extra chromosome were produced as the result of non-disjunction by irregular distribution of univalents to the offspring of the asynaptic plants. The above plants were brought about by gene mutation, produced artificially by the atomic bomb explosion. This has the same effect on these plants as X-rays and radium.

In *Datura*, when self-pollinated, the trisomic character was mainly transmitted at the rate of about 25% from the ovules. The "Globe" character of the trisomic "Globe" type was inherited by 3% through the pollen, while 25% through the ovules.[1]

On the other hand, the line which produced trisomic plants only was discovered among the offspring of "dwarf mutant plant × normal plant" in *Oenothera*[4] and of "*T. polonicum* × *T. spelta*."[15]

It is a characteristic of the inheritance in trisomics that the ratio of segregation depends not upon the chance, but upon the result of the control of the various conditions; namely, in most cases, the production rate of trisomic plants is usually less than that of normal ones.

Only two seeds germinated out of 12 seeds obtained from the self-pollination of plant No. 21–8. One of them (No. 21–8–1) is very similar to the parent (No. 21–8) in morphological and cytological character, while the other resembles the normal one, as stated previously. To his regret, the author could not investigate the inheritance of the former (No. 21–8–1) because of its death during the flowering time. In the following generation, no gigas plant was produced from the latter (No. 21–8–2), so that the inheritance of the trisomic plant can not be assured in this paper.

Usually trisomic types were characterized by decreasing the growing vigour, according to one extra chromosome.[9,10] But these two gigas plants and one offspring of plant No. 21–8, having one extra chromosome respectively, increased the size of various parts (Figs. 1 and 2; Table 1).

VIII. Summary

1). Morphological investigations and cytological studies were performed on the two gigas mutants, produced in the offspring of the asynaptic wild senna in 1949. Cytological studies have determined that these gigas mutants are trisomic plants with 27 chromosomes (2n+1).

2). These gigas mutants were characterized by gigas type, delay of flowering time and high sterility.

3). In the pollen mother cells, $1_{III}+12_{II}$ and $13_{II}+1_{I}$ were observed in most cases; and $1_{IV}+1_{III}+10_{II}$ in fewer cases.

4). At, and after the first anaphase, various irregularities could be observed. Lagging chromosomes and irregular sporads were often noticed, but bridge, regression, and tripolarity were rarely seen.

5). In the cytological examination of two offspring, which were produced from the self-pollination of plant No. 21–8, observations showed that one resembled the parent, while the other resembled the normal plant.

IX. Acknowledgments

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