KARYOTYPIC POLYMORPHISM IN *BEGONIA TUBEROHYBRIDA* VOSS.*

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Introduction

Karyotypic polymorphism in intraspecies of *Begonia* was first described by Matsuura and Okuno (1936, 1943). They reported that diversity of external characters in *Begonia semperflorens* and *B. Rex* is correlated with differences in their chromosomal organization and suggested that such a karyotypic polymorphism as noted in *B. semperflorens* and *B. Rex* is also expected to be found in other *Begonia* species. In a plant of *B. Rex* having 44 somatic chromosomes, a very peculiar behavior of meiotic chromosomes was also noted by them.

In the present study, the same phenomenon was discovered also in *B. tuberohybrida*. And chromosomes in pollen grains of *Begonia* were first demonstrated by the present study. Up to the present time, several different chromosome numbers were found in pollen grains of No. 22. But its cytological details were not accomplished yet sufficiently, and further work is now planned to be done on a larger scale.

Materials and methods

Materials for study were taken from plants growing in the greenhouse of our Laboratory, which originally came from various sources.

For determining the somatic chromosomes of the root tips, the usual paraffine method was employed, the fixative being Nawaschin's chromoacetic solution or La Cour 2 BE and the stain being iron-alum haematoxyline. For the meiotic chromosomes in PMCs, the iron-acetocarmine smear technique was used and either Nawaschin's chromoacetic formalin solution or Carnoy's fluid was made in addition to the acetocarmine smear method. The photomicrographs in this paper were made with the aid of a Leitz-Makam camera.

Observations

Various different chromosome numbers were found in 18 examined (Nos. 4, 7, 6, 5, A, 100, 3, 9, 27, 16, 17, 19, 101, 22, 21, D, C, E).

As will be seen in Table 1, chromosome numbers show a wide range of variation. Eighteen plants examined vary from one another with respect to size and amount of anthocyanin of the leaf and stem and other minor characters. They were also found to be strikingly polymorph with respect to the flower type, namely they vary from one another with respect to number of petals in male flowers. Thus they range from single to double

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Photo. A. 1—3. Representing 3 plants of *B. tuberohybrida*. 1, single flowering plant; 2, intermediate plant between 1 and 3; 3, double flowering plant. 4½.

Table 1. List of the chromosome numbers in 18 plants of *B. tuberohybrida* found by the present study.

Materials	Chromosome numbers $2n$	Figures
No. 4	46	1
No. 7	47	2
No. 6 and 5	48	3
No. A	49	4
No. 100 and 3	52	5
No. 9	54	6
No. 27, 16, 17 and 19	56	7
No. 101	58	8
No. 22	60	9
No. 21	64	10
No. D and C	66	11
No. E	(72)*	

* In meiotic metaphase of No. E, 42 (30II+12I) chromosomes were found (Fig. 22).

(Photo. A). These striking differences in characters strongly suggest the existence of karyotypic differentiation in these plants. But on account of the complicated chromosomal organization, the relation between the diversity of their external characters and their karyotypic differentiation has not ascertained yet by the present observation.

As is obvious in reference to the annexed figures (Figs. 1—11), the chromosomes of these individuals show isomorphic nature; and vary in length from rods to spherules. It seems impossible to identify the homologous pairs, because of the considerably large number of chromosomes with slight difference.

Meiotic chromosome configurations were studied in 7 plants, No. 5, 3, 9, 27, 22, 21 and D; the results are represented in Table 2.

As will be seen in Table 2, meiotic configuration in these 7 plants were statistically studied. The four or five modes of chromosome associations were found to occur.

It must be noted that in no case there occurs multivalent formation. In the first division, univalents usually split at the equator after the bivalents had passed to the poles and random distribution seems to take place at the second division (Figs. 27 and 28).

The univalents are distributed at random in the first division, and frequently a few fail to be included in the two daughter nuclei. In the second metaphase, many cells show unequal numbers on the two plates (Figs. 30—34), and later phases frequently show smaller

Table 2. Meiotic configurations at MI in 7 plants of *B. tuberoshybrida*.

Material	Meiotic configurations	Frequency	Percentage
No. 5 ($2n=48$)	24II	100	30.6
	23II+2 I	197	60.2
	22II+4 I	27	8.3
	21II+6 I	3	0.9
No. 3 ($2n=52$)	26II	120	40.1
	25II+2 I	137	44.8
	24II+4 I	40	13.4
	23II+6 I	3	1.0
	22II+8 I	2	0.7
No. 9 ($2n=54$)	27II	7	9.1
	26II+2 I	20	26.0
	25II+4 I	31	40.3
	24II+6 I	7	9.1
	23II+8 I	2	2.6
	more than 10 I	10	12.9
No. 27 ($2n=56$)	28II	72	57.6
	27II+2 I	39	31.2
	26II+4 I	11	8.8
	25II+6 I	2	1.6
	more than 8 I	1	0.8
No. 22 ($2n=60$)	30II	31	19.4
	29II+2 I	78	48.8
	28II+4 I	38	23.7
	27II+6 I	9	5.6
	26II+8 I	4	2.5
No. 21 ($2n=64$)	32II	31	36.9
	31II+2 I	39	46.4
	30II+4 I	14	16.7
No. D ($2n=66$)	33II	219	78.2
	32II+2 I	45	16.1
	31II+4 I	14	5.0
	30II+6 I	1	0.4
	29II+8 I	1	0.4

chromosome groups in addition to the four major groups (Figs. 39 and 40). In the first telophase, chromosome bridges have been observed rarely (Fig. 29). In anaphase and telophase, lagging chromosomes were often observed and laggards at this stage are mainly due to univalents present at the first metaphase (Fig. 26). More often, the laggards fail to be included in the two daughter nuclei and remain outside the nuclei. Laggards have been also observed at the second division. Chromosome groups which fail to be included in

Photo. B. Representing somatic chromosome in No. 27, meiosis in PMCs in No. 9, 27 and 22, pollen grain mitosis in No. 22 and marked difference in pollen abortion in No. 6 and 22. 1, somatic metaphase plate (No. 27) showing 56 chromosomes; 2, meiotic metaphase plate (No. 27) showing 28 bivalent chromosomes; 3-6, showing univalents at AI (No. 9 and 27); 3, 2I; 4, 4I; 5, 6I; 6, 8I;

Figs. 1—11. Various chromosome numbers in 11 plants. 1, No. 4 ($2n=46$); 2, No. 7 ($2n=47$); 3, No. 6 ($2n=48$); 4, No. A ($2n=49$); 5, No. 100 ($2n=52$); 6, No. 9 ($2n=54$); 7, No. 27 ($2n=56$); 8, No. 101 ($2n=58$); 9, No. 22 ($2n=60$); 10, No. 21 ($2n=64$); 11, No. D ($2n=66$). $\times 2600 \times 2/3$.

the main nuclei enter the telophase, forming a number of various sized microcytes. The number of microcytes per sporad ranges from 3 to 10 (Figs. 46—52), as given in Table 3.

Table 3. Number of microcytes per sporad of No. 9.

No. of microcytes	3	4	5	6	7	8	9	10
No. of cases	2	43	49	41	33	25	6	1

In five plants, Nos. 6, 9, 27, 22 and 21, the percentage of abortive pollens was measured. The results are generally in accordant with the expectation from the meiotic configuration, namely the difference in pollen sterility is parallel to the difference in univalent chromosome

7 and 8, MII (No. 27); 7, normal MII; 8, showing some surviving univalents in cytoplasm; 9 and 10, AII (No. 27) showing chromosome bridge; 11, AII (No. 27) having three plates; 12—14, pollen grain mitosis in No. 22; 12, metaphase showing 29 chromosomes; 13, side view; 14, anaphase; 15 and 16, second equilibrium stage from multiple plates (first equilibrium stage); 17 and 18, representing marked difference in pollen abortion in two plants (No. 22 and 6); 17, nearly lacking abortive pollen grains (No. 22); 18, nearly lacking of normal pollen grains (No. 6). These photographs were taken from acetocarmine preparations, excepting Fig. 1. 1, $\times 2000$; 2—11, 15 and 16, $\times 1000$; 12, 13 and 14, $\times 1250$; 17 and 18, $\times 150$.

Figs. 12-25. Various chromosome configurations at MI in 7 plants. 12, No. 4 ($n=24$, $22\text{II}+2\text{I}$); 13, No. 5 ($n=24$, 24II); 14, No. 4 ($n=25$, $21\text{II}+4\text{I}$); 15, No. 9 ($n=27$, 27II); 16, No. 9 ($n=31$, $23\text{II}+8\text{I}$); 17, No. 27 ($n=28$, 23II); 18, No. 27 ($n=29$, $27\text{II}+2\text{I}$); 19, No. 27 ($n=30$, $26\text{II}+4\text{I}$); 20, No. 27 ($n=31$, $25\text{II}+6\text{I}$); 21, No. 21 ($n=33$, $31\text{II}+2\text{I}$); 22, No. E ($n=42$, $30\text{II}+12\text{I}$). 23 and 24, formation of multiple plates (first equilibrium stage) in No. 9. 25, 6 univalents at MI in No. 9. $\times 2600 \times 2/3$.

Figs. 26-37. AI, TI and MII in PMCs of No. 9 and No. 27. 26, abnormal AI; 27, 4 I splitting at AI; 28, 5 I splitting and 3 undivided univalents at AI; 29, AI with 1 bridge and 1 univalent; 30, unbalanced telophasic nucleus having 16 chromosomes in cytoplasm; 31-34, MII with 1, 2 and 3 chromosomes lying outside the equatorial plates (Fig. 32 shows two plates of polar view having unbalanced chromosome numbers); 35, side view of MII with many chromosomes lying outside the equatorial plate; 36, MII having 3 nuclear plates; 37, normal MII. $\times 2600 \times 2/3$.

Figs. 38—45. Representing AII and abnormal meiotic divisions in PMCs of No. 9 and No.27. 38, AII showing 2 univalents; 39, AII with 4 and 2 chromosomes lying out the main groups; 40, AII with 2 telophasic micronuclei; 41, formation of multiple plates in the first equilibrium stage; 42, AII with 3 nuclei; 43, third equilibrium stage; 44, polycaric conditions of PMC having some chromosome bridge; 45, polycaric condition. $\times 2600 \times 2/3$.

Figs. 46—52. Polycaric conditions in No. 9. 46, 3 microcytes; 47, normal tetrad; 48, 5 microcytes; 49, 6 microcytes; 50, 7 microcytes; 51, 8 microcytes; 52, 9 microcytes. **Figs. 53—60.** Chromosomes in pollen grain mitosis in No. 22. 53, 23; 54, 29; 55, 30; 56, 31; 57, 35; 58, 10; 59, anaphase; 60, anaphase with 2 laggards. $\times 2600 \times 2/3$.

number and its frequency. Some of these are represented in Photo. B, 17 and 18. Although the difference in the meiotic configurations of chromosomes may be responsible for the sterility, it may, however, depend on the environmental conditions, especially on air temperature. When the pollens of the same plant, No. 9, which was fixed on different days were examined separately, it was found that they were different in fertility. The results are represented in Table 4.

Table 4. Marked difference in pollen abortion in same plant (No. 9) which on fixed on different days.

Normal pollen	Abortive pollen	Fixed day
37.9%	62.1%	August 3, 1953
30.4	69.6	„ 11, „
87.9	12.1	September 24, „

In one of the plants of *B. tuberohybrida* under investigation, another abnormal meiosis was noted. Before the separation of chromosomes at the first division takes place, the normal bipolarity of the spindle is considerably broken down, and double or more plates are formed, each containing a group of chromosomes which are in number (Fig. 41). The bivalents in each groups then begin to divide, and univalents appear to migrate in various directions (Fig. 42); thus the whole situation comes to take a very confusing appearance. After this, the chromosomes enter the second equilibrium stage, at which more numerous plates are formed scattered in cell. From this stage onwards, univalents begin to split and again a confusing situation results. After the splitting of univalents and the migration of their halves were accomplished, the third equilibrium stage was attained (Photo. 15 and 16. Fig. 43).

In the present study, chromosomes in pollen grain division were first demonstrated. Owing to the meiotic irregularities mentioned above, pollen grains with various chromosome numbers should be made. It is rather difficult to get the polar views; for the division takes place along the vertical axis of the ellipsoid and cone or egg-shaped pollen. Fortunately, however, some polar views have been observed and several different chromosome numbers were found in No. 22. The results of chromosome counts were as follows; 28 (Fig. 53), 29 (Fig. 54), 30 (Fig. 55), 31 (Fig. 56), 35 (Fig. 57), 10 (Fig. 58). Probably other chromosome numbers in addition to the above-mentioned numbers are also expected to be found. Studies on pollen grains division is of a preliminary nature, and further work is now planned to be done on a larger scale.

Discussion

The genus *Begonia* has attracted particular notice in its remarkable aneuploidy in intraspecies. Two such notable instances, *B. semperflorens* and *B. Rex*, have been already reported by Matsuura and Okuno (1936, 1943). In the case of *B. semperflorens*, seven plants, Nos. I—VII, were first selected; they vary from one another with respect to size and thickness of the leaf, pubescence, and amount of anthocyanin, and other minor charac-

ters. These striking differences in characters strongly suggest the existence of karyotypic differentiation in these plants, and actually were ascertained when five plants among them were cytologically examined. In *B. Rex* likewise, eight different chromosome numbers were found in eight plants examined. As seen in Table 1, in *B. tuberohybrida* likewise, twelve different chromosome numbers were found in fifteen plants examined, and six materials (No. 5, 3, 9, 27, 22 and D) meiotic configurations were statistically studied (Table 2). In *B. semperflorens* and *B. Rex* likewise, the diversity of external characters was also found in several plants of *B. tuberohybrida* examined. The flower character, doubleness and singleness, may be not, however, correlated with differences in their chromosomal organization.

In any case where meiosis of eighteen plants was studied, the multivalent formation did not occur. From this result, there may exist several different genomes within plants of *B. tuberohybrida*. Concerning the basic chromosome number in the genus *Begonia*, 6, 7 and 13 have been advanced by Matsuura and Okuno (1936, 1943), and they suggested that the last may be of secondary origin derived from the former two. Although it may certainly be dangerous to arrive at the conclusion, it is more probable that the present eighteen plants of *B. tuberohybrida* may be polyploids or aneuploids which are multiples of either 6, 7 and 13 or hybrids induced by the crosses between polyploids having 6, 7 and 13 basic chromosomes. Especially the meiotic chromosome configurations seem to indicate that plants of No. 5 and 2 are octoploid derived from 6 and 7 basic chromosomes, and a plant of No. D is of a form derived from 13 basic chromosomes plus 7 chromosomes. Its meiotic configuration may be presented as follows; $26\text{II} + 7\text{II}$.

In a plant of No. 27 having 56 somatic chromosomes, a very peculiar type of abnormal meiosis was observed. It is characterized by the formation of multiple plates posterior to the first metaphase, and during the course of this division, a tendency of one genome to behave as an individual entity is shown here and there. The same observation on a plant of *B. Rex* was first demonstrated by Matsuura and Okuno (1943). They suggested that this mode of division may be of significance in the possibility of formation of gametes with various chromosome numbers. From these results, it will be realized how a large range of chromosome diversity in one species can easily arise through intraspecific crosses between such different chromosomal gametes. The new varieties in *Begonia* are ordinarily produced either from hybridized seeds or from propagation by cuttings. Even sterile plants which would otherwise perish, would survive by means of vegetative reproduction. Therefore, it may be expected that aneuploids in *Begonia* are found more often. In a hybrid, *Solanum dimissum* $\times$ *S. tuberosum*, formation of multiple plates and a tendency of one genome (6 or 12 chromosomes) to behave as an individual entity were also reported by Okuno (1951, 1952). The same observation on tetraploid *Primula* was made by Upcott (1939). In pollen mitosis of tetraploid *Primula kewensis*, he found the frequent occurrence of double plate formation. In some of the cases two equal plates were formed, each with 9 chromosomes which seems to be the ancestral set of chromosome.

Observations on chromosomes in pollen grain division were carried out with an aim to test, to what extent a variation in chromosome in pollen grains is in accord with expecta-

tion from the meiotic irregularities mentioned above and subsequent distribution of univalents just described. But it is rather difficult to get good metaphase plates, especially the polar view; for the division takes place along the vertical axis of the ellipsoid and cone or egg-shaped pollen. Several different chromosome numbers, 28, 29, 30, 31, 35 and 10, however, were found in No. 22 having 60 somatic chromosomes (Figs. 53—58). From these results, it will be realized how a large range of chromosome diversity can easily arise from hybridization between such different chromosome gametes. Probably such a karyotypic polymorphism as noted *B. semperflorens*, *B. Rex* and the present *B. tuberohybrida* is also expected to be found in other *Begonia* species, when one deals with material on a large scale. The present study in pollen grains division is of a preliminary nature, and its detailed report will be described in the next paper.

Summary

Twelve different chromosome numbers were found in eighteen plants of *B. tuberohybrida* examined (Table 1). Meiotic chromosome configurations were studied in seven plants, No. 5, 3, 9, 27, 22, 21 and D (Table 2). They were found to be striking polymorph with respect to the nuclear organization.

In a plant of *B. tuberohybrida* under investigation, a very peculiar type of abnormal meiosis was noted. It is characterized by the formation of multiple plates posterior to the first metaphase. And during the course of this division, a tendency of one genom to behave as an individual entity is shown.

In *Begonia*, chromosomes in pollen grain division were first demonstrated by the present study. Several different chromosome numbers were found. The results of chromosome counts were as follows; 28, 29, 30, 31, 35 and 10.

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