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CHARACTERISTICS OF COLCHIPLOID *PHLOX DRUMMONDII*

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ABSTRACT

Colchitetraploids were produced in a pure white flowered variety of *Phlox drummondii*, a garden ornamental, and studied for morphological and cytological characters for three successive generations. Flowers were bigger and their longivity was increased by 4–5 days in the colchiploids, thus, increasing their ornamental value. There was a sudden drop in quadrivalent frequency from C_0 to C_1 generation and this tendency was maintained in C_2 also which lead to rapid cytological diploidization of the colchiploids in the second generation itself.

Key words: Colchiploid, *Phlox*.

The genus *Phlox*, family Polemoneaceae, is a common garden ornamental throughout the world. The plants produce beautiful flowers in various colours. In India, the most commonly grown ornamental species of *Phlox* is *P. drummondii*. Colchitetraploidy has been induced with the objective to develop a new variety which has bigger flowers of better ornamental value. The tetraploid plants have been characterized. Their chromosome behaviour during meiosis was studied in successive generations.

MATERIALS AND METHODS

A pure white flowered variety of *P. drummondii* was selected for the induction of colchitetraploidy.

Colchicinization. Two methods, seed immersion and cotton swab, were used. In the former, seeds were immersed in 0.1, 0.15 and 0.2% colchicine (Sigma) solution for different durations, washed in running tap water, and sown in the pots. In the latter, cotton swabs soaked in 0.1, 0.15 and 0.2% colchicine solution were placed on the emerging apical tip between two cotyledonary leaves for 2 or 3 days, 5/6 h each day.

Chromosome studies. For meiotic study, young flower buds were fixed in 1:3 acetic acid : alcohol mixture for 24 h. Anthers stained in leucobasic fuchsin by the usual method were

squashed in 1% iron acetocarmine. For mitotic study, healthy root tips were pretreated in saturated solution of p-dichlorobenzene for 3 h at about 25°C and then fixed in 1:3 acetic acid : alcohol mixture for 24 h. Root tips were stained in leucobasic fuchsin in the usual manner and squashed in 1% iron acetocarmine. Photographs were taken from temporary preparations.

RESULTS

Efficiency of colchicinization. Tetraploidy was induced by the cotton swab method using 0.15% colchicine for 12 h spread equally over 2 days. Two colchiploids were obtained out of 100 seedlings treated. Primary screening of colchiploids was done by comparing their stomata with the diploid and were found to be bigger in the former. This was later confirmed by chromosome count. Seed immersion method failed completely as the seeds did not germinate.

CHARACTERISTICS OF COLCHIPLOIDS

Growth rate. As compared to the diploid, the colchiploids were slower in growth and the flowering was delayed by about 20 days.

Cell size. The increase in cell size was reflected in a pronounced increase in the size of stomata and pollen grains of colchiploids. However, the number of stomata per unit area in the tetraploid was 40% less..

Morphology. The colchiploids showed significant gigantism as they were about 1.5 times bigger than the diploids. They had thicker stem and dark green leaves, and were more fleshy and broader. The flowers were conspicuously larger in size (Fig.1A, B). Due to appreciable increase in the size of the leaves and flowers, the colchiploids were much better ornamentals than their diploid parents. Another significant feature was that the flowers were retained for 4–5 more days than in the diploids which gave a better longivity of flowering.

CYTOLOGY

Diploids. All the PMCs analysed at diakinesis/metaphase I had seven bivalents (Fig. 1C) which, on an average, resolved into 4.96 ring and 2.04 rod bivalents, the range being 1-7 and 0-6, respectively (Table 1). The chiasmata per cell ranged from 9–17, mean being 12.52, out of which 11.34 were terminalized giving terminalization coefficient of 0.90 (Table 2). Anaphase I had normal distribution of chromosomes (7:7) (Fig. 1 D). Subsequent course of meiosis was normal, resulting in 95% stainable pollen grains and good seed set.

COLCHITETRAPLOIDS

Co meiosis. Meiosis in the colchitetraploid was typical of autotetraploid nature. The average number of associations per cell was 2.76 IV + 0.04 III + 8.12 II + 0.61 I, the range

being 0–7, 0–1, 0–14 and 0–4, respectively (Fig. 1E; Table 1). Chiasmata per cell ranged from 20–29, mean 24.1, out of which 22.8 were terminalized giving terminalization coefficient of 0.94 (Table 2). Anaphase I was quite abnormal. Out of 25 cells analyzed, 15 had 14:14 distribution of chromosomes (Fig. 1F), followed by 15:13 in 8, and 16:12 in 2 cells.

Table 1. Average number and range of associations at diakinesis/metaphase I in diploid and tetraploid plants of *Phlox drummondii*

Material	No. of cells analysed	Quadrivalents		Trivalents		Ring bivalents		Rod bivalents		Total bivalents		Univalents	
		mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
2x	100	—	—	—	—	4.9	1–7	2.0	0–6	7.0	—	—	—
C_0	25	2.8	0–7	0.04	0–1	5.1	0–12	3.04	0–8	8.1	0–14	0.6	0–4
C_1	25	0.7	0–3	—	—	8.9	5–14	3.7	0–7	12.6	8–14	—	—
C_2	25	0.6	0–2	—	—	5.9	2–10	6.5	3–10	12.4	9–14	0.6	0–4

Comitosis. The root tip cells of 6 out of 8 seeds had 28 chromosomes (Fig. 1 H), aggregated into 7 groups of 4 chromosomes each. The remaining 2 seeds had 30 chromosomes and the two extra chromosomes tallied with the 5th and 7th group of chromosomes.

C_1 meiosis. As compared to C_0 plants, significant drop in the mean and range of quadrivalents was observed in C_1 plants, with corresponding increase in bivalents. The average number of associations per cell was 0.68 IV + 12.64 II, range being 0–3 and 8–14, respectively (Fig. 1G; Table 1). The number of chiasmata ranged from 21–29, mean 25.31, out of which 23.3 were terminalized giving terminalization coefficient 0.91. At anaphase I, equal distribution of chromosomes (14:14) was observed in all the 25 cells.

Table 2. Mean and range of chiasmata, terminalization coefficient, and anaphase I distribution in diploid and tetraploid plants of *Phlox drummondii*

Material	No. of cells analysed	Chiasmata				Teminali-zation coefficient	Anaphase		
		mean	range	termina-lized	untermi-nalized		distri-bution	No. of cells	(%)
2x	100	12.5 ± 2.3	9–17	11.3	1.2	0.9	7:7	25	(100)
C_0	25	24.1 ± 2.5	20–29	22.8	1.3	0.9	14:14	15	(60)
							15:13	8	(32)
							16:12	2	(8)
C_1	25	25.3 ± 2.2	21–29	23.3	2.0	0.9	14:14	25	(100)
C_2	25	21.6 ± 1.9	18–25	18.1	3.5	0.8	14:14	25	(100)

C_2 meiosis. The drop in the mean (0.64) and range (0–2) of quadrivalents was maintained in this generation also, but to a lesser extent. The average number of associations per cell

was 0.64 IV + 12.40 II + 0.64 I (Table 1). Chiasmata per cell ranged from 18–25, mean 21.56, out of which 18.1 were terminalized giving terminalization coefficient of 0.83 (Table 2). Chromosome distribution at anaphase I was normal (14:14) in all the cells analysed.

Pollen stainability. In the diploid, pollen stainability was 95%. In C_0 it was 30% which rose to 50% in C_1 and 57% in C_2.

Seed set. Seed set was very poor in C_0. Only 14 seeds per plant were obtained and they too gave only 50% germination. In C_1 the average seed set per plant was 44 which significantly rose to 75 in C_2 generation.

DISCUSSION

In the present study, the cotton swab method of colchicinization was found to be successful. The same method produced successful results in other studies [1–3]. The seed treatment method was a complete failure as the seeds failed to germinate.

The colchitetraploids showed all the characteristic features of induced polyploidy. Their slower growth rate, though initially disadvantageous, ultimately proved to be an exceptionally desirable character in the form of longivity of flowers for 4–5 more days than in the diploids. The initial slow rate of growth could be because of reduced rate of cell division [4], lower amount of growth hormone [5] or lower rate of metabolic activities [6].

The increase in cell size was reflected by bigger size of stomata and pollen grains in the colchiploids. These two criteria could be used for primary screening of colchiploids. The increase in cell size resulted in the increase of other plant parts, such as, leaves, internodal distance, flowers and seeds, thus, leading to overall gigantism. These characters imparted better ornamental value to the colchiploid. Though the seed set was very poor in C_0 (which improved in subsequent generations), this hardly underscores the ornamental value of the colchiploids.

The colchiploids were characterized by the presence of high quadrivalent frequency of C_0 (mean 2.76, range 0–7). However, there was significant reduction in quadrivalents in C_1 (mean 0.68, range 0–3), and C_2 (mean 0.64, range 0–2). The corresponding range of bivalents increased from 0–14 in C_0 to 8–14 in C_1 and 9–14 in C_2. Though the downword trends in quadrivalent frequency in the succeeding generations has been reported in some autotetraploid lines [7], the sudden drop in the very next generation, as observed in the present case, has rarely been reported. The trend of reduction in quadrivalent frequency was maintained in C_2 generation also to a lesser extent as compared from C_0 to C_1. Further studies in the succeeding generations might reveal whether this important meiotic

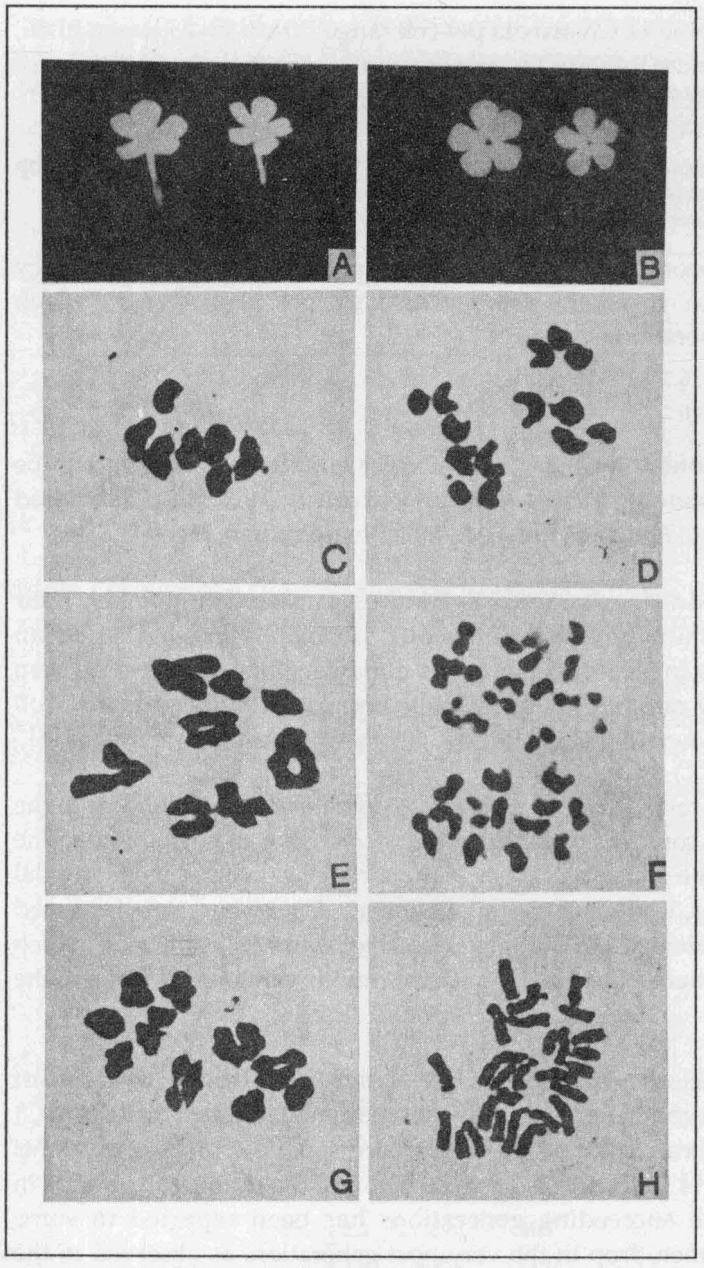

Fig. 1. A, B) Flowers of 4x and 2x; C) meiotic metaphase I of 2x, 7II; D) anaphase I of 2x, 7:7 distribution; E) meiotic metaphase I of 4x, 6 IV + 2II; F) anaphase I of 4x, 14:14 distribution; G) meiotic metaphase I of 4x (C_1), 14 II; and H) mitotic metaphase of 4x, 28 chromosomes.

behaviour leading to cytological diploidization is maintained. Our observations are contrary to those of Raghuvanshi and Pathak [2], who observed increase in mean quadrivalent number from 1.61 in C_1 to 2.65 in C_2 with corresponding decrease in bivalents. It is interesting that the same species behaves so differently at chromosomal level to the same treatment at different places. This could possibly be due to the change in environmental condition or may be due to some minor genotypic differences in the materials used.

Another point of interest in the present study is that with a downword trend in quadrivalent frequency from C_0 to C_2, a noticeable difference was observed at anaphase I. While unequal distribution (15:13, 16:12) was observed in 40% cells in C_0, all the cells had equal (14:14) distribution of chromosomes in C_1 and C_2. The pollen stainability also increased from 30% in C_0 to 57% in C_2 plants, resulting in good seed set. The average seed set/plant which was 14 in C_0 increased to 44 in C_1, and 75 in C_2 plants. Therefore, it may be concluded that sterility in C_0

plants was due to segregational errors of quadrivalents [8, 9].

The present investigation has shown that the colchiploids produced in *P. drummondii* has many morphological features which would establish them as better ornamentals. The cytological features as observed in C_1 and C_2 lend support to the establishment of improved morphological characters of the colchiploids.

REFERENCES

1. T. S. Dhillon. 1970. *Phlox drummondii.* I. Cytogenetics of colchicine induced autotetraploids. Japan J. Genet., **45**: 305–312.

2. S. S. Raghuvanshi and C. S. Pathak. 1975. Polyploid breeding and possibility of raising double varieties in *Phlox drummondii* Hook. Cytologia, **40**: 355–363.

3. S. Rama Rao, S. N. Raina and P. K. Srivastava. 1982. Induced autotetraploidy in *Phlox drummondii* Hook. J. Cytol. Genet., **17**: 53–58.

4. O. Y. Eigsti. 1947. The pollen tube method for making comparisons of differences in mitotic rates between diploid and tetraploid. Genetics, **32**: 85.

5. P. Larsen and S. Mintung. 1950. Growth promoting and growth retarding substances in pollen from 2n and 3n apple varieties. Bot. Gaz., **3**: 436–447.

6. F. Schwanitz. 1951. Untersuchungen an polyploiden pflanzen. XII. Der Giges. Charakter der Kulturflanzen und scine Bedeutung fur die polyploidie Zuchtung. Züchter, **22**: 273–275.

7. W. Gottschalk. 1978. Open problems in polyploidy research. The Nucleus, **21**: 99–112.

8. C. D. Darlington. 1965. Recent Advances in Cytology (2nd ed.). J. and A. Churchill Ltd., London.

9. M. H. Hazarika and H. Rees. 1967. Genotypic control of chromosome behaviour in rye. X. Chromosome pairing in autotetraploids. Heredity, **22**: 317–332.