Polyloid Breeding and Possibility of Raising Double Varieties in *Phlox drummondii*, Hook.

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Phlox drummondii, a winter ornamental of family Polemoniaceae, is represented by many varieties differing in flower colour, flower morphology and general habit of the plant. Owing to successful polyploid breeding among ornamentals like *Torenia fournieri* (Joshi and Raghuvanshi 1966), *Catharanthus roseus* (Raghuvanshi and Chauhan 1968), *Thunbergia alata* (Raghuvanshi and Singh 1973) a programme was launched to investigate the potentialities of polyploid breeding in this species.

Materials and methods

Seeds of different varieties were sown in separate pots. Shoottips of young seedlings were treated with 0.2% aqueous colchicine solution in the evening for six hours. This resulted in checking the growth for a few days and later sprouts were either tetraploid or diploid. Reverted shoots were again given similar treatment, thus stabilized tetraploids were obtained. For cytological investigation flower buds were fixed in 1:3 acetic alcohol fortified with iron for 24 hours. Later on the buds were transferred to 70% alcohol. Acetocarmine squashes were studied in temporary preparations and slides were made permanent by ethanol-butanol schedule.

Hybridizations between $4n$ and $2n$ plants were carried out to raise triploids. Buds of proper size were emasculated in the evening and pollinated next morning when the stigma become receptive. To check cross pollination petals were tied with thread. C_2 generation of tetraploids was raised for selecting lines having improved ornamental value as well as higher percentage of seed setting.

Observation

In $2n$ and $4n$ plants the average height was 55 and 54 cm, number of branches 15 and 11, flower diameter 2.1 and 2.8 cm (Fig. 2) length of corolla tube 1.3 and 1.6 cm, petal length 1.0 and 1.2 cm, petal breadth 0.9 and 1.3 cm and anther length was 0.15 and 0.2 cm respectively. The general size of plant at tetraploid level was more or less similar to diploids. There was general increase in leaf size in tetraploids (Fig. 1), but among tetraploids themselves gradation of leaf size was noted. Flower size was also increased over diploids (Fig. 1). One of the achievement of polyploid breeding in this plant is improvement in the shades of flower colour leading to tremendous increase in ornamental value. Flowering was little

Fig. 1. Diploid, tetraploid and triploid leaf and flower.

delayed in tetraploids but the flowering period was prolonged.

In C_1 generation the tetraploids showed leaf distortions in the beginning but later the leaves became normal. Initial flowers were also abnormal, they were small and usually having fringed petal lobes but later on the flowers became normal, whereas, in C_2 generation the flowers and leaves were normal from the beginning.

In yellow variety a tetraploid plant of C_1 generation was observed in late season having one branch with double flowers. In comparison to normal five petal lobes, this branch had flowers with ten petal lobes arranged in two whorls (Fig. 2). Other floral parts were normal and also there was good pollen fertility.

Our *Phlox* breeding programme included building of triploids for raising aneuploids for detailed studies. For raising these triploids reciprocal crosses between diploids and tetraploids were carried out. Normally crosses are more successful when diploids are used as male parents, but in this species viable seeds could be obtained from crosses

Fig. 2. Double flower of yellow variety.

using diploids as females and tetraploids as males. In its reciprocal cross, though fruit formation took place in rare cases but all the seeds were shrivelled and inviable. So far it has been possible to raise only one hybrid, which though had good number of triploid cells, yet other cells displayed variation in chromosome number as described later. The plant was 63 cm in height and had 20 branches. Length and breadth of leaf was 3.7 and 1.5 cm respectively. Flower diameter was 1.7 cm in the beginning of flowering but in late season the diameter was 2.0 cm.

Pollen fertility in diploids was 94.9% and in tetraploids it was 88.5% and 94% in C_1 and C_2 generations. In triploid pollen fertility was 39.06%.

Cytological: The chromosome number of *Phlox drummondii* is $2n=14$ (Meyer 1944, Fig. 3) and the cytological behaviour of different varieties was found to be normal. Difference in cytological behaviour was observed in C_1 and C_2 generations of autotetraploids. Various configurations at metaphase I are summarised in Table I. Quadrivalent (Figs. 4, 6, 11, 12) and trivalent (Fig. 5) increased in C_2 generation, whereas the univalent frequency decreased. Among the bivalents there was sharp increase in rings and decrease in rods, but the total frequency of bivalents decreased. One plant in C_1 generation showed very low frequency of quadrivalents (0.05 per PMC), and most of the cells showed bivalents only (Fig. 13). Unfortunately there was no seed setting in this plant despite good pollen fertility (84.0%).

Table 1. Showing different types of chromosomal associations, chiasma frequency and pollen fertility

Level of ploidy		Quadrivalent		Trivalent	Bivalent		Univalent	Chiasma frequency	Pollen fertility
		Ring	Chain		Ring	Rod		Per chromosome	
2n	Per cell	—	—	—	3.52	3.42	0.06	0.75	94.9%
	Range	—	—	—	6-7	2-14	0-4	0.60-0.80	
3n	Per cell	0.41	0.10	3.25	3.52	0.41	2.4	0.82	39.06%
	Range	0-2	0-1	0-7	0-7	0-2	0-8	—	
4n (C_1 generation)									
	Per cell	1.31	0.3	0.06	4.17	5.14	0.95	0.73	88.5%
	Range	0-6	—	0-2		2-14	0-4	—	—
4n (C_2 generation)									
	Per cell	2.07	0.58	0.22	6.23	1.81	0.67	0.88	94.0%
	Range	0-5	0-3	0-2	6-23	0-5	0-2	.87-0.92	—

Anaphasic separation in tetraploids showed laggards ranging from 1 to 3, unequal separation (Fig. 14) and dividing univalents were also observed. The frequency of these anomalies was higher in C_1 than C_2 .

Besides the normal tetraploids two plants in C_1 generation were mixoploid. One plant had $2n=27$ (83.3% PMCs, Fig. 16) and $2n=25$ (16.7% PMCs, Fig. 15) chromosomes at metaphase I. At AI lagging chromosomes and at T_2 more than four groups were observed. This plant later on stabilized at tetraploid level. The

chromosomes in the other plant varied from 10 to 71. Out of 9 cells studied all the cells had different number of chromosomes such as 10, 14, 15, 21, 24 and 71 (Fig. 17, 18, 19, 20 and 21). Besides these 1 to 2 fragments were also observed in 3 cells. AI and AII were also disturbed leading to the formation of many groups of chromosomes at T₂. Pollen fertility was 40%. Before this plant could stabilize at particular level of ploidy it died without setting any seed.

The hybrid plant of violet variety obtained by crossing diploid and tetraploid displayed variation in chromosome number from 21 to 24. Different PMCs had 21 (30.9% Figs. 22, 23, 24), 22 (30.9%, Figs. 7, 25, 27), 23 (23.6%, Figs. 8, 9, 10) and 24 (14.6%, Fig. 26) chromosomes. Frequency of trivalent was high with 3.25 per PMC, in few cases all the chromosomes were involved in trivalent configuration (Fig. 24). Quadrivalent frequency was very low as compared to tetraploids, and in any case not more than two quadrivalents were observed in any PMC. It seems that there is no correlation between number of chromosomes and quadrivalents in a PMC, because in one case although the PMC had only 21 chromosomes (3n) even then there were two quadrivalents (Fig. 22). Univalent frequency was 2.4 per PMC, and they range from 0 to 8 (Fig. 27).

At AI unequal separation with 10+11 (7.14%), 9+14 (1.7%, Fig. 28), 13+10 (1.7%) and one (46.05%), two (4.3%) and four laggards (9.3%) were observed. At AII besides the normal 4 groups, cells with 2 laggards (17.8%), 1 laggard (22.1%) and 3 laggards (2.1%) were observed. All these anomalies led to the formation of different number of spores at sporad stage. Normal 4 spores were observed in 21.2%, 5 spores in 16.9% and 6 spores in 1.7% sporads.

Meiosis in diploids were similar in both the years with regular bivalent formation and normal anaphasic separation leading to the formation of 4 spores per sporad.

Chiasma frequency in 2n, 4n (C₁ generation), 4n (C₂ generation) and 3n (mixoploid) was 0.75, 0.73, 0.88 and 0.82 per chromosome respectively. Increase in chiasmata per chromosome between 2n and 3n, 2n and 4n (C₂ generation), 4n (C₁ generation) and 3n, 3n and 4n (C₂ generation) and C₁ and C₂ generations of auto-tetraploids was statistically significant. In comparison to these in 4n (C₁ generation) and 2n the increase in chiasmata per chromosome was statistically insignificant (Table 2).

Table 2. Showing mean value of chiasmata, standard deviation and standard error at different levels of ploidy

Level of ploidy	2n	3n (Mixoploid)	4n (C ₁ generation)	4n (C ₂ generation)
Analysis of chiasmata				
Actual mean (M)	0.75	0.86	0.73	0.88
Standard deviation (S.D.)	0.0884	0.0826	0.0071	0.0099
Standard error (S.E.)	0.0125	0.0116	0.0010	0.0098

Figs. 3-10. 3, diploid, metaphase I. 4-6, tetraploid. 4, MI, showing 1_(IV) 1_{III} 10_{II} 1_I configurations. 5, MI, (4_(IV) 1_{IV} 2_{III} 1_{II}). 6, MI, (3_(IV) 8_{II}). 7-10, Triploid.—7, MI, 3X=22, (1_(IV) 2_{III} 5_{II} 2_I). 8, MI, 3X=23, (1_(IV) 5_{III} 2_{II}). 9, MI, 3X=23, (4_{III} 5_{II} 1_I). 10, MI, 3X=23, (2_(IV) 5_{III}).

Seed setting was normal in diploids, whereas, tetraploids in C_1 generation showed poor seed setting but in C_2 generation there was definite improvement. Different varieties had shown gradation in seed setting. There was 95% seed setting in yellow tetraploids while the white variety had only 5% seed setting. In red, violet and dark pink varieties the seed setting was 80%, 52% and 32% respectively. Number of seeds per fruit was reduced in tetraploids. In diploids normally 4 seeds were formed in a fruit, but in tetraploids it ranged from 1–3. Tetraploid seeds were bold, slightly bigger than diploids. Mixoploid plant had only 1% seed setting and there was only one seed per fruit.

Discussion

Polyploidy is known to increase the size of organs of determinate growth like leaf, flower etc. and in certain cases there may be increase in total plant size as reported in *Catharanthus roseus* (Raghuvanshi and Chauhan 1968), *Thunbergia alata* (Raghuvanshi and Singh 1973) and *Tropaeolum majus* (Raghuvanshi and Pathak 1974), but in present case the polyploids besides increase in the flower size, showed astonishing change in the shades of flower colour, which certainly improve their ornamental value over their diploid progenitors.

Among ornamentals double flowering varieties are considered to be of great value. In one of the C_1 tetraploids of yellow variety during late season one of the branches had flowers with ten petals instead of the five in the normal flowers. These petals were arranged in two whorls of five each. All the flowers in this particular branch were of double type. Other floral parts like androecium and gynoecium were normal. Pollen fertility was like other tetraploids, even then there was no seed setting. However, it may be mentioned that when this double flowering branch was observed it was fairly late in season and temperature had gone up and even in controls the seed setting was extremely poor. It is suggested that this double flowering branch may have originated as a result of mutation. This observation opens up the possibility of synthesizing double flowering varieties of *Phlox* which will certainly be of great ornamental value.

Quadrivalent frequency was higher in C_2 generation of autotetraploids of *Phlox*, however, Gills and Randolph (1951) had reported decreased quadrivalents in succeeding generations of polyploids induced in maize, resulting in increased bivalent frequency and fertility. In the present case also there was increased pollen and seed fertility, although there was high quadrivalent frequency. It was observed in natural occurring polyploids of *Dactylis glomerata* that despite high multivalent frequency there was complete fertility (Brix and Quardt 1953).

Figs. 11–28. 11–14. Tetraploid. 11, MI, $(5_{(IV)} 1_{IV} 2_{II})$. 12, MI, $(6_{(IV)} 2_{II})$. 13, MI, $(11_{(II)} 2_{II} 2_I)$. 14, AI, showing unequal separation $(15+13)$. Fig. 15–21. $4n$ (Mixoploid). 15, MI, $2n=25$, $(2_{(IV)} 1_{IV} 3_{(II)} 3_{II} 1_I)$. 16, $2n=27$, $(1_{(IV)} 1_{IV} 7_{(II)} 2_{II} 1_I)$. 17, MI, $2n=10$ $(4_{II} 2_I)$. 18, $2n=15$ $(4_{(II)} 3_{II} 1_I)$. 19, $2n=21$, metaphase I showing two spindles. 20, MI, $2n=24$ showing 12 bivalents. 21, MI, $2n=71$, $(33_{II} 5_I)$. 22–28. Triploid. 22, $3X=21$, MI, $(2_{(IV)} 3_{III} 2_{II})$. 23, MI, $3X=21$, $(4_{III} 3_{II} 3_I)$. 24, $3X=21$. Metaphase I, showing 7 trivalents. 25, MI, $3X=22$ $(1_{(IV)} 2_{III} 4_{(II)} 4_I)$. 26, MI, $3X=24$ $(8_{II} 8_I)$. 27, $3X=22$ $(2_{III} 4_{II} 8_I)$. 28, AI, $3X=22$, unequal separation $(14+9_I)$.

Tetraploids of *Phlox drummondii* raised by Dhillon (1970) showed 4.7 quadrivalent per PMC, which is quite high in comparison to 2.5 per PMC observed in our material. Chromosomal pairing can be affected by a number of factors like change in temperature, moisture content of soil etc. In *Tabernaemontana divaricata*, var. I. effect of temperature on chromosomal pairing was clearly demonstrated by Raghuvanshi and Chauhan (1970). Difference in our observation and those of Dhillon (1970) could be either due to effect of environmental condition or may be the two materials differ in genetic constitution specially regarding multivalent association. Work of Raghuvanshi and Singh (1973) in *Trigonella foenum-graecum* has clearly demonstrated that different varieties of same species may show great variation in chromosome association at tetraploid level. However, whether the difference in multivalent frequency is as a result of environment or genetic in nature can be varified by growing different strains under similar environmental conditions and by further genetic analysis.

The hybrid raised by crossing $2n$ and $4n$ plants, although displayed variation in chromosome number, still the variation was near triploid level. Mixoploid nature is unstable condition and usually a plant may gradually stabilize at more balanced chromosomal level. Keeping this in view the chromosomal behaviour of this plant was studied at the beginning, in the middle and at the end of its flowering period, but same level of chromosomal variability was noted throughout.

The first formed flowers in this plant were small but later on there was improvement in size. Though pollen fertility was low (39.06%), seeds which appear to be viable have been obtained and these will be grown in the next season for the study of its progeny to explore the possibility of raising aneuploids.

Chiasma frequency increased in C_2 generation of autotetraploids, but in C_1 generation it was lower than diploids. Mixoploid plant revealed increased chiasma frequency over diploids and C_1 tetraploids.

Pollen fertility in tetraploids was slightly reduced in C_1 , but in C_2 generation it reached near about the diploid level, thus in this respect it differs from the induced polyploids of other plant species, where there is high pollen sterility at tetraploid level such as *Torenia fournieri* (Joshi and Raghuvanshi 1960), *Catharanthus roseus* (Raghuvanshi and Chauhan 1968), *Thunbergia alata* (Raghuvanshi and Singh 1973) and *Tropaeolum majus* (Raghuvanshi and Pathak 1974) Polyploids of *Tabernaemontana divaricata* (Raghuvanshi and Chauhan 1970) and *Arrhoxystylum elegans* (Raghuvanshi and Pathak 1973), displayed increase in pollen fertility over their diploid parents, but in these cases it was due to genic mutations and changed incompatibility behaviour respectively.

Although there was more or less similar pollen fertility in tetraploids, still there was gradation in seed setting, in yellow (95%), red (82%), violet (52%, dark pink (32%) and white (5.0%) varieties. The plants having high seed sterility had normal development of gynoecium and other floral parts. There was normal pollen germination over stigma.

It is interesting that response of different varieties with regard to seed fertility showed tremendous variation at tetraploid level. It may be emphasized again that as far as meiotic behaviour is concerned all the varieties displayed more or less

similar pattern at tetraploid level so effect on seed setting may be the result of some genetic factor(s).

Summary

Polyploids of *Phlox drummondii*, Hook. raised in white, yellow, dark pink, red and violet varieties exhibited improved flower size, blooming pattern and also displayed improvement in shade of colour over their diploid progenitors. While C_1 tetraploids had lower chiasma frequency than diploids, in C_2 there was increase in chiasma frequency which was higher than diploids. Result of efforts to raise triploids as a result of hybridizations between $2n$ and $4n$ have been discussed. Differential response towards seed setting had been exhibited by different varieties at tetraploid level. Possibility of raising double varieties has been discussed.

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