Induced Polyploidy in Legumes III. *Phaseolus vulgaris* L.

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Phaseolus vulgaris L., the French bean, is an important vegetable crop plant. Induced polyploidy is reported in this species (Šiškovic 1957, Eberle 1961, Monge *et al.* 1963, Sheopuria and Tiwari 1970). The present communication is a detailed cytological and morphological investigation on the colchicine induced tetraploidy in three cultivated varieties of *P. vulgaris*.

Materials and methods

Seeds of three cultivated varieties of *Phaseolus vulgaris* L. viz. varieties Premier, Prince and Climbing were collected from a local nursery. Polyploidy was induced by treating the seedlings with 0.25% and 0.5% aqueous solution of colchicine for different durations by cotton-plug method before the emergence of the third leaf (Table 1).

Cytological techniques

Meiotic chromosomes were studied by fixing the flower buds between 7.00 to 8.00 hours in propiono-alcohol (1:1) for 30 minutes and preserved in 70% alcohol. Anthers were smeared in 1% propiono-carmin solution. Fertility of pollens was also studied by staining the pollens in 1% propiono-carmin solution. Excess stain was avoided by passing 45% propionic acid between the slide and the cover slip. Stomatal studies were made by peeling off the surface layer of the leaf. Figures were drawn with the magnification of $\times 1400$, and $\times 300$. Photomicrographs were taken from suitable plates.

Observations

Treatments with 0.25% for 6 hours 1 day and 6 hours 2 days yielded polyploidy in all the three varieties while the same with 0.5% for 6 hours 1 day induced polyploidy in the variety Prince only.

In the polyploids of all the three varieties, growth was checked just after the treatment but after some days normal growth was resumed. First pair of leaves in these plants became wider, dark green and thicker as compared to the respective diploids. Other leaves emerged after the first pair of leaves were found to be deformed. Apices in the leaf-lets of these leaves were acuminate instead of acute apices in the respective diploids. Gradually normal conditions were restored in these plants. Leaf-buds have been found to sprout in some cases from other sites

than the treated region and were nipped off. Effect of colchicine was found to be significant in these plants as compared to the plants with those unwanted leaf-buds. In the variety Climbing, the climbing habit was found to be checked just after the treatment but gradually it was regained.

In the tetraploid plants of all the varieties, frequency of stomata was decreased while the mean size of the guard cells increased significantly. Variation in the stomatal size in control and tetraploid of the variety Premier have been observed (Table 2, Figs. 1 and 3). Flowering was found to be delayed in these plants and the frequency of flowers was low in comparison to the respective diploids (Table 2).

Table 1. Frequency of polyploids in different treatments on *P. vulgaris*

Name of plant	Concentration	Duration	No. of seedlings treated	No. of them survived	No. of polyploids obtained	% polyploids in treated plants	% polyploids in survived plants
<i>P. vulgaris</i> variety Prince	0.5	6 hrs. 1 day	15	7	1	6.6	14.2
	0.5	6 hrs. 2 days	15	4	0	—	—
	0.25	3 hrs. 3 days	10	8	0	—	—
	0.25	6 hrs. 1 day	10	7	0	—	—
	0.25	6 hrs. 2 days	10	5	1	10	20
<i>P. vulgaris</i> variety Premier	0.5	6 hrs. 1 day	10	4	0	—	—
	0.5	6 hrs. 2 days	10	2	0	—	—
	0.25	6 hrs. 1 day	10	7	1	10	14
	0.25	6 hrs. 2 days	15	8	2	13.3	25
<i>P. vulgaris</i> variety Climbing	0.5	6 hrs. 1 day	10	3	0	—	—
	0.5	6 hrs. 2 days	10	2	0	—	—
	0.25	6 hrs. 1 day	10	7	1	10	14
	0.25	6 hrs. 2 days	10	6	1	10	16

Only a few fruits came to appear in the tetraploids of the variety Prince but none of them attained maturity. Matured fruits were produced by a tetraploid plant of the variety Premier. The frequency of these fruits was low as compared to that of the diploid plants. Most of the seeds of these fruits were abortive. The abortive seeds were also found to occur in the control plants but their frequency was comparatively low. Number of seeds per pod has been found to be reduced in this plant. One of the seeds produced from this plant was provided with incomplete seed-coat and failed to germinate.

Tetraploidy has not been maintained in the variety Climbing where diploidy was restored in the later stages.

Sterility of pollens in the tetraploids of all the varieties has been found to be increased in comparison to that of the respective diploids. A marked change in the pollen-size has also been noticed (Table 2, Figs. 2 and 4). Only five seeds were obtained from tetraploid plant of the variety Premier. The tetraploid plant of the variety Prince did not set seed.

Cytology

Meiotic study in the normal diploid plants of all the varieties revealed $n=11$ bivalents (Figs. 5–6). In the tetraploids of the variety Premier, PMC study

Figs. 1–4. 1–2, stomata and a pollen grain in diploid. $\times 300$. 3–4, stomata and a pollen grain in tetraploid. $\times 300$.

Table 2. Comparative data of diploid and tetraploid *P. vulgaris* variety Premier

	Diploid	Tetraploid
Stomatal frequency/unit area (lower surface)	29.0 ± 0.25	18.0 ± 0.11
Size of guard cell (μ) lower surface		
length	20.6 ± 0.285	21.61 ± 0.19
width	12.89 ± 0.21	14.70 ± 0.96
Pollen size (μ)	49.82 ± 0.34	60.71 ± 0.19
Sterility (%)	12.86	59.47
Time required for flowering (range in days)	35–42	51

showed $n=22$ bivalents although very rarely clear twenty-two bivalents have been found. In all the varieties different combinations of uni-, bi-, tri-, and quadrivalents were observed in the tetraploids (Figs. 7–9). Quadrivalents ranged from 0–4, trivalents from 0–3 and univalents from 0–10 in the tetraploid of the variety Premier (Table 3). Univalents were found to occur in most of the cells. Frequency of quadrivalents was 1.14 per cell in the tetraploid of the variety Premier (Table 3). In anaphase I, 22–22 chromosomes in both the poles have been observed. Laggards were noticed in 53.3% cells of these plants and in 13.3% cells formation

of bridges was observed (Fig. 10). Unequal separation of chromosomes has also been observed.

Meiotic study in the tetraploid of the variety Prince was meagre due to its fewer number of flowers. However, different combinations of uni-, bi-, tri- and quadri valents were noticed (Fig. 8). Range of quadrivalents varied from 0-1, that of trivalents varied from 0-2 and that of univalents varied from 0-10. The occurrence of univalents was prevalent in most of the PMCs.

Tetraploid number of chromosomes was observed in lower frequency in the

Figs. 5-11. 5, metaphase I ($n=11$) in diploid (variety Premier). 6, metaphase I ($n=11$) in diploid (variety Prince). 7, metaphase I ($n=22$) with uni-, bi-, tri- and quadrivalents in tetraploid (variety Premier). 8, metaphase I ($n=22$) with bi- and quadrivalents in tetraploid (variety Prince). 9, metaphase I ($n=22$) with bi- and univalents in tetraploid (variety Climbing). 10, anaphase I ($n=22$) with laggard in tetraploid (variety Premier). 11, anaphase I ($n=22$) showing one pole in tetraploid (variety Climbing).

Table 3. Chromosome association at metaphase I in tetraploid
P. vulgaris variety Premier

Types of association															Frequency per cell
IV	—	—	4	1	1	—	—	2	1	1	—	1	—	3	1.14
III	1	—	—	1	—	—	1	3	2	—	1	1	—	1	0.806
II	18	18	13	15	15	19	18	13	16	18	20	15	22	14	15.39
I	5	8	2	7	10	6	5	1	2	4	3	3	—	1	4.14
No. of cells	1	6	5	1	1	5	2	6	1	2	3	1	1	1	Total 36

variety Climbing. In this plant also, due to lack of sufficient number of flowers, detailed meiotic study could not be made. Prevalence of univalents was observed in the treated plants of this variety. In the anaphase I, separation of 22-22 chromosomes has been observed (Fig. 11). Unequal separation and formation of bridges were noticed in 9.7% and 48.7% cells respectively.

Polyspores with the formation of 5-11 spores in the mother cells in addition to normal tetrads was observed in the tetraploids of all the three varieties.

Discussion

In all the three varieties, treatments with 0.25% colchicine for 6 hours 2 consecutive days yielded maximum number of polyploids. Although treatment with 0.5% colchicine solution gave polyploid plant in one case, this concentration was found to be toxic in almost all cases.

In the tetraploids, the immediate effects observed, were thick, dark green and deformed leaves and the apices of the leaf-lets were acute instead of acuminate as in the control diploids. The arrestation of growth and development just after the treatment has been attributed to some physiological disturbances and slower rates of cell division (Swanson 1957, Gunckel 1957).

In some treated plants of all the varieties, leaf-buds have been found to emerge from other sites than the treated regions and nipped off. In such plants, polyploids have been obtained in more cases than the other plants where these leaf-buds have not been removed as found in other plant species (Das and Mukherjee 1967, Biswas and Bhattacharyya 1972). Increase in size of guard-cells has been reported by Šišković (1957), Monge *et al.* (1963) and Sheopuria and Tiwari (1970) in *Phaseolus vulgaris*. Similar increase in stomatal sizes has been observed in the tetraploids of the variety Premier. Stomatal size and frequency together with pollen sterility and meiotic disturbances have been taken as criteria of detecting polyploids as pointed out by Eigsti and Dustin (1955).

Flowering was late and frequency of flowers was low in the tetraploids of variety Premier. Delayed flowering has also been reported by Sheopuria and Tiwari (1970) in *P. vulgaris*. The delayed flowering may be due to the slower rate of metabolic activities in the tetraploid plants (Biswas and Bhattacharyya 1971).

Pollen sterility and pollen size have been found to be increased in the tetraploids of the variety Premier as reported earlier in *P. vulgaris* (Šišković 1957 and Monge *et al.* 1963).

Meiotic study in the normal diploids of all the varieties revealed $n=11$ bivalents. In the tetraploids, $n=22$ bivalents have been observed. The exact 22 bivalents have been noticed in rare cases but various combinations of uni-, bi-, tri- and quadrivalents have been observed. In the variety Premier, the frequency of quadrivalents was 1.14 per cell. PMCs with complete set of quadrivalents have not been observed in any case. As many as 4 quadrivalents have been observed while the previous report of Monge *et al.* (1963) shows 5-10 quadrivalents in the autotetraploid of this species. Occurrence of high frequency of bivalents has been attributed to the short size of chromosomes (Riley and Chapman 1958). Raghuvanshi

and Joshi (1966), however, attributed it to a gene favouring bivalent formation. In the variety Climbing, the chromosome number was found to be increased upto tetraploid level but mostly it was hypotetraploid.

Summary

Seedlings of three cultivated varieties of *Phaseolus vulgaris* L. e.g., Premier, Prince and Climbing have been treated with aqueous solution of colchicine.

Polyploid plants were obtained in all the three varieties and treatment with 0.25% colchicine solution produced maximum number of polyploids. In the tetraploids, deformed, thick and dark green leaves, stunted growth, delayed flowering, changes in size of stomata and pollen grains and reduced fertility have been observed and discussed with reference to previous observations.

Meiotic studies of all the tetraploids have revealed exact doubling but different combinations of uni-, bi-, tri- and quadrivalents have been observed. PMCs with complete set of quadrivalent formation have not been observed. Occurrence of high frequency of bivalents has been discussed. Maximum number of quadrivalents was 4.

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