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Comparative Morphophenological studies and genetic improvement in *Ocimum Basilicum* Var *Purpurascens* Benth. (Lamiaceae) through induced autopolyploidy

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Abstract

Colchiploids of *Ocimum basılicum* var *purpurascens* Benth (Lamiaceae), a herb of high medicinal and commercial value, yielded significant results with regard to various morphophenological characters, such as root-stem ratio, branches, leaves, inflorescence, fresh and dry weights of different parts, biomass, height, seed, etc. Secondary branches bearing leaves increased from 2.5 per primary branch in the third generation autopolyploids (C_2). Total leaf area (total number of leaves x area of a leaf) produced by C_1 plants also considerably improved from 10884.60 cm^2 to 29308.70 cm^2. Various characters of the inflorescence and an important source of phytochemical constituents in addition to leaves, such as total number and length of inflorescence, the total number of floral whorls in an inflorescence, diameter of a floral whorl, and length between two whorls improved a lot in C_2 herbs. Also, biomass, height at different stages of growth and seed output, its weight, germination percentage, etc. showed a significant increase. After an initial decline in C_0 and C_1 plants, an overall improvement in the above characters was clearly noticed in C_2 colchiploids of the herb understudy. Commercial exploitation of the plant thus produced is therefore recommended.

Keywords: Autopolyploidy, colchiploidy, *Ocimum basılicum* var *purpurascens*, Lamiaceae

Introduction

Of the 160 species of *Ocimum* L. belonging to the mint family Lamiaceae and distributed throughout the world (WILLIS 1973) *Ocimum basılicum* var *purpurascens* Benth., a natural tetraploid with 2n=4x=48 chromosomes, is an important herb from medicinal and commercial viewpoints. Its essential oil contains linalool (Sobti *et al.* 1976, Thoppil and Jose 1994, Gupta 1996, Sinha and Singh. unpublished) [3, 8, 9, 12], methyl chavicol (Sobti *et al.* 1976, Sinha and Singh unpublished) [8, 9], and methyl cinnamate (Sobti 1976, Gupta 1996, Sinha and Singh unpublished) [3, 8, 9] as main constituents in addition to a number of other terpenoids (citronellal, thujone, cis-ocimene, etc.) and is sold at a high price in the international market. An added advantage with the species under study is that it can grow luxuriantly in any part of the world right from sea-shores to high altitudes. Taking into consideration its commercial significance and its worldwide distribution, the present investigation was undertaken in order to see improvement if any, in total herbage yield and related characters of the species through autopolyploidy. Leaves and inflorescences are the two chief sources of the chemical constituents in the species.

Material and Method

A survey of Lamiaceae herbs had been conducted by Kamat (1992) [4] in the revenue division of Darbhanga of north Bihar (India). During the present investigation, only *O. basilicum* was vigorously searched and two varieties namely *O. basilicum* var *purpurascens* and *O. basilicum* var. *thyrsiflorus* was collected in north Bihar consisting of revenue division of old Kosi (Saharsa) and old Tirhut (Muzaffarpur) in addition to Darbhanga division. Herbaria were kept in the Department of Botany R.K. College (L.N. Mithila University), Madhubani, Bihar. Identification of the two varieties was confirmed at the National Botanical Research Institute, Lucknow. Of the two varieties, *O. basilicum* var *purpurascens* was grown without applying any chemical fertilizer for a year in the Experimental Gardens. Seeds were collected & packed in polythene bags and kept in desiccators. Plants raised from these seeds and possessing good qualities were taken for further experimental studies.

Various qualitative and quantitative morphological characters, as mentioned in Tables 1 and 2. were studied in the diploid parent and its autopolyploids. Characters were studied and data collected from 25 plants divided into 5 groups in all four generations-the first diploid and the rest three its autopolyploids.

Three concentrations of colchicine (E Merck (India) Ltd.), namely, 0.10%, 0.20%, and 0.30%, were prepared for raising polyploids. For colchicine treatment plants raised from seeds of the 'elite plant' were transferred to earthen pots at the 4-6-leaved stage and allowed to stabilize and grow to the 6-8-leaved stage. These plants, 25 each was treated with the above freshly prepared three aqueous concentrations of colchicine for 12,24, 36, and 48 h. Treatment of school apices was done with the help of sterilized cotton balls soaked in desired colchicine solutions by carefully separating and inserting the soaked balls in between the two young leaves closely covering the shoot bud. Cotton balls were regularly soaked with the appropriate concentrations of colchicine with the help of a clean plastic dropper in order to avoid drying. All the plants were washed in tap water after the required period of treatment and were kept in diffused light and growth was noted.

Lateral diploid branches beneath the treated tip were carefully removed to avoid any serious injuries to the main plants and also the development of any diploid portion. They were transferred to the Experimental Gardens after they looked healthy and a pair of leaves appeared on the treated plant.

Table 1: Comparative morphological characters of diploid (2n=4x=48) and polyploids (2n=4x x 2=8X=96) of *Ocimum basilicum* var. *purpurascens*: Vegetative

Characters		Mean±SD				+/-*
		Diploid	C_0	C_1	C_2	
Area cm²	Root	624.92±126.01	612.28±134.41	524.24±101.23	799.04±65.65	+
	Shoot	5447.60±1993.81	4754.20±490.28	5088.00±1374.74	10250.60±3614.37	+
	Leaf	11.72±0.73	14.96±1.96	12.04±0.77	11.96±1.07	+
Root/Shoot ratio		1:8.53	1:7.92	1:9.82	1:13.08	+
Canopy (cm)		5.92±0.43	11.54±0.68	4.40±0.33	6.04±0.17	+
Total	Leaves	928.72±84.22	1047.76±119.34	891.36±226.42	2450.56±164.79	+
	Pr.br.	25.00±9.27	14.84±0.61	7.04±0.79	17.88±0.46	-
	Sec.br.	63.80±11.81	172.52±36.43	138.68±21.27	284.96±21.43	+
Weight (g)	Fresh Stem	257.80±65.91	214.92±10.29	42.96±30.10	498.72±23.75	+
	Dry Stem	131.92±35.26	87.40±20.46	13.56±10.02	81.72±3.71	-
	Fresh Root	28.24±9.35	25.48±2.10	44.76±22.21	49.80±0.47	+
	Dry Root	12.84±3.11	10.08±1.47	9.20±3.32	9.40±0.00	-
	Fresh Leaves	110.16±9.39	121.20±7.19	48.64±11.85	278.08±8.36	+
	Dry Leaves	21.96±9.77	35.24±2.73	13.88±1.69	37.84±1.59	+
Height (cm)	At 1st Lat. br.	5.62±0.32	0.00±0.00	4.56±0.36	5.31±0.11	-
	At 1st Infl.	39.76±4.51	32.66±1.42	38.93±3.15	42.80±1.24	+
	At 1st flower	49.36±4.34	39.50±1.46	44.48±2.43	50.63±2.72	+
Biomass		271.84±59.79	242.50±23.08	159.76±49.63	784.89±21.86	+

*C_2 = When compared with diploid

Table 2: Comparative morphological characters of diploid (2n=4x=48) and Polyploids (2n=4x x 2=8x=96) of *Ocimum basılicum* var. *purpurascens*: Floral

Characters		Mean±SD				+/-*
		Diploid	C_0	C_1	C_2	
LENGTH in cm	Inflorescence	15.82±2.66	23.52±1.67	26.58±2.45	27.34±1.66	+
	Between whorls	0.93±0.12	1.90±0.17	2.15±0.11	2.37±0.30	+
	Pedicellate Flower	1.68±0.13	1.96±0.08	1.98±0.07	1.68±0.05	=
	Unpedicellate flower	1.22±0.09	1.40±0.06	1.16±0.05	1.17±0.07	-
	Pedicles	0.44±0.04	0.58±0.05	0.54±0.04	0.51±0.03	+
	Upper pair of stamen	0.98±0.06	1.05±0.03	1.05±0.02	0.70±0.05	-
	Lower pair of stamen	1.16±0.07	1.19±0.04	1.04±0.03	0.67±0.03	-
	Carpel	1.05±0.02	1.04±0.05	1.02±0.07	0.94±0.04	-
Dia. of whorls (cm)		1.05±0.20	1.96±0.07	2.04±0.11	2.10±0.07	+
No. of whorls/Infl.		15.56±0.79	17.56±1.13	18.56±0.54	18.68±1.03	+
Total no. of in/plant		248.44±154.22	34.88±18.30	154.52±51.53	412.84±12.31	+
Fresh wt. of info./plant		77.84±55.39	16.84±4.07	62.40±22.28	150.44±8.99	+
Dry wt. of infl./plant		35.36±27.11	3.26±0.92	22.36±2.86	63.20±8.04	+

*C_2 when compared with diploid

Observations

Detailed morphological and palynological studies were made in the diploid and three succeeding autopolyploid generations.

Diploid plants

Qualitative morphological characters

Morphological characters were studied in the diploid parent and polyploids raised from the latter. The polyploids resembled most of the characters with their parent except the following. All the three generations (C_0, C_1, and C_2) studied showed swollen and knot-like nodes. In C_2 plants, nodes were so close that internodes were almost eliminated. However, in the succeeding two generations, nodes were normally interrupted with internodes of normal lengths. Interestingly, nodes and internodes both of the C_1 and C_2 plants bore adventitious roots. Branches were weak as they got detached from the main plant body when the load on them increased with growth. Leaves were thick, dark-green, and incised on margın above the treated site and entire below.

Quantitative morphological characters

The height of the diploid plant at first flowering was 49.36±4.34 cm. Shoot and root areas were studied to see how much of the latter is required to support the former. The ratio of the two data was 1:8.53. The total number of leaves per plant leaf area fresh and dry weights of leaves per plant were 928.72±84.22, 11.72±0.73 cm^2, 110.16±9.39g and 21.96±9.77g, respectively, On the other hand, the total number of inflorescences per plant and their fresh and dry weights were 248.44 ±154.22, 77.84±55.39 g and 35.36±27.11g, respectively. The length of inflorescence was calculated to be 15.82±2.66 cm while the number of whorl per inflorescence was 15.56±0.79. Length between two whorts in an inflorescence and diameter of whorls was 0.93±0.12 cm and 1.05±0.20 cm. respectively (Table 1)

An interesting study was made regarding the number of seeds per fruit. The percentages of 0-4 seeded fruits varied from 3.10 to 0.80 to 21.89 to 9.88%. The total number of seeds per plant and weight of 100 seeds were 680.12±302.15 and 124. 84±2.82 mg, respectively. The percentage of germination was observed to be 75.36±6.55% (Table 3).

Palynological characters

The area of pollen mother cells and the diameter of their nucleolus were studied in order to see changes, if any, arising during polyploid formation. The data in diploid was determined to be 1107.41±97.96 and 4.70±0.74 cm^2. Since pollen sterility is a common feature among the various species of *Ocimum* and as usually synthetic polyploids lead to sterility it was studied and data were obtained separately in lower and upper pair of stamens. The lower pair of stamens exhibited 30.16 ±5.72% and the upper pair 24.33±5.51% of sterility (Table 3).

First polyploid generation (Co)

Colchicine treatment

Young herbs were treated with 0.10 %., 0.20% and 0.30% concentrations of colchicine for 12, 24, 36 and 48h. Treatments with 0.10% showed no effect while 0. 30% proved lethal. On the other hand, while treatment with 0.20% gave rise to plants bearing polyploid-like characters in 12, 24, and 36h of treatments, the 48h of exposure proved lethal. Other concentrations, other than 0.10 and higher than 0.30% were also tried but were found either quite ineffective or highly lethal, respectively.

Of the 25 plants treated for 12h with 0.2%, 21 (84%) survived which upon chromosome analyses showed only 3 polyploids (12%). In 24h of treatment 19 plants (76%) survived of which 8 (32%) were polyploids while in 36h of treatment only 15 plants (60%) survived but the number of plants with polyploid status was 8 (32%). Treatment of 48h proved 100% lethal (Table 4).

Table 3: Comparative palynological characters of diploid (2n =4x=48) and polyploids (2n=4x x 2=8x=96) of *Ocimum basilicum* var. *purpurascens*

Characters		Mean ±SD				+/-*
		Diploid	**C_0**	**C_1**	**C_2**	
Area μ m^2	PMC	67.17±1.30	75.20±1.29	61.28±0.22	58.38±1.67	-
	Nucleolus	52.56±0.74	61.07±0.20	48.11±1.10	45.41±0.23	-
	Fertile Pollen	67.17±1.30	75.20±1.29	61.28±0.22	58.38±1.67	-
	Sterile Pollen	52.56±0.74	61.07±0.20	48.11±1.10	45.41±0.23	-
Pollen Sterility %	Lower Stamens	30.16±5.72	51.61±6.25	21.12±2.20	28.43±3.44	-
	Upper Stamens	24.33±5.51	52.41±6.45	14.76±1.38	23.96±3.62	-
	Per flower	33.87±4.94	54.61±9.90	22.71±1.52	26.12±1.54	-
%	4-seeded fruit	21.89±9.88	2.50±1.80	20.14±0.27	14.75±0.75	-
	3-seeded fruit	3.97±1.74	1.54±0.61	3.12±0.27	4.74±0.37	+
	2-seeded fruit	3.63±2.24	4.81±0.54	0.86±0.06	3.01±0.13	-
	1-seeded fruit	3.87±2.61	8.79±2.55	0.31±0.05	1.37±0.30	-
	0-seeded fruit	3.10±0.80	7.46±0.66	0.43±0.11	0.71±0.8	-
	Germination	75.36±6.55	70.16±5.06	85.12±2.90	72.24±6.74	-
Wt. of 100 seeds (mg)		124.84±2.82	141.76±1.90	129.04±2.84	128.84±4.36	+

*C_2 when compared with diploid

Table 4: Results of treatment with 0.20% of colchicines on *Ocimum basilicum* var. *purpurascens* (2n=4x=48)

Treatment (h)	Treated Plants No.	Plants survived No./%	Phenotypically altered plants No./ %	Polyploids No./%	Polyploids out of phenotypically altered plants (%)
12	25	21/84.00	5/23.80	3/12.00	60.00
24	25	19/76.00	15/78.94	8/32.00	53.33
36	25	15/60.00	12/80.00	8/32.00	66.66
48	25	…	…	…	…

Qualitative morphological characters

The overall qualitative morphological characteristics of the plants were almost the same except a few described with characters of a diploid parent.

Quantitative morphological characters

The height of the plant in Co at first flowering was 39.50±1.46cm and as expected the first polyploid generation (Co) showed stunted growth, except foliar characters. The total number of leaves per plant increased from 928.72±84.22 to 1047.76±119.34. Leaf area showed an increase of 3.24 cm^2. Fresh (121.20 ±7. 19g) and dry weights (35.24±2.73g) of leaves also exhibited an increasing trend.

The ratio of root and shoot areas, found to be 1:7.92 was slightly lesser than that studied for the diploid plants (1:8.53), possibly because of the fact that while the former remained a diploid the latter became a polyploid after colchicine treatment of the shoot apex. Inflorescence, a character of phytochemicals importance, was the worst Victim in Co plants as its total number declined from 248.44±154.22 in the normal to only 34.88±18.30. Its fresh and dry weights also considerably decreased. Exhibiting a fall by 5 and 11 times,

respectively. It was, however interesting to note that the above loss in inflorescence was compensated to some extent by its length, the number of whorls in each inflorescence, the length between two whorls, and the diameter of whorls all indicating an upward trend. (Table 2).
As far as percentages of seeds/fruit were concerned, 0-2-seeded fruits increased and 3-and 4-seeded fruits declined by 8.76 times. The total number of seeds per plant fell to nearly half. But, the weight of 100 seeds increased from 124.84±2.82 to 141.76±1.90. On the other hand percentage of germination exhibited a slight downward trend as generally expected from first-generation induced polyploidy. (Table 3).

Palynological characters
Areas of pollen mother cells and its nucleolus were larger than the ones studied for the diploid plant. The two areas of the former were more than or almost double those of the latter. Area of fertile and sterile pollens also increased. On the other hand, sterility in both the upper (52.41±6.45 cm^2) and lower (51.61±6.25cm^2) pairs of stamens exhibited an upward trend. However, the common trend of a higher percentage of sterility in lower pair of stamens as reported earlier in a number of species of *Ocimum* studied by Singh (1981, 1990) [8] was seen to have been reversed in this generation.

Second polyploid generation (C_1)
Qualitative morphological characters
Plants of the C_1 generation also had the same characters as observed for C_0 plants.

Quantitative morphological characters
The height of the plant at first flowering was 44.48±2.43 CM. It was interesting to note that the plants of the C_1 showed a general decline instead of exhibiting improvement in vanous characters. Root area declined by 14.38%, while that of the shoot, increased by 7.02%, resulting in a major shift in the root-shoot ratio (1:9.82). The only improvement observed was in the fresh weight of root and the characters related to the inflorescence. While the fresh weight of root improved by 75.66%, its dry weight declined by 8.73%. The percentage of the total number of inflorescences per plant was up by 343.00 in comparison to plants of Co. Its fresh (270.54%) and dry (585.88%) weights also increased considerably. Other characters, such as the number of whorls per inflorescence, length between whorls, and diameter of whorls, slightly increased except for the length of inflorescence which showed an upward trend of 13.01%.
The leaf another important part from the phytochemical viewpoint exhibited an overall decline. The total number of leaves per plant declined in C_1 by 14.03% while leafing area by 99.20%.
The plants of C_1, generation exhibited a good seed-set with a high percentage of 4- seeded fruit, that is 20.14±0.27. While the total number of seeds (955.68±87.98) considerably increased by 213.01%, the weight of 100 seeds fell to 129.04±2.84 from 141.76±1.90 observed for the C_0. These plants however showed a high percentage of germination, that is 85.12±2.90. (Table 3)

Palynological characters
There was no increase in the areas of PMC, its nucleolus, and fertile and sterile pollens in comparison to its parent plants, that is, those of Co However, areas of PMC and its nucleolus were higher than those of the diploid plant. Sterility in lower and upper pairs of stamens considerably declined to 21.12±2.20 and 14.76± 1.38% respectively, maintaining the common feature of the genus *Ocimum* in particular and Labiatae in general (Singh lc).

Third polyploid generation (C_2)
Qualitative morphological characters
Plants of this generation showed almost the same characters as observed in Co and C_1.

Quantitative morphological characters
The mean height of the C_2 plants was 50.63cm±2.72 exhibiting an increase by 13. 83%. There was a remarkable improvement in the overall quantitative characters of C_2 plants. Both root and shoot areas considered increased by 53.42% and 101.47%. However, the root-shoot ratio was seen to deviate tremendously from the normal and also from the first two polyploid generations (Co and C_1.), the ratio being 1:13. 08 (Table 1).
Leaf characters showed quite encouraging results with the total number of leaves reaching 2450.56±164.79 and its fresh and dry weights 278.08±8.36 and 37.84±1.59, respectively. It may be mentioned that the above three characters showed an increase of 174.92%, 471.71%, and 172.62%, respectively when compared with the preceding generation (C_1,). A declining trend, though very slight, was exhibited with regard to leaf area which fell by 0.67% in comparison to C_1. It was, however, 2. 05% higher than the diploid parent (Table 1)
The inflorescence exhibited remarkable improvement in its number (412.84±12.31), fresh (150.44g±8.99), and dry weights (63.20g±8.04) showing an increase by 167.18%, 141.08%, and 182.65%, respectively. Other characters of the inflorescence as well such as length of the inflorescence, the length between whorls, and diameter of whorls, showed improvement of various degrees (Table 2).
The number of seeds per fruit varied from those of C_1, plants and exhibited a decline Number of 4-seeded fruits fell to 14.75±0.75 from 20.14±0.27 in Ci plants. The total number of seeds per plant and percentage of germination too, declined by 12.31% and 15.33%. Respectively, however, the weight of 100 seeds (128. 84mg±4.36) remained almost the same when compared with that of the C_1 plants.

Palynological characters
Areas of PMCs and their nucleolus showed an upward trend. However, the area of fertile pollens decreased. Pollen sterility in the two pairs of stamens considerably increased to 28.43±3.44 in lower and 23.96±3.62 in upper. The two pairs of stamens maintained the same characteristic difference in pollen sterility, that is, the lower showing the higher percentage of sterility than its upper counterpart.

Conclusion
Root - shoot system
In all the higher plants, there is a balance between the shoot system above and a supporting root system below. In the diploid *O. basilicum* var *purpurascens* Benth. This ratio was found to be 1:8.53. After doubling of the chromosome number, in the raw autopolyploid (Co), it showed a decline, that is, 1:7.92. The ratio exhibited an upward trend in $C_{1,}$ that is 1:9.82. This trend was further maintained by the third generation (C_2) as well and the ratio was raised to 1:13.08. It was therefore clear from the above facts that a gap has developed between the root and the shoot system and the former has not undergone a concomitant increase with the latter.

Branches

The species is a highly medicinal and aromatic one and the chief sources of chemical compounds are its leaves and inflorescences it was, therefore, desirable to investigate its primary and secondary branches. In the diploid, the number of primary and secondary branches were 25±9.27 and 63.80±11.81, respectively and the ratio of the two was 1:2.5, meaning thereby that there were 2.5 secondary branches per primary branch. In the Co, the ratio came to 1:11.63, while in the C the increased to 1:19.69 and in the C_2 it fell to 1:15.94. The number of primary branches has been studied in autotetraploid of some medicinal plants, such as *Hyoscyamus albus* L. (Srivastava and Lavania 1990) [6, 10, 11] and *H. niger* L (Lavinia and Srivastava 1991) [6, 10, 11]. Both of the plants showed an increase in the number of primary branches. Srivastava and Tripathi (1990) [6, 10, 11] reported both primary and secondary branches in the C_1 generations of synthetic autotetraploid of fodder grasses *Atylosia albicans* W.A. Benth. and *A volubilis* (Blanco) Gamble. It was observed in the three colchiploid generations that the primary branches fell and got detached from the main plant body when the herbs were fully grown perhaps due to the excess load of an increasing number of secondary branches on them. At the same time, leaves at the lower half of the calchiploid plants were seen to fall, which might be due to their close planting in the Experimental Gardens and lack of sunlight penetrating the bottom of the polyploid plant. The fall of the primary branches, it was suggested, might be overcome to some extent, if the plants are cultivated at the required distance.

Leaves

The total number of leaves per plant was 928.72±84.22 in the diploid parent, 1047. 76±119.34 in Co, 891.36±226.42 in C_1, and 2450.56±164.79 in C_2 generations. The highest number of leaves obtained from the C_2 was a good sign as the leaves are one of the two main sources of medicınal and aromatic compounds. The other source being inflorescence. As it was evident from the data collected, leaf area increased with the increase in the number of leaves in the first autopolyploid generation (C_0) both these characters decreased in the second, and the area through decreased slightly, the total number of leaves per plant increased remarkably in the third generation, C_2. It was noted that though leaf area in C_2 was lower than Ci and Co plants, it was only slightly higher than that observed for the diploid. There was, however, a net gain in the total leaf area of a C_2 plant (18424.60 cm^2) if all the leaves of a single plant of diploid (10884.60cm^2) and C_2 (29308.70cm^2) are together taken into consideration. Bose and Choudhury (1962) [2] made similar studies on the number and size of leaves of the colchiploids of *Ocimum kilimandscharicum* Guerke. Bahl and Tyagi (1988) [1] also reported an increase in the number and area of leaves in the synthetic autotetraploid of *Coleus forskolin* (Willd) Briq. of the same family which supported the present investigation. An increase in the leaf area was noted in *Hyoscyamus niger* L. by Lavania and Srivastava (1991) [6, 10, 11] also, but the authors found the leaf area to decline in the autotetraploid of another species of the same genus *H. albus* L. (Srivastava and Lavinia 1990) [6, 10, 11]. Shrivastava and Tripathi (1990) [6, 10, 11] also reported higher values of the leaf in *A. albicans* W. A. Benth and *A. Volubilis* (Blanco) Gamble.

Inflorescence

The inflorescence is one of the two main sources of medicinal and aromatic compounds in *Ocimum* spp. As far as its total number per plant is concerned, it was found that after a sudden decline in C_0 it improved a lot in C_2 herbs. Besides this, the upward trend was observed in various other characters of the inflorescence as well such as length of inflorescence, the total number of floral whorls in an inflorescence, the diameter of a floral whorl, and length between the two whorls. All the above characters showed remarkable improvement in C_2 generation. The C_2 colchiploids was therefore undoubtedly a superior one compared to its diploid parent as far as inflorescence was concerned. In the synthetic autopolyploids of *O kilimandschancum* Guerke. Though a natural aneuploid (2n=6x+4=76 chromosomes), larger and taller inflorescence axes were observed by Bose and Choudhury (1962) [2]. Contrary to this, autotetraploid of Coleus forskohii (Willd). Showed compact and shorter inflorescence (Bahl and Tyagi 1988) [1].

Fresh and dry weights

It was found that while the fresh weight of root gradually increased from diploid to C2 except for C_0, its dry weight decreased gradually. While the dry weight of the diploid root was 45.47% of its first weight, it was only 18.88% of the fresh weight in C_2 showing an increase in moisture contents of roots in the synthetic polyploids.

The fresh weight of stem decreased and reached the lowest point in C_1 (257.80g±65.91 in diploid parent to 42.96g±30.10 in C_1 and again increased considerably to reach 498.72g±23.75 in C_2. Like the roots, its dry weight decreased from diploid to C_2 generation. The percentages of dry weights of the plants in the four generations including the parent, were 51.17, 40.67, 31.56, and 16.39 of the fresh weights in diploid, Co C_1, and C_2 respectively. It is interesting to note that while fresh weight went on decreasing up to C_1 it abruptly increased in C_2 but its dry weight maintained a gradual declining trend irrespective of the increase in fresh weight.

The above two weights of the leaves indicated a trend different from those of root and stem. Their fresh weight increase in Co had a record low of 48.64g±11.85 in C_1 and again took a big leap of 278.08g±8.36 in C_2. Fresh weight leaves increased in *O kilimandschancum* Guerke autopolyploids as well (Bose and Choudhury 1962) [2]. On the other hand, dry weight showed no correlation with fresh weight, while diploid showed 19.93%, C_0 29.08%, C_1 28.54%, and C_2 only 13.60% of their fresh weights. It, therefore, clearly indicated that high growth was not accompanied by thickening of the cell wall or deposition of cell materials, rather moisture content increased in the C_2 colchiploids. Though the fresh weight of leaves has been found to increase in colchiploids of other plants as well, like *H albus* L. (Shrivastava and Lavania 1990) [6, 10, 11] and *H. niger* L. (Lavania AND Srivastava 1991) [6, 10, 11], its dry weight also increased at the same time in the former species.

As far as fresh and dry weights of the inflorescence of the dıploid and polyploid plants are concerned, they did not exhibit any correlation between them. On becoming polyploid C_0 herbs showed a sharp decline in fresh weight. It again took an upward trend in the next generation (62.40g±22.28 in C_1) and reached its peak (150.44g±8.99) in C_2, On the other hand, dry weights of inflorescences of plants of the four generations had a different story to tell. The dry weight of. diploid was 45.43% of its fresh weight while thone of C_0, C_1, and C_2 were 19.36, 35.83%, and 42.01% of their fresh weights, respectively. It was, therefore, noticed that gradually consecutive polyploid generations recovered their dry weights eliminating moisture contents, probably in their efforts to restore normalcy.

The behavior of the above characters, therefore, presented two important facts. The first was that while C_0 or C_1 showed depression, C_2 exhibited a remarkable recovery. The other interesting point exhibited by them was that the lowest value of fresh weight resulted in a comparatively higher dry weight value.

Biomass

Biomass of the diploid (271.84 g±59.79) and the three autopolyploids showed a decreasing trend in Co and C_1 but recovered considerably in C_2 (784.89g±21.86) and reached a value more than three times of the diploid. It might have improved as a result of stabilization of the treated plant and subsequent generations may be expected to show more encouraging results.

Height

Height of the plants was taken at three different stages of growth, namely, at the emergence of the first lateral branch, at the first inflorescence, and at the first flower. Growth behavior at these three stages exhibited a similar pattern. The height of the herbs at the emergence of the first lateral branch in C_1 declined to 4.56cm + 0.36 from 5.62cm±0.32 in the diploid. Since the C_0 plants bore the first lateral branch from below the untreated or diploid part of the plant, those were removed in order to leave ultimately only a polyploid one. And hence, the height at the exit of the first lateral branch was considered to be zero. Herbs of the C_2 generation further recovered and the height was noted to be 5.31cm±0.11. The height before the emergence of the first inflorescence also declined in Co (32.66cm±1.42) improved to 38.93cm±3.15 in C_1 and reached its maximum, that is, 42. 80cm±1. 24 in C_2.

A similar trend was observed regarding the growth of the plant at the first anthesis. The height declined in Co (39.50 cm±1.46). Improved in C_1 (44.48cm±2.43) and finally reached a value of 50.63 cm±2.72 in C_2. The growth pattern, therefore, seemed to be based on the gradual stabilızation of the colchiploids.

When the growth patterns of C_1 and C_2 plants were compared with those of the generations immediately before them and diploid progenitor, the former showed a little higher initial growth of 0.67% (between the first lateral branch and the first inflorescence) and declined in the second stage (between the first inflorescence and the first flower) by 18.86% and 42.19% over the Co and diploid ones. On the other hand, the C_2 performed well at both stages and showed 9.08% and 9.80% of initial growth and 221.26% and 85.73% of growth at the second stage over their C_1 and diploid counterparts, respectively. However, Bahl and Tyagi (1988) [1] reported slow initial growth and delayed flowering but an overall increase in plant height of the tetraploids of *Coleus forskohlii* by 11.56%. Shrivastava and Tripathi (1990) [6, 10, 11] also noted an overall increase in 4x plants of the two spp. of *Atylosia*. On the other hand, in the synthetic tetraploids of many a plant namely *H. albus* L. (Srivastava and Lavania 1990) [6, 10, 11] and *H. niger* L. (Lavania and Srivastava 1991) [6, 10, 11], etc. increased.

Seed

Percentages of properly grown fruit that is, 4-seeded one, also declined after treatment with colchicine in Co (2.50±1.80) from diploid (21.89±9.88) but improved in C_1 (20.14±0.27). It, however, exhibited a fall once again in C_2 reaching a value of 14.75±0.75%. The total seed output per plant maintained the same behavior of decline in Co (305.32±95.90), rise in C_1 (955.68±87.98), and again a fall in C_2 (837.28±26.14) against a total output of 680.12±302.15 in diploid.

It was interesting to note that the weight of 100 seeds increased in C_0 (141. 76mg±1.90) in comparison to its diploid source (124.84mg±2.82), but was found to be almost stabilized in C_1 (129.04mg±2.84) and C_2 (128.84mg±4.36). This study found support from the works done on *H. albus* L. (Srivastava and Lavania 1990) [6, 10, 11] and *H. niger* L. (Lavania and Srivastava 1991) [6, 10, 11]. The rise in seed weight in the first autopolyploid generation (C_0), cytogenetically an abnormal one was probably due to a high number of one-seeded (8.79±2.55%) and 2-seeded (4.81±0.54%) fruits in comparison to 3-seeded (1.54±0.61%) and 4-seeded ones (2.50±1.80%). Since fruits with a low number of seeds had experienced abortion of the remaining seeds the surviving ones might have received a better supply of nutrition thereby increasing the size and weight of individual seed.

Germination of seeds in the source plant was calculated as 75.36±6.55%. while in C_0 it fell down to 70.16±5.06%, improved to 85.12±2.90% in C_1, but again saw a downfall in C_2 (72.24±6.74%). Though the above germination percentage of C_2 was lower than its diploid source, it might be considered a higher value as far as the success of the colchiploids in terms of seed fertility was concerned. Similar studies have been made in *H.albus* again by Srivastava and Lavania (1990) [6, 10, 11], where though the percentage of seed fertility calculated in terms of filled seeds declined in the 4x plants (83.78% against 94.44% in diploids), its mean value was still higher. Germination percentage also fell to 67.13% in tetraploid of this species (*H. albus*) from 98.07% in diploid progenitor. Since this percentage was more than 65%, it was considered higher (Lavania 1991, Srivastava, Lavania and Mishra 2000) [6, 10, 11], which may be expected to improve in subsequent generations.

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