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Varietal Response to Chromosomal Doubling in *Coleus forskohlii Briq.*

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ABSTRACT. Tubers of Coleus forskohlii Briq. are the only source of diterpenoid forskolin used in the treatment of glaucoma, congestive cardiomyopathy, asthma and certain cancers. Induction of autotetraploidy holds promise in this vegetatively propagated crop as enhanced expression in vegetative characters and forskolin content, if any, can be exploited commercially unhindered by adverse effect on seed fertility.

Both seed and shoot treatments with aqueous colchicine were successfully carried out to induce chromosome doubling in three tuberous (A, D & K) and three non tuberous (E, H & I) collections. Increased pollen and stomatal size and decrease in stomatal number aided in detection of tetraploid sector. Tetraploid K showed slow growth. Varietal differences in the expression of morphological and fertility characters following chromosomal doubling were observed. Increase in forskolin content in the autotetraploid of high forskolin containing 'K' deserves commercial exploitation.

INTRODUCTION

Coleus forskohlii Briq. (Syn. *C. barbatus* Benth.) belonging to family *Lamiaceae* is a perennial aromatic herb with tuberous roots. The tuberous roots have been identified as a rich source of coleonol (forskolin) which is being developed as a drug for glaucoma, congestive cardiomyopathy, asthma and certain cancers (De Souza *et. al.*, 1986). The plant is reported to have originated in the Indian subcontinent and is distributed in subtropical Himalayas, Southern India and in Sri Lanka. A variety of *C. forskohlii* is cultivated in Western India for its edible roots, which is pickled.

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Induced autotetraploidy has been attended by enhanced content and yield of secondary metabolites in various medicinal plant species like *Papaver somniferum*, (Andreev, 1963), *Rauvolfia serpentina* (Janaki Ammal, 1962), *Catharanthus roseus* (Krishnan, *et. al.*, 1985), *Hyoscymus muticus* (Lavania, 1986) and others. There is hence, a case for breeding autotetraploid cultivars in *C. forskohlii*, with higher content of active principle.

The commercial crop of *C. forskohlii* is raised from stem cuttings. It is, therefore, an ideal candidate for induced autotetraploidy, as lowered seed fertility will not pose problems in its commercial exploitation.

Since the success of autotetraploid breeding programme is determined by the genetic response of diploid progenitors, production of autotetraploids using a wide genetic base is imperative. The present study was initiated with these considerations in view.

MATERIALS AND METHODS

Three tuberising accessions (A, D and K) and three non-tuberising accessions (E, H and I) of *C. forskohlii* collected from different parts of India were used. Autotetraploids were induced using seed and shoot-tip treatments.

Seed treatment

Seeds were treated for 12 and 24 hours with 0.1% colchicine in all accessions except the seed sterile K. The 25 seeds treated in each concentration were sown in seed pans along with untreated control. Forty five day old seedlings were transplanted in poly-bags and field planted after 60 days.

Shoot apex treatment

Ten rooted shoot cuttings in A, E, I and K were treated with 0.25% aqueous colchicine for three hours for three consecutive days and

transplanted in the field after 45 days. Sectors exhibiting 'gigas' characters were vegetatively multiplied.

Suspected autotetraploid sectors from both treatments were initially compared with diploid for stomatal and pollen characters to ascertain their ploidy status (Table 1). Vegetatively propagated diploids and induced autotetraploids were compared for exomorphic and other characters in two field trials. In the first trial rooted cuttings of diploid (2n) and induced autotetraploid (4n) of K were planted in five rows each, alternately, adopting 60x30 cm spacing. Each row consisted of 100 plants. Two guard rows of diploid were also planted. Five samples were drawn (*vide* Table 2) and in each sampling ten diploid and ten autotetraploid plants were harvested with roots intact. Mean values of characters were compared using students' 't' test (Table 2).

In the second trial, five (20 in case of E) rooted shoot cuttings of diploid (2n) and induced autotetraploid (4n) of D, E, H and I were field planted in 3 m long rows, adopting 60x60 cm spacing. Data were recorded at 210 days in four plants each. The differences in diploid and autotetraploid are expressed as percentage over diploid values (Tables 4 and 5).

RESULTS AND DISCUSSION

Induction of autotetraploids

In *C. forskohlii*, Bahl and Tyagi (1988) reported production of two autotetraploids following treatment of seeds with 1.0% colchicine solution for 48 hours. Shoot apex treatments for 24 hours and 48 hours with 0.05% and 1.0% colchicine failed to yield autotetraploids. In the present study, however, both seed and shoot apex treatments were successfully used for induction of autotetraploids in six accessions. The lower concentrations tried in the present study of 0.25% for shoot tip treatment and 0.1% for seed treatment, were found to be effective despite shorter duration of treatments (for seeds 12 and 24 hours and for shoot tips 3 hours for 3 days). The efficacy of seed treatment of colchicine in small seeded crop species for chromosomal doubling is well documented (Sen and Vidhyabhusan, 1960). The small seeded plant taxa unlike large seeded ones do not absorb colchicine in quantities toxic to germination.

Table 1. Stomata and pollen characters in 2n and 4n of six Coleus forskohlii accessions.

Accession No	Ploidy status	Stomata			Pollen		
		Length (μ)	Breadth (μ)	No./ m.field	Length (μ)	Breadth (μ)	Fertility (%)
A	2n	31.6	18.8	42.4	46.8	42.4	97.5
	4n	47.6	20.8	22.8	59.6	53.2	88.5
D	2n	36.0	20.4	23.4	47.2	40.4	97.0
	4n	52.4	23.6	16.9	60.0	52.4	96.4
E	2n	28.0	15.2	42.8	45.2	41.2	98.0
	4n	48.0	18.0	24.4	57.2	50.7	89.6
H	2n	34.0	19.6	81.4	48.4	42.4	93.7
	4n	51.6	19.6	25.0	54.4	46.4	96.4
I	2n	38.0	17.6	23.0	49.2	44.4	97.0
	4n	50.8	20.0	17.0	60.4	56.0	76.2
K	2n	32.8	17.2	17.0	-	-	00.0
	4n	54.8	19.6	9.8	-	-	-

Table 2. Morphological characters in 2n and 4n of K.

Character	Ploidy	DAYS				
		50	90	120	150	175
Plant height (cm)	2n	29.0	43.7	39.7	49.7*	45.8*
	4n	24.8	38.1	37.9	44.3	40.0
No. of branches	2n	15.2**	19.7*	16.7**	16.8**	19.5**
	4n	7.9	9.5	11.5	14.0	13.0
Spread E-W (cm)	2n	40.9*	59.8*	49.5	69.7**	55.1
	4n	35.4	47.3	46.5	50.5	49.1
Spread N-S (cm)	2n	35.7	66.7**	48.7	55.6	50.7
	4n	33.6	42.4	45.1	50.3	46.1
Stem dia (mm)	2n	13.6	18.6**	16.4	30.1**	28.6**
	4n	12.4	16.4	17.6	22.9	21.4
No. of leaves	2n	198.4**	675.2*	666.9**	1013.6**	510.0**
	4n	118.4	326.8	372.8	667.8	263.0
Leaf length (cm)	2n	8.8	8.9	6.9**	3.8	5.3
	4n	8.9	8.8	6.6	3.7	5.2
Leaf breadth (cm)	2n	4.4	4.5	3.4	2.0	2.3
	4n	5.4**	5.3**	4.1	2.4*	2.7*
Petiole length (cm)	2n	1.6	2.3	1.6**	1.3	1.2
	4n	1.6	2.0	1.3	1.3	1.2
Internodal length (cm)	2n	2.9**	3.8	2.9**	3.1	2.1
	4n	2.3	3.6	1.3	3.0	2.5*

**, * denote significance of 't' values at P = 0.01 and 0.05 respectively.

C. forskohlii being small seeded, also responded to seed treatment of colchicine.

The present study, established the feasibility of producing autotetraploids in *C. forskohlii* using both seeds and shoot cuttings. The latter method of induction is very important especially for non-flowering accessions as well as in genotypes where risk of segregation through sexual reproduction is not desirable. This is well demonstrated from the present study as one of the accessions, namely K, where colchiploids have been obtained, is shy flowering and seed sterile. This accession is commercially important because of its high tuber yielding ability and high forskolin content.

General characters of autotetraploids

In autotetraploids of *C. forskohlii*, Bahl and Tyagi (1988) reported inferior expression for number of primary branches, leaves per plant and pollen stainability. Characters for which induced autotetraploid recorded superior expression were, leaf area per plant, number of stomata per mm, size of stomata and pollen diameter. In the present study, for the identification of tetraploid sectors in all the six accessions, increased stomatal size (length and breadth), decrease in number of stomata (per microscopic field) and increased pollen size (length and breadth) were found to be of diagnostic value (Table 1).

One of the consistent observations in induced autotetraploids is, increase in cell size as reflected in the size of pollen and stomata (Stebbins, 1971). This was found to be true for *C. forskohlii* autotetraploids induced in the present study and earlier by Bahl and Tyagi (1988). Disagreement in the data on stomata number and pollen stainability in our studies and of Bahl and Tyagi (1988), might have arisen due to genotypic differences of diploid progenitors used.

Varietal response to chromosomal doubling

Growth behaviour of diploid and autotetraploid was compared in accession K by monitoring expression of various parameters at intervals

commencing from 50 up to 175 days. Slow initial growth in autotetraploid of K was evident at 50 days and persisted even until 150 days. Autotetraploid K was inferior in plant height (at 150 and 175 days), number of branches (at all the five harvests), N – S plant spread (at 90 days), stem diameter (at 90, 150 and 175 days), leaf number (at all the harvests), leaf length (at 120 days), petiole length (at 120 days), internodal length (at 50, 120 and 175 days), number of tubers at 50, 90, 120 and 150 days, tuber diameter (at 90 days), fresh and dry weight of tubers (at 120, 150 and 175 days) and dry matter per cent at 90 and 150 days (Tables 2 and 3). Leaves in autotetraploid K was broader at 50, 90, 150 and 175 days. In general, autotetraploid was inferior for most of the characters studied till 175 days. Similar slow intital growth is reported for induced autotetraploids of several medicinal plants *eg. Solanum vianum* (Krishnan, 1988), *Papaver bracteatum* (Milo *et. al.*, 1987), *Catharanthus roseus* (Krishnan *et. al.*, 1985).

As in accession K, autotetraploids of D, E, H and I were also inferior for most of tuber characters to their diploid progenitors (Tables 4 and 5). This was noted in tuber length, fresh weight of tubers per plant, volume of tubers per plant and dry weight of tubers per plant. However varietal differences were observed in the expression of other characters. While root number was decreased in E and H following chromosomal doubling, increase in tuber number in D and roots in I was observed. Diameter of root was increased in E (which has thickened roots), but was decreased in H and I as well as in tubers of D. While tuber density was *on par* in diploid and induced autotetraploid of D and H, reduction in density was observed in I and increase in E tetraploids.

Varietal differences for the expression of morphological characters were also evident. An increase in number of branches was observed in all four autotetraploids (D, E, H and I). Autotetraploids exhibiting higher expression for other morphological characters (as compared to diploid progenitors) were: D for plant height, spread in N – S direction, number of leaves, stem diameter, number of inflorescence and I for plant height and number of leaves. Diploids and autotetraploids were *on par* for plant height in E and H; for plant spread in E – W direction in D, E and H and for stem diameter in E and H. Autotetraploids exhibited inferior expression for one or more characters. This was so, for plant spread in both directions for I; in N – S direction and number of leaves in E and H and number of inflorescences in H. Similar

Table 3. Tuber characters in 2n and 4n of K.

Character	Ploidy	DAYS				
		50	90	120	150	175
Number	2n	13.0**	19.0**	36.5**	28.4**	19.2
	4n	9.0	13.3	18.4	19.5	15.9
Length (cm)	2n	-	7.2	10.8	13.8	12.3
	4n	-	7.7	10.2	13.5	13.8
Diameter (mm)	2n	-	8.8	14.4	14.4	17.2
	4n	-	10.8*	14.2	15.2	17.0
Fresh weight (g)	2n	-	56.0	141.5*	257.0*	271.5*
	4n	-	59.5	90.0	203.0	182.0
Dry weight (g)	2n	2.3*	9.0	25.7**	39.2**	40.1*
	4n	1.4	7.5	15.5	25.4	27.7
% Dry matter	2n	-	16.5**	19.4	16.1**	14.9
	4n	-	13.5	17.5	12.4	15.3

**, * denote significance of 't' values at P = 0.01 and 0.05 respectively.

Table 4. Morphological characters in 2n and 4n of four C. forskohlii accessions at 210 days.

Accessions	D			E			H			I		
Character	2n	4n	% over 2n	2n	4n	% over 2n	2n	4n	% over 2n	2n	4n	% over 2n
Plant height (cm)	24.3	33.8	39.1	43.8	46.5	7.3	43.3	46.5	7.4	37.5	49.0	30.7
Spread E-W (cm)	58.8	56.5	-3.9	66.8	65.3	-2.0	51.5	49.0	-4.9	130.3	91.3	-29.3
Spread N-S (cm)	47.5	61.3	29.1	81.0	66.8	-17.5	57.0	33.5	-41.2	123.8	91.3	-26.3
No. of branches	13.8	38.3	177.5	20.3	80.3	297.0	25.0	58.0	132.0	79.3	103.3	30.3
No. of leaves	433.3	967.0	123.2	1985.3	1374.3	-30.8	971.5	806.5	-17.0	3200.0	3998.0	24.9
Stem dia (mm)	14.0	27.5	96.4	23.3	24.5	5.2	23.5	39.0	6.3	27.3	28.3	3.7
No. of inflorescences	25.5	60.5	137.3	49.5	56.0	13.1	84.0	50.5	-39.9	-	-	-

Table 5. Tuber/root characters in 2n and 4n of four C. forskohlii accessions at 210 days.

Accessions	D			E			H			I		
Character	2n	4n	% over 2n	2n	4n	% over 2n	2n	4n	% over 2n	2n	4n	% over 2n
Number	6.0	7.8	30.0	13.0	9.5	-26.9	11.0	9.5	-13.6	10.0	12.0	20.0
Length (cm)	10.5	8.8	-16.9	17.2	13.7	-25.5	16.4	12.8	-21.9	24.3	22.8	-6.2
Diameter (mm)	14.0	11.3	-19.3	8.0	12.2	52.5	7.3	5.5	-24.7	6.5	4.4	-32.3
Fresh weight (g)	103.8	86.3	-16.9	148.8	72.5	-51.3	97.5	30.0	-67.2	92.5	66.3	-28.3
Volume (cc)	95.0	77.5	-18.4	158.8	83.8	-47.2	98.8	35.0	-64.6	83.8	73.8	-11.9
Density	1.09	1.12	2.8	0.95	1.16	22.1	0.99	1.11	12.0	1.29	0.88	-31.8
Dry weight (g)	23.0	16.8	-26.9	27.4	20.0	-27.0	16.8	7.0	57.8	23.6	15.6	-33.9
% dry matter	22.1	19.5	-12.2	18.8	20.7	10.1	14.6	20.2	38.4	26.6	25.5	-4.1

varietal differences in response to chromosomal doubling was reported for *Cicer arietinum* (Srivasthava, 1955) and *Ribes nigrum* (Vaarama, 1947).

One of the consistent observations in induced autotetraploids is the reduction in fertility. In *C. forskohlii*, pollen fertility was decreased in autotetraploids of A, D, E and I, but was *on par* in H. Autotetraploid of K did not flower. But percentage seed set (as estimated in 50 flowers each of both ploidy types) in autotetraploid of D (2.5%), E (8.1%) and H (3.1%) was poor as compared to their diploid progenitors (D = 44.4%; E = 46.9% and H = 40%).

However, 100 seed weight in autotetraploids was higher in all the three accessions. The respective diploid and autotetraploid values being 58 mg and 113 mg for D, 55 mg and 148 mg for E, 50 mg and 65 mg for H. Increase in seed weight in autotetraploids is reported in *Solanum vianum* (Krishnan, 1988) and *Catharanthus roseus* (Krishnan *et. al.*, 1985). The observed difference in the magnitude of increase in seed weight among *C. forskohlii* accessions need to be verified by density tests to exclude aborted and shrivelled seeds in the sample drawn.

Root samples of diploid and autotetraploid K were assayed for forskolin at Hoechst India Limited, Bombay, along with those of diploid A. Forskolin content in 'K' diploid was found to be three times higher than that of 'A'. Roots of autotetraploid K had 17 per cent higher forskolin than diploid K. This desirable response in content of active constituent on chromosomal doubling in accession 'K' of *C. forskohlii* is in agreement with those reported for several other medicinal plants *eg. Rauvolfia serpentina* (Janaki Ammal, 1962), *Catharanthus roseus* (Krishnan *et. al.*, 1985), *Hyocymus muticus* (Lavania, 1986).

The observed increase in forskolin content will enhance the utility of tetraploid K as commercial source for user industry. Studies are underway to compare tuber yield beyond 180 days of diploid and autotetraploid K.

CONCLUSION

In three tuberous and three non-tuberous accessions of *C. forskohlii* autotetraploidy was induced both by seed and shoot tip

treatments with colchicine. Increase in size of stomata and pollen grain and decrease in stomatal number characterised all autotetraploids. Autotetraploids were found to be inferior for root characters. Morphological characters generally showed superior expression in autotetraploids. However, genotypic differences in the expression of several morphological characters and some of the tuber or root characters, following chromosomal doubling were evident among the accessions. Forskolin content in K autotetraploid increased by 17 per cent.

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