III RESULTS AND DISCUSSIONS

RESULTS AND DISCUSSIONS

A) Karyotypes :

Every species of plant or animals, by and large, is characterised by definite somatic chromosome number. It was first emphasized by a Russian Scientist Nawaschin (1932). Karyotype was first defined in 1926 by Delaunay as a group of species resembling each other in the morphology and number of their chromosomes. However, Lewitsky (1931) defined it as a phenotypic appearence of the somatic chromosomes in contrast to their genotype. According to Swanson (1960), karyotype is defined as the basic chromosome set of a species and is further characterised to form and size of chromosomes as well as to their number. When represented in a diagramatic fashion it is referred to as idiogram. The analysis of karyotype is known as karyosystematics (Sato 1942). After realizing the importance of karyotypic study in both animals and plants as a powerful mean of characterization of a species, several workers have elaborated a method. One of the first to do this job is Heitz (1925). He developed an elaborate system of representing a karyotype using different symbols. However, it soon waded away as it did not contribute to develop the concept of karyotype. Almost 30 years later Tjio and Levan's (1950) have suggested an expression for somatic chromosome merely on the position of centromere, secondary constriction and satellite. To express the karyotype they used Roman letters such as :

Type A - Long chromosome with a satellite

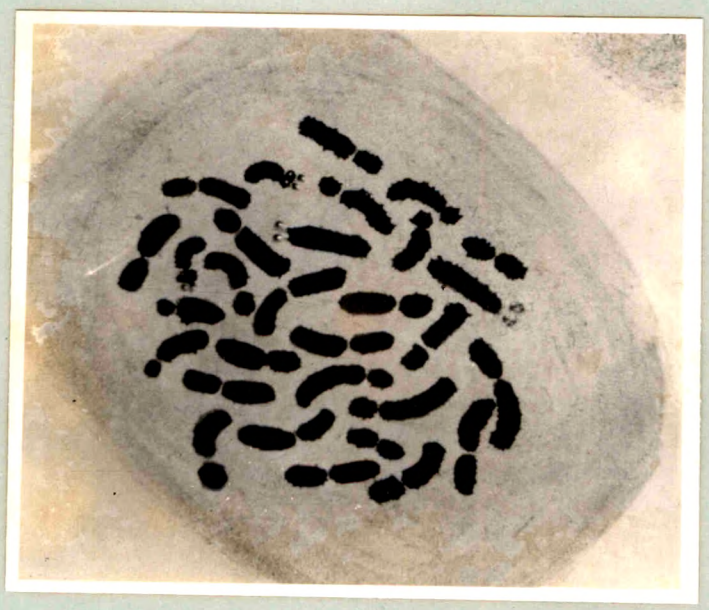
Type B - Long chromosome with secondary constriction on chromosome arm

Type C - Long chromosome with median primary constriction and so on.

The number behind the letter indicated the number of chromosome in their particular category. However, Battaglia (1955) an Italian Cytologist suggested another method based on chromosome morphology and position of centromere. He classified the chromosomes as median centromere type, submedian centromere type, terminal centromere type, isobrachial chromosome and so on. The limitation of this method is very evident. This terminology, though used in practice could not be effectively used for formulation of karyotype, which needed symbolization and brevity. Ising (1962) based on Heitz's method evolved a system of karyotype expression. In this system 5 groups of chromosomes were distinguished and they were symbolized by following letters, which indicated both centromeric location and relative chromosome size. They are as follows :-

V, L, j, l, i ; but again this has the limitation of expression. The whole system thus, has eventually been revised by Levan et al. in 1964. In a comprehensive paper, they suggested a nomenclature for centromeric position of a chromosomes.

Plate 5 : Somatic chromosomes from root tip cells of *Coleus forskohlii*



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Although, discussion of this comprehensive paper is beyond the scope of this dissertation. The gist of recommended nomenclature is as follows.

Location of the centromere :

If the total length of the chromosome is denoted by "C" and the length of long arm and short arm "l" and "s" respectively, the location of the centromere is expressed as the difference (d) = l - s or as ratio (R) = $\frac{s}{l}$. The ratio between the arm is often calculated as centromeric index (i) = 100 $\frac{s}{C}$. If the total length of the chromosome is "C" and "r" or "i" are given the "l" and "s" are calculated as follows :-

$$l = C / (r + s)$$

$$s = C / (r + 1)$$

$$l = C (100 - i) / 100$$

$$s = iC / 100$$

After Levan et al. (1964) have suggested a method of karyotypic expression it was still found rigid to make it applicable. The several workers have modified, thus, the expression of karyotype. The better formulation of chromosome classification has been suggested by Abraham and Prasad (1983). This is one of the easy way of expressing/classification karyotypes. This classification of chromosome karyotype is adopted here.

TYPE A : (Chromosome I)

A pair of long chromosomes (5.04 μ) with sub-terminal centromere. This is the longest pair.

TYPE B . (Chromosome II)

A pair of long chromosomes (4.95 μ) with median centromere.

TYPE C : (Chromosome III)

A pair of long chromosomes (4.59 μ) with sub-median centromere.

TYPE D : (Chromosome IV and VIII)

Two pairs of median chromosomes (3.86 and 3.50 μ) with median centromeres.

TYPE E : (Chromosomes V to VII)

Three pairs of median chromosomes (3.82 μ, 3.82 μ and 3.68 μ) with submedian centromeres.

TYPE F : (Chromosome IX)

A pair of median chromosome (3.18 μ) with terminal centromere and satellite on short arm.

TYPE G : (Chromosomes X and XI)

Two pairs of median chromosomes (3.18 μ and 3.00 μ) with sub-terminal centromeres.

TYPE H : (Chromosome XII)

A pair of short chromosome (2.70 μ) with median centromere.

TYPE I : (Chromosome XIII)

A pair of short chromosome (2.55 μ) with subterminal centromere and satellite on short arm.

TYPE J : (Chromosome XIV)

A pair of short chromosome (2.22 μ) with subterminal centromere.

TYPE K : (Chromosome XV)

A pair of short chromosome (1.50 μ) with submedian centromere. This is the shortest pair.

Observations and Discussions :

The Coleus forskohlii member of Labiatae has chromosome number n = 30 (Plate 5). The idiogram of the karyotype that has been studied in this species is given in the Fig.1. The morphological features of somatic complement such as chromosome length, arm ratio, 'i' value, centromeric position are given in the Table 1. The karyotype formula is :-

$$K_{(n)} = A^{st} + B^{m} + C^{sm} + 2D^{m} + 3E^{sm} + F^{t} + 2G^{st} + H^{m} + I^{st} + J^{st} + K^{sm}$$

The range of variation of chromosome length in u is from largest to smallest, 5.04 to 1.5 μ.

In other words being dicotyledonous member, the chromosome size is relatively small. Eight pairs of chromosomes in

Table 1 : Morphological features of somatic chromosomes of Coleus forskohlii.

Chromosome pair	Chromosome length (μ)			Arms ratio (s/l)	'i' value	Centromere position
	Total	Short arm (s)	Long arm (l)			
I	5.04	0.90	4.14	0.22	17.86	st.
II	4.95	2.13	2.82	0.75	43.03	m.
III	4.59	1.68	2.91	0.58	36.60	sm.
IV	3.86	1.59	2.27	0.70	41.19	m.
V	3.82	1.05	2.77	0.38	27.48	sm.
VI	3.82	1.09	2.73	0.40	28.53	sm.
VII	3.68	1.30	2.36	0.55	35.33	sm.
VIII	3.50	1.41	2.09	0.67	40.28	m.
IX	3.18	0.27 sat.	2.91	0.09	08.49	t.
X	3.18	0.54	2.64	0.20	16.98	st.
XI	3.00	0.40	2.60	0.15	13.33	st.
XII	2.70	1.29	1.41	0.91	47.78	m.
XIII	2.55	0.10 + 0.27 sat.	2.18	0.17	14.51	st.
XIV	2.22	0.43	1.77	0.24	19.37	st.
XV	1.50	0.58	0.92	0.63	38.67	sm.

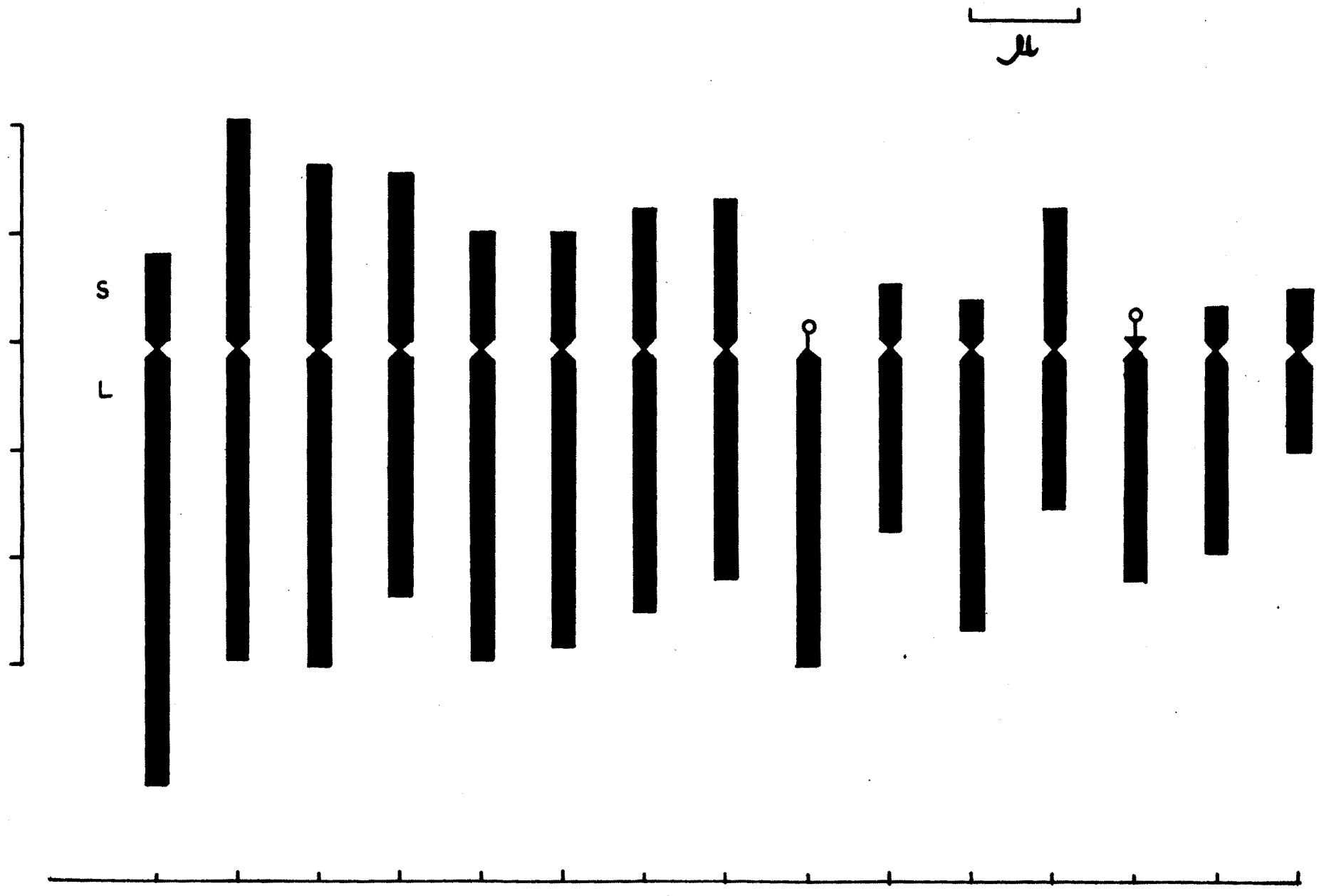


Fig. 1 : IDIOGRAM OF THE SOMATIC COMPLEMENT OF Coleus forskohlii Briq

the complement has more or less median or submedian centromere. The 9th pair has a subterminal centromere and 13th pair is a SAT chromosome. The complement does not appear to have nucleolar organizer or secondary constriction.

Because of the importance of family Labiatae some members from Central India have been studied for their cytological investigation by Bir and Saggoo (1982). They collected 16 economically important species of Pachamarhi hill of M.P. for the cytological investigation. Out of which 5 species namely Leucas mollissima, n = 14; Micromeria capetellata, n = 24; Plectranthus mollis, n = 14; Pogostemon purpurescens, n = 16 and Salvia coccinea, n = 11; Coleus barbatus, n = 17; Lavandula bipinnata, n = 20; Ocimum canum, n = 40; Ocimum sanctum, n = 18; Hyptis sauveolens, n = 14.

It is noteworthy to mention here even different species belonging to the same genus do not have the same chromosome number and these collections of Bir and Saggo do not include Coleus forskohlii which has n = 15 chromosome. That means Coleus forskohlii and Coleus barbatus both are known to be very closely related. Two species which do not have the same chromosome number reflecting thereby the possible role of polyphoidy. More precisely with n = 17 in Coleus barbatus and n = 15 in Coleus forskohlii is a clear reflection of aneuploidy playing a role.

Bahl and Tyagi (1988) made a karyotypic study of Coleus forskohlii, not based on the somatic preparation but on pachytone chromosomes. Because of the length of the pachytene chromosome, it is not possible to compaire the karyotypic pattern with respect to the size of the chromosome. Nonetheless variation in chromosome among the known species of Coleus provides hope of identifying a genetic marker for high terpenoid content used by pharmaceutical industry as a drug. Alternatively it also opens up a new horizon for raising interspecific hybrids for higher forskolin content. As such these plants can easily be propogated by clonal cuttings.

B) Inorganic constituents :

As mentioned earlier some of the minerals profoundly influence the alkaloid content or the, drug in question, of the plant. In the recent years a sharp manipulation of drug yield with nutritional manipulation of the mineral is accomplished. The role of trace element by and large has a profound influence to play on the drug content of the plant. For instance Gasic et al. (1978), in Zeckoslovakia raised collection of Colchicum autumnate along with the soil samples. They analyse the mineral content especially the trace element such as Cu, Zn, Mn of the soil and the colchicine content of the tuber. They establish a positive correlation between these trace elements

on the total alkaloid content. In order to manipulate the forskolin content of Coleus forskohlii under artificial condition, the both root tuber as well as leaf mineral contents have been analysed. The minerals that analysed Cu, Co, Ca, Mg, Mn, Fe, Zn.

Calcium :

Calcium content of both leaf and the root analyse separately, are given in the Table 2. It is evident that calcium content of the leaf is much higher than the root. In other words leaf of the Coleus forskohlii has 3 times higher amount of calcium than in the roots, this may be possibly because root tuber in Coleus forskohlii mainly functions as a storage organ of reserved food. The mineral absorption, though is carried out by the roots as well as advantitious roots it is transported to the leaves because of the pulling force of transpiration stream. The leaf is a metabolic center. Whether it is a stored food or any other compound the basic skeleton is being built in the leaves and subsequently transported to the storage organ. The low calcium content of the root means the amount of calcium pumped in the leaf is partly secured in the root. In other words the calcium requirement of the plant appears to be much higher i.e. 4.75% in the leaf and 1.52% in the root (Table 2). This is indicative of the fact that Ca^{2+} rich soil perhaps facilitates

better growth of the Coleus forskohlii. It is needless to mention here that Ca^{2+} is a very important mineral which influences wide variety of plant metabolism.

Magnesium :

Magnesium is another divalent cation which is indispensible for plants. Nonetheless unlike Ca^{2+} it's requirement is much low. It is highly mobile element. The analytical data of Mg^{2+} of roots and leaves are given in Table 2. The leaf has 1.21% Mg^{2+} as expressed on the dry wt. basis while the root has 0.85% (Table 2). Although, leaf is a photosynthetic organ, where the chlorophyll is there with a Mg^{2+} and porphyrin ring, there is relatively less difference between Mg^{2+} content of the leaf and the root. This reflects on the fact that greater amount of Mg received at the leaf site through transpiration is being retraslocated to the roots possibly because of its requirement.

Grunwald (1975) while reviewing the work on plant sterol pointed out that, mavalonic kinase and phosphomavalonic kinase enzymes are key enzymes of sterol synthesis and their activities are dependent upon Mg^{2+} and Mn^{2+} . Obviously therefore, the high Mn content of a non-photosynthetic organ, the root tuber of Coleus forskohlii may be correlated with the sterol synthesis.

Manganese :

Mn^{2+} is a member of the trace element group and hence its requirement is in trace only. Although, it's requirement is in trace, it is an indespensible divalent cation. The vary electron transport process of photosynthesis is dependent upon Mn^{2+} . Besides number of reactions such as amino acid metabolism, alkaloid and steroid metabolism are dependent upon Mn^{2+}.

The analytical data of leaf and root organ of Coleus forskohlii are given in Table 2. Similar to that of Mg^{2+} there is greater proportion of Mn^{2+} distributed in the leaf while in the root it is only 1/4th of the total Mn^{2+} content of the leaf. As mentioned earlier the leaf is a centre of metabolic activities and hence its requirement of mineral is always higher. Nonetheless the root Mn^{2+} content of 0.0128% is a sizeble amount. Similar to that of Mg^{2+}, Maganese is known to be required as a co-factor for mavalonic kinase and phosphomavalonic kinase enzyme. According to Tchen (1958) and Williamson and Kekwick (1965) amongst the two elements Mg^{2+} and Mn^{2+} which are known to promote enzyme activity, the Mn^{2+} has been shown to be more effective than Mg^{2+}. This means it is a very important mineral so far as sterol biosynthesis is concerned.

Iron :

Iron is also the member of trace element group. Amongst the trace elements Fe participate in number of reactions. The very structural integrity of cytochromes

is dependent upon Fe^{2+} . It is powerful member of the redox system. The analytical data of root and leaf, iron content are given in Table 2. The values of iron content indicates that the leaf has slightly higher amount than the root. That means the leaf has 0.5% iron while the root has 0.3%. In other words much of the iron received at the leaf site is retranslocated through phloem to root system.

Zinc :

This member of the minor element group is very important one; which profoundly influences the synthesis of growth hormone and auxins. The analytical data of leaf and root given in the Table 2 clearly indicates that unlike other elements, root has high proportion of 0.091% while leaf has only 0.074%. In other words, the greater amount of Zn^{3+} is translocated to the root. It is needles to mention here that Zn^{3+} controls the auxin precursor tryptophane. Now a cyclic aromatic amino acids also play a pivotal role to the formation of carbon skeleton in complex sterol alkaloids and terpenes.

Copper :

Copper is another important trace element whose requirement is in very very small quantity. The root and leaf Cu content are given in Table 2. The leaf has 0.038% while the root of Coleus forskohlii has 0.03%. In other words the distribution of Cu^{2+} in the leaf and root is more

or less on par in as much as its requirement. Gasic et al. (1978) have shown the correlation between the soil copper content and colchicine content of the Colchicum autumnale. This study clearly indicates that Cu^{2+} has certain critical role to play in alkaloid metabolism. It is well known that alkaloid or sterols or such other secondary metabolites, are basically synthesized in the organ other than leaf, possibly in the root systems. This may be the reason as to why copper is traceble in sizable quantity in the root.

Cobalt :

Although cobalt has no much greater significant role to play in alkaloid metabolism, it decides the very integrity of enzyme nitrogenase. The root and leaf Co^{3+} analysed is given in Table 2. Leaf has 0.018% while the root has 0.014%. In other words this trace element is stored in the sizable quantity in root system. Greater correlation exists between Co^{3+} content and nodulation in leguminous plant. Nonetheless, there are evidences to show that nonnodulus plants also fix the nitrogen. In the absence of sufficient evidences the Cobalt can only be linked with nitrogen metabolism of this plant.

b) Nitrogen content :

Nitrogen status of a plant is one of the important decisive factors which profoundly influence the yield and

Table 2 : Inorganic constituents of root and leaf of Coleus Forskohlii Briq. (Expressed in percent dry weight).

Plant parts	Ca	Mg	Mn	Fe	Zn	Cu	Co
Roots	1.525	0.855	0.013	0.313	0.092	0.030	0.014
Leaf	4.750	1.210	0.051	1.500	0.074	0.038	0.018

the quality. The yield may be a sink product or biomass, both are important from point of view of quantitative determination. Therefore, the nitrogen content of the leaf and root tuber has been estimated from the dry samples of this plant and presented in Table 3.

Table 3 : Nitrogen content of the leaf and roots of Coleus Forskohlii.

Organ	Moisture percentage	N content (mg/100 g dry tissue)
Leaf	90.2	418.0
Root	87.8	744.0

The results clearly indicate that the fleshy leaves of Coleus forskohlii have very high moisture content of 90.20% while the roots have low moisture content. So far as nitrogen content is concerned it is 418 mg/100 gm dry tissue in the leaf, while it is 744 mg/100 gm dry tissue in the roots. In other words the values of nitrogen though appear to be low in the leaves this is only because of high moisture content, while the roots are also fleshy and functions as a storage organ. Nitrogen is relatively higher but the overall status, if not discouraging it is not very encouraging. In conclusion it may be said that the nitrogen status of Coleus forskohlii is very

poor. Therefore, application of nitrogen may improve the nitrogen status of plant.

C) Effect of NPK trial on the root and shoot biomass yield :

It is needless to mention here in Coleus forskohlii the sink organ is root for the drug, forskolin is mainly extracted from the root. Nevertheless the aerial part that is shoot is the main biomass earning region through photosynthesis. As a corollary, therefore, the growth of the aerial part in any given genotype, by and large can be used as an index, determining the biomass yield of the root, unless it is genetically defined as a nontuberous type. The optimization of NPK is essential for maximization of biomass yield. Therefore, in the present investigation NPK trials has been taken to specify the dose. The results are presented in Table 4.

a) The root biomass yield

The three main NPK trials taken are in the proportion of 70:50:50, 50:70:50 and 50:50:70 Kg/hect. respectively. These trials have been taken in three replicates. The mean values are computed for their significance. Amongst, the three doses of NPK trial the 1st dose 70:50:50 has yielded 550 grams of root/plant, the second dose 50:70:50 yielded 650 gram/plant and the third dose with 50:50:70 yielded 825 grams of root/plant, while the control yielded 6kg8 grams/plant (Table 4). The results clearly indicate the high nitrogen content and relatively low phosphorous and potash content has reducing effect, while the

Table 4 : Effect of different doses of NPK on the root and shoot biomass yield of *Coleus forskohlii* (Expressed in g fresh tissue per plant).

Treatment	Root yield grams	't' value	Shoot yield grams	't' value
Control	608 (28.80)	n.s	558 (19.50)	n.s
N : P : K 70 : 50 : 50 kg/ha.	550 (29.30)	n.s	625 (21.80)	n.s
N : P : K 50 : 70 : 50 kg/ha.	650 (31.20)	n.s	560 (28.90)	n.s
N : P : K 50 : 50 : 70 kg/ha.	825 (38.50)	3.42 *	870 (22.80)	4.12 *

* Significant at 5%.

Values in parenthesis are S.E.

high phosphorous has a beneficial effect. On the other hand keeping the nitrogen and phosphorous level low, if the potash level is increased the root biomass yield significantly increases. This leads to conclude that Coleus forskohlii requires more K or yields better root biomass in the K rich soil. This observation has certain bearing even with the nitrogen status of the plant. Our earlier result with the nitrogen status determination has clearly indicated both leaves as well as the roots have low nitrogen content and high moisture content. Perhaps the plant species is not a nitrophilous one, therefore, the very nitrogen requirement of the plant itself is low, and hence high nitrogen content of the soil is not required for better cultivation.

Mothes (1955) reviewing the work on alkaloid metabolism in plants emphasized that nutritional conditions greatly influence the alkaloid concentration and total alkaloid content of any plant. He pointed out that K has a positive effect on alkaloid production and N influences the alkaloid content. This justifies therefore, in the present investigation as to why high K application leads to high yield of roots.

b) Shoot biomass yield

The effect of NPK trial on shoot biomass yield is presented in Table 4. The values given in the table clearly indicate that, amongst the three different doses of NPK namely 70:50:50, 50:70:50 and 50:50:70, the 3rd dose resulted

Table 5 : Effect of different doses of NPK on relative forskolin content of *Coleus forskohlii*.

Treatment Kg/ha.	Forskolin content (spot intensity)
Control	+
70 : 50 : 50 N : P : K	++++
50 : 70 : 50 N : P : K	++
50 : 50 : 70 N : P : K	+++++
Standard forskolin	+++

Plate 6 : TLC plate of forskolin content exhibiting effect of different doses of NPK

1) Standard

2) Control

3)

N	P	K
70	50	50

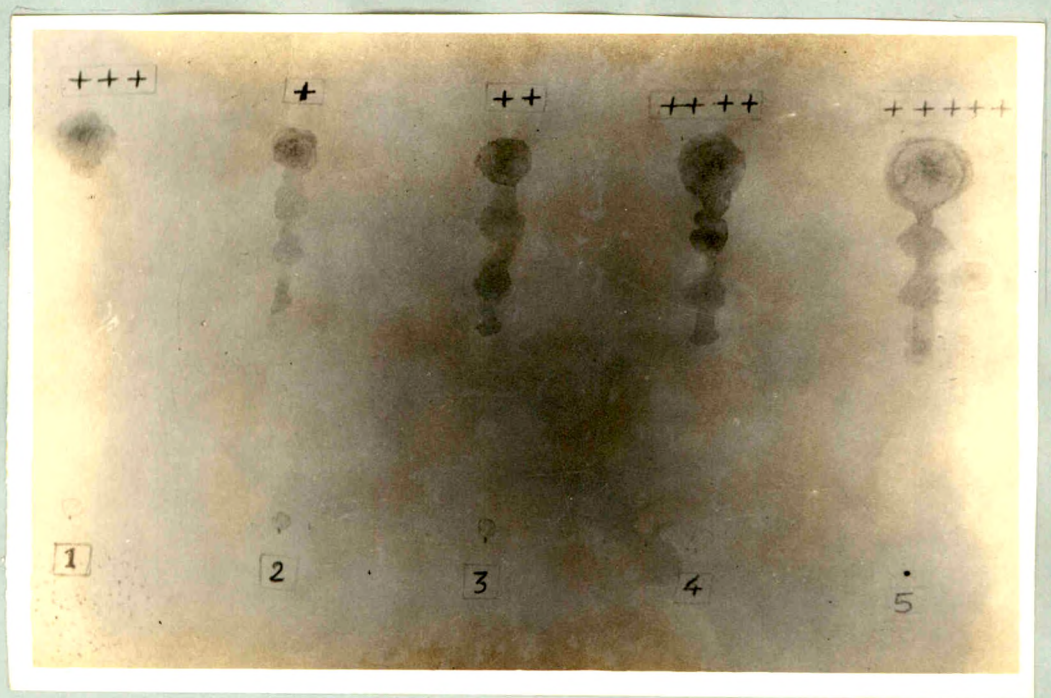
4)

N	P	K
50	70	50

5)

N	P	K
50	50	70

Plate 7 : Plate showing effect of Potassium on root tuber



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into 870 gram/plant average shoot biomass yield, while 50:70:50 and 70:50:50 have yielded respectively 560 and 625 grams/plant. The control has yielded 558 gram/plant, this means neither the increased nitrogen content nor the increased phosphorous content stimulated the biomass yield of shoot, but the increased K content, similar to that of root biomass also stimulates the shoot biomass. Perhaps the better growth of the aerial part with increased photosynthetic leaf area may also be contributing to the increased biomass of the shoot as well as the root.

As indicated by Mothes (1955) nutritional conditions especially with K greatly influence the alkaloid content. Coupled with the fact that, increased biomass with increased alkaloid content especially with the K nutrition greatly benefit the yield.

c) Effect of NPK nutrient on the forskolin content

The forskolin content analysed from the root of the plants raised on different doses of NPK by TLC method is presented in Table 5 and Plate 6. It is clear from the result that those which have received high potassium content rather than those which have received more nitrogen or more phosphorous. In other words K nutrition stimulates not only high biomass yield, but even the sink product of the root forskolin. Therefore, application of high K content and low N and P is recommendatary in harvesting the better yield of roots and high forskolin content.

D) Mutation breeding :

Since the discovery that high energy radiations such as x-rays and Gamma radiations double the rate of spontaneous mutation (Muller 1927), the importance of radiation in isolating useful mutants both from seeds as well as cuttings has greatly enhanced the improvement of crops. Isolation of the clonal mutants by irradiating the buds in vegetatively propogated crops has been in vogue and this has resulted into many horticultural varieties of ornamental plants, as well as plants of medicinal importance. With a view to isolating a useful mutant either in giving a high biomass yield or increased forskolin content in the root tubers, the cuttings of Coleus forskohlii having axillary buds have been subjected for irradiation.

The entire schedule of mutation breeding study is as follows.

To identify ideal dose which could yield useful mutation without causing lethality in the first instance, following doses were chosen. They are, 500 R, 1000 R, 1500 R, 2000 R, and 2500 R, for each of the dose 60 freshly harvested cutting were exposed at Bhabha Atomic Research Centre, Trombay, Bombay. No sooner were they received they were planted. Out of the 5 doses chosen only those lots which received 500 Rad servive. Amongst the rest few sprouted but long before they establish, died. In other words the mortality is very high in the cutting which have

Plate 9 : Plant [illegible] irradiated with 500 R

Plate 9 : Plant Gamma-irradiated with 1000 R

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Plate 10 : Mutants raised from Gamma-irradiated stock

Plate 11 : TLC plate showing separation and content of forskolin exhibiting the effect of Gamma-radiation

1) Standard
2) Control
3) Mutant No.1
4) Mutant No.2
5) Mutant No.3

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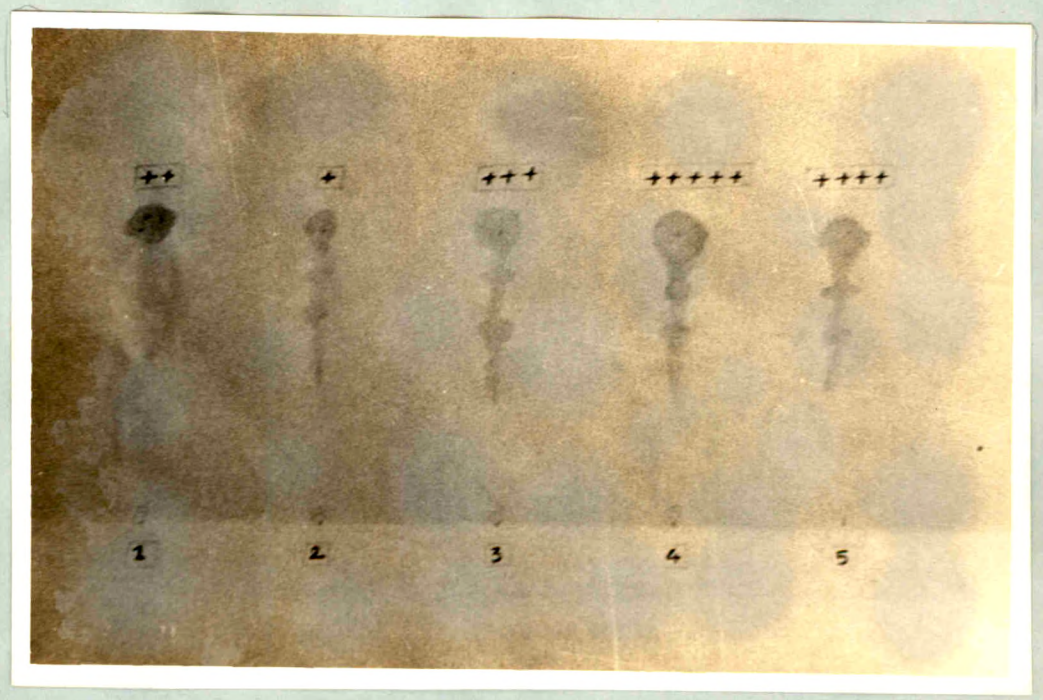

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received more than 500 Rad . Amongst those which received 500 R the servival rate is 97%. These were raised in the same way as the check plants as M_1 generation. They did not exhibit any external morphological deformities in the M1 phase.

M2 population :

From the M1 population which have received 500 Rad cuttings were raised for the M2 generation and they were kept under close observation for any external morphological mutant characters. However, no altered morphology could be seen from the 6 month old M2 clonal population. When harvested after 6 month, the roots and shoots were separated and the biomass yield/plant was recorded. The forskolin alkaloid from the root of M2 population was chromatographycally isolated by TLC method. The values of biomass yield and forskolin is given in Table 6. From the table it is evident that there is increase in the root yield/plant upon the control when irradiated, while there is no much greater difference in control and irradiated stock so far as shoot biomass is concerned. On the contrary with radiation exposure there is reduction in the aerial shoot biomass.

a) Effect of Gamma-irradiation on forskolin content :

The forskolin content in the roots of M2 population has been isolated by TLC method and depending upon the colour intensity they have been quantified and the values are given in the Table 7. It is evident from the result that the

Table 6 : Effect of 500 R Gamma-radiation on root and shoot yield in Coleus forskohlii.

Plant organ	Expressed as g fresh weight/plant			
	Moisture percentage	Control	Irradiated 500 R M_2 generation	't' value
Root	87.0	689	748	2.12 *
Shoot	89.4	695	629	n.s.

* Significant at 5% level.

Table 7 : Effect of 500 R Gamma-radiation on relative forskolin content of roots of Coleus forskohlii.

Samples	Content of forskolin (spot intensity)
Control	+
Standard	++
Mutant 1	+++
Mutant 2	+++++
Mutant 3	++++

forskolin content in the irradiated stock has increased almost 4 times of the control (Plate 11). This raises the hope of isolating this mutant for high forskolin content. The precise quantitative determination, either by the GLC or by HPLC method will better project light on the reality of the situation. The increased forskolin content in the irradiated stock may either be due to stimulated activity of enzymes concern or due to high transcription level at the genetic site. Stability of the mutant character with respect to forskolin persuaded for the future clonal generation may clarify the possibility.

b) Effect of Gamma-irradiation on the stomatal behaviour :

It is well established with the mutation study that the primary effect of mutagens is readily exhibited by the stomatal behaviour and morphology. Therefore, it has been a practice to examine stomata primarily, before the irradiated stocks are assessed for their chromosome morphology and the other effects. Therefore, with the help of steady state porometer the stomatal behaviour of irradiated stock and the non-irradiated stock has been assessed and given in Table 8. For the purpose in each of the case 4 matured leaves at random were chosen. The stomatal parameters recorded are diffusive resistance for water vapour, transpiration rate and diffusive resistance for CO_2.

What is evident from the result is that in the irradiated stock, diffusive resistance for CO_2 has increased, while the transpiration rate has decreased. This is a clear indication that,

Table 8 : Effect of 500 R Gamma-radiation on stomatal behaviour, transpiration rate and gas exchange in Coleus forskohlii.

Treatment	Leaf surface	Diffusive resistance for H_2O vapour Scm^{-1}	Diffusive conductance for water vapour cmS^{-1}	Transpiration $ugS^{-1}\ cm^{-1}$	Diffusive conductance for Co_2 cmS^{-1}	Diffusive resistance for Co_2 Scm^{-1}
Control	Lower	2.49	0.44	1.72	0.942	1.24
	Upper	12.11	0.15	1.33		
Irradiated	Lower	34.15	0.04	1.19	0.116	9.55
	Upper	53.97	0.03	0.84		

although, the radiation effect of 500 R is not persievable externally by way of morphological alteration, the change in the stomatal behaviour, is indicative of the penetrance level of radiation. However, what concerns is, increased, diffusive resistance to CO_2 is not a desirable effect.

E) Foliar application of Mg^{2+} and Mn^{2+} :

It has been shown that some of the minerals especially of the trace element group profoundly influence secondary products of the plants such as alkaloids, steroids, and terpenes etc. Gasic et al.(1978) have shown a good correlation between Cu, Zn and Mn content of the soil and colchicine content in Colchicum autumnale. Tchen (1958), Williamson and Kekwick (1965), have also shown that Mg^{2+} and Mn^{2+} profoundly influence a activity of enzymes of sterol metabolism. These studies open a new vistas in the direction of acheiving increased secondary product metabolism by facilitating foliar absorption of required trace elements. As such there is exhaustive literature available on the effectiveness of the foliar application of micronutrient and accomplishment of yield.

The result of such study have been presented in Table 9. For foliar application only Mg^{2+} and Mn^{2+} at a concentration of 50 and 100 ppm have been chosen. The results indicate that the root and the shoot yield in control are 600 and 612 gram/plant respectively. Whereas, the root yield when 50 and 100 ppm of

Table 9 : Effect of foliar application of Mg^{2+} and Mn^{2+} on shoot and root yield of *Coleus forskohlii*.

Plant organ	Yield in grams fresh tissue/plant					
	Control	Mg^{2+} 50 ppm	Mg^{2+} 100 ppm	Mn^{2+} 50 ppm	Mn^{2+} 100 ppm	Mg^{2+} 50 ppm + Mn^{2+} 50 ppm
Root	660 (41.50)	558 (30.15)	625 (19.34)	652 (35.68)	664 (32.40)	810 (22.45)
Shoot	612 (29.74)	586 (39.00)	556 (28.55)	602 (42.55)	684 (36.60)	734 (22.44)

Values inparenthesis are S.E.

Mg^{2+} sprayed is, respectively, 558 and 625 grams/plant and the shoot biomass yield is respectively 586 and 556 grams/plant. In other words the Mg^{2+} foliar application does not appear to be encouraging, although with 100 ppm Mg^{2+} the root yield has increased by 25 gram over the control/plant. So far as Mn^{2+} foliar application is concern the root biomass yield is 652 and 664 grams/plant respectively in 50 and 100 ppm sprayed plants. When mixture of 50 ppm Mg^{2+} and 50 ppm Mn^{2+} is sprayed the average root yield has increased to 810 gram/plant which is more than 30% that of the control. Similarly the shoot biomass yield has increased from 612 gram/plant in the control to 734 grams/plant. This increase is 20%. The significant increase in biomass is certainly going to contribute for the over all yield of forskolin content and raises the hope of increasing productivity in terms of drug yielding organs. Since, it is known that some of the key enzymes of sterol biosynthesis such as mavalonic kinase and phosphomevalonic kinase are stimulated by Mg^{2+} and Mn^{2+} (Tchen 1958, Williamson and Kekwick 1965), quantitative determination of forskolin has been carried out by TLC method and presented in Table 10. Although TLC method is not as precise method of quantification as GLC and HPLC, in absence of these later facilities TLC method has been adopted. Nevertheless fairly good quantification by the intensity of spot could be achieved, it is clear from the Plate 12 as well as table 10 that, 100 ppm foliar application of Mg^{2+} has increased forskolin content 4 times of the control while the

Plate 12 : TLC plate exhibiting the effect of foliar spray of Mg^{2+} and Mn^{2+} on forskolin content

1) Standard

2) Control

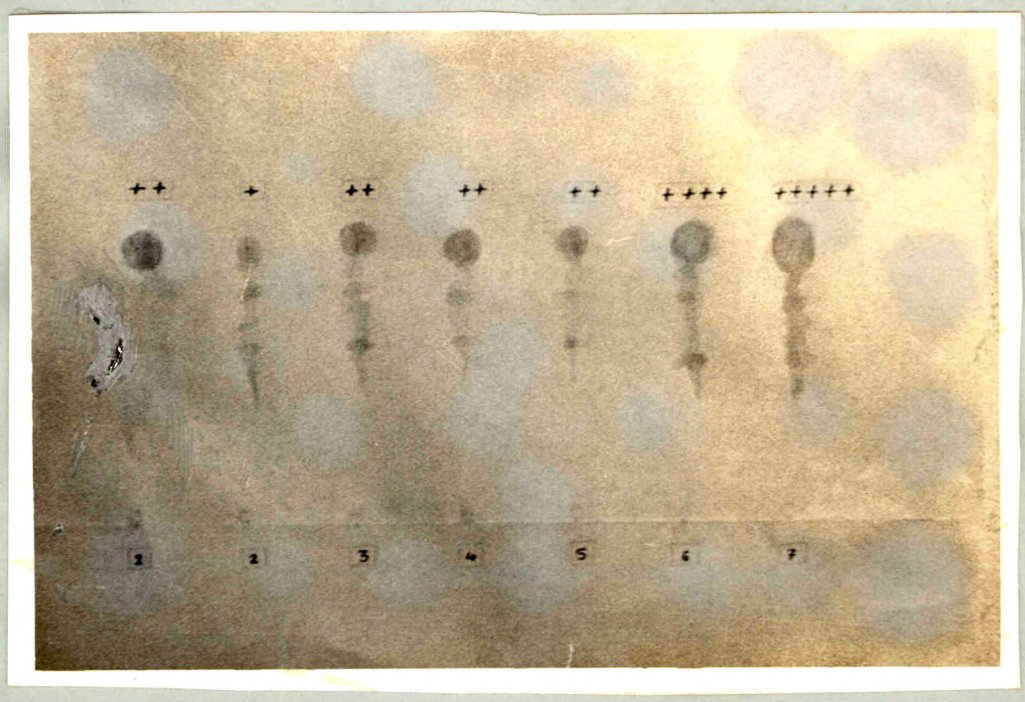
3) Mn (50 ppm)

4) Mg (50 ppm)

5) Mn (100 ppm)

6) Mg (100 ppm)

7) Mn (50 ppm) +
   Mg (50 ppm)



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mixture of Mg^{2+} and Mn^{2+} 50 ppm each has stimulated 5 times of the control (Plate 12).

Table 10 : Effect of foliar application of Mg^{2+} and Mn^{2+} on relative forskolin content of roots Coleus forskohlii.

Treatments	Content of forskolin (spot intensity)
Control	+
Standard forskolin	++
Mg^{2+} (50 ppm)	++
Mg^{2+} (100 ppm)	++++
Mn^{2+} (50 ppm)	++
Mn^{2+} (100 ppm)	++
Mg^{2+} (50 ppm) + Mn^{2+} (50 ppm)	+++++

The increase in the root biomass and the shoot biomass with the increase forskolin content raises the hope that, by foliar application of essential nutrient accomplishes increased yield of the drug and biomass. Lugade (1987) tried with foliar application of Mn^{2+} and Zn^{2+} and achieved increased colchicine

content in the tubers of Gloriosa rothschildiana. It is therefore, concluded that rather than applying at the root zone requisite nutrient applied to the plant over leaf foliage has a better effect.