

Studies on correlation and path analysis in mutants of Coleus (*Coleus forskohlii* Briq.) for yield and forskolin content in V₂M₁ generation

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ABSTRACT

The present investigation was carried out during 2003-2007 involving terminal cuttings of coleus ecotype 'Garmai'. Genotypic correlation coefficient between yield and its components in mutants of coleus was studied, viz., plant height, number of branches plant⁻¹, number of leaves plant⁻¹, number of tubers plant⁻¹, tuber length and tuber girth were found to have positive and highly significant correlation with yield. However, forskolin and essential oil content showed negative correlation with yield. Path analysis of component characters on yield of Coleus in V_2M_1 generation exerted positive direct effect through the characters plant height, number of leaves plant⁻¹ and number of tubers plant⁻¹. Similarly, direct effect was observed to be negative through number of branches plant⁻¹ (-0.930), total amount of alkaloids (-0.066) and forskolin content (-0.026). The current investigation resulted in residual effect of 0.158 indicating the accuracy and appropriate selection of component character for crop improvement programme. Weightage must be given to component characters exhibiting positive attributes towards fresh tuber yield in Coleus. However, some traits with negative attributes are also chosen for getting improved quality, *i.e.*, forskolin content, without much inhibition on fresh tuber yield plant⁻¹.

Key words: Coleus forskohlii, correlation, Path analysis

INTRODUCTION

Medicinal plants traditionally occupied an important position in rural and tribal lives of India and are considered as one of the most important sources of medicines since the dawn of human civilization. One such an important medicinal plant is medicinal coleus (Coleus forskohlii Briq.). The tuberous roots of coleus are rich source of forskolin, a diterpenoid activates Cyclic Adenosine Monophosphate or AMP in the cells (Bhat et al, 1977). Coleus forskohlii is the only known natural source of forskolin. Due to its multifaceted pharmacological effects, forskolin is used for treatment of eczema (atopic dermatitis), asthma, psoriasis, cardiovascular disorders and hypertension, where decreased intracellular cAMP level is believed to be a major factor in the development of disease process (Rupp et al, 1986). Extent of genetic variation in Coleus forskohlii is limited. Continuous vegetative propagation for many years has reduced the vigour and tolerance to biotic and abiotic stress, causing low yields. Hence, yield and quality is enhanced possibly by developing

Present address: 1 Agricultural College and Research Institute, Madurai 2 Agricultural Research Station, Aliyarnagar a mutant in this species with high tuber yield and improved forskolin content through induced mutations. The ultimate goal of crop improvement in coleus is to improved tuber yield and forskolin content. Being a complex trait, the tuber yield is largely influenced by many component characters. Information on strength and direction of correlation of these component characters on tuber yield and inter se association among them would be useful in designing breeding programmes for yield improvement. The relationship between yield and its component characters is likely to vary according to the genetic material used and environment under which the material is evaluated as well as due to interaction of these factors. Therefore, it is worthwhile to study the heritable association between variables (Genotypic correlation) for identification of important yield components so that weightage can be given to these characters of importance in further breeding programmes (Johnson et al, 1955). The current investigation confines to correlation and path analysis in mutants of coleus in V_2M_1 generation.

MATERIAL AND METHODS

The present investigation was carried out at Medicinal plant unit, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2003-2007. Terminal cuttings of coleus ecotype 'Garmai' was obtained from Manjini in Salem district of Tamil Nadu, where this crop is grown commercially by the farmers in a larger extent. Based on preliminary experiments, it is concluded that the LD₅₀ value for gamma rays was 3.00 kR and for EMS it was noticed at 1.00 % concentration which was exposed for a period of 3.00 h. Based on the sensitivity studies, mutagenic treatments were formulated viz., AT_1 – Control, AT_2 - 2.50 kR gamma rays, AT_3 - 3.00 kR gamma rays, AT_4 - 3.50 kR gamma rays, AT_5 - 0.50 % EMS, AT₆ - 1.00 % EMS, AT₇ - 1.50 % EMS, AT₈ - 2.50 kR gamma rays + 0.50% EMS, AT_{o} - 2.50 kR gamma rays + 1.00% EMS, AT₁₀ - 2.50 kR gamma rays + 1.50% EMS, AT_{11} - 3.00 kR gamma rays + 0.50% EMS, AT_{12} - $3.00 \text{ kR gamma rays} + 1.00\% \text{ EMS}, \text{AT}_{13} - 3.00 \text{ kR gamma}$ rays + 1.50% EMS, AT₁₄ - 3.50 kR gamma rays + 0.50% EMS, AT_{15} - 3.50 kR gamma rays + 1.00% EMS, AT_{16} -3.50 kR gamma rays + 1.50% EMS. While imposing the treatments, terminal cuttings were treated with Gamma rays and EMS separately. But for combination of mutagenic treatments, cuttings were initially treated with respective EMS concentration and immediately then they were exposed to Gamma radiation and planted in field in Randomized Block Design (RBD) with 600 plants in each treatment.

Total number of branches (including primary, secondary and tertiary branches) and leaves produced from planted terminal cutting following mutagenic treatment was represented as first vegetative generation and designated as V_1M_1 generation plants. Secondary shoots were considered as the second vegetative generation. Secondary shoots were obtained by cutting back the primary shoot and planted for the study of V₂M₁ generation. The mutants were evaluated by adopting standard recommended cultural practices (Hegde, 2001 and Rajamani, 2003) for crop cultivation. The biometrical traits viz., plant height, number of branches plant⁻¹ and number of leaves plant⁻¹ were observed at 180 days after planting. Similarly, yield parameters like length and girth of tuber and fresh tuber yield plant⁻¹ were also recorded. After the harvest of tubers, the quality traits like forskolin (Mersinger et al, 1988), essential oil (A.S.T.A, 1960) and total alkaloids (Kokate et al, 2001) were estimated by adopting standard procedures. In V₂M₁ generations, the genotypic correlation coefficients and phenotypic correlation coefficient were estimated according to Johnson *et al* (1955). The significance of the genotypic correlation coefficients was tested by referring to the standard table given by Snedecor and Cochran (1967). Path coefficient analysis was carried out according to Dewey and Lu (1959) by partitioning the genotypic correlation into direct and indirect effects.

RESULTS AND DISCUSSION

The correlation coefficients between yield and its components and inter correlations among various yield attributes were estimated. In general, genotypic correlation coefficients were of higher in magnitude than phenotypic correlation indicting the lesser influence of environmental factors. Being a complex trait, tuber yield is largely influenced by many component characters. The relationship between yield and its component characters is likely to vary according to the genetic material used and environment under which the material is evaluated as well as due to interaction of these factors (Table 1 and 2).

The highest positive and significant genotypic correlation of yield was observed with tuber girth (0.997) and it was closely followed by number of leaves plant⁻¹ (0.962) and number of tubers plant⁻¹ (0.958). Other traits exhibited positive and significant genotypic correlations with yield are tuber length (0.934), plant height (0.906) and number of branches plant⁻¹ (0.847). While the characters viz., forskolin (-0.782) and essential oil content (-0.167) showed negative correlation with yield. Intercorrelation showed that the plant height had positive and highly significant association with number of branches plant⁻¹, number of leaves plant⁻¹, number of tuber plant⁻¹, tuber length and tuber girth. Number of tuber plant⁻¹ exhibited positive and highly significant association with tuber length and tuber girth. Each of these characters not only had positive association with each other but also highly significant with yield.

The yield exhibited positive and significant phenotypic correlation with plant height, number of branches plant⁻¹, number of leaves plant⁻¹, number of tuber plant⁻¹, tuber length and tuber girth. However, the forskolin and essential oil showed negative correlation with yield. This apparent negative correlation at genetic level would have arisen from repulsion linkage of gene(s), controlling the direct and indirect effects. Conversely, positive association was due to the coupling phase of linkage. This is in agreement with the earlier findings of Geetha and Prabhakaran (1987),

Statistics on Coleus mutants and yield

Table 1. Effect of gamma rays (kR) and EMS on mean values for different traits in V,M, generation in Coleus

	Treatment	Biometrical traits at 180 Days from planting			Yield trait				Quality trait		
		Plant height (cm)	Days from p Number of branches plant ⁻¹	0	Number of tubers plant ⁻¹	Length of tuber (cm)	Girth of tuber (cm)	Fresh Tuber-yield plant ⁻¹ (g)	Total alkaloid content(g)	Essential oil content (ml)	
AT1	Control	63.50	53.50	235.00	24.50	24.70	3.45	510.00	1.20	0.10	0.40
AT2	2.50 kR gamma rays	62.00	51.00	222.50	21.00	22.50	3.25	505.00	1.19	0.09	0.52
AT3	3.00 kR gamma rays	60.55	50.50	216.50	20.50	20.69	3.00	471.66	1.25	0.11	0.50
AT4	3.50 kR gamma rays	59.20	49.95	211.00	19.00	19.00	2.95	430.85	1.25	0.09	0.48
AT5	0.50 % EMS	58.00	49.00	229.50	23.50	23.50	3.30	497.55	1.19	0.10	0.42
AT6	1.00 % EMS	56.50	48.65	215.00	21.00	21.00	3.00	462.90	1.15	0.11	0.45
AT7	1.50 % EMS	55.00	48.05	203.00	18.00	19.80	2.85	433.20	1.10	0.09	0.40
AT8-	2.50 kR gamma rays + 0.50% EMS	55.10	46.50	210.50	21.50	20.90	3.20	492.40	1.21	0.07	0.40
AT9	2.50 kR gamma rays + 1.00% EMS	53.60	45.00	200.00	18.50	19.10	3.18	477.60	1.20	0.18	0.45
AT10	2.50 kR gamma rays + 1.50% EMS	53.00	44.95	192.00	18.00	18.45	3.12	445.18	1.17	0.09	0.62
AT11	3.00 kR gamma rays + 0.50% EMS	52.50	44.00	182.00	16.50	17.65	3.00	420.00	1.14	0.12	0.46
AT12	3.00 kR gamma rays + 1.00% EMS	50.00	43.70	178.50	14.50	17.00	2.95	409.28	1.10	0.10	0.46
AT13	3.00 kR gamma rays + 1.50 % EMS	49.00	42.80	160.00	13.00	16.20	2.65	389.30	0.96	0.09	0.55
AT14	3.50 kR gamma rays + 0.50% EMS	45.50	41.20	148.00	12.00	15.00	2.45	375.50	1.10	0.10	0.50
AT15	3.50 kR gamma rays + 1.00% EMS	43.10	40.15	131.50	10.50	14.33	2.30	305.00	1.26	0.14	0.61
AT16-		42.35	39.80	108.00	9.00	13.40	2.05	290.55	1.09	0.10	0.63

Bhandari and Gupta (1991), Prabhakar *et al* (1994) and Shanmugasundaram (1998). Correlation coefficients between the characters revealed that those characters exerted positive association among others are prone for improvement and underlined the fact that one component character leads to the concurrent improvement of the other component characters. The present findings are concurrent with Shanmugasundaram (1998) in turmeric and Kavitha (2005) in coleus. The present information on strength and direction of correlation of these component characters on tuber yield and *inter se* association among them would be useful in designing breeding programmes for yield improvement.

Correlation coefficient between any two characters would not give a complete picture for a situation like yield, which is controlled by several other traits, either directly or indirectly. In such situations, path coefficient analysis furnishes a means of measuring direct effect of each trait as well as indirect effect via other characters on yield. So information on direct and indirect effect on yield is important, which is explicable by path analysis proposed by Wright (1921) and illustrated by Dewey and Lu (1959). The interrelationships of the component characters on yield provide the likely consequences of their selection for simultaneous improvement of desirable characters with yield. The path analysis of component traits on yield of coleus mutants showed positive direct effects through the characters viz., plant height (0.979), number of leaves plant- 1 (0.422), number of tubers plant 1 (0.169), tuber length (0.386), tuber girth (0.048) and essential oil content (0.008)(Table 3). The direct effect was the highest for plant height (0.979) followed by number of leaves plant⁻¹(0.422), while the trait, number of branches plant⁻¹ had the highest indirect effect (-0.930). Since correlation of these characters with yield is positive, preference should be given to these characters in selection programme to isolate superior mutants with genetic potential for improving yield. A similar line of work was reported by Viswanathan et al (1993) in Abrus precatorius and Srivastava and Chauhan (1998) in Bauhinia variegata.

The direct effect was observed to be negative through number of branches plant⁻¹ (-0.930), total alkaloids (-0.066) and forskolin content (-0.026). This vivid conflict

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	\mathbf{X}_{1}	X_2	X ₃	X_4	X ₅	X ₆	X ₇	X ₈	X 9	X ₁₀
1	1	0.983**	0.958**	0.943**	0.934**	0.888**	0.415	-0.250	-0.697**	0.906**
	1	0.983**	0.959**	0.929**	0.938**	0.852**	0.471	-0.140	-0.435	0.909**
		1	0.928**	0.919**	0.930**	0.818**	0.418	-0.284	-0.688**	0.847**
		1	0.931**	0.910**	0.933**	0.785**	0.465	-0.181	-0.445	0.854**
			1	0.980**	0.955**	0.968**	0.406	-0.197	-0.805**	0.962**
			1	0.972**	0.957**	0.901**	0.449	-0.108	-0.552*	0.963**
				1	0.985**	0.981**	0.467	-0.192	-0.751**	0.958**
				1	0.973**	0.852**	0.453	-0.152	-0.590*	0.951**
					1	0.924**	0.381	-0.233	-0.779**	0.934**
					1	0.875**	0.436	-0.130	-0.514*	0.937**
						1	0.291	-0.178	-0.897**	0.997**
						1	0.464	0.055	-0.328	0.919**
							1	0.215	-0.247	0.321
							1	0.331	0.046	0.370
								1	0.008	-0.167
								1	0.192	-0.085
									1	-0.782**
									1	-0.541*
K ₁₀										1
U										1

Table 2. Effect of gamma rays (kR) and EMS (per cent) on genotypic and phenotypic correlation coefficient in Coleus mutants in VM generation

Upper values refers to genotypic correlation coefficient * Significant at 5 % level ** Significant at 1 % level # Lower values refers to phenotypic correlation coefficient

 X_1 : Plant height

 X_2^1 : Number of branches plant⁻¹ X_3^2 : Number of leaves plant⁻¹

 X_4 : Number of tubers plant¹ X_5 : Length of tuber

 X_{ϵ} : Girth of tuber

 X_7 : Total alkaloids

- X_8 : Essential oil
- X_{0} : Forskolin

Table 3. Effect of gamma rays (kR	R) and EMS (per cent) on path analysis in	Coleus mutants in V ₂ M ₁ generation
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	X ₁	X ₂	X ₃	X_4	X ₅	X ₆	X ₇	X ₈	X ₉	Yield
X ₁	0.979	0.318	0.338	-0.179	-0.386	-0.048	-0.043	-0.004	0.018	0.993
X,	0.952	-0.930	0.392	0.155	0.359	0.040	-0.028	-0.002	0.018	0.956
X,	0.968	-0.989	0.422	0.166	0.368	0.047	-0.027	-0.002	0.021	0.974
X	0.896	-0.991	0.414	0.169	0.380	0.048	-0.031	-0.001	0.019	0.903
X ₅	0.944	-0.981	0.403	0.166	0.386	0.045	-0.025	-0.002	0.020	0.956
X ₆	0.897	-0.882	0.409	0.166	0.356	0.048	-0.019	-0.001	0.023	0.997
X ₇	0.419	-0.451	0.171	0.079	0.147	0.014	-0.066	0.002	0.006	0.321
X ₈	-0.252	0.306	-0.083	-0.032	-0.090	-0.009	-0.014	0.008	0.000	-0.166
X ₉	-0.704	0.742	-0.340	-0.127	-0.301	-0.043	0.016	0.000	-0.026	-0.783

Residual effect: 0.158 Diagonal element - Direct effects

 X_1 : Plant height

 X_4 : Number of tubers plant⁻¹ X_2^1 : Number of branches plant⁻¹ X_3^2 : Number of leaves plant⁻¹

 X_{5}^{4} : Length of tuber X_{6}^{4} : Girth of tuber

between the correlation and path coefficient analysis arouse largely from the fact that correlation simply measures the mutual association without regard to causation, while path specifies the relative importance of each causal factor. So information on direct and indirect effect on yield is important which is explicable only by means of path analysis. It is also evident from the study that direct selection can be made on tuber characters as they are true components relating to yield and selection on these will be rewarding. The current investigation resulted with the residual effect of 0.158 X_7 : Total alkaloid content X_{10} : Fresh tuber yield plant⁻¹

 X_8' : Essential oil content X_9 : Forskolin content

indicating precision on selection of component characters. Most of the breeding programmes preferred with residual effect lesser than one. It indicates that accuracy and appropriate selection of component character for crop improvement programme. This is supported by the earlier works of Nandi et al (1992), Maurya et al (1998), Shanmugasundaram (1998), Ushanandhinidevi (2004) in turmeric and Kavitha (2005) in coleus. On a wholesome, the weightage must be given to component characters exhibiting positive attributes towards the fresh tuber yield

X₁₀: Fresh tuber yield plant⁻¹

of coleus. However, certain traits with negative attributes are also chosen for getting improved quality *i.e.*, forskolin content without much inhibition on fresh tuber plant⁻¹.

REFERENCES

- A.S.T.A. 1960. Official analytical methods of the American spice trade association, New York, pp. 41-42
- Bhandari, M.M. and Gupta, G.S. 1991. Association analysis of *Opium poppy* Linn. *Int'l. J. Trop. Agri.*, **9** (1): 42-44
- Bhat, S.V., Bajwa, B.S., Dornauer, H., DeSouza, N.J. and Fehlilhaber, H.W. 1977. Structure and stereochemistry of new labdane diterpenoid from *Coleus forskohlii. Tetrahedron Lett.*, **19**:1669-1672
- Dewey, D.R. and Lu, K.H. 1959. A correlation and path coefficient analysis of components of crested wheat grass seed production. *Agron. J.*, **51:**515-518
- Geetha, V. and Prabhakaran, P.V. 1987. Genotypic variability, correlation and path coefficient analysis in turmeric. *Agril. Res. J. Kerala*, **25**:249-254
- Hegde, L. 2001. Crop improvement in *Coleus forskohlii* Briq. **In :** National seminar on transfer of technology of medicinal and aromatic crops, held on 20-22 February, Bangalore, pp. 92-96
- Johnson, H.W., Robinson, H.F. and Comstock, R.E. 1955. Estimation of genetic variability in soybean. *Agron. J.*, **47:**314-318
- Kavitha, C. 2005. Genetic diversity studies in *Coleus* forskohlii Briq. Ph.D thesis, TNAU, Coimbatore
- Kokate, C.K., Prohit, A.P. and Gokhale, S.B. 2001. Pharmacognosy, Nirali Prakasam Publ., Warangal, India.
- Maurya, K.R., Kumar, R., De, N. and Singh, S.N. 1998.
 Correlation and path analysis performance studies of some turmeric (*Curcuma domestica* Val.) genotypes.
 In: National Seminar on recent development in spices production technology, Bihar Agricultural College, Sabour. pp. 11-12

- Mersinger, R., Donauer, H. and Rainhard, E. 1988. Formation of forskolin by suspension cultures of *Coleus forskohlii. Planta Med.*, **54:**200-204
- Nandi, A., Lenka, D. and Singh, D.N. 1992. Path analysis in turmeric. *Ind. Cocoa, Arecanut and Spices J.*, **17:**54-55
- Prabhakar, M., Singh, J. and Srivastava, L.J. 1994. Genetic variation and correlation studies for some important characters associated with solasodine yield in *Solanum khasianum* Clarke. *Ind. J. Forestry*, 17:26-31
- Rajamani, K. 2003. Cultivation technology of *Coleus forskohlii*. **In:** State level sem. Medicinal Plants, held at Trichy, pp: 53-58
- Rupp, R.H., De Souza, N.J. and Dohadwalla, A.N. 1986.
 In: Proceedings of the International Symposium on Forskolin: Its chemical, biological and medical potential. Hoechst India Ltd., Bombay, pp. 19-30
- Shanmugasundaram, K.A. 1998. Evaluation and selection for certain quantitative and qualitative characters in turmeric (*Curcuma domestica Val.*). M.Sc. (Ag.) thesis, TNAU, Coimbatore
- Snedecor, G.W. and Cochran, C.W.G. 1967. Statistical methods. The Iowa State University Press, Iowa, U.S.A.
- Srivastava, A. and Chauhan, K.C. 1998. Path coefficient analysis between shoot dry weight and other characters in *Bauhinia variegata* Linn. progeny. J. *Tree Sci.*, **15**:111-113
- Ushanandhinidevi, H. 2004. Induction of mutagenesis in turmeric (*Curcuma longa* L.) through gamma rays for variability and quality improvement. Ph.D thesis, TNAU, Coimbatore
- Vishwanathan, T.V., Sunil, K.P. and Sujatha, R. 1993. Path analysis for lethality in gamma irradiated population of Indian liquorice (*Abrus precatorius* L.) *South Ind. Hort.*, **41**:101-105
- Wright, S. 1921. Correlation and causation. J. Agril. Res., 20:557-585

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