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Studies on alkaloid yield in mutants of *Catharanthus roseus* (L.) G. Don. through character correlation and path analysis

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ABSTRACT

Study on the relationship between alkaloid yield and it's components will improve the efficiency of breeding programs to select the appropriate mutant with higher economy to fulfill the world demand for vincristine and vinblastine, with a view of it's clinical importance. The comparative study on the morphometric variations on the mutants of *C. roseus* revealed that plant height and pollen diameter is positively correlated with each other through character correlation. The relative importance of each agronomical component in determining alkaloid yield in mutants of *Catharanthus roseus* (L.) G. Don. is achieved by partitioning into direct and indirect effects through path co-efficient analysis. The number of leaves and dry matter yield has direct effect on alkaloid yield with increased number of follicle/ plant. The increment in number of leaves/ plant resulted in increase dry matter yield with higher alkaloid yield. The vindoline, catharanthine and vincristine have direct effect but vinblastine pose indirect effect on alkaloid yield.

Introduction

The *Catharanthus roseus* (L.) G Don. with 2n=16 chromosome, is a perennial, highly self-pollinated plant belonging to family Apocynaceae (Stern,1975). The Vinca alkaloids are useful in the treatment of both malignant and nonmalignant diseases. These alkaloids are useful in platelet and platelet associated disorders. The use of vincristine to treat thrombotic thrombocytopenic purpura is reported to be successful (Walia *et al*, 2011). One tonne of *C. roseus* leaves yield 50 gms of Vincristine sulfate in crude form. On further purification 40gms of vincristine obtained, thus very low yield from source material making it very expensive. Vinblastine present at a level of 1000 times higher than vincristine in the leaves makes the cost one third that of vincristine. Therefore vinblastine is being used as parent drug to obtain vincristine through structural modifications (Hamada & Nakazawa, 1991). However Electrometric titration values and nuclear magnetic resonance spectra of vincristine and vinblastine show structurally related but chemically unsymmetrical dimeric compounds with a chemical formula C_{46} H_{56} N_4 O_7 (Neuss *et al*, 1959). A mutation breeding programme was conducted on the seeds of *C. roseus* with gamma rays and ethyl methane sulfonate single and in combination and six different morphological forms of mutants have been isolated to develop high yielding varieties (Maithy Roy *et al*., 2008). The mutagenesis resulted in the change in plant morphology, particularly the epidermal structures and distribution of stomata and trichomes on leaves with reference to photosynthetic efficiency and transpiration rate of plants (Maithy Roy *et al*., 2007). Production of a stable quality and quantity of these plants is important for growing world market, which make it necessary to breed varieties with high yield and quality. Previous workers have shown that, a randomized block design experiment of progenies of *C. roseus* of 71 single plants showed broad sense heritability high for plant height and low for plant weight (Kulkarni *et al*., 1984) whereas 26 genotypes of Periwinkle, was recorded very high genetic advance for all the four alkaloids and it is very low for plant height and stem width (Diwedi *et al*., 1999).

The 26 genotypes isolated from different mutagenic treatment in M_2 generation, classified in six different morphological mutants were critically evaluated in randomized block design in three replications for morphometric and agronomic traits in M_3 generation and their heritability and genetic advance were recorded (Maithy Roy *et al*, 2009). The correlation of a particular character with other characters contributing to alkaloid yield, is of great importance in indirect selection of genotypes. Simple correlation analysis that relates with yield to a single variable may not provide a complete understanding about the importance of each component (Dewey and Lu, 1959). Path coefficient analysis is a statistical technique of partitioning the correlation coefficients into its direct and indirect effects, so that the contribution of each character towards yield could be estimated (Dewey and Lu, 1959). Path coefficient analysis have been widely used in plant breeding programs to determine the nature of the relationship between yield and yield components that are useful as selection criteria to improve crop yield but interpretation of the actual contribution of each variable is difficult (Hair *et al*, 1995). The present study focused on interrelationship among alkaloid yield with other characteristics in mutants of *C. roseus* for developing selection criteria for higher yield of vincristine and vinblastine.

Materials and methods

In order to determine the most important characteristics which has affecting single plant yield, 26 genotypes of six morphological forms, raised after gamma rays (5, 10, 15, 20 kR) and ethyl methane sulfonate (0.2, 0.4 and 0.6%) were evaluated in randomized block design with three replications in M_3 and M_4 generation. Plants were grown 70 cm apart with each other from row to row and 40 cm. apart from plant to plant to provide space

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for better proliferation. Before transplantation, nearly 20 Kg./ha. of DAP (Di ammonium phosphate) were applied. A combination of NPK fertilizer at rate of 100 Kg /ha urea (N), 40 Kg /ha DAP (P_2O_5), and 40 Kg /ha. of murate of potash (K_2O) were applied, when plants were four month old after transplantation. Irrigation is required after every fortnight during active growing period of a plant. Observations on these plants were made after seven months of transplantation.

The obseravable data were collected on the following characters. The plant height (cm.) was measured from just above the soil surface where plants stand up to the tip of flowers. The main stem circumference (cm.) was measured. The number of leaves/ plant were counted. The leaf area (cm^2) of five leaves per plant was measured with the help of leaf area meter. Net assimilation rate (NAR) is a measure of the amount of photosynthetic product going into plant material (carbon assimilated by photosynthesis minus carbon lost by respiration). The NAR was worked out by measuring plant dry weight with a gap of one month interval (root plus shoot). The leaf harvest index is the ratio of economic yield to biological yield that is the fresh weight of leaves divided by total weight of a plant. The epidermal peelings of fresh leaves were observed under microscope for counting stomata and cell density. The total weight of plant (gm.) was measured with hand weighing machine before harvesting of leaves. The dry matter yield is the ratio of dry weight of leaves against fresh wt. of leaves. The pollen grains were dusted on a clean glass slide on a drop of 1% aceto-carmine. The pollen diameter at equatorial and polar axis was measured with the help of occular micrometer under 40 X objective. The number of bloomed floral buds at the time of observation against the total number of floral buds present in a plant, were counted for flowering percentage. The siliqua length (cm.) was measured from base where it attaches, to the tip of siliqua. The extraction of total crude alkaloid and estimation of alkaloid Profile were done using Reversed Phase High Performance Liquid Chromatography (Singh *et al*., 1999). In order to determine the relationship between examined traits the correlation coefficients, path coefficients analysis were estimated using their corresponding formulas (Sharma,1999).

Results

The observation of morphometric traits of agronomic components were recorded in table 1.The plant height in vt mutants were taller whereas smaller in dwob and mtsl mutants than control. The circumference of stem ranged between 4.0 to 6.0 cm. The nt 25 has higher number of leaves/ plant followed by vt 22. The dwob and mtsl mutants have lesser number of leaves. The leaf area was larger in nt mutants and smaller in mtsl mutants in compare to control. The NAR is an estimate of photosynthesis engaged in photosynthetic process. The upel mutants have higher NAR varying 1.49-2.31 gm cm^2 followed by dwob mutants. The leaf harvest index was higher in dwob mutants and lower in spl mutants in compare to control. The net assimilation rate and harvest index was recorded highest in dwob 134.The higher number of epidermal cell was recorded in mtsl 160 with smaller epidermal cell size whereas stomata frequency were higher in nt mutants due to absence of trichome. The total weight of plant were recorded highest in nt 121 followed by vt 22. The mtsl mutants have higher value of percentage dry matter yield. However nt, dwob and vt mutants have lower percentage of dry matter yield therefore these mutants have higher moisture content in their leaves. The spl 112, upel 153 also have higher dry matter yield. The vt 81 has larger pollen diameter and flowering percentage whereas dwob 148 with smaller pollen diameter and mtsl 2 with lower flowering percentage. The follicle length was smaller in mtsl and dwob mutant and larger in vt and nt mutants in compare to control. The tall plants like vt and nt mutants provide more space for flower and siliqua formation as leaves are distantly arranged on branches. The upel 154 has higher NAR and leaf crude alkaloid but lower VCR, VLB, vindoline and catharanthine percentage (Table 2). The upel 153 and nt 51 also have higher percentage of leaf crude alkaloid. The nt 25 has lower VCR and VLB percentage in comparision to control. However dwob 132 and mtsl 116 has higher VCR, VLB percentage and mtsl 2 have higher vindoline and catharanthine percentage. The dwob 102 has higher vindoline and vincristine percentage but dwob 148 with higher percentage of vindoline only.

The positive correlation exists between pollen diameter and plant height that indicates that genes governing these traits are in coupling phase of linkage (Table 3). The plant height increase with increase in pollen diameter. The total weight of plants, harvest index has positive correlation with vindoline, catharanthine, vincristine and vinblastine. The Harvest index influences percentage increase in vindoline, catharanthine, vincristine and vinblastine in a positive manner and also exerts negative influence on crude alkaloid. Leaf area/ plant has positive correlation between crude alkaloid, vindoline, catharanthine and total weight of plant confirms the direct effect of leaf area on crude alkaloid and primary precursor of dimeric indole alkaloid. The positive correlation between NAR with total weight of plants, number of follicle/ plant and alkaloid percentage indicates that the total photosynthetic product largely contributes between these two traits. The increment in number of leaves/ plant and leaf area has greater influence on stomata frequency positively and negatively on the cells of upper epidermis. The larger number of leaves and larger leaf area has higher stomata frequency but increased number of cells on upper epidermis of leaves reduces stomata frequency. The plant height has positive correlation with flowering and siliqua formation therefore increased plant height results in higher percentage of siliqua formation.

The relative importance of each agronomical component in determining alkaloid yield is achieved by partitioning into direct and indirect effects through path co-efficient analysis (Table 4). The number of leaves has highest direct effect on alkaloid yield with increased number of follicle/ plant. The leaf area/ plant and flowering percentage have largest indirect effect on alkaloid yield. The dry matter yield has positive direct effect on alkaloid yield. The increment in number of leaves/ plant resulted in increase dry matter yield with higher alkaloid yield. The harvest index, stem thickness, stomata frequency and weight of plant have indirect effect on alkaloid yield. However net assimilation rate, number of epidermal cell, pollen diameter and follicle length also have direct effect on alkaloid yield. The vindoline, catharanthine and vinblastine have direct effect but vincristine pose indirect effect on alkaloid yield.

Discussion

The mutants show vigorous growth in comparison to control, it may or may not promise better agronomic performance. Leaves may either be larger and thicker or smaller and thinner. The mutant shows an increase or decrease in plant height, number of leaves/ plant and pods/ plant.

Table 1. Mutation affecting morphometric traits of vegetative component related with biomass yield

Serial No.	Plants	PH	MSG	NLV	LA	NAR	HI	CELL	STOM	WT	DMY	FP	DIP	SIL
1	CC	78.3	4.5	255.7	6.6	0.127	14.6	96.7	23.9	875.6	19.4	23.5	80.1	2.51
2	vt 22	80.8	6.1	535.8	5.9	0.164	11.6	73.9	13.4	890.6	28.9	51.6	86.4	2.82
3	vt 81	81.8	5.8	454.7	6.6	0.264	18.8	73.4	14.7	769.2	27.4	57.2	89.4	2.89
4	dwob 96	42.3	2.7	108.7	6.2	0.285	20.6	110.3	20.9	538.7	26.4	33.7	76.8	1.74
5	dwob102	46.5	3.4	117.4	5.7	0.29	32.6	72.1	15.2	537.2	31.5	26.6	73.4	1.94
6	Dwob128	42.5	4.8	127.5	5.7	0.13	37.9	78.6	25.6	633.5	29.3	28.6	79.9	2.16
7	dwob 134	46.9	4.8	77.1	5.1	0.344	36.4	80.2	18.3	502.3	22.4	30.6	65.7	1.82
8	dwob 132	38.6	4.2	67.1	5.9	0.33	29.7	77.9	21.2	509.6	25.6	34.6	79.8	1.75
9	dwob 148	46.9	4.8	87.1	6.3	0.201	31.9	92.1	17.7	472.6	32.9	29.4	50.2	2.29
10	mtsl 2	49.9	4.5	121.3	4.9	0.052	28.3	97.7	12.4	360.4	39.8	15.3	79.3	2.31
11	mtsl 116	42.7	4.1	157.1	4.3	0.058	29.7	115.7	12.7	427.9	29.9	18.9	75.2	1.64
12	mtsl 159	45.5	4.4	105.9	4.9	0.09	25.1	104.5	20.1	416.3	36.6	15.3	72.6	2.24
13	mtsl 160	47.8	4.6	128.9	4.8	0.04	22.1	120.3	18.3	408.6	40.1	17.4	77.1	1.72
14	nt 6	46.7	3.9	347.5	9.3	0.135	14.3	84.1	23.9	715.6	28.8	34.6	69.8	2.61
15	nt 13	65.8	5.8	426.9	8.5	0.145	12.5	87.4	24.3	704.6	42.4	30.1	67.1	2.94
16	nt 14	53.1	4.5	419.9	10.7	0.43	18.3	81.6	25.1	448.7	25.9	38.1	72.7	2.48
17	nt 21	57.9	5.3	294.5	8.4	0.164	14.6	91.6	27.2	694.2	26.8	40.8	68.7	2.96
18	nt 25	61.5	5.4	512.7	8.8	0.173	15.5	101.9	22.2	726.2	25.1	37.2	63.6	3.03
19	nt 51	70.1	5.3	458.1	6.2	0.143	13.2	109.7	25.9	741.5	30.6	42.4	72.2	2.36
20	nt 101	52.7	5.1	294.3	3.1	0.15	12.9	94.1	26.7	464.9	30.7	20.1	74.2	1.65
21	nt 121	71.2	5.8	289.7	8.9	0.185	17.3	87.7	24.1	1110.3	21.2	32.9	87.6	2.73
22	upel 135	71.6	5.7	172.1	4.8	1.77	17.9	81.7	17.4	797.1	38.4	35.7	64.3	2.27
23	upel 151	67.3	4.4	166.9	5.7	2.31	18.3	79.4	15.7	856.6	40.3	38.9	54.9	2.51
24	upel 153	77.4	4.9	184.6	4.9	1.49	14.1	74.3	12.2	834	24.6	36.2	62.6	2.42
25	upel 154	70.4	5.9	203.5	5.1	1.92	19.7	62.7	15.1	848.6	33.9	35.2	55.1	2.75
26	spl 112	70.2	4.6	306.3	4.4	0.127	7.12	110.3	12.7	546.1	38.1	34.8	81.9	2.45
27	spl 119	69.4	4.1	289.1	5.2	0.115	12.3	112.5	10.9	663.7	34.8	28.9	82.2	2.42

PH-plant height (cm), MSG- Main stem circumference (cm), NOL- number of leaves, LA- leaf area (cm^2), NAR- net assimilation rate (gm cm^2), HI- harvest index(%), CELL- number of cell on epidermis, STOM- stomata density, WT- weight of plant (gm), DMY- dry matter yield (%), FP- flowering percentage (%) , DIP- diameter of pollen (μ), SIL

Table 2. Mutation affecting chemometric traits of alkaloid yield and it's profile

Plants	Catharanthine(%)	Vindoline (%)	Vincristine (%)	Vinblastie (%)	Leaf crude alkaloid (%)
CC	0.0033	0.0078	0.00073	0.0011	1.31
vt 22	0.0027	0.0128	0.00085	0.0019	1.03
vt 81	0.0035	0.0121	0.00095	0.0026	1.35
dwob 96	0.0052	0.0174	0.00013	0.0036	1.44
dwob 102	0.0054	0.0296	0.00143	0.0026	1.49
dwob 128	0.0018	0.0167	0.00033	0.0065	1.51
dwob 132	0.0005	0.0004	0.0058	0.0106	1.27
dwob 134	0.0025	0.0009	0.00065	0.0017	0.99
dwob 148	0.0055	0.0348	0.00045	0.0025	0.85
mtsl 2	0.0116	0.031	0.00135	0.0028	1.42
mtsl116	0.0081	0.0158	0.00165	0.0043	0.68
mtsl159	0.0022	0.0046	0.00065	0.0012	1.37
mtsl160	0.0063	0.0193	0.00045	0.0036	1.77
nt 6	0.0091	0.0313	0.0008	0.0016	2.04
nt 13	0.0096	0.0213	0.0011	0.0026	1.26
nt 14	0.0135	0.0135	0.0008	0.002	1.46
nt 21	0.0057	0.0162	0.0006	0.0018	2.23
nt 25	0.0047	0.0125	0.0012	0.0014	1.51
nt 51	0.0013	0.0129	0.00045	0.0006	1.71
nt 101	0.0036	0.0009	0.00088	0.0024	0.99
nt 121	0.0034	0.0091	0.00071	0.0023	1.1
upel 135	0.0017	0.0107	0.00053	0.0013	1.9
upel 151	0.0019	0.0065	0.00123	0.0026	1.06
upel 153	0.0019	0.0066	0.00085	0.0026	2.23
upel 154	0.0019	0.0061	0.00028	0.0018	1
spl 112	0.0006	0.0125	0.00068	0.0024	0.87
spl 119	0.0036	0.0054	0.00055	0.0028	1.26

Table 3. Genotypical and Phenotypical correlation of agronomic traits of mutants of *C. roseus*

	PH	ST	NOL	LA	NAR	HI	CELL	STOM	WT	DMY	FP	DIP	SIL	VIN	CAT	VCR	VLB	ALK
PH		0.787	0.733	0.152	0.315	-0.760	-0.846	-0.053	0.854	-0.359	0.631	0.793	0.654	0.722	-0.399	-0.296	-0.465	0.034
		0.715	0.654	0.148	0.307	-0.693	-0.688	-0.066	0.733	-0.295	0.532	0.704	0.594	-0.356	-0.319	-0.248	-0.425	0.039
ST			0.662	0.180	0.345	-0.597	-0.582	0.241	0.756	-0.162	0.708	0.557	0.692	-0.655	-0.399	-0.177	-0.310	0.156
			0.571	0.159	0.352	-0.527	-0.457	0.173	0.628	-0.157	0.624	0.485	0.646	0.316	-0.327	-0.160	-0.283	0.142
NOL				0.609	-0.087	-0.738	-0.829	0.566	0.694	-0.618	0.724	0.888	0.682	0.305	0.068	-0.285	-0.402	0.072
				0.462	-0.072	-0.621	-0.061	0.429	0.544	-0.503	0.590	0.766	0.582	0.153	0.052	-0.237	-0.343	0.089
LA					-0.136	-0.365	-0.493	0.775	0.362	-0.402	0.229	0.637	0.629	0.240	0.307	-0.122	-0.293	0.145
					-0.119	-0.321	-0.391	0.635	0.288	-0.296	0.239	0.548	0.559	0.062	0.247	-0.103	-0.240	0.139
NAR						-0.059	-0.062	-0.469	0.441	0.522	0.315	0.023	0.427	-0.255	-0.294	-0.042	-0.100	0.362
						-0.054	-0.055	-0.396	0.397	0.450	0.273	0.019	0.040	-0.112	-0.248	-0.041	-0.097	0.323
HI							0.844	-0.174	-0.650	0.550	-0.548	-0.887	-0.758	0.655	0.068	0.403	0.568	-0.329
							0.690	-0.132	-0.516	0.428	-0.463	-0.790	-0.693	0.309	0.068	0.367	0.503	-0.277
CELL								-0.217	-0.700	0.468	-0.515	-0.938	-0.670	0.465	0.073	0.285	0.422	-0.163
								-0.145	0.592	0.385	-0.346	-0.753	-0.562	0.050	0.039	0.236	0.355	-0.112
STOM									0.098	0.474	0.244	0.330	0.368	0.234	0.235	0.020	-0.135	-0.021
									0.112	-0.316	0.150	0.285	0.264	0.177	0.192	0.007	-0.114	-0.030
WT										0.171	0.621	0.734	0.777	-0.503	-0.460	-0.232	-0.408	0.131
										-0.084	0.421	0.605	0.658	-0.249	-0.391	-0.207	-0.365	0.113
DMY											-0.201	-0.588	-0.291	0.233	-0.195	0.402	0.413	-0.035
											-0.171	-0.455	-0.237	-0.007	-0.171	0.337	0.312	-0.030
FP												0.605	0.603	-0.256	-0.040	-0.484	-0.106	0.065
												0.469	0.599	-0.106	-0.025	-0.338	-0.078	0.086
DIP													0.776	-0.359	-0.009	-0.403	-0.492	0.102
													0.667	-0.179	0.015	-0.394	-0.440	0.119
SIL														-0.348	-0.024	-0.308	-0.566	0.484
														-0.221	-0.050	-0.281	-0.514	0.404
VIN															0.462	-0.158	0.762	0.061
															0.172	-0.077	0.381	0.059
CAT																-0.049	0.064	0.063
																-0.064	0.052	0.089
VCR																	-0.047	-0.016
																	-0.039	-0.158
VLB																		-0.344
																		-0.272
ALK																		

Table 4. Path matrix of crude alkaloid yield (%)

	PH	ST	NOL	LA	NAR	HI	CELL	STOM	WT	DMY	FP	DIP	SIL	VIN	CAT	VCR	VLB
PH	0.498	0.385	0.385	0.074	0.154	-0.371	-0.413	-0.026	0.417	-0.176	0.308	0.388	0.320	0.353	-0.195	-0.145	-0.227
ST	-0.121	-0.153	-0.101	-0.027	-0.053	0.091	0.089	-0.036	-0.115	0.025	-0.108	-0.086	-0.106	0.100	0.061	0.027	0.047
NOL	4.462	4.027	6.083	3.706	-0.553	-4.495	-5.047	3.441	4.227	-3.750	4.409	5.405	4.149	-1.860	-0.418	-1.733	-2.451
LA	-0.562	-0.669	-2.254	-3.705	0.506	1.351	1.824	-2.867	-1.341	1.491	-0.817	-2.358	-2.328	-0.889	-1.138	0.451	1.084
NAR	0.165	0.182	-0.046	-0.071	0.523	-0.030	-0.032	-0.245	0.230	0.273	0.165	0.021	0.223	-1.339	-0.154	0.022	-0.052
HI	0.632	0.496	0.614	0.303	0.049	-0.831	-0.702	0.144	0.541	-0.457	0.455	0.738	0.630	0.544	-0.057	-0.335	-0.472
CELL	-0.244	-1.684	-2.399	-1.426	-0.179	2.443	2.894	-0.629	-2.027	1.354	-1.491	-2.717	-2.020	1.354	0.212	0.827	1.222
STOM	0.004	-0.018	-0.044	-0.060	0.036	0.013	0.017	-0.078	-0.007	0.037	-0.019	-0.025	-0.028	-0.018	-0.018	-0.001	0.011
WT	0.868	-0.769	-0.705	-0.386	-0.448	0.661	0.712	-0.099	-1.016	0.174	-0.632	-0.746	0.785	0.511	0.468	0.236	0.415
DMY	-0.462	-0.209	-0.794	-0.518	0.672	0.707	0.602	0.610	-0.227	1.280	0.259	-0.756	-0.374	0.299	-0.251	0.517	0.531
FP	-2.659	2.983	-3.053	-0.930	-1.330	2.308	2.171	-1.029	-2.618	0.848	-4.215	-2.535	-2.655	1.081	0.168	1.720	0.448
DIP	0.442	0.311	0.495	0.355	0.013	-0.490	-0.523	0.184	0.410	-0.328	0.335	0.557	0.432	-0.200	-0.005	-0.240	-0.223
SIL	2.145	2.552	2.515	2.320	1.576	-2.796	-2.574	1.357	2.848	-1.073	2.325	2.862	3.688	1.283	-0.091	-1.136	-2.050
VIN	-0.980	-0.889	-0.414	0.326	-0.346	0.888	0.637	0.318	-0.682	0.316	-0.348	-0.486	-0.472	1.354	0.627	-0.214	1.034
CAT	-0.332	-0.333	0.057	0.255	-0.254	0.056	0.061	0.199	-0.383	-0.162	-0.033	-0.007	-0.021	-0.384	0.832	-0.040	0.053
VCR	0.017	0.010	0.016	0.007	0.002	-0.023	-0.016	-0.001	-0.013	-0.023	0.023	0.024	-0.017	0.009	0.003	-0.057	0.003
VLB	-0.156	0.104	0.135	-0.098	0.033	0.191	0.141	-0.045	-0.137	0.138	0.035	-0.165	-0.186	0.256	0.216	-0.016	0.335

PH= plant height, ST= stem thickness, NOL= number of leaves, LA= leaf area, NAR= net assimilation rate, HI= harvest index, CELL= number of cell on epidermis, STOM= number of stomata on epidermis, WT= weight of plant, DMY= dry matter yield, FP=flowering percentage, DIP= diameter of pollen, SIL= follicle length, VIN= vindoline, CAT= catharanthine, VCR= vincristine, VLB= vinblastine, ALK= alkaloid

Induced mutationl studies on *Vigna mungo* were carried out using different doses of gamma rays and two alkylating agents viz. DES and DMS, isolated various types of mutants like dwarf, excessively branched, hairy pod and round leaves show quantitative variation (Raisinghanni and Mahna, 1994). Mutation displayed increase in many quantitative characters like plant height, number of leaves per plant, follicle length, dry matter yield per plant, leaf area, and percentage of catharanthine, vindoline, vincristine and vinblastine. The dwarf plant possesses profuse branching, broad thick leaves with short pods. The ideal plat type should have bushy growth producing higher biomass (Singh and Rai, 1993). Leaf harvest index, NAR, dry matter yield and percentage crude alkaloid are indication of economic yield of plant. Mutation affects plant morphology as well as quantitative variation in vegetative components related to alkaloid yield which leads to selection of mutant with higher percentage of biomass and alkaloid yield.

The estimates of correlation coefficients measure the degree of relationship between pair of characters. The phenotypic correlation coefficient was larger than their corresponding genotypic values. This could be either due to modifying effect of environment or the strong association of the characters under genic level (Tyagi and Sharma, 1991). The negative correlation of plant height with VCR, VLB, catharanthine, vindoline and harvest index indicate plants with reduced height will have higher biological yield containing more percentage of monomeric and dimeric alkaloids. As alkaloids are synthesized and present in leaves, therefore, increase in leaf area corresponds to higher percentage of crude alkaloid, vindoline and cathranthine. The negative correlation of plant height with leaf/ stem ratio and oil content in *Mentha* indicates that plants with reduced height will have higher leaf biomass, containing more oil (Tyagi and Sharma, 1991). In *C. roseus* significant genetic and environmental correlations were found between the leaf and root dry weight but not between the genetic components and ajmalicine content of roots (Levy *et al*., 1983). The positive genotypic correlation of seed yield and opium yield with high direct selection gain and appreciable correlated response with each other indicated that increase in one is directly associated with increase of other (Yadav *et al*, 2004). The Correlation in *Carum copticum* showed that biological yield has high positive correlation with single plant yield (Dalkani *et al*, 2011). Hence the knowledge of correlation between such characters is essential with the aim of improvement in alkaloid yield.

The relative importance of each agronomical component in determining alkaloid yield is achieved by partitioning into direct and indirect effects through path co-efficient analysis, thus allowing relative weightage of importance given to each character, selected and manipulated. The choice of agronomic traits such as number of leaves/ plant, leaf area, dry matter yield will be rewarding for improvement in alkaloid yield. The highest direct contribution to alkaloid yield is made by number of leaves/ plant and plant height. The leaf area has maximum indirect effect on alkaloid yield. Hence selection for number of leaves/plant, dry matter yield confirmed strong association and direct influence on alkaloid yield. In *Mentha* oil content exhibited highest direct effect on oil yield followed by herb yield and menthol content. The indirect effects were found to be regulating the character association in most cases (Tyagi and Sharma, 1991). The path analysis revealed the highest direct contribution to seed yields by husk yield, panicle length followed by days of flower in *Plantago* (Lal *et al*., 1999). In Soyabean the total direct and indirect effects were 17.61, 1.32, 42.34, 2.86 and 15.51% for plant height, number of branches per plant, number of pods/ plant, number of seeds per pod and 100 seed weight respectively (El-Badaway and Mehasen, 2012). Higher biomass production relates with productive capacity of plant with improved plant frame of desirable growth habit with proper leaf canopy, high harvest index with maximum utilization of total dry matter production. The above study about morphometric variations, correlation coefficient and path analysis inferred that the **dwob** and **nt** mutants may be an important consideration for future exploitation in field of commercial production of increased percentage of VCR, VLB, catharanthine and vindoline. Due to higher doses of mutagenic treatment in dwob mutants result in shorter plant height, smaller leaf area with higher economic yield showing increased percentage of VCR and VLB in intercostal cells of leaves were enhaced. However, nt mutants induced through lower doses of mutagen resulted in larger leaf area which inhances plastidial pathway of metabolites formation in leaves.

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