Indian J. Genet., **67**(1): 62-65 (2007)

Genetic analysis of important morpho-ecomonic traits in periwinkle [*Catharanthus roseus* (L.) G. Don]

Maneesha Singh, Alka Srivastava[1], Ajay Pratap Singh[2], Vandana Singh, Archana Srivastava, Srikant Sharma, G. C. Uniyal and Samresh Dwivedi

Central Institute of Medicinal and Aromatic Plants, Lucknow 226 015
[1]Botany Department, Lucknow University, Lucknow 226 007

(Received: December 2005; Revised: August 2006; Accepted: August 2006)

Abstract

The experiment was conducted with six diverse genotypes including one 0.5% EMS induced mutant and one released white with cream center cultivar 'Nirmal' and one recently released white petal with pink center cultivar 'Prabal' of periwinkle [*Catharanthus roseus* (L.) G. Don] to seek the genetic information on leaf alkaloid yield by using diallel analysis. Analysis of variance for 6 × 6 diallel cross progenies revealed highly significant differences among treatments (inclusive of direct and reciprocal F_1s and the parents) for all the traits. This study revealed that medium × medium and medium × high performing parents provided the best cross combination for the production of high leaf alkaloid yielding lines. Based on three analyses, namely graphical, variance component and combining ability, both additive and non-additive genetic variances were found important in the inheritance of alkaloid yield and other yield contributing characters although non-additive component was higher than the additive component for most of the characters except for leaf-stem ratio.

Key words: Periwinkle, diallel analysis, combining ability, correlation coefficient.

Intoduction

Genetic constitution of the population is essential for formulating an efficient breeding programme. Besides using genetic markers of classifying the exiting variability in the germplasm, combining ability studies are useful in classifying parental lines in terms of their hybrid performance. In self-pollinated crops, these studies are useful in assessing the nicking ability of parents and thus help in selecting parents which when crossed would give rise to more desirable segregants. The choice of the parents is the first important step in any plant breeding programme aimed at genetic improvement of plant.

The present investigation was carried out for selecting superior genotype combinations with the objective of gaining more knowledge about the genetic architecture and inheritance patterns of leaf alkaloid yield. Need was also felt to identify the genotypes showing better combining ability for further varietal improvement work in this important medicinal crop periwinkle [*Catharanthus roseus* (L). G. Don], yielding life saving anticancerous alkaloids-vincristine and vinblastine.

Materials and methods

The present investigation was carried out during 1996-1999 at Experimental Farm of Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow [located 26.9°N latitude 85.5°E longitude with an elevation of 120 m above the mean sea level]. Six genotypes including the check 'Nirmal', 0.5% EMS induced mutant recently cultivar 'Prabal' were hybridized in all possible combination (F_1 including reciprocals) resulting in 30 F_1's The selfed seeds of six genotypes, 15 hybrids and 15 reciprocals (30 F_1's) of diallel were sown in earthen pots under glass house condition [during February 1998 and seedling emerged 10 days after sowing. Two month old seedlings - 15-20 cm tall were transplanted in the field arranged in randomized complete block design with three replications each/in plots of 4 m × 3 m size. The plants were relatively widely spaced at 60 cm for maximum expression of yield. Morphometric data were recorded on five competitive plants per plot for the following morphometric traits such as plant height (cm), number of primary branches, number of secondary branches, leaf area (cm), leaf stem ratio. After 10 months of transplanting plants were cut about 8 cm above the ground. Total herbage yield (fresh) was taken. Leaves were separated from the stem and dried at 40°C for 48 to 72 hrs in an incubator. Total alkaloid was extracted from the dried leaves. Plot means (averaged over samples) were subjected to treatment on CIMAP's in house developed computer program package-STATPACK diallel analysis.

[2]Present address: Deptt. of Biochem. & Mol. Bio, Univ. Nebraska Medical Center, Omaha, USA

Results and discussion

The ANOVA for 6 × 6 diallel cross progenies revealed highly significant differences among progenies inclusive of reciprocal F_1s and the parents for all the eight traits (Table 1). The mean performance of the parents revealed that CR-6 was the best parent for important alkaloid yield related traits such as plants height, number of primary branches, leaf-steam ratio, total herbage yield, leaf alkaloid content (%), total leaf alkaloid yield followed by CR-2 for leaf-steam ratio (Table 2).

Table 1. Test of null hypothesis for the eight characters in periwinkle

Character	't' test of heterogeneity (d.f. = 4)	't' test of 'b' on the null hypothesis	
	t^2 value	Ho:b=0	Ho:b=1
Plant height (cm)	2.99	1.013	3.55*
No. of primary branches	3.63	0.682	0.26
No. of secondary branches	0.46 × 10-7	3.860*	0.49
Leaf area (cm^2)	1.99	9.62*	1.72
Leaf stem ratio	0.55	2.76	1.47
Total herbage yield (q/ha)	8.64*	3.24*	4.51*
Leaf alkaloid content (%)(w/w)	3.29	2.69	–0.694
Total leaf alkaloid yield (q/ha)	3.06	2.280	2.97

,Significant at 5%and 1% level of probability, rest are non-significant

Diallel analysis helps to acquire knowledge to formulate appropriate breeding methods and to estimate heritability values, which can be used to predict progress through selections. The first two assumptions i.e. diploid segregations and homozygosity were satisfied as *Catharanthus roseus* self pollinated indicated and genotypes used are homozygous. However, the non-significance of 't^2 for total herbage yield indicated the failure of atleast one of the assumptions. Significant deviations of regression coefficient 'b" from zero but non-significant deviation of regression coefficient from unity as well as from 't^2' for number of secondary branches and leaf area showed the fulfillment of all the assumptions of diallel analysis. Plan height and total leaf alkaloid yield coefficient from unity showed the presence of epistasis (Table 3).

As regards the variances for *gca*, *sca* and reciprocal, they were highly significant, thus signifying the role of both additive and non-additive genetic variance as well as variance due to cytoplasmic differences (Table 4). The positive association between the *per se* performance of the parents and the estimates of their *gca* effect were detected in case of plant height, number of primary branches, leaf length, leaf area, leaf breadth/length ratio, leaf-stem ratio and total herbage yield. CR-6 was the best general combiner for maximum number of traits followed by CR-1 among the genotypes studied (Table 4).

Table 2. Estimation of genetic variance components and related statistics for the eight characters in periwinkle (Hayman's model, 1954)

S.No.	Component/related statistics	A	B	C	D	E	F	G	H
1.	D (Additive components)	0.51 ±4.99	3.30** ±0.65	11.77** +3.27	1.24** +0.09	0.0001** +0.0001	974.26* ±213.58	0.149** ±0.017	0.086 ±0.032
2.	H_1 (Dominance components)	47.91* ±12.67	4.88* +1.64	16.10** +3.27	1.36** +0.25	–0.009* +0.0003	1242.88 ±542.19	0.219** ±0.04	0.262* ±0.08
3.	E (Environment components)	0.76 ±1.89	0.22 +0.24	0.69 +0.49	0.112* +0.04	0.00002 +0.00003	19.62 ±80.73	0.001 ±0.006	0.0006 ±0.012
4.	F (Mean cov. of D & H_1)	–4.11 ±12.19	3.71 +1.58	9.22 +3.15	1.00* +0.24	0.001** +0.0002	139.48 ±521.78	0.108 ±0.04	0.031 ±0.07
5.	H_2 (Proportion of ± genes)	42.89* ±11.32	3.63* +1.40	13.81* +2.93	1.05** +0.22	0.0006* +0.0002	1074.46 ±484.36	0.152* ±0.04	0.192 ±0.07
6.	H_2 (Overall dominance effect)	52.36** ±7.62	2.27 +0.90	23.06** +1.97	1.99** +0.15	0.00009 +0.0001	529.69 ±326.00	0.056 ±0.03	0.071 ±0.048
7.	$(H_1/D)^5$ (Mean degree of dominance)	9.68	1.22	1.17	1.05	0.86	1.13	1.21	1.75
8.	($H_2/4H_1$ (Proportion of dominant genes with ± effects)	0.224	0.186	0.214	0.194	0.166	0.216	0.199	1.180
9.	$(4DH_1)^5 + F\ 4DH_1)^5 + F$ (Proportion of recessive genes)	0.41	2.72	20.1	2.26	2.81	1.14	1.85	1.23
10.	($h^2//H_2$) (No. of dormant gene blocks)	1.220	0.625	1.670	1.890	0.150	0.490	0.370	0.368
11.	r.yr (wr +vr)	–0.745	0.750	–0.866	0.620	0.708	0.157	0.023	0.121
12.	H^2 (ns) (%) (heretibility in narrow sense)	29.50	27.04	36.93	41.88	59.99	63.51	58.24	56.04

*,** Significant at 5% and 1% level of probability; A = Plant height (cm), B = Number of primary branches, C = Number of secondary branches, D = Leaf area (cm^2), E = Leaf-stem ratio, F = Total herbage yield (q/ha), G = Leaf alkaloid content (%), H = Total leaf alkaloid yield (q/ha)

Table 3. ANOVA (mean sum of square) for combining ability analysis for the eight characters in periwinkle (Diallel analysis-Griffing, 1956) (Method 1, model 11)

S. No.	Characters	*gca** (d.F. = 5)	*sca** (d.F. = 15)	Reciprocal* (d.f. = 15)	Error (d.f. = 70)	gca/*sca*
1.	Plant height (cm)	29.656	22.213	6.681	0.7840	1.34
2.	Number of primary branches	20713.000	2.030	1.730	0.2210	1.34
3.	Number of secondary branches	15.239	7.593	6.400	0.6950	2.01
4.	Leaf area (cm^2)	1.736	0.639	0.388	0.1120	2.72
5.	Leaf stem ratio	0.002	0.0001	0.00001	0.0001	20.0
6.	Total herbage yield (q/ha)	3029.300	556.850	157.159	18.1480	5.44
7.	Leaf alkaloid content (%)(w)	0.329	0.077	0.043	0.0010	4.27
8.	Total leaf alkaloid yield (q/ha)	0.373	0.097	0.097	0.0010	3.85

*Significant at 1% level of probability

Table 4. General combining ability (*gca*) for the eight characters in six parents of periwinkle

S. No.	Parents	A	B	C	D	E	F	G	H
1.	CR-1	0.009	–0.157	–0.667*	0.070	0.004*	9.287**	0.018	0.103**
2.	Cr-2	–0.379	–0.546**	–0.917**	0.106	0.009**	12.537**	0.095**	0.119**
3.	CR-3	–0.435	–0.019	–1.194**	–0.600	–0.006**	–12.935**	–0.068**	–0.127**
4.	CR-4	–1.324**	0.065	1.361**	0.063	–0.019**	–24.213**	–0.210**	–0.239**
5.	CR-5	–0.935**	–0.213	1.333**	–0.698**	0.003	–0.935	–0.099**	–0.082**
6.	CR-6	3.065**	0.870**	0.083	0.464**	0.003**	16.259**	0.263**	0.226**
	S.E.(g)	0.232	0.122	0.22	0.088	0.001	1.12	00.900	0.007
	S.E. (g_1-g_1)	0.362	0.190	0.34	0.136	0.002	1.74	0.014	0.010
	C.D.1%	1.46	0.770	1.37	0.550	0.008	7.01	0.420	0.403

*,** Significant at 5% and 1% level of probability; A = Plant height (cm), B = Number of primary branches, C = Number of secondary branches, D = Leaf area (cm^2), E = Leaf-stem ratio, F = Total herbage yield (q/ha), G = Leaf alkaloid content (%), H = Total leaf alkaloid yield (q/ha)

These studies show that, for most of the morphological traits the high × low general combination crosses exhibited greater *sca* effect, most likely because of the concentration of opposing alleles in the parents, which showed high allelic interaction along with their genetic diversity in F_1s as well as interaction effect (Table 5). Such crosses are ideally suited for exploitation either though pedigree selection or recurrent selection. In some of the crosses graded under poor combiners on the basis of parents, highly desired level of traits improvement was observed which emphasizing [5] poor combining parents lacked the additive effect of good inbred, but were highly responsible to heterozygosity in the way of non-additive effects. Reciprocal differences were significant for all characters examined as observed earlier [5]. Many of the crosses such as CR-2 × CR-1, CR-3 × CR-1, CR-6 × CR-1, CR-4 × CR-2 and CR-6 × CR-3 showed significant effects which were either due to cytoplasmic factors or to cytoplasmic and genetic interactions.

Yield as a plant character is very complex having number of components. It is influenced considerably by the interaction of several factors that are directly or indirectly related to it. Knowledge of the various factors that contribute significantly to yield will effectively help in the breeding programme. Sampson [4] stressed that correlation among progenies should be good indicators of or pleiotropism within an elite gene pool, hi the present case, for most of the traits, phenotypic correlation coefficient exhibited lower value that the corresponding estimates at the genotypic level whereas Kulkarni [5] showed that phenotypic correlation were higher than genotypic correlation in periwinkle for the morphological traits. Based on the estimates of character associations, it was suggested that tall and bushy plants with thin stem having high leaf-stem ratio are likely to produce high herbage yield with high leaf alkaloid yield (q/ha). The main emphasis of this study was to increase the amount of leaf alkaloid constituents such as vinblastine and vincristine along with high total leaf alkaloid yield. Thus, this study revealed that plants posessing high herbage yield, high total leaf alkaloid yield, (q/ha) could also produce high leaf alkaloid constituents.

Table 5. Specific combining ability (*sca*) of 6 × 6 diallel crosses for the eight characters in periwinkle

S.No.	Crosses	A	B	C	D	E	F	G	H
1.	CR-1 × CR-2	–0.815	1.269**	2.289**	0.008	0.007*	41.46**	0.14**	0.26**
2.	CR-1 × CR-3	4.074**	1.074**	0.833	0.339	–0.008*	–7.40*	–0.040	–0.09**
3.	CR-1 × CR-4	0.630	–0.176	–0.889	–0.137	0.024**	–0.62	–0.07**	–0.17**
4.	CR-1 × CR-5	2.074**	0.102	0.972	0.589**	–0.011**	–14.57**	0.14**	0.031*
5.	CR-1 × CR-6	–3.093**	–0.815*	0.389	0.006	0.014**	17.74**	0.23**	0.43**
6.	CR-2 × CR-3	–1.037	–0.537	0.883	0.504*	0.006	4.32	0.15**	0.17*
7.	CR-2 × CR-4	3.852**	1.713**	2.361**	–0.169	–0.009	–8.04**	0.12**	–0.01
8.	CR-2 × CR-5	–1.537	–0.509	0.889	0.158	–0.0091	–3.82**	0.28**	–0.24**
9.	CR-2 × CR-6	2.963	–1.259**	–2.194*	0.102	–0.001	–2.18	0.10**	0.09**
10.	CR-3 × CR-4	–0.593	0.519	0.972	–0.603**	0.009	16.10**	–0.30**	0.27**
11.	CR-3 × CR-5	–1.648	0.630*	1.833**	–0.421	0.001	7.16*	–8.99**	–0.03
12.	CR-3 × CR-6	2.852**	0.046	0.250	0.292	0.017**	–10.37**	–0.03	–0.16**
13.	CR-4 × CR-5	–2.093**	–0.620*	–20.56**	0.734**	0.006	13.94**	–0.05*	–0.01
14.	CR-4 × CR-6	–0.926	0.463	2.208**	0.175	0.0003	–8.09**	–0.19**	0.10**
15.	CR-5 × CR-6	6.195**	0.407	–0.611	0.556*	0.002	7.80**	–0.07**	–0.06**
	S.E. (s_1)	0.53	2.28	0.50	0.20	0.003	2.56	0.02	0.015
	S.E. (S_{ij}-S_{jk})	0.81	0.43	0.76	0.30	0.004	3.89	0.03	0.02
	C.D.1%	2.39	1.27	2.24	0.89	0.011	11.48	0.09	0.07

*,**Significant at 5% and 1% level of probabitily; A = Plant height (cm), B = Number of primary branches, C = Number of secondary branches, D = Leaf area (cm^2), E = Leaf-stem ratio, total herbage yield (q/ha), G = Leaf alkaloid content (%), H = Total leaf alkaloid yield (q/ha)

Acknowledgements

The authors acknowledge the help, support and constant words of encouragement of the Director, CIMAP. The financial support to the CIMAP's Gene Bank by Department of Biotechnology is gratefully acknowledged.

References

1. **Jinks J. L.** 1954. The analysis of continuous variation of diallel cross of *Nicotiana rustica*, Genetics, **39**: 767-788.
2. **Falconer D. S.** 1961. Introduction to quantitative gentics. Oliver and Boyd, Edinburgh.
3. **Durrah L. L. and Hallauerr A. R.** 1972. Genetic effects estimates from generation mean in 4-diallel set of inbred Crop Sci., **12**: 615-621.
4. **Sampson D. R.** 1971. Additive and non-additive genetic variances and genotypic correlation for yield and other traits in oats. Can. J. Genet. Cytol., **13**: 864-872.
5. **Kulkarni R. N., Dimri B. P. and Rajgopal K.** 1984. Variability for quantitative character in periwinkle. Indian Drugs, **22**: 61-64.