



Influence of heterosis, genetic association and variance on crude tropane alkaloids in black henbane (*Hyoscyamus niger* L.)

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Black henbane (*Hyoscyamus niger* L.) is a *Solanaceous* medicinal plant [1, 2] used in both modern and traditional systems of medicine [3]. Its leaves and flowering tops in dried form are used in tincture extracts, they constitute the drug *Henbane Herba*. Black henbane suffers from low tropane alkaloid content and poor herb yield. With a view, therefore the magnitude of heterosis provide a basis for genetic diversity and a guide to the choice of desirable parents for developing superior F_1 hybrids so as to exploit hybrid vigour and/or for building better gene pool to be employed in population improvement. To attain these objectives, a priori knowledge of the nature of gene action and heterosis is of paramount significance in crop improvement. To gather this information in the black henbane a 6x6-diallel analysis of six variable and distinguished selfed lines of *Hyoscyamus niger* was carried out.

Diallel (6 × 6) method I model II [4] was followed to cross the six inbred lines (Table 1). The materials comprised 15 F_1 s, 15 reciprocals, 6 parents, making a total of 36 entries. Thirty six n^2 diallel cross progenies involving ($n = 6$) parents, 15 direct and 15 reciprocal crosses were grown in randomized block design replicated twice with single row plots of 3 m length, each 50 cm apart. Morpho-metric observations were recorded on 5 random competitive plants per plot for days to flower, plant height (cm), shoot numbers, leaves on main shoot, leaf length (cm), width of midrib (cm),

fresh herb yield (g/plot), and crude tropane alkaloid(%). Heterosis was computed over desirable and economic parents for each character. For chemical analysis, plants were harvested at mid flowering stage and dried in shade. Crude tropane alkaloid content was analyzed by gravimetric method following Cromwell [5].

Highly significant differences among treatment (6 × 6) cross progenies inclusive of direct and reciprocal F_1 s and the parents were observed for all the traits. As regard the variances for *gca*, *sca* and reciprocals, they were all highly significant, thus signifies the role of both additive and non-additive genetic variance differences. When we compared the relative magnitude of *gca* and *sca* variances in the progenies due to *sca* (non-additive genetic variances) was invariably larger than *gca* (additive genetic variance) for all the characters except width of midrib. The reciprocal differences in relation to *gca* or *sca* variances revealed that variances due to reciprocals were lesser than *sca* variances for all the characters except number of leaves, width of midrib and crude alkaloid content. This clearly shows that the role of both fixable and the non-fixable components of genetic variance were operative for the expression of these traits in the progenies. There was manifestation of high and positive heterosis for length of leaves as is evident from the superiority over economic and desirable parents both up to 187.33% and 158.48%,

Table 1. General combining ability effects (gi) and mean performance ($\bar{X}$) of six parents for the eight characters in black henbane

Parents	Days to flower		Plant height		No. of shoots		Leaves on main shoot		Leaf length		Width of midrib		Fresh herb yield		Crude alkaloid content	
	gi	mean	gi	mean	gi	mean	gi	mean	gi	mean	gi	mean	gi	mean	gi	mean
Normal (P1)	-3.51	74.00	-10.07**	80.34	0.67**	5.84	1.08	50.84	-4.01**	6.63	-0.04**	0.31	-437.28**	1225.00	-0.007**	0.07
Rb (P2)	6.74**	85.00	-10.21**	114.34	-0.44	2.83	-3.41*	74.50	4.29**	22.44	0.01**	0.49	328.97**	2350.00	0.0001	0.17
Aela (P3)	-7.51**	71.00	-18.94**	62.83	0.10	6.34	-0.07	57.84	-2.31**	6.82	-0.10**	0.28	304.56**	2825.00	0.0162**	0.17
Early (P4)	-2.10	80.00	-4.93**	135.34	0.01	6.84	-5.27**	50.25	-1.89**	6.90	-0.02**	0.31	-83.28**	1275.00	-0.0129**	0.08
Late (P5)	-0.31	62.50	22.50**	129.00	0.79**	10.2	8.37**	73.34	-0.51**	7.37	-0.08**	0.37	-329.40**	1325.00	0.0126**	0.09
Erect (P6)	6.69**	111.5	21.67**	218.67	-1.13**	1.00	-0.70	40.84	4.43**	18.84	0.15**	0.61	216.43**	1725.00	-0.0093**	0.21
S.E.(gi)±	1.69	-	1.39	-	0.27	-	1.72	-	0.09**	-	0.002-	-	7.07	-	0.001	-
S.E.(gi-gi)±	2.61	-	2.16	-	0.41	-	2.66	-	0.15**	-	0.005-	-	10.95	-	0.001	-

*-P < 0.05, ** -P < 0.01, respectively

respectively in the cross $P4 \times P6$. The highest positive and significant economic heterosis was manifested by the cross $P2 \times P4$ (261.16%) followed by the crosses $P5 \times P6$ (256.11%), $P2 \times P5$ (234.92%) and the cross $P1 \times P6$ (233.03 %), respectively for the length of leaves. The cross $P4 \times P6$ was also manifested the high economic and desirable heterosis for herb yield (57.04% DP and 121.14% EP), leaves on main shoots (51.88% DP and 50.12% EP) and crude alkaloid (148.57 % EP). High economic and desirable heterosis exhibited by eight crosses, namely $P2 \times P3$ (73.47**), $P2 \times P4$ (105.71**), $P2 \times P5$ (75.31**), $P2 \times P6$ (79.59**), $P3 \times P4$ (23.27*), $P3 \times P6$ (121.02**), $P4 \times P6$ (121.14**) and $P5 \times P6$ (47.59**) and one cross $P4 \times P6$ (57.04**) all in percent for high herb yield and alkaloid content twelve and 3 crosses exhibited high economic and desirable heterosis in order.

In some crosses, heterosis for herb yield and crude alkaloid was due to heterosis for other characters, for instance in cross $P4 \times P6$, heterosis was significant not only for yield and crude alkaloids but also for days to flower, economic heterosis (with low *sca* and low $\times$ high *gca*); plant height, economic heterosis (with high *sca* and high negative $\times$ high *gca* combination); leaves on main shoot, both desirable and economic heterosis (with low *sca* and high negative $\times$ low negative *gca* combinations); leaf length both desirable and economic heterosis (with high *sca* and high negative $\times$ high *gca* combinations) and width of midrib economic heterosis (low *sca* and high negative $\times$ high *gca* combinations). However, shoot number does not exhibit this relationship i.e. no significant heterosis (with low *sca* and low $\times$ negative high *gca* combinations). The entire cross showing high heterosis for crude alkaloids also exhibited heterosis for herb yield. This indicates that herb yield was responsible for heterosis of crude alkaloids. Thus, heterosis for herb yield can be used reliably for predicting heterosis for crude alkaloids between lines.

Specific combining ability (*sca*) was also computed in all the 15 crosses under study. The crosses with high *sca* for days to flower, plant height, length of leaves, width of midrib, and crude alkaloid exhibited high heterosis. However, crosses with high *sca* effects were also the combinations with high $\times$ high, high $\times$ low and low $\times$ low *gca* lines. A comparison of heterosis of the check line ($P1$) with another lines in different crosses for economic traits provide distinct and encouraging results for the use of lines in hybridization with suitable parents. Considering days to flowering, out of five superior crosses for desirable heterosis hybrid in $P3 \times P5$ (35.34 %) and out of 10 superior crosses for economic heterosis in the cross $P2 \times P6$ (28.11%). For plant height $P2 \times P6$ (60.99%) DP and cross $P4 \times P6$ (78.32 %) EP; for shoot number $P1$

$\times P5$ (-44.25) DP and $P2 \times P4$ (-38.6%) for EP. Here we have considered the negative heterosis for shoot numbers because the erect growth habit coupled with non-overlapping leaf-disposition is useful for high crop density to obtain higher biomass yield and, in turn, more crude drug production per unit area. For leaves on main shoot the cross $P4 \times P6$ for DP and EP (51.88 and 50.12); length of leaves $P4 \times P6$ (158.48%) for DP and $P2 \times P4$ (261.26) for DP; width of midrib $P1 \times P4$ (25.81%) DP and for the crosses $P4 \times P5$ and $P5 \times P6$ (131.15%); herb yield $P4 \times P6$ (57.04%) DP and 121.14% EP and alkaloid content $P4 \times P5$ (121.98% DP and 188.57% for EP second high orders of heterosis. As a consequence of heritability in narrow sense (h_{ns}^2 %) were low for flowering date, shoot number, leaves on main shoot, medium for plant height, herb yield, crude alkaloid and high for leaf length and width of midrib. However, the corresponding genetic advance in this study was rather very low for all the characters except fresh herb yield. Low values of genetic advance in the presence of moderate heritability in narrow sense might be obviously due to prevalence of non-additive genetic variance (Table 2). The real worth of selection underlies the high heritability coupled with high genetic advance for this, herb yield is important where the heritability in narrow sense is 32.11% and genetic advance percent over means is 10.65. Hence, it is suggested to calculate the other parameters with heritability in narrow sense and genetic advance for determining the success of selection practices.

In addition to heritability narrow sense and genetic advance, nature and magnitude of character associations also have a direct bearing on the success of selection. The leaf length had antagonistic associations with width of midrib and fresh herb yield (Table 2). However, crude alkaloid manifested significant correlation with number of leaves and herb yield. Plant height was significantly correlated with number of shoots and number of leaves. So are widths of midrib and fresh herb yield which may prove to be the direct selection parameter for genetic improvement of black henbane. The negative correlations were for plant height, shoot numbers and number of leaves with fresh herb yield. The plant height may not be a suitable parameter of selection to realize high leaf yield due to not owing to high genetic advance associated with its heritability narrow sense.

References

1. Chopra R. N., Nayar S. L. and Chopra I. C. 1956. Glossary of Indian Medicinal Plants CSIR, New Delhi, pp.138.
2. Sharma J. R., Lal R. K., Misra H. O., Gupta M. M. and Ram R. S. 1989. Potential of gamma radiation enhancing the biosynthesis of tropane alkaloids in black henbane (*Hyoscyamus niger* L.). *Euphytica*, 40: 253-258.

Table 2. Genetic associations between some important economic traits and others allied genetic parameters in black henbane

Characters	Days to flower	Plant height (cm)	Shoot No.	Leaves on main shoot	Leaf length (cm)	Width of midrib (cm)	Fresh herb yield (cm)	Crude alkaloid (%)
Days to flower	-	0.025	-0.174	-0.305	0.225	-0.035	0.122	-0.114
Plant height (cm)		-	0.361*	0.507**	0.108	0.165	-0.373*	0.215
Shoot No.			-	-0.007	-0.273	0.113	-0.481**	-0.116
Leaves on main shoot					-0.225	-0.112	-0.388*	0.300
Leaf length (cm)					-	0.645*	0.542**	0.215
Width of midrib (cm)							0.149	-0.091
Fresh herb yield (g)							-	0.320*
Crude alkaloid (%)								-
Others allied genetic parameters:								
$\wedge \sigma^2 g$	9.17	196.75	0.18	7.03	10.09	0.01	82931.65	0.0002
$\wedge \sigma^2 s - \wedge \sigma^2 D$	144.83	819.47	1.74	83.99	18.41	0.01	200898.20	0.003
$\wedge \sigma^2 r$	82.70	357.78	0.88	119.11	4.69	0.01	148989.90	0.001
$(\wedge \sigma^2 s / \wedge \sigma^2 g)^{0.5}$	3.97	2.04	3.11	3.46	1.35	0.81	1.56	3.61
$\wedge h^2$ (ns) %	6.39	24.61	9.03	5.41	46.50	49.83	32.11	10.83
G.A.	0.93	7.60	0.21	0.79	3.06	0.10	191.31	0.003
G.A.%/ $\bar{X}$	1.00	7.32	3.36	1.46	20.54	23.81	10.65	2.04

*-P < 0.05 and ** P < 0.01, respectively

3. **Bianchini F., Corbetta F. and Pistoia M.** 1977. Health plants of the World (Atlas of Medicinal Plants). Newsweek Books, New York. pp. 120 and 210.
4. **Griffing B.** 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci., **9**: 463-493.
5. **Cromwell B. T.** 1955. The alkaloids. In: Modern Methods of Plant Analysis, Peach K. and M.V. Tracey (Eds.). **4**: 499.