



Induced heterotic genetic diversity in black henbane (*Hyoscyamus niger* L.)

R. K. Lal¹, S. P. S. Khanuja² and H. O. Misra¹

¹Department of Quality Genetic Material Production and Crop Improvement

²Department of Genetic Resources and Bio Technology, Central Institute of Medicinal and Aromatic Plants P.O. CIMAP, Lucknow 226 015

(Received: January 2005; Revised: March 2005; Accepted: March 2005)

The *Hyoscyamus niger* L., is an important solanaceous medicinal plant used in both modern and traditional systems of medicine. The main chemical constituents of the crude drug, viz., hyoscyamine and hyoscyne (1 - scopolamine) also manifest sedative, mydriatic, antispasmodic and anticholinergic properties. Since the study of genetic variability for diverse morpho-economic and secondary metabolic traits in the parents and its hybrids populations is a prelude to potential crop improvement, genetic divergence among them was quantified by multivariate analysis with the objectives: i) to assess the proximity of parents and hybrids with each other thus was classifying them in different clusters/groups. ii) to identify highly divergent clusters/promising parents/hybrids for higher herb of better quality.

The mutagenic treatments proved effective in producing a wide range of morphological mutants but only five important and stable mutant (M6) lines derived from gamma irradiation (source ⁶⁰Co) on dried seeds on commercial lines were used in this study. Diallel (6 × 6) Griffing's, method I model II [1, 2] was followed to cross the mutant lines including one check of *Hyoscyamus niger*. The material for 6 × 6 diallel design comprised 15 F₁'s, 15 reciprocals and 6 parents (5 mutant lines with one check), making a total of 36 entries.

Genetic divergence among 36 hybrid populations of *Hyoscyamus niger* were studied. The plants were grown in randomized block design with two replications at the research farm of the Central Institute of Medicinal and Aromatic Plants, Lucknow-India in the year 1998-99. Each treatment consists of single row 5.00 m long and 1.75m apart (plant to plant distance was kept 1.0 m). The plants received normal intercultural operations, irrigation, and fertilizer applications (60 kg N, 30 kg P₂O₅, and 30 kg K₂O per hectare). Observations were recorded on the 8 economic traits (days to flower, plant

height (cm), shoot numbers, leaf on main shoot, leaf length (cm), width of midrib (cm), herb yield (green bio-mass in gm) and crude tropane alkaloid (%). Mean values for all the eight characters were subjected to D²-statistics and canonical analyses according to [3, 4, 5]. For chemical analysis, plants were harvested at mid-flowering stage and dried in shade. Crude tropane drug (alkaloid content) was analyzed by gravimetric method.

Induced heterotic genetic diversity among the 36 hybrid populations of *Hyoscyamus niger* was relatively large, although 22.22 (%) of the hybrids could be grouped within one cluster 1st (8 hybrids) followed by clusters 2nd (7 hybrids) 19.44 (%), 3rd (7 hybrids) (16.67 %), cluster 4th (6 hybrids) 16.67 (%) and cluster 5th (5 hybrids) 13.89 (%), respectively. Only 8.34 % of the accessions were highly divergent forming two different clusters VI and VII including 2 and 1 hybrid only (Table 1).

Highly significant differences ($P = 0.01$) for all of the eight traits, indicating the presence of considerable divergence among the 36 hybrids of *Hyoscyamus niger* crop. Hybrids could be grouped into seven clusters and enormous diversity among the hybrids were indicated by wide range of D² - value ($D^2 = 68.19 - 12858.33$). The high heritability in narrow sense (h^2 (NS) 46.50, 49.83 %) was observed for the traits leaves length and width of midrib followed by medium heritability 24.61 and 32.11 % in plant height and herb yield. Other characters were in low heritability ranges (Table 2). The mean intra- and inter-cluster D² values indicated the highest divergence between clusters designated VII and I ($D^2 = 10714.90$) followed by clusters designated VI and VII. ($D^2 = 8193.07$). Divergence was also noted between clusters designated IV and VII ($D^2 = 7431.13$), IV and V ($D^2 = 7253.59$) and between I and II ($D^2 = 5921.97$). The clustering pattern was also confirmed by

Table 1. Intra- (**Bold**) and inter-cluster divergence (\bar{D}^2) in *H. niger*

Clusters	I	II	III	IV	V	VI	VII	\bar{D}^2	Parents and hybrids included in clusters
I (8)	254.58	5921.97	4501.01	2189.66	5228.29	1811.97	10714.9	5062.30	P1,1×2,1×3,1×4,1×5,4×1,P4,P5
II (7)		889.15	2157.11	5537.05	1474.78	3392.20	1798.19	3380.22	1×6,2×1,P2,2×3,3×6,4×6,6×1
III (7)			1326.85	2943.24	3511.33	4486.58	2679.29	4056.91	2×5,3×2,P3,4×3,5×3,P6,6×5
IV (6)				612.27	7253.59	4883.02	7431.13	5039.62	1×5,3×1,3×4,4×5,5×1,5×4
V (5)					559.91	1875.19	4357.62	3950.13	2×4,2×6,4×2,5×6,6×4
VI (2)						530.11	8193.07	4107.01	6×2,6×3
VII (1)							0.00	5862.37	5×2

spatial distribution of accessions under the canonical analysis. The cluster VII was highly divergent and unique including only one hybrid (5 × 2).

The herb yield (0.823) followed by width of midrib (0.392) and leaf length (0.309) at the primary axis (Z1) and the crude tropane alkaloid (0.904) followed by plant height (0.099) and leaves on main shoot (0.003) at the secondary axis (Z2), respectively were the largest contributors to genetic divergence. The least contributors to genetic divergence were shoot number (−0.006) and width of midrib (−0.398) at the primary and secondary axis in order (Table 2). The distribution pattern of hybrid populations of diverse origin, a single cluster indicates that genetic diversity observed within *H. niger* was not related to geographic origin. Noted differences in plant characters probably occurred due to hybridization within the parents. Thus, in this set of *H. niger* parents to be used as parents for hybridization to induced heterotic genetic diversity in recombination breeding programme should be selected on the basis of the quantified degree of divergence as opposed to geographic origin of the *H. niger* hybrids. In this context, prospective parents to develop inter se single crosses (Hybrids) could be: hybrid labeled No. 5 × 2, 6 × 2, 6 × 3, 2 × 4, 2 × 9, 4 × 2, 5 × 6, 6 × 4, 1 × 5, 3 × 1 and 3 × 4. These hybrids are divergent and possess complementary characters to create superior hybrids for high herb of better quality.

Identifying promising accessions based on *per se* performance is useful until, suitable hybrids are developed and standardized. Hybrid labeled No. 5 × 2, 6 × 2, 6 × 3, 2 × 4, 2 × 9, 4 × 2, 5 × 6, 6 × 4, 1 × 5, 3 × 1 and 3 × 4 had desirable characteristics. Furthermore, all of the characters influencing high herb yield and tropane alkaloid in plant were medium to highly heritable ($^h^2$ (NS) ranges from 32.11 - 49.83

Table 2. Range of variations and heritability in narrow sense percent ($^h^2$ (NS %)) of selected economic characters in *H. niger*

Traits	Z1 vector	Ranks	Z2 vector	Ranks	$^h^2$ (BS)%
Days to flower	0.013	VI	−0.007	IV	6.39
Plant height	0.025	V	0.099	II	24.61
Shoot Nos.	−0.006	VIII	−0.012	V	9.03
Leaves on main shoot	0.004	VII	0.003	III	5.41
Leaf length	0.309	III	−0.096	VII	46.50
Width of midrib	0.392	II	−0.398	VIII	49.83
Herb yield	0.823	I	−0.075	VI	32.11
Crude alkaloid (%)	0.271	IV	0.904	I	10.83

$^h^2$ (NS) - heritability in narrow sense

%). Hence, the authenticity of the selection differential is employed to obtain selection gains more quickly through repeated selection followed by hybridization. These indicating that variability/genetic diversity among this medicinal crop can be increased either through induced mutation or hybridization/heterosis breeding followed by selection.

References

1. **Griffing B.** 1965a. A generalized treatment of the use of diallel cross in quantitative inheritance. *Heredity*, **10**: 31-35.
2. **Griffing B.** 1956b. Concept of general and specific combining ability in relation to diallel crossing system. *Aust. J. Bio. Sc.*, **9**: 463-493.
3. **Lal R.K., Sharma J. R., Singh N., Misra H.O. and Naqvi A. A.** 2001. Genetic associations and diversity in the genetic resources of curry neem (*Murraya koenigii*). *J. Med. Aromat. Plant Sci.*, **22** and **23**: 216-221.
4. **Mahalanobis P. C.** 1936. On the generalized distance in statistics. *Proc. Nat. Inst. Sci.*, **2**: 49-55.
5. **Rao C. R.** 1952. *Advanced Statistical Methods in Biometrics Research*. John Wiley and Sons, New York. 1-104.