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CODEN: IAASCA **ORIGINAL ARTICLE**

Study of Genetic Divergence of *Hyoscyamus muticus* L. by using of various genotypes under different environmental conditions

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ABSTRACT
Hyoscyamus muticus L. is a herbaceous plant. It belongs to family- solanaceae. It is a valuable source of different tropane alkaloids, particularly Hyoscyamine having traces of hyoscine and atropine plants used in pharmaceutical products. We have utilizing different types of genotype at different environmental conditions. There were a significant variation among 21 genotypes chosen for all the 7 characters studied in both the environments. The range and coefficient of variation were the maximum for fresh herb-yield followed by leaf area and the length of main inflorescence. Days to 50% flowering had the least variation. This pattern was observed under both the environmental conditions. However, while examining the performance of individual genotypes (S_1 to HM-2) vis-à-vis the commercial check (S_{21}) for important traits, like flowering time, fresh herb-yield and alkaloid content; it is evident that only one genotype, i.e., S_{10} was the earliest, significantly later under the first environmental condition. . In the second environment, none of the genotype was significantly earlier but S_1 and IC-66 were significantly later than the check, whereas for fresh herb yield noneof the genotype in both the environments was significantly better than the check. Only Tetraploid-2 was numerically better than the check; it escaped significance by a narrow margin. For alkaloid content, HM-2 followed by S_7 and Tetraploid-1 was significantly better ($p < 0.01$) than the check in environment I, whereas only S_1 was found to be perceptibly superior over the check ($p < 0.05$) in environment II. A clearer picture with respect to alkaloid yield which is a product of fresh herb yield and alkaloid content, emerges as follows : Tetraploid-2 was the best genotype in both the environments yielding 1.90 g/plant and 2.46 g/plant crude alkaloid as compared to 1.40 g/plant and 1.93 g/plant by the check in the two environments, respectively. Besides S_7 (2.40 g/plant) S_{10}(1.58 g/plant) in environment I and IC-51 (2.12 g/plant) in environment II were also promising genotypes.
Keywords : *Environment, Genotypes, Hyoscyamus muticus L. Medicinal plants*

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INTRODUCTION

Medicinal plant always plays as a key role in human life. Rural people and forest dwellers are utilizing medicinal plants for preventive and curative purpose of various ailments. Plants have been used medicinally since antiquity.Plant based medicines are being used in various systems i.e. Ayurveda, Siddha, Unani, Homoeopathy and even Allopathy at some extent also. Nearly 90 per cent plant based medicines are obtained from the nature, while 10 per cent are medicinal plants cultivated in farms. Egyptian henbane (Hyoscyamus muticus L.) is a herbaceous plant. It belongs to solanaceae family. It is a valuable source of tropane alkaloids, particularly Hyoscyamine having traces of hyoscine and atropine plants used in pharmaceutical products.[6,9] Recently, rising efforts have been devoted to reducing mineral fertilizer supply, production cost, and environmental pollution via decreasing the doses of nitrogenous fertilizers and adopting biofertilizer farming systems. In India, it is mainly found growing in temperate to subtropical regions and is grown largely in Kashmir and Uttar Pradesh. As a consequence of development of its appropriate agro-technology at Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, it has now been recommended for commercial cultivation as *Rabi* season crop in the tropical and subtropical agro-climatic zones of India.[8] Henbane is a cool season crop and it does not tolerate very cold temperature, as the growth and development of these plants is appreciably slow below 20^0C and becomes almost static if the temperature remains below 10^0C. The plants perish under frosty

conditions. Low temperature has also been reported to affect the seed germination. Seeds of *H. muticus* germinate best in 8-10 days if a temperature between 20-30^{0}C is maintained with adequate moisture. Since *H. muticus* is a cross-pollinated crop, the breeding strategy may likely be the same as adopted for such crops. But in view of the modern awareness on the equally important role played by past history of selection and nature of gene action as by the mating system; it would be a rational proposition to adopt any breeding procedure irrespective of its so-called application to self or cross-pollinated crop. As such, it is essential to have an *a priori* knowledge of the variance components and allied genetic parameters coupled with inherent-associations for economic traits. Development of inbred lines (S_5) and their possible exploitation after their adequate evaluation for genetic differentiation and mutagenic treatment of a few inbreeds for generating wider spectrum of variation would be a sound complement to the imminent breeding programme for crop improvement.[10] The aim of study the different genotypes of Hyoscyamus muticus L. by different environmental conditions and genetic variations for improve the yield of seeds.The present investigation on *H. muticus*L. comprised two experiments Experiment- I: Study of genetic variation (a) Genetic divergence among populations (b) Character associations among traits.

MATERIAL AND METHODS

The present investigation was carried out using different genotypes of Hyoscyamus muticus L. collected from various locations at Botanical Garden and Agricultural Research Farm of Narain (P.G.) College, Shikohabad, Firozabad, Dr. B.R.A. Univ., Agra, (U.P.) India, during the years 2005-2006 and 2006-2007. In first experiment Twenty one diverse genotypes of *H. muticus*, fifteen obtained from CIMAP, Lucknow, five from NBPGR, New Delhi and Local bulk, comprised the material for genetic divergence and correlation studies. These genotypes were selfed for 2-3 generations to make them nearly homozygous and homogenous lines. The heterogenous local population was used as check. Salient features of these 21 (S_1 – S_{21}) genotypes are given in Table 1.

Table 1 : Pedigree, sources and salient features of 21 strains/ varieties in *H. muticus*.

Sl.No.	*Strains/ Varieties*	*Pedigree*	*Sources*	*Salient features*
1	S_1	Introduced from Egypt	CIMAP, Lucknow	Erect; corolla yellow, violet tinged; white anthers.
2	S_2	Introduced from Egypt	CIMAP, Lucknow	Erect; thick leaves; corolla violet, yellow tinged; white anthers.
3	S_3	Introduced from Egypt	CIMAP, Lucknow	Prostrate; thin stem; small, long leaves; corolla yellow, violet. tinged; anthers violet.
4	S_4	Introduced from Persia	CIMAP, Lucknow	Erect; thin stem; Flowers and pods small; corolla and anthers violet.
5	S_5	Introduced from Persia	CIMAP, Lucknow	Erect; very thick (leathery) and brittle big leaves; inflorescence compact; corolla yellow with violet eye; anthers violet.
6	S_6	Introduced from Sudan	CIMAP, Lucknow	Erect; pigmented ridged stem; corolla yellow with violet eye; anthers violet.
7	S_7	Introduced from Sudan	CIMAP, Lucknow	Erect; green ridged stem; corolla violet with yellow tinge on tip; anthers violet.
8	S_8	Selection from S_2	CIMAP, Lucknow	Erect; big and leathery leaves; corolla violet, yellow tinged; anthers violet; capsules large.
9	S_9	Selection from S_5	CIMAP, Lucknow	Corolla yellow; anthers white; fused (double) flowers frequently occurring.
10	S_{10}	Selection from S_6	CIMAP, Lucknow	Corolla yellow with violet tinge.
11	CIMAP-1	Advanced breeding lines	CIMAP, Lucknow	Corolla yellow with violet tinge.
12	CIMAP-2	Released variety	CIMAP, Lucknow	550 q/ha fresh herd yield, 150 days growing period, high

				alkaloid content.
13	CIMAP-3	Released variety	CIMAP, Lucknow	Short duration (120 days) crop, yield 35 q/ha dry herb, 0.07 to 0.15% alkaloid field.
14	Tetraploid -1	Released variety	CIMAP, Lucknow	200 days growing period, 645 q/ha fresh herb yield, 43.2 q/ha dry herb yield and high alkaloid content.
15	Tetraploid -2	Released variety	CIMAP, Lucknow	180 days crop duration, 45-50 q/ha dry herb yield, 0.11 to 0.15% tropane alkaloids.
16	IC-66	Variety	NBPGR, New Delhi	Short duration (100 days), yield 25 q/ha dry herb yield, 0.05 to 0.10% tropane alkaloids
17	IC-101	Variety	NBPGR, New Delhi	Long duration period (225 days), 625 q/ha fresh herb yield and high tropane alkaloid.
18	IC-51	Inbred line	NBPGR, New Delhi	Prostrate, thin stem, medium fleshy leaves, corolla yellow.
19	IC-55	Inbred line	NBPGR, New Delhi	Erect item, thick leaves anthers pale, high alkaloid content
20	HM-2	Advanced breeding line	NBPGR, New Delhi	185 days crop duration, high alkaloid content.
21	S-21 (check)	Commercial Bulk		220 days crop duration, 40.0 quintal/ha dry plant yield, medium alkaloid content.

The heterogenous local population was used as check. In second experiment, material consisted of seven morphologically divergent parents.Days to 50% flowering, Plant height, Number of primary branches / plant, Leaf area (cm^2), Main inflorescence length (cm), Herb yield (g/plant),Crude drug (%).
The mean values of the two years were statistically analyzed for the mean ($\bar{X}$), range, standard error etc and subjected to D^2-statistics and canonical analysis according to Mahalanobis [16].

RESULT AND DISCUSSION

Thesalientfeaturesoftheresultsobtainedfromthe present investigation can be presented as all the 21 genotypes examined differed significantly among themselves for all the seven traits in both the environments as also confirmed by a wide range of D^2 values (D^2 = 9.0 to 1677.1). Flowering time was strongly correlated in negative direction with plant height and main inflorescence length. Alkaloid content did not manifest a perceptible degree of correlation with any of the traits. But primary branches were positively correlated ($P < 0.5$) with herb yield. The dominance deviation was much more important than the additive variance for majority of characters. Over dominance was most prevalent for all the traits. While alkaloid content was poorly heritable, the herb yield was having moderate h^2 leaf area, flowering time, number of primary branches and main-inflorescence length manifested moderate to high habitability. [6,14]

Genetic differentiation at different axis was found to be consummated by diverse forces of differentiation. For instance, leaf area, main inflorescence length and herb yield were the important characters, in that order, at the primary axis of differentiation; while plant height followed by inflorescence length and herb yield were so at secondary axis of differentiation. Thus, the characters, which manifested the highest coefficient of variation, i.e. herb, yield, leaf area and main inflorescence length (Table 2) were responsible for major differentiation among the test genotypes.Days to 50% flowering had the least variation. This pattern was least variation. This pattern was observed under both the environmental conditions. However, while examining the performance of individual genotypes (S_1 to HM-2) vis-à-vis thecommercial check (S_{21}) for important traits, like flowering time, fresh herb-yield and alkaloid content; it is evident that only one genotype, i.e., S_{10} was the earliest, significantly later under the first environmental condition. In the second environment, none of the genotype was significantly earlier but S_1 and IC-66 were significantly later than the check, whereas for fresh herb yield none of the genotype in both the environments was significantly better than the check. Only Tetraploid-2 was numerically better than the check; it escaped significance by a narrow margin. For alkaloid content, HM-2 followed by S_7 and Tetraploid-1 was significantly better ($p < 0.01$) than the check in environment I, whereas only S_1 was found to be perceptibly superior over the check ($p < 0.05$) in environment II. A clearer picture with

respect to alkaloid yield which is a product of fresh herb yield and alkaloid content emerges as follows: Tetraploid-2 was the best genotype in both the environments yielding 1.90 g/plant and 2.46 g/plant crude alkaloid as compared to 1.40 g/plant and 1.93 g/plant by the check in the two environments, respectively. Besides S_7 (2.40 g/plant) S_{10}(1.58 g/plant) in environment I and IC-51 (2.12 g/plant) in environment II were also promising genotypes (Table 3).

Table 2.ANOVA for seven economic characters of *H.muticus* grown in two environments.

Traits	ENV. I		ENV. II	
	Treatment (20)	Error (20)	Treatment (20)	Error (20)
Days to 50% Flowering	205.95**	55.41	183.05**	14.43
Plant height	228.65**	23.95	187.43**	17.50
Number of primary branches/plant	6.88**	1.70	2.60*	1.14
Leaf Area	6203.43**	60.15	11369.77**	3860.40
Main inflorescence length	166.81*	58.28	144.40**	16.11
Fresh herb yield	143546.27*	67177.16	187043.57*	81251.12
Alkaloid content	0.102**	0.025	0.052*	0.023

* & ** Significant at 5% and 1% levels, respectively; values in parentheses are corresponding degrees of freedom.

Table : 3: Mean performance of 21 genotypes of *H.muticus* in two environments.

Strains / varieties	Environment	days to 50% flowering	Plant height (cm)	Number of primary branches/	Leaf Area (cm^2)	Main inflorescence length	Herb yield (g/plant)	Alkaloid content (%)	Calculated alkaloid
		X_1	X_2	X_3	X_4	X_5	X_6	X_7	Y
S_1	Env. I	134.5	60.5	11.5	263.4	37.5	785.9	1.387	1.45
	Env. II	143.0	65.7	9.3	250.1	52.6	857.9	1.595	1.74
S_2	Env. I	157.3	51.9	5.8	168.7	37.5	445.2	1.320	0.77
	Env. II	126.2	54.3	6.3	226.6	43.7	610.3	1.135	0.89
S_3	Env. I	131.2	58.4	11.2	133.2	33.0	970.0	1.176	1.47
	Env. II	108.3	56.6	11.0	175.5	50.5	1275.0	1.119	1.85
S_4	Env. I	133.0	59.0	12.6	264.8	33.2	1085.5	0.945	1.35
	Env. II	128.5	51.2	7.7	219.4	34.7	774.5	0.973	0.96
S_5	Env. I	127.5	74.5	8.5	237.5	55.0	720.5	1.065	0.95
	Env. II	116.5	63.5	7.3	344.6	51.5	930.0	1.198	1.49
S_6	Env. I	131.5	47.5	12.1	251.5	34.4	763.0	1.320	1.30
	Env. II	117.5	60.3	7.0	180.2	47.0	572.0	1.343	1.01
S_7	Env. I	133.0	44.3	7.5	374.2	27.5	1120.5	1.650	2.40
	Env. II	120.5	63.4	7.4	284.4	48.6	879.5	1.433	1.64
S_8	Env. I	152.0	57.2	9.5	263.5	33.4	520.5	1.085	0.75
	Env. II	112.0	83.6	7.5	375.5	72.3	811.0	1.155	1.21
S_9	Env. I	144.5	52.4	7.3	173.7	42.0	629.6	1.075	0.89
	Env. II	114.0	65.5	6.8	184.5	55.0	676.0	1.105	0.96
S_{10}	Env. I	111.5	74.0	8.7	273.3	61.3	1084.5	1.113	1.58
	Env. II	107.5	70.0	7.8	191.0	59.4	1110.5	1.132	1.66
		X_1	X_2	X_3	X_4	X_5	X_6	X_7	Y
CIMAP-1	Env. I	145.0	53.8	6.5	191.5	30.5	200.0	1.275	0.35
	Env. II	126.0	64.3	8.3	170.6	84.7	500.0	0.947	0.60
CIMAP-2	Env. I	127.6	80.5	6.5	295.0	47.3	675.0	1.190	1.13
	Env. II	114.4	74.3	8.6	267.1	51.6	885.0	1.460	1.60
CIMAP-3	Env. I	143.0	57.0	8.2	241.0	36.7	620.0	1.035	0.83
	Env. II	110.0	82.6	8.5	261.9	69.2	440.0	1.213	0.70
Tetraploid-1	Env. I	134.5	61.0	7.6	296.5	37.8	680.0	1.610	1.42
	Env. II	109.0	86.5	9.5	261.4	62.4	860.0	1.105	1.23
Tetraploid-2	Env. I	133.2	76.5	9.5	241.0	44.0	1370.0	1.045	1.90
	Env. II	124.8	75.6	10.7	413.0	50.2	1646.5	1.169	2.46
IC-66	Env. I	142.5	58.5	10.9	163.2	34.0	774.5	1.510	1.50
	Env. II	137.0	62.8	8.7	185.4	45.5	1058.8	1.165	1.61

IC-101	**Env. I**	138.0	67.2	7.0	248.5	44.2	659.0	1.265	1.07
	Env. II	123.0	79.4	8.7	237.5	55.4	566.0	1.308	0.98
IC-51	**Env. I**	129.0	75.6	9.5	190.8	47.3	645.0	1.150	0.97
	Env. II	124.5	72.7	8.4	321.5	61.0	1260.0	1.300	2.12
IC-55	**Env. I**	125.7	78.0	8.7	222.4	52.0	980.0	0.975	1.25
	Env. II	118.3	74.3	7.9	298.8	55.2	520.0	0.998	0.67
HM-2	**Env. I**	145.0	51.0	7.6	209.7	32.7	615.0	1.710	1.27
	Env. II	118.5	73.6	8.5	169.6	53.5	740.0	1.230	1.28
S-21	**Env. I**	132.5	64.0	7.2	195.0	45.0	968.5	1.155	1.40
(Commercial)	**Env. II**	114.5	72.1	9.2	233.5	53.2	1180.0	1.230	1.93
C.D.	**Env. I**	20.65**	21.75**	3.75**	113.37**	18.55*	545.35*	0.455**	
	Env. II	19.50**	19.77**	2.35*	153.56**	17.34**	622.58*	0.327	

& ** Significant at 5% and 1% levels, respectively.

A clearer picture of the nature and magnitude of genetic variation among the 21 populations chosen could be depicted by Mahalanobis. D^2 statistic, wherein the divergence between any pair of populations was quantified in terms of D^2 value for the first environment only (Table 4). The highest D^2 value (D^2> 1000) was found between the pair of populations S_3 and S_7 (1676.9) followed by S_7 and IC-66 (1342.1), S_7 and IC-51 (1183.2), S_7 and S_9 (1142.8) and between S_7 and CIMAP-1 (1045.1). The lowest value (D^2< 20) was observed between Tetraploid-2 and IC-55 (9.2) followed by S_2-S_9, S_5-IC-101, S_8-CIMAP-3, S_1-S_4, CIMAP-3–IC-101, S_1-S_6, S_4-S_8, S_9-CIMAP-1, IC-55-S_{21}, and S_1-S_{10} (D^2 values ranging from 10.7 to 19.8).

Table 4 Genetic divergence (D^2 values) among 21 genotypes of *H. muticus* grown .

Genotypes	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	-	252.5	429.3	12.8	52.3	16.7	422.6	22.6	200.8	19.8	165.4
2		-	78.2	259.0	121.2	260.7	1239.3	207.3	10.7	323.8	19.5
3			-	428.5	252.1	431.1	1676.9	419.6	79.9	511.6	100.8
4				-	52.5	29.6	456.6	16.9	199.5	29.4	177.7
5					-	92.6	711.3	43.2	91.7	62.5	72.6
6						-	443.2	45.0	196.4	50.4	177.5
7							-	480.4	1142.8	385.8	1045.1
8								-	168.3	48.9	141.5
9									-	261.4	18.8
10										-	228.3
11											-
Genotypes	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	$\overline{D}^2$
1	9.0	5.0	39.7	103.7	270.9	35.2	201.3	105.6	77.4	145.1	133.2
2	361.7	126.6	424.6	134.6	32.4	150.5	43.4	95.5	71.5	34.5	212.4
3	590.4	301.1	685.4	224.5	45.2	321.2	65.1	176.3	194.8	104.8	355.4
4	67.5	30.3	68.5	92.6	283.3	43.4	203.1	98.5	106.7	147.3	140.3
5	80.8	19.6	140.4	23.2	140.2	10.9	68.6	13.4	50.3	40.4	107.1
6	131.3	53.2	74.1	166.1	282.9	79.8	234.9	153.9	88.5	175.6	159.2
7	390.5	611.6	233.4	833.5	1342.1	594.5	1183.2	891.4	771.5	1011.6	792.8
8	60.8	11.4	68.0	92.8	265.5	25.5	188.7	93.8	83.8	127.8	130.7
9	326.2	94.67	375.7	116.3	35.1	127.7	36.4	74.2	59.7	26.5	182.3
10	38.4	64.6	39.1	115.5	346.0	49.8	244.0	119.7	130.5	182.1	162.7
11	257.8	77.5	306.7	89.3	37.0	89.2	30.1	59.2	30.6	18.2	157.2
Genotypes	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	$\overline{D}^2$
12	-	88.3	42.4	108.4	402.6	47.2	275.5	134.2	169.4	210.3	192.2
13		-	110.2	60.4	175.5	15.4	115.2	51.7	44.6	66.4	107.6
14			-	204.4	471.6	88.3	373.0	225.3	171.2	284.5	221.8
15				-	129.7	35.1	59.6	9.2	80.7	38.3	136.2
16					-	183.5	22.4	93.8	86.8	40.4	233.2
17						-	109.8	37.2	46.1	64.3	108.1
18							-	31.3	74.2	13.4	178.9
19								-	64.5	19.1	127.3
20									-	38.5	123.0
21											139.5

However, on an average of n-1 pairs (where each population is paired with other n-1 populations), the parent S_7 was found to be the most divergent genotype with $\overline{D}^2$ = 792.8 (see last column of Table 3). This was followed, though with considerably less magnitude by S_3 (355.4), IC-66 (233.2), Tetraploid-1 (221.8)

and S_2 (213.8), all with $\bar{D}^2$ >200. The least divergent populations were S_5, CIMAP-3 and IC-101, all with $\bar{D}^2$ > 107.0 rest of the parents were showing $\bar{D}^2$ from 123.0 to 200.0. A further examination of Table 4 revealed that the frequency of genotype pairs showing maximum divergence (D^2> 1000) was 7 and very low divergence (D^2 < 20) was recorded for only 16 pairs of genotypes. Majority of the genotype pairs manifested low (D^2 = 21-100) and medium (D^2 = 100-500) degree of divergence. [Table 4].

CONCLUSION

In conclusion the analysis of variance revealed the presence of sufficient variability among the all genotypes for different characters. Maximum range of variation indicated the presence of wide variation for the characters. The present research work on *Hyoscyamus muticus* entitled "Genetic studies of seed yield, was done, employing two types of experimental approaches, namely, genetic divergence and diallel analysis. A set of 21 diverse genotypes (S_3inbreds) of *Hyoscyamus muticus* L. were subjected to multivariate analysis for genetic divergence and character-association studies in this investigation. Out of these, eight diverse parents were used for diallel analysis to obtain information on genetic variance components and allied genetic parameters.

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