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Induced polygenic changes occurring simultaneously with major gene changes in black henbane (*Hyoscyamus niger* L.)

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Summary. Two macromutants [unbranched (Ub) and restricted branched (Rb)], one point mutation [yellow flowerd ('Aela')] and two flowering variants (early and late) were isolated in the M_2 generation of gamma-irradiated progenies of black henbane (*H. niger*) – a medicinal plant belonging to family Solanaceae. These major gene changes were coupled with simultaneous changes in several polygenic systems conditioning continuous variation for flowering time, plant height, numbers of shoots and leaves, biomass and crude drug content. Such quantitative variation in qualitative mutants were measured for both per se means and cross-bred performance in terms of: (1) shifts in univariate means, (2) changes in pooled divergence, (3) alterations in gca effects, and (4) changes in heterotic capacity. The mutants were significantly divergent from the parental base for all the six metric traits, both individually as well as collectively. Divergence among mutants was wider ($D^2 = 1491$ to 11 469) than that for mutants versus source parent ($D^2 = 117$ to 7120). Conspicuous alterations in both the nature and the magnitude of the gca effects associated with all mutants, except L-2013 (late flowering), relative to the parental base were observed for all of the metric traits examined (gca $\simeq$ per se means). Similarly, the mutants manifested greater heterotic potential than the parental base. This is even more strongly reflected in mutant × mutant hybrids for all of the characters. The amount of economic heterosis for diverse traits followed the pattern: crude drug > biomass > flowering time $\simeq$ plant height > number of shoots $\simeq$ number of leaves.

Key words: *Hyoscyamus niger* – Macromutants – Polygenic changes – gca effects – Average heterosis – Mutant heterosis

Introduction

Macromutants (distinct qualitative genetic variants) are easily identified and can, therefore, be selected for promptly. Should they be agronomically viable, they are channelled through varietal development programmes or otherwise exploited through cross-breeding (Micke et al. 1987; Sinhamahapatra 1986). In the former case, a macromutant can often be associated with many qauantitative changes in the genetic background of the mutated genotype that are independent of the mutated loci but which occur simultaneously, thus enlarging the genetic base (Sinha and Joshi 1986; Giriraj et al. 1990). Two such major mutations – the unbranched (Ub) mutant with a non-overlapping disposition of thick and large leaves and floral modifications and the restricted branching (Rb) mutant with similar features to the Ub mutant but with three to four closely inclined branches arising from the upper half of the single shoot – have been recovered in gamma-irradiated M_2 progenies of *Hyoscyamus niger* (Sharma and Singh 1983; Sharma et al. 1986; Anonymous 1988). A yellow-flowered point mutation with pale-yellow tubular flowers and thinner branches has also been isolated in the same material (Sharma et al. 1989). Christened as var 'Aela', it has been subsequently released for commercial cultivation in India (Anonymous 1987). One early (E-2010) and one late (L-2013) deviant, also with all of the other characteristic features of the normal parental base (P) material except flowering duration, have also been sorted out in the M_2 generation.

H. niger (black henbane), a solanaceous plant, is a rich source of tropane alkaloids, mainly hyoscyamine and hyoscine, which manifest antispasmodic, anticholinergic and mydriatic properties. These alkaloids

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are widely employed in modern medicine as therapeutic agents against respiratory and intestinal disorders (Morton 1977; Bianchini et al. 1977; Anonymous 1982). An open growth habit with fragile and thick (woody) branches arising right from the ground level, voilet-veined flowers with deep purple eyes and overlapping leaf orientation are the most common morphological features of this plant.

All of the five major mutants, Ub, Rb, 'Aela', E-2010 and L-2013, isolated in the M_2 generation of this plant have shown substantial variation for several quantitative (metric) characters, possibly due to a repatterning of the polygenic system under the influence of gamma irradiation. In the investigation presented here the degree of quantitative (polygenic) changes in these qualitative mutants has been measured and discussed in terms of: (1) shifts in per se performance, (2) changes in quantified pooled divergence, (3) changes in combining ability and (4) alterations in the magnitude of heterosis.

Materials and methods

Three stable major mutations, Ub, Rb and 'Aela', and two deviants, E-2010 (early flowering) and L-2013 (late flowering), all derived from gamma irradiation, plus one parental base (normal – P) in black henbane (*Hyoscyamus niger* L.) formed the basic material for the present study. All six of these true breeding parents were crossed in diallel fashion to give rise to $n(n+1)/2 = 21$ progenies, including six parents and 15 direct F_1s. During 1989–90, these were grown in randomized block design replicated twice. The plot size was paired rows 3 m long and 50 cm apart. The inter-plant distance for Ub and Rb was 15–20 cm, while that for the others was 50–60 cm, approximated by selective thinning at the seedling stage. A fertilizer schedule for N, P and K at concentrations of 40, 30 and 30 kg/ha, respectively, was followed with irrigation as per requirement.

Metric observations were recorded on five plants per plot at the mid-flowering stage (about 14 days after the first anthesis), which is the optimal period of maximum alkaloid biosynthesis in the inflorescence. Six characters, namely days to 50% flowering, plant height (cm), numbers of shoots and leaves on the main shoot, biomass (green herbage yield, g/plant) and crude drug content (%) were measured as accurately as possible. The crude drug was analysed gravimetrically by the method outlined by Cromwell (1955).

The metric data averaged over samples ($n = 5$) were statistically analysed for simple ANOVA (analysis of variance), multivariate D^2-statistic (Rao 1952), general combining ability (gca) effects (Griffing 1956) and for heterosis (%) over the best commercial check – standard var 'Aela' (included in the experimental materials).

Results and discussion

That radiation mutagenesis can generate both qualitative and quantitative mutants is an established fact (reviewed by Micke et al. 1987). What is, however, more interesting is the remarkable changes that occur simultaneously for characters showing continuous variation in qualitative mutants (macromutants). This is reflected in the present study on both per se and cross-bred performance in addition to changes in major genes.

Changes in per se performance

Alterations in per se performance for quantitative traits in qualitative mutants due to physical mutagenesis were perceptible for the following two parameters:

Shifts in univariate means

Mutants (M) were significantly different ($P < 0.01$) from their parental base (P) for all of the six metric traits studied (Table 1). Thus, the two macromutants Rb and Ub were markedly later in flowering, taller in height, lower in branching and higher in both biomass and crude drug content than P. The point muation 'Aela' was, however, greater ($P < 0.01$) than the parental base for biomass and crude drug content only, and not much different for other characters. Among the two deviants for flowering time, the early deviant, E-2010, besides being significantly earlier than P was also highly different ($P < 0.01$) from it for plant height and numbers of shoots and leaves; no change in biomass and crude drug (%) was observed. On the

Table 1. Shifts in per se means for six characters in henbane derived upon gamma irradiation

Parent/mutant	Days to flower	Plant height (cm)	Number of shoots/plant	Number of leaves on main shoot	Biomass (g/plant)	Crude drug (%)
P_1-P	74.0	80.3	5.8	50.8	1225	0.070
P_2-Rb	85.0	114.3	2.8	74.5	2350	0.173
P_3-Aela	71.0	62.8	6.3	57.8	2825	0.172
P_4-L-2013	80.0	135.3	6.8	50.2	1275	0.081
P_5-E-2010	62.5	129.0	10.2	73.3	1325	0.091
P_6-Ub	111.5	218.7	1.0	40.8	1725	0.217
$\bar{M}$ (P_2–P_6)	82.0	132.0	5.4	59.3	1900	0.150
$CD_{1\%}$	10.53	24.59	1.25	9.90	437.97	0.035
CV (%)	25	44	59	23	37	46

other hand, the late deviant, L-2013, did not manifest any changes in these traits except that it was taller ($P < 0.01$) and had more shoots per plant ($P < 0.05$) than P.

On an overall basis (M versus P), the mutants were 11% later (82 days versus 74 days), 64% taller (132.02 cm versus 80.3 cm) and 55% better yielders of biomass (1900 g versus 1225 g/plant) than the parental base; they also had a 11.4% higher percentage of crude drug content (0.150% versus 0.070%). There was no change in numbers of shoots and leaves (see Table 1), however the range indicated that the number of shoots varied from 1.0 to 10.2 and the number of leaves from 50.2 to 74.8 in the mutants while being 5.8 and 50.8, respectively, in the parent. As a result, the coefficient of variation (CV) was highest (59%) for number of shoots, followed by crude drug content (46%), plant height (44%) and biomass (37%). Hence, based on the range of values, all of the mutants were higher for biomass and crude drug content while for other traits, both poor and better mutants than the parent were recovered. In other words, changes in biomass and drug content were positive unidirectional; while for the other characters ambidirectional alterations were recorded. This is clearly reflected in the percentage alterations brought out by mutagenesis in various metric traits relative to the untreated parental base (Fig. 1).

Thus, gamma radiation substantially altered the polygenic background of different qualitative mutants, thereby enlarging the spectrum of continuous variation in *H. niger*. The literature is replete with reports on such quantitative changes in different crops (Gaul 1965; Scossiroli 1970; Sinha and Joshi 1986; Giriraj et al. 1990), but the present investigation reveals concurrent changes of a continuous nature (micromutations) along with that of a discrete nature (macromutations). Therefore, macromutations are not always of academic significance alone; they may exhibit a better agronomic performance, as both Rb and Ub mutants have shown here. In fact, owing to their mono-stem nature with non-overlapping leaf-arrangement, they are physiologically the most efficient plant types, being capable of yielding a very high biomass per unit area under high population pressure. As such, one of them (Ub mutant) is in the pipeline for release, whereas the point mutant 'Aela' has already been released for commercial cultivation (Anonymous 1987).

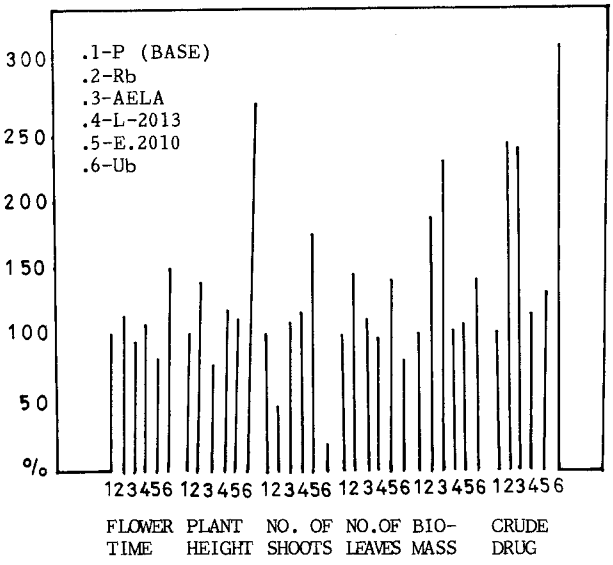

Fig. 1. Relative changes (%) in mutants when compared to the parental base (*P*) (100) for six traits in *H. niger*

Changes in pooled divergence

Shifts in the mean performance of mutants for individual traits could be transformed into pooled divergence for all of the six traits quantified in terms of D^2 values (Rao 1952) and averaged for each pair of genotypes – parental base versus mutants and mutants versus mutants (Table 2). The mutants manifested a very wide range of divergence from the base (P): $D^2 = 117 (P_4)$ to $7120 (P_2)$. Thus, while the two flowering deviants (E-2010 and L-2013) were the least divergent from the parental base ($D^2 = 117–239$); Rb, Ub and 'Aela', in that order, were highly divergent ($D^2 = 4432–7120$). Obviously, such a marked divergence of macromutants from their parent material is the potential consequence of physical mutagenesis influencing a number of polygenic systems together with major genes.

Divergence among the mutants was even more vivid, the range being $D^2 = 1491$ (Rb-'Aela') to 11469 (L-Ub). However, while Rb and 'Aela' ($D^2 = 1491$) followed by 'Aela' and L-2013 (1828) and Rb and E-2010 (2060) were close to each other, L-2013 and Ub (11469) followed by 'Aela' and Ub (9272) and 'Aela' and E-2010 (8443) were the most divergent from each other (see Table 2). This naturally suggests that the effectiveness of mutagenesis was highly differential in affecting

Table 2. Changes in pooled divergence (D^2) for six characters in P and mutants in *H. niger*

Mutant versus mutant	D^2	P versus mutant	D^2
Rb-Aela	1 491	P-Rb	7 120
Rb-L-2013	3 329	P-Aela	4 432
Rb-E-2010	2 060	P-L-2013	117
Rb-Ub	4 404	P-E-2010	239
Aela-L-2013	1 828	P-Ub	7 002
Aela-E-2010	8 443	Range (parent-P)	117–7 120
Aela-Ub	9 272	Range (mutants)	1 491–11 469
L-2013-E-2010	8 226		
L-2013-Ub	11 469		
E-2010-Ub	2 354		

the different polygenic systems that created (pooled) divergence among the mutants.

Changes in cross-bred performance

Changes in per se performance is often confounded with environmental influence, but true genetic changes are reflected well in cross-bred progeny performance. As a sequel to this, all of the five mutants/deviants together with their single parental base were cross-bred in a diallel fashion resulting in 15 F_1 hybrids. Marked changes in terms of gca effects and magnitude of heterosis involving both the parental base (P) and its resultant five mutants were recorded in this study.

Alterations in gca effects

Conspicuous changes in both the nature (+ or − direction) and magnitude (size) of gca effects associated with mutants versus the parental base were observed for all six characters under study (Table 3). Thus, the Rb mutant manifested significant shifts in its gca effects for flowering time, numbers of shoots and leaves, biomass and crude drug content; it remained unchanged for plant height. The Ub mutant was likewise for all traits except crude drug. Both these macromutants exhibited drastic alterations of gca effects not only in size but also in its nature as compared with P. This is duly corroborated by their corresponding per se performance and is hence highly reliable (see Table 1). Therefore, the predominance of a fixable (additive) component of genetic variation for these traits in the two macromutants is most likely. The point mutation, var 'Aela', also maintained a similar trend for all six characters. However, number of leaves in 'Aela' was not precisely under additive genetic control (gca $\not\simeq$ per se mean). On the other hand, the late flowering deviant (L-2013) did not reflect any changes in gca effects corresponding to the means for any trait and is hence unreliable. The early deviant E-2010 did register reliable changes in gca effects for all characters except flowering time and biomass: gca $\simeq$ per se mean performance (see Tables 1 and 3).

Thus, it is apparent that except for L-2013 all of the mutants exhibited significant changes vis-a-vis the parent (P) in their gca effects for nearly all of the

Table 3. Changes in general combining ability (gca) effects for six characters caused by irradiation in *H. niger*

Parents/mutants	Flowering time	Plant height	Number of shoots	Number of Leaves	Biomass	Crude drug content
P_1 (P)	−3.5	−10.1	0.70	1.08	−437.3	−0.0070
P_2 (Rb)	6.7	−10.2[a]	−0.44	−3.41[a]	328.9	0.0001
P_3 (Aela)	−7.5	−18.9	0.10	−0.07[a]	304.6	0.0160
P_4 (L-2013)	−2.1[a]	−4.9[a]	0.01[a]	−5.27[a]	−83.3[a]	−0.0130[a]
P_5 (E-2010)	−0.3[a]	22.5	0.79	8.37	−329.4[a]	0.0130
P_6 (Ub)	6.7	21.7	−1.13	−0.70	216.4	−0.0090[a]

[a] Not corresponding with per se mean performance

Table 4. Economic heterosis over 'Aela' (the standard parent) as manifested by different mutants in *H. niger*

Cross[a]	Days to flower	Plant height	Shoot number	Leaves on main shoot	Biomass	Crude drug content
$P_1 \times P_2$	4.05	15.35	34.08	−8.20	16.33	35.71
$P_1 \times P_3$	27.70	−11.31	19.86	−16.72	8.86	78.57
$P_1 \times P_4$	5.41	24.88	59.76	35.08	−24.49	57.14
$P_1 \times P_5$	17.57	19.70	−3.00	32.78	−21.43	171.43
$P_1 \times P_6$	−8.11	15.35	14.21	31.14	7.14	150.00
$P_2 \times P_3$	22.97	15.77	11.30	5.57	73.47	145.71
$P_2 \times P_4$	41.22	−39.52	−38.61	−43.44	105.71	27.86
$P_2 \times P_5$	50.00	32.57	28.42	−3.28	75.31	141.43
$P_2 \times P_6$	58.11	6.18	−11.56	11.41	79.59	58.57
$P_3 \times P_4$	13.51	−1.87	−5.82	24.48	23.27	166.43
$P_3 \times P_5$	50.00	2.69	22.69	18.69	−23.80	26.43
$P_3 \times P_6$	−2.70	−23.72	17.04	−10.16	121.02	142.14
$P_4 \times P_5$	4.05	59.68	19.86	16.06	−23.63	188.57
$P_4 \times P_6$	23.65	78.32	11.30	50.12	121.14	148.57
$P_5 \times P_6$	52.70	59.13	14.13	−21.31	47.59	4.29

[a] P_1–P_6 as in Table 1

characters. Such genetic alterations are the natural consequences of physical mutagenesis interfering directly with the basic genetic apparatus, i.e. the DNA content of the parental cells.

Changes in heterotic capacity

Induced mutations can alter the heterotic potential of their carriers for various traits (Konotop 1968; Morgun et al. 1973; Romer and Micke 1974; Stoilov and Daskaloft 1976; Rawat and Tyagi 1989). In the present case, we have compared the economic heterosis of the mutants and the source material (P) against a standard variety, 'Aela', which itself is one of the mutants from the same source (Table 4). This has been examined in three ways: (1) heterosis over 'Aela' in parental base (P) × mutants hybrids; (2) heterosis over 'Aela' in mutant × mutant hybrids; (3) average heterosis over 'Aela' as manifested by individual mutants and P for different traits.

Heterosis in hybrids of P × mutants. A variable degree of heterosis was observed for various characters in different hybrids involving the base parent (P) and the mutants. The highest percentage of heterosis was recorded for crude drug content in hybrids P × E-2010 (171.4%) and P × Ub (150%). The P × Rb hybrid registered maximum positive heterosis for biomass (16.33%), while P × E-2010 resulted in negative heterosis (−21.43%). Similarly, hybrid P × L-2013 produced highly heterosis for numbers of leaves (35.08%) and shoots (59.76%) and plant height (24.88%), whereas P × 'Aela' gave maximum heterotic effect for flowering time (27.70%). Thus, mutants are genetically highly different insofar as their capacity to yield heterotic hybrids with P is concerned.

Mutant heterosis. The impact of mutagenic treatment on the heterotic potential of diverse mutants is even more strongly reflected in mutant × mutant hybrids. Thus, the highest heterosis over 'Aela' was registered again for crude drug content by hybrids L-2013 × E-2010 (188.57%), followed by 'Aela' × L-2013 (166.43%), L-2013 × Ub (148.57%), Rb × 'Aela' (145.71%), 'Aela' × Ub (142.16%) and Rb × E-2010 (141.43%). Interestingly, var 'Aela' occurred more frequently in these hybrids than other mutants. Similarly, hybrids L-2013 × Ub, 'Aela' × Ub and Rb × L-2013 for biomass; Rb × Ub, E-2010 × Ub, 'Aela' × E-2010 and Rb × E-2010 for flowering time; L-2013 × Ub for number of leaves were substantially heterotic hybrids, with more potential than those involving P as one of the parents.

Average heterosis of parents (half-sib heterosis). The average heterosis of all of the crosses where a particular parent was commonly involved (i.e. mean heterosis of half-sibs) revealed a differential heterotic potential of each parent (Table 5). The contribution of all five mutants (Ub, Rb, E-2010, 'Aela' and L-2013, in that order) to economic heterosis for flowering time was more, and that for numbers of shoots and leaves less, than that of the parental base (P). On the other hand, mutants 'Aela', E-2010 and Ub, for crude drug, and L-2013, Ub and E-2010, for plant height, contributed more, while Rb and L-2013 for the former and 'Aela' and Rb for the latter trait contributed less than P to heterosis. Further, while none of the mutants, like the parental base, caused negative heterosis for crude drug content (Rb, L-2013 and E-2010 for flowering time, E-2010 for plant height and Rb and Ub for biomass also) did not cause negative heterosis, all of them, including P, contributed to negative heterosis for numbers of shoots and leaves per plant.

Thus, irradiation of a parental base could lead to major gene alterations coupled with a tangible degree of simultaneous changes in the genetic background of qualitative/macro-mutants influencing quantitative or continuous variation in *H. niger*. This has also been

Table 5. Half-sib heterosis (mean of all hybrids with one common parent) over the commercial standard var 'Aela' for six characters in *H. niger*

Parent/ mutants	Flowering time		Plant height		Number of shoots		Number of leaves		Biomass		Crude drug content	
	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)
P_1 (P)	13.7	8.1	18.8	11.3	32.0	3.0	33.0	12.5	10.8	23.0	98.6	–
P_2 (Rb)	35.3	–	17.5	39.5	24.6	25.1	8.5	18.3	71.1	–	81.9	–
P_3 (Aela)	28.5	2.7	9.2	12.3	17.7	5.8	16.2	13.4	56.7	23.8	111.9	–
P_4 (L-2013)	17.6	–	54.3	20.7	30.3	22.2	31.4	43.4	83.4	24.1	84.0	–
P_5 (E-2010)	34.9	–	34.8	–	21.3	3.0	22.5	12.3	61.5	23.0	106.4	–
P_6 (Ub)	44.8	5.4	39.7	23.1	14.2	11.6	30.9	27.0	75.3	–	100.7	–
Average	29.1	5.4	29.0	21.5	23.3	11.8	23.8	21.2	59.6	23.4	97.2	–

reported in jute (Sinhamahapatra and Rakshit 1981; Sinhamahapatra 1986). Our result axiomatically implies that in addition to macromutations, micromutations also occur, most likely as a result of concurrent alterations in different polygenic systems within the same genotype. Such changes are independent of pleiotropic mutations of a qualitative nature as has been observed in Rb and Ub mutants in this crop (Sharma and Singh 1983).

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