Evaluation of Quantitative Characteristics in Some Mutant Lines of *Nigella sativa* L.

Animesh K. Datta and Amal K. Biswas

Department of Botany, University of Kalyani, Kalyani 741235, India

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Induction of mutation forms an important part of breeding programme which aims at the improvement of crop plants through manipulation of genetic variation (Gaul 1964, Micke 1969, Sigurbjörnsson 1976, Brock 1977). By the mutation research work of the present authors for last several years, creation of genetic diversities was possible in *Nigella sativa* L. (black cummin), a spice yielding member of the family Ranunculaceae, and several mutant lines have been recovered (Datta and Biswas 1985). In this communication, the findings on the quantitative characteristics observed in the mutants have been presented.

Material and methods

Quantitative traits were studied in the M₂, M₃ and M₄ generations of the mutant lines. Selfed seeds of individual M₂ viable mutants (cited in tables) were sown for M₃ and subsequent generations. The true breeding mutant plants were grown in randomized block design with three replications in the experimental plots during M₃ and M₄ generations. But in *prostrate*, *brown seed-coat* and *dwarf* mutant plants, three replications could not be performed due to paucity of selfed seeds. Selfed seeds of individual mutants were sown in lines keeping a uniform distance 15 cm between the plants and 25 cm in between the lines. In each plot, the mutants were alternatively sown in lines with control seeds and in all cases border effect was avoided by growing control plants along the boundary line of the plot.

Morphological variations were studied in the mutants as well as in the control. The variations were assessed from seed germination, seedling survivability, plant height, number of primary and total branches, number of capsules per plant, capsule length per fruit, number of compartments per capsule, seed-set per fruit (fitted seeds), 100-seed weight, seed size (length and thickness) and harvest index. Harvest index was calculated by using the formula:

$$\text{Harvest index (HI)} = \frac{\text{Economic yield (seed yield/plant)}}{\text{Biological yield (total dry wt./plant)}} \times 100.$$

Seed size was measured by using a diallel gauze. All sterility types (flower, capsule, pollen and seed) were represented in percentages.

In addition to these observations, the extent of variations among control plants of different years and between control and mutant lines growing under uniform environmental conditions were studied by means of analysis of variance.

Results and discussion

Germination and survival

Since the mutants were identified in M_2 generation frequency of germination and survival of the individual mutants could be recorded in the M_3 and M_4 generations (Table 1). As compared to the respective controls, the *feathery leaf* mutant has demonstrated increased frequency of germinability and survivality; while minimum percentage of germination and survival were noted in the *prostrate* mutant plants (Table 1). Percentage of germination and survival of the *brown seed-coat* mutant could not be recorded at M_3 generation, because the mutant trait was not possible to identify before harvest; obviously artificial self pollination has not been made possible in the unidentified mutant. Consequently, seeds from only open pollinated mutant plants were recovered in the M_2 generation. Slightly higher frequencies of germinability and survivality were recorded in most of the mutants at

Table 1. Germinability and survivality of seedlings of control and mutant plants

Plant types	M ₃ generation					M ₄ generation				
	No. of seeds sown	Germination		Survival		No. of seeds sown	Germination		Survival	
		Fre-quency	%	Fre-quency	%		Fre-quency	%	Fre-quency	%
Control	200	164	82.00	164	82.00	2620	1863	71.30	1868	71.30
<i>Lax branching</i>	556	308	55.40	271	48.74	180	108	60.00	87	48.33
<i>Feathery leaf</i>	129	119	92.25	108	83.72	120	100	83.33	94	78.33
<i>Bushy</i>	80	36	45.00	31	38.75	120	68	56.67	68	56.67
<i>Dwarf</i>	19	9	47.37	05	26.32	50	29	58.00	21	42.00
<i>Viridis</i>	100	66	66.00	61	61.00	160	118	73.75	105	65.63
<i>Early flowering</i>	50	26	52.00	25	50.00	120	69	57.50	66	55.00
<i>Brown seed coat</i>	—	—	—	—	—	80	69	86.25	61	76.25
<i>Prostrate</i>	15	5	33.33	02	13.00	25	06	24.00	04	16.00

Table 2. Rate of germination of M_3 seeds in control and mutant plants at field condition

Plant types	No. of seeds sown	Germination (%)					
		15 days	20 days	25 days	30 days	35 days	40 days
Control	2620	45.95	70.34	71.30	71.30	71.30	71.30
<i>Lax branching</i>	180	52.77	60.00	60.00	48.33	48.33	48.33
<i>Feathery leaf</i>	120	82.50	83.33	78.33	78.33	78.33	78.33
<i>Bushy</i>	120	—	4.17	9.17	42.50	56.67	56.67
<i>Dwarf</i>	50	22.00	28.00	48.00	58.00	42.00	42.00
<i>Viridis</i>	160	38.13	73.75	73.75	67.50	65.63	65.63
<i>Early flowering</i>	120	57.50	55.83	55.00	55.00	55.00	55.00
<i>Brown seed coat</i>	80	58.75	86.25	86.25	82.50	76.25	76.25
<i>Prostrate</i>	25	—	—	8.00	12.00	16.00	16.00

M₄ generation as compared to their respective control (Table 1).

The rate of germination was observed in the M₄ generation after 15, 20, 25, 30, 35 and 40 days of sowing (Table 2, Fig. 1). Maximum germination in the control plants was recorded after 25 days of sowing, while in *feathery leaf*, *lax branching*, *viridis*, *early flowering* and *brown seed coat* mutants germination was found to be completed within 20 days of sowing but delayed rate of emergence was noted in *bushy*, *dwarf* and *prostrate* mutants (Table 2, Fig. 1). The first count of germinated seedlings in the field was made after 15 days of sowing but the *prostrate* and *bushy* mutants failed to germinate within this period, although the *feathery leaf* mutant has shown maximum frequency of germination within the said period (Table 2).

Fig. 1. Graph shows rate of seed germination in control and in different mutant plants (M₄ generation) of *N. sativa*.

Table 3. Records on height, branches and fruit characters in control and mutant lines

Plant types	Height (cm)				No. of primary branches				No. of capsule/plant				Septa/capsule				Capsule length/plant (cm)			
	M ₂	M ₃	M ₄		M ₂	M ₃	M ₄		M ₂	M ₃	M ₄		M ₂	M ₃	M ₄		M ₂	M ₃	M ₄	
Control	47.42 ±1.30	48.66 ±1.53	51.84 ±2.92		5.80 ±0.34	6.26 ±0.53	6.04 ±0.51		17.13 ±0.91	17.99	17.18		5.12 ±0.13	5.20	5.60		1.14 ±0.06	1.20	1.27	
<i>Lax branching</i>	49.70	49.65	47.90		8.33	9.70	7.80		49.0	54.0	39.50		5.30	5.23	5.80		1.39	1.31	1.36	
<i>Feathery leaf</i>	49.00	47.64 ±5.01	52.27 ±2.77		7.00	6.29 ±0.69	5.47 ±0.40		34.0	26.29 ±4.76	18.57		5.42 ±0.08	5.74 ±0.08	6.30 ±0.33		1.27 ±0.03	1.25 ±0.05	1.29 ±0.05	
<i>Bushy</i>	35.60	34.92	31.68		8.00	7.33	6.97		39.0	40.5	28.00		5.02	5.16	5.3		1.16	1.0	0.93	
<i>Early flowering</i>	50.5	48.05	36.65		7.00	6.10	3.53		19.0	17.3	7.43		5.20	4.93	5.40		1.15	1.32	1.27	
<i>Brown seed coat</i>	36.0	43.27	34.14		5.00	6.00	4.17		23.0	28.10	28.50		5.0	5.21	5.40		1.05	1.18	1.12	
<i>Viridis</i>	38.5	27.72	24.25		2.00	3.50	2.57		4.0	7.75	4.90		5.0	4.89	5.00		1.00	1.07	1.04	
<i>Prostrate</i>	34.0	29.06	22.10		7.00	6.30	5.70		24.0	29.40	25.50		6.3 ±0.36	5.30	5.40		1.06	1.06	1.01	
<i>Dwarf</i>	16.50	15.32	16.25		3.00	2.60	2.56		5.0	4.20	4.67		5.00	5.00	5.00		0.84	0.90	0.76	

Table 4. Records on seed characters in control and mutant lines

Plant types	Seeds/capsule			100 seeds weight (gm)			Seed length (mm)			Seed thickness (mm)			Harvest index (%)		
	M ₂	M ₃	M ₄	M ₂	M ₃	M ₄	M ₂	M ₃	M ₄	M ₂	M ₃	M ₄	M ₂	M ₃	M ₄
Control	65.60 ±4.24	66.85 ±8.54	65.27 ±10.77	0.2511 ±0.013	0.2254 ±0.018	0.1393 ±0.023	1.41 ±0.02	1.38 ±0.01	1.30 ±0.01	1.02 ±0.02	1.00 ±0.02	0.92 ±0.02	34.85	24.31	17.68
<i>Lax branching</i>	61.03 ±5.14	60.06 ±6.21	58.60 ±5.64	0.1754 ±0.002	0.1807 ±0.006	0.1573 ±0.002	1.46 ±0.05	1.48 ±0.039	1.35 ±0.03	1.05 ±0.02	1.00 ±0.021	1.03 ±0.03	25.24	16.85	15.12
<i>Feathery leaf</i>	63.17 ±4.85	64.26 ±4.00	75.90 ±5.87	0.1781 ±0.004	0.2541 ±0.011	0.1443 ±0.003	1.47 ±0.03	1.56 ±0.04	1.42 ±0.04	1.16 ±0.03	1.25 ±0.03	0.94 ±0.02	26.32	28.00	18.31
<i>Bushy</i>	30.63 ±8.60	36.65 ±4.57	29.1 ±7.40	0.1641 ±0.003	0.1791 ±0.004	0.1712 ±0.004	1.42 ±0.01	1.40 ±0.02	1.46 ±0.01	1.07 ±0.02	1.15 ±0.03	0.94 ±0.02	6.12	8.72	4.45
<i>Early flowering</i>	57.63 ±4.48	61.57 ±5.46	56.3 ±7.26	0.2230 ±0.007	0.1851 ±0.011	0.1414 ±0.006	1.46 ±0.04	1.60 ±0.04	1.38 ±0.02	1.13 ±0.03	1.18 ±0.02	0.98 ±0.02	27.45	22.57	13.36
<i>Brown seed coat</i>	47.20 ±3.21	37.50 ±6.71	44.0 ±9.50	0.2732 ±0.004	0.2567 ±0.026	0.1600 ±0.018	1.48 ±0.04	1.53 ±0.04	1.46 ±0.04	1.19 ±0.02	1.15 ±0.02	1.07 ±0.02	22.50	18.40	15.29
<i>Viridis</i>	22.00 ±8.12	39.72 ±8.12	45.93 ±7.65	0.1463 ±0.008	0.1614 ±0.008	0.1585 ±0.013	1.36 ±0.09	1.52 ±0.06	1.42 ±0.02	1.01 ±0.02	1.12 ±0.03	0.99 ±0.04	10.04	14.20	17.05
<i>Prostrate</i>	22.60 ±10.32	26.42 ±10.32	41.60 ±10.32	0.1441 ±0.007	0.1374 ±0.007	0.1482 ±0.007	1.35 ±0.05	1.24 ±0.02	1.28 ±0.03	0.97 ±0.01	1.01 ±0.02	1.04 ±0.05	6.15	7.35	19.48
<i>Dwarf</i>	24.40 ±2.14	25.12 ±2.14	28.11 ±2.14	0.1273 ±0.002	0.1215 ±0.002	0.1309 ±0.002	1.12 ±0.01	1.17 ±0.0	1.23 ±0.03	0.89 ±0.03	0.95 ±0.02	1.04 ±0.02	17.50	20.00	22.19

Fig. 2. Bar histograms represent different sterility types in control and in different mutants of *N. sativa* at M₂, M₃ and M₄ generations.

Seedling mortality at younger stages was a common phenomenon in the mutant plants, but none of the seedlings of the *bushy* and *prostrate* mutants died even after 40 days of sowing as in case of control (Table 2, Fig. 1)

Post-harvest findings and sterility types

Tables 3 and 4 demonstrated a comparative account of different parameters recorded after harvest in control and eight mutant plants during M₂, M₃ and M₄ generations. The number of primary branches was found to be increased in *lax branching* and *bushy* mutants, while total number of capsules per plant enhanced in *lax branching*, *feathery leaf*, *bushy*, *brown seed-coat* and *prostrate* mutant plants. Length and thickness of seed were found to increase in *lax branching*, *feathery leaf*, *brown seed-coat* and *early flowering* mutants, while septa per capsule and capsule length per fruit increased only in the *feathery leaf* and *lax branching* mutants. As compared to controls of the respective generations, reduced seed-setting was evidenced in the mutant plants, although the *feathery leaf* mutant of the M₄ generation manifested increased seed setting. Considering the three generations studied, it is apparent that seed-setting is more or less normal in *feathery leaf*, *lax branching* and *early flowering* mutant lines. The *brown seed-coat* mutant was unique in producing heavier seeds than the control in all the generations; increased seed weight was,

Table 5. Comparative account of different sterility types in control and mutant lines

Plant types	Sterility (%) in plant types in different generations											
	Flower			Capsule			Pollen			Seed		
	M ₂	M ₃	M ₄	M ₂	M ₃	M ₄	M ₂	M ₃	M ₄	M ₂	M ₃	M ₄
Control	21.83	29.12	27.33	30.61	28.07	31.64	3.50	2.67	2.33	29.82	25.40	33.31
<i>Lax branching</i>	11.33	12.24	14.54	45.00	46.28	40.48	6.74	2.31	3.38	41.89	36.50	27.85
<i>Feathery leaf</i>	19.20	10.68	16.75	26.15	20.67	11.81	6.28	6.23	6.19	19.84	14.84	14.22
<i>Bushy</i>	61.52	53.42	47.46	76.92	57.61	53.36	51.52	47.12	61.62	67.22	65.89	63.30
<i>Early flowering</i>	17.63	22.28	22.13	28.42	23.79	25.76	3.11	2.76	0.47	25.82	31.83	26.33
<i>Brown seed coat</i>	28.70	30.92	29.59	38.96	34.43	37.59	11.34	9.70	5.48	54.32	49.73	33.26
<i>Viridis</i>	56.62	42.39	41.78	72.12	64.79	38.31	11.34	12.32	11.49	41.66	34.16	33.00
<i>Prostrate</i>	34.88	26.79	25.68	51.60	34.92	30.38	79.66	38.76	22.93	68.21	44.30	26.63
<i>Dwarf</i>	24.22	19.64	14.29	24.76	24.12	19.23	7.82	8.42	8.12	35.64	29.97	33.92

Fig. 3.

however, noted in the M_4 generation of all but the *dwarf* mutant.

Thus, the survey of the mutant lines has disclosed that the mutants have exhibited superiority over the control plants in some of the characters only but not in all the parameters. This observation, therefore, opens up the possibility of direct selection of the desired mutant plant types as well as the scope of improvement through hybridization followed by proper selection, although the sterility barriers in the mutant plants needs to be minimized through repeated screening of the population. Compared to control, in the present investigation, all the sterility types (flower, capsule, pollen and seed) have been found to be conspicuously increased in the *bushy*, *viridis* and *dwarf* mutant plants; but all the sterility types, in general, have reduced gradually in the following generations (Table 5, Fig. 2).

Variation in quantitative characteristic in control and mutant plants

Significant year to year phenotypic changes have been recorded in the control plants of *N. sativa* regarding most of the traits excepting capsule length and filled seeds per capsule, when grown in the same season each year (Table 6). Certainly, environmental variations could be attributed for the differential responsiveness of the different parameters studied. Since the different characters of the control plants exhibited variations during different years, it would be logical to compare the mutant plant types (cited in Table 8) with control when grown in the same year under uniform agro-climatic conditions.

Comparison among the control and six different mutant plant types was restricted to ten different characters and mean values of the characters, $F\%$, CD at 5%, C.V. (coefficient of variation) and heritability (broad sense) were estimated. Significant variations have been noted in the different plant types for most of the traits excepting seed length and seed weight per plant (Table 7). In the control and mutant plant types, maximum and minimum variations as measured by co-

Table 6. Analysis of variance of different parameters studied in four successive generations in the control plants of *Nigella sativa*

Source	d.f.	Mean sum of squares (M.S.S.)										
		Plant height (cm)	No. of primary branches	No. of total branches	No. of total capsule	Septa per capsule	Capsule length/fruit (mm)	Filled seeds/capsule	Seed length (mm)	Seed thickness (mm)	Seed weight (gm)	Harvest index
Replications	4	4.22	0.025	19.36	19.36	0.01	0.0049	169.01	0.0010	0.0042	0.000007	4.87
Year	3	302.74**	0.85*	31.24	31.24	0.26**	0.0155	10.18	0.0161**	0.0193**	0.011578**	291.19**
Error	12	16.91	0.17	11.61	11.61	0.02	0.0047	43.28	0.0026	0.0016	0.000235	5.77

* and **: significant at 5% and 1% level respectively

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Table 7. Analysis of variance of different characters in control and six mutant types of *Nigella sativa*

Source	d.f.	Mean sum of squares (M.S.S.)										
		Plant height (cm)	No. of primary branches	No. of total branches	No. of total capsule	Septa/capsule	Capsule length/fruit (mm)	Filled seeds/capsule	Seed length (mm)	Seed thickness (mm)	Seed weight/plant (gm)	Harvest index
Replications	2	29.33	0.92	38.70	38.70	0.06	0.0006	112.15	0.0064	0.0034	0.0000095	16.43
Samples	6	357.96**	10.71**	418.36**	418.36**	0.53**	0.0751**	705.36**	0.0103	0.0088*	0.0004115	67.20**
Error	12	24.50	1.27	67.04	67.04	0.03	0.0078	117.46	0.0056	0.0021	0.0001729	9.77

* and **: significant at 5% and 1 % level respectively

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Table 8. Mean, F-value, C. D., C. V. and heritability in different parameters of control and mutant plants of M_4 generation

Samples	Characters (mean values)										
	Plant height (cm)	No. of primary branches/plant	Total branches/plant	Total capsule/plant	No. of septa/capsule	Capsule length/fruit (cm)	Filled seeds/capsule	Seed length (mm)	Seed thickness (mm)	100-seed weight (gm)	Harvest index (%)
Control	51.85	6.03	16.37	17.37	5.7	1.28	65.1	1.30	0.92	0.1393	17.68
<i>Lax branching</i>	47.90	7.80	38.50	39.50	5.3	1.36	58.6	1.35	1.03	0.1573	15.12
<i>Feathery leaf</i>	52.27	5.47	17.57	18.57	6.3	1.29	75.9	1.42	0.94	0.1443	18.31
<i>Bushy</i>	31.68	6.97	27.00	28.00	5.3	0.93	29.1	1.46	0.94	0.1712	4.45
<i>Early flowering</i>	36.65	3.53	6.43	7.43	5.4	1.27	56.3	1.38	0.98	0.1414	13.36
<i>Brown seed coat</i>	34.14	4.17	27.50	28.50	5.4	1.12	44.0	1.46	1.07	0.1600	15.29
<i>Viridis</i>	24.25	2.57	3.90	4.90	5.0	1.04	45.9	1.42	0.99	0.1585	17.05
F-value	14.61**	8.41**	6.24**	6.24**	17.99**	9.63**	6.00**	1.84	4.19*	2.38	6.87**
C. D. at 5%	8.80	2.01	14.56	14.56	0.30	0.16	19.28	0.13	0.09	0.02	5.56
C.V. %	12.43	21.63	45.04	42.69	3.11	7.47	20.23	5.35	4.67	8.59	21.61
Heritability % (broad sense)	81.94	71.17	63.59	63.59	84.97	74.09	62.52	22.32	50.77	31.50	66.19

** : significant at 1 % level. * : significant at 5 % level

efficient of variations were recorded for total number of branches per plant and number of septa per capsule, while septa per capsule and seed length revealed highest and lowest value of heritability respectively (Table 8). In the M₄ lines, different parameters of different mutants have exhibited superiority over control plants (Table 8).

Finally it may be concluded from the present investigation that there exist variations for most of the traits among the control and mutant plant types when grown in a uniform agro-climatic conditions; and furthermore different sterility types have reduced gradually in most of the mutant lines in the subsequent generations. Thus, the present study has not only facilitated the scope of selection of the mutant types but also brightened the possibility of proper utilization of the plant types for better yield through intercrossing.

Summary

Quantitative variations regarding germination, survival, plant height, number of primary and total branches per plant, capsule length and chamber per fruit, filled seeds per capsule, seed size (length and thickness), 100-seed weight, harvest index and sterility types (flower, capsule, pollen and seed) were assessed in control and eight mutant types—*lax branching*, *feathery leaf*, *bushy*, *early flowering*, *prostrate*, *dwarf*, *brown seed-coat* and *viridis* in M₂, M₃ and M₄ generations. Year to year variations in yield attributing traits among control plants were studied. Analysis of variance performed in control and M₄ mutant lines revealed significant variations in most of the phenotypic traits.

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