



Appraisal of variability for agro-morphological traits in M₂ and M₃ generation of *Nigella sativa* L. induced through gamma irradiation.

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Abstract:

Nigella sativa L. (kalonji or black cumin) is an economically and medicinally important seed spice with considerable potential for improvement in seed yield and other agronomic traits. However, the narrow phenotypic variability in genetic resource limits the effectiveness of conventional breeding programme. The present study was undertaken to create and characterize induced variability through gamma-ray mutagenesis. A dose-dependent reduction in seed germination was observed, with reductions of 6.7-78.2% over the control. Based on the fitted logistic dose-response model, the LD₅₀ for gamma irradiation was estimated to be approximately 146 Gy. In the M₃ generation mean values for plant height, total branches, capsules per plant, locules per capsule, seeds per capsule, seed yield per plant, and 1000-seed weight increased by 4.6, 13.8, 16.8, 35.8, 21.5, 14.8, and 28.9%, respectively, compared with the control. The coefficient of variation ranged from 14.7% (1000-seed weight) to 24.6% (locules per capsule), indicating substantial variability and scope for further selection. A distinct macro-mutant was identified in the M₂ generation, characterized by complete absence of gynoeceum, induction of large number of greenish-yellow petals compared with five whitish petals in the parental type. Further evaluation of 45 selected M₃ progenies revealed substantial variation in yield-associated traits. Correlation analysis showed that seeds per capsule were positively and significantly associated with locules per capsule (0.69) and seed weight per capsule (0.52), whereas test weight showed significant negative associations with locules per capsule (-0.66). The number of seeds per locule was also negatively associated with locules per capsule (-0.55). Based on the correlation studied, up to seven locules/capsule appeared to provide an optimum balance between seed number/capsule and seed weight. Overall, the study demonstrated that gamma irradiation effectively broadened the variability in *N. sativa* L.

Keywords: *Nigella sativa* L., black cumin, gamma irradiation, induced mutation, phenotypic variability, LD₅₀, macro-mutant, yield attributing traits, correlation.

Introduction

Nigella sativa L., commonly known as black cumin or Kalonji, is an annual diploid herb (2n=2x=12) belonging to the family Ranunculaceae. In India, it is cultivated mainly in Madhya Pradesh, Telangana, Karnataka, Maharashtra, Tamil Nadu, Jharkhand, Bihar, West Bengal, Assam, Punjab, Uttar Pradesh, and Rajasthan. The seeds of *N. sativa* are an important source of bioactive compounds and are widely used as a spice and food preservative as well as in traditional

and therapeutic applications for various disorders (Sultana et al., 2015; Lal et al., 2020). The seeds contain approximately 30-40% fatty oil, comprising both saturated and unsaturated fatty acids, and 0.40-0.50% volatile oil, which has considerable industrial and nutritional importance (Gómez-Candela et al., 2011). Thymoquinone is the major bioactive constituent of *N. sativa* and has been associated with several biological activities, particularly antioxidant and anti-inflammatory effects. The volatile oil is also utilized as

a flavouring agent in food and beverages and in pharmaceutical industries (Abdel-Fattah et al., 2000). Despite its economic, nutritional, and medicinal importance, *N. sativa* productivity remains relatively low hence, considerable improvement is required in important agronomic, and quality attributes. The identification and development of genetically superior genotypes with high seed yield and desirable and quality characteristics are therefore important objectives of *N. sativa* breeding. However, the available germplasm exhibits relatively narrow genetic variability for seed yield and several yield-attributing traits which limits the rate of genetic improvement through selection. Induced mutation breeding approach is particularly useful in crops having a narrow genetic base, where naturally occurring variability are inadequate for the development of superior cultivars. Important traits requiring improvement include seed and oil yield, oil quality, maturity duration and tolerance to abiotic stress etc.

Mutation breeding has previously been employed in *N. sativa*, and mutants exhibiting desirable economic and morphological characteristics have been developed through gamma irradiation (Datta et al., 1985; Mitra & Bhowmik, 1997; Saha & Datta, 2002; Kumar & Gupta, 2007). In black cumin (*Nigella sativa* L.), some superior mutant lines were developed by induced mutagenesis through X-rays and gamma radiations and proper selection to improve the many yield attributing characters like lax branching, feathery leaf mutant, bushy, male sterile, crumpled leaf, early flowering, brown seed coat, etc. (Datta, 2005).

Gamma radiation can induce heritable changes in a wide range of traits, including plant architecture, flowering behaviour, capsule characteristics, test weight and seed yield. Similar improvements in yield-related traits and morphological diversity following gamma irradiation have also been reported in several aromatic and medicinal plant species (Rang et al., 2001, Agrawal and Kumar, 2021).

Despite previous efforts, information on the extent and nature of induced variability in *N. sativa* remains comparatively limited, particularly with respect to the systematic evaluation of mutant populations for agronomic traits. Therefore, there is a need to generate and characterize novel variability to facilitate the identification of superior genotypes. In this context, the present study was undertaken to generate and evaluate the phenotypic variability in morphological and agronomic traits to develop valuable genetic resources for their potential utilization in future breeding programmes.

Material and Methods

The *N. sativa* variety Ajmer Nigella-20 (AN-20), developed at the ICAR-National Research Centre on Seed Spices, Ajmer, was used for this mutation breeding programme. Approximately 10,000 dry seeds were irradiated for each treatment dose with seven doses of gamma radiation, viz., 50, 75, 100, 125, 150,

175 and 200 Gy, using ^{60}Co as the radiation source at the Bhabha Atomic Research Centre, Mumbai, in 2021. The irradiated seeds were subjected to germination tests to determine the reduction in germination percentage caused due to different doses of gamma radiation. The LD₅₀ was estimated using a two-parameter logistic dose-response model at 95% confidence interval, in which irradiation dose was the independent variable and reduction in germination (%) was the dependent variable. The dose corresponding to a 50% reduction in germination was estimated from the fitted curve (Ritz et al., 2015). The M₁ seeds from each treatment were planted separately at wider spacing than the recommended spacing. During the rabi season of 2022-23, the individually harvested M₁ plant progenies were grown as plant-to-row progenies in paired rows of 4.0 m length to raise the M₂ generation along with control. The M₂ generation was screened throughout the cropping period for phenotypic variations. The following observations were recorded to identify putative mutants: plant height, days to anthesis, days to maturity, number of capsules per plant, number of locules per capsule, number of seeds per locule, seed yield per plant, and 1000-seed weight.

The observed morphological variations were broadly classified into two categories viz. i.) Macro-mutants, involving conspicuous structural changes in traits such as flowers, capsules, and number of locules per capsule and ii.) Micro-mutants, involving quantitative or less conspicuous changes in traits such as days to flowering, days to maturity, 1000-seed weight, and disease resistance. Bulk of forty to fifty plants from M₂ progenies possessing desirable traits of interest were harvested in from different doses of irradiation separately. These progenies were subsequently evaluated in paired rows in M₃ generation during 2023-24. In M₃ generations, major agronomic traits, including number of fertile branches, number of capsules per plant, number of seeds per capsule, seed yield and 1000-seed weight, were evaluated. Statistical analyses, including mean, coefficient of variation and Pearson's correlation coefficient, were performed to assess the phenotypic variation in the quantitative traits.

Results and Discussion

Effect on seed germination

The seed germination test was conducted within one month of gamma irradiation using the top-of-paper method in petri plates under ambient laboratory conditions during November. The experiment was laid out in a randomized complete block design with four replications, each consisting of 25 seeds and germination was recorded on the seventh day. The temperature during the experimentation ranged from 27 to 32 °C. Gamma irradiation resulted in a significant dose-dependent reduction in seed germination, with differences among irradiation doses exceeding the critical difference of 6.4% at the 5% significance level (data not shown). Germination decreased by 6.7, 18.2, 38.5, 45.7, 52.6, 76.4, and 78.2% at irradiation doses of

50, 75, 100, 125, 150, 175, and 200 Gy, respectively (Fig. 1). The median lethal dose (LD_{50}) was estimated to be approximately 146 Gy by fitting a two-parameter logistic dose-response model. The present findings are consistent with those of Tasmin et al., (2022) who evaluated the effects of gamma irradiation at 50, 100, 150, 200, 250, and 300 Gy on *N. sativa*. They reported the highest seed germination (94.5–97.1%) at 50 Gy and the lowest germination (18.2–22.0%) at 300 Gy. Among the doses tested, LD_{50} close to 150 Gy was observed, resulting in approximately 51–55% reduction in seed germination and about 50% reduction in most seedling growth parameters. In the other studies by Kumar and Gupta (2007) identified 100 Gy as LD_{50} dose for inducing desirable morphological and agronomic variations in *N. sativa*. Amin et al. (2018), who evaluated gamma radiation doses of 50, 75, and

100 Gy in two varieties of *N. sativa* (AN-1 and VN-1), also reported reductions in seed germination of up to 40%. The response of seed germination to gamma irradiation is generally dose-dependent. Low doses may stimulate or slightly enhance germination by activating physiological and enzymatic processes, whereas higher doses progressively inhibit germination, germination rate, and seedling growth because of physiological disturbances and chromosomal damage (Maamoun et al., 2014). A similar dose-dependent reduction has been reported in fenugreek, where gamma irradiation reduced M_1 seed germination by 8.2, 17.3, and 26.5% and seedling length by 10.3, 23.3, and 42.2% at 250, 300, and 350 Gy, respectively (Meena et al., 2025).

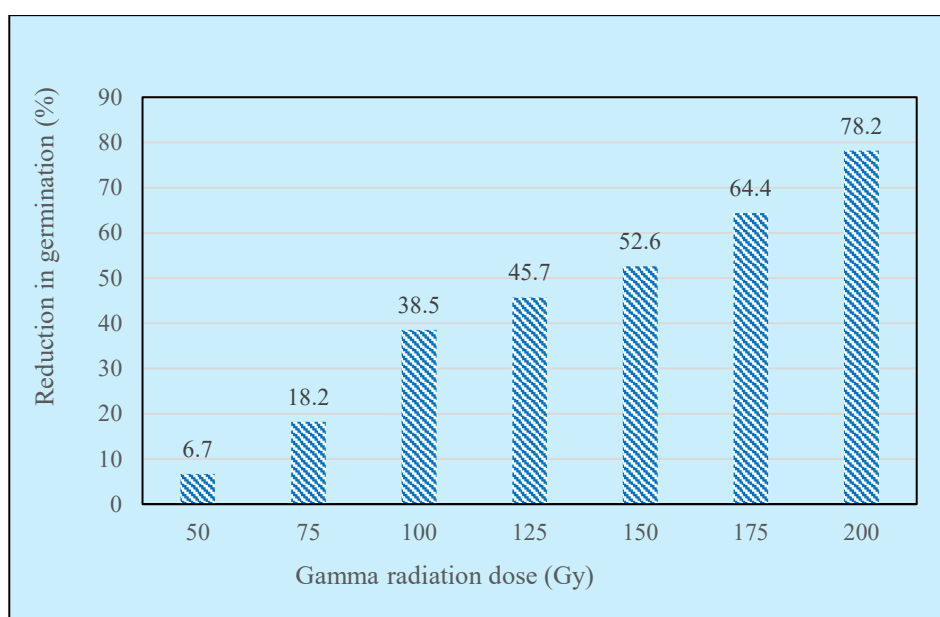


Fig. 1 Effect of gamma radiation dose on germination

Assessment of induced variability for agro-morphological traits:

Approximately 1000 M_1 individual plants from each dose viz. 75, 100, 125, 150, and 175 Gy of gamma irradiation were harvested separately. Plant-to-row progeny testing of all harvested plants were evaluated in paired rows of 4 m length. In the M_2 generation, each progeny were carefully evaluated for induced of variability for different agro-morphological traits. One hundred forty-five M_2 progenies were selected, and plants within each selected progeny were harvested in bulk. These selected progenies were subsequently planted in paired rows of 4 m length to raise the M_3 generation during 2023-24. From M_3 generation, 45 promising progenies were identified and observations were recorded for vital agro-morphological traits. The number of capsules per plant ranged from 68 to 98, with a mean of 72.4, compared with mean of 49.7 in the control. The number of locules/capsule varied

from 4 to 14, with a mean of 7.1, whereas in the control recorded a mean of 5.2. The 1000-seed weight ranged from 2.4 to 3.2 g, with a mean of 2.9 g compared with 2.2 g in the control. Similarly, seed yield per plant ranged from 15.4 to 26.6 g, with a mean of 20.4 g, compared with mean of 16.7 g in the control. A positive shift in the mean value was observed for all these traits. The increase in mean values ranged from 4.6% for plant height to 35.8% for the number of locules per capsule. The gains in mean values 1000-seed weight, number of seeds per capsule, number of capsules per plant, and seed yield per plant were 28.9, 21.5, 16.8, and 14.8%, respectively, compared with the parental variety. These results indicates that gamma irradiation successfully generated substantial variability in the segregating populations of *N. sativa* which may provide opportunities for further selection for enhancing seed yield through attributing traits. The coefficient of variation (CV) ranged from 14.7%

(1000-seed weight) to 24.6% (number of locules/capsule) indicating considerable variation among the selected progenies. The relatively high magnitude of variability suggests sufficient scope for undertaking additional cycles of selection to improve desirable traits in subsequent generations (Table 1).

The present findings are supported by Elhafez et al. (2025), who evaluated gamma irradiation doses of 50, 100, and 150 Gy in *N. sativa*. They reported that 100 Gy significantly increased plant height, number of branches, fresh and dry weight/plant, number of capsule/plant, 100-seed weight, and seed yield/plant, whereas 150 Gy significantly enhanced oil and thymoquinone contents. Gholizade et al. (2019) observed substantial variation in essential oil ranging from 0.65 to 1.36% and fatty oil content ranging from 25.30 to 35.02%. Similarly, Kumar and Gupta (2007) reported that gamma-ray treatments ranging from 50 to 300 Gy induced substantial morphological variation in *N. sativa*. Notably, a hexapetalous mutant was identified at 100 Gy, which showed increased seed number and seed weight compared with the control. Overall, the results demonstrate that gamma irradiation is an effective tool for inducing and expanding novel variability in *N. sativa*.

Detection of induced macro-mutants:

A macro mutant AN-20/150/126 was observed in M₂ generation where the petals have transformed and increased radically in number petals more than hundred as compared to five and colour become greenish yellow as compared to white in parental line. The gynoecium was absent in all the plants of this progeny hence no seed set was taken placed hence the variant could not be carried forward (Fig. 3). It is evidenced that the *N. sativa* is very much responsive to the mutation through gamma irradiation. A number of induced morphological traits, yield components in

the nine selected mutants (Mt1-Mt9) have been reported in M₃ and M₄ of *N. sativa* to determine the best genotype to release as a new cultivar. The results showed that Mt2 plants were taller in height. Mutants Mt5 and Mt6 are promising high yielding genotypes identified as new cultivars, whereas Mt3 and Mt7 possess an improved quality for ornamental use (El-Mahrouk et al.; 2020). The variations in the plant morphology such as rudimentary and poor development of leaflets, stunted and poorly-branched plants, reduction in height, and number of pods and number of seeds per pod were commonly observed in fenugreek with gamma irradiation at 300 and 350 Gy (Meena et al. 2025).

On the other hand, four mutant progenies viz. AN-20/150/54, AN-20/150/77, AN-20/150/118 and AN-20/175/102 exhibited varied number of locules/capsule ranged from 4-12 across the plants within and between progeny (Fig. 3). In the direction of selection of progenies with optimum number of locules/capsules further selection cycles are needed to develop genotypes with enhance seed yield through increasing of locules/capsule. Asif and Ansari (2019) studied effect of gamma radiation (50, 100, 150, 200 and 250 Gy) and combination of EMS in *N. sativa* variety Ajad Kalonji-1 and reported wide variability in M₂ generation. Variability for floral colour (yellow and pink coloured flowers), organ phenotypes like the shape of petaloid sepals, number of carpels, colours of anther (black-coloured type, multi-coloured type and brown-coloured types), number of locules/capsules ranged from 2 to 20 and number of seeds/capsule. Their findings demonstrated that induced mutagenesis can effectively increase the frequency and spectrum of useful variation in *N. sativa*.

Table 1: Range, mean, coefficient of variation and shift in mean based on 145 M₃ progenies as compared to control in *N. sativa* variety AN-20

Gamma dose	Parameters	Plant height (cm)	Total no. of branches	No. of capsules/plant	No. of locules/capsule	No. of seed/capsule	Seed yield/Plant (g)	1000 seed weight (g)
Control (Untreated)	Minimum	49.5	11.5	38.9	4.0	71.5	14.5	1.9
	Maximum	62.8	22.4	56.8	6.0	93.7	19.7	2.8
	Mean	58.4	17.2	49.7	5.2	77.2	16.7	2.4
	CV (%)	9.5	11.8	16.4	9.8	13.4	8.7	8.9
145 progenies of 75, 100, 125, 150 and 175 Gy	Minimum	35.4	3.4	18.5	4.0	52.1	15.4	1.6
	Maximum	75.6	32.8	78.3	14.5	109.5	26.6	3.2
	Mean	61.1	19.6	58.0	7.1	93.8	19.2	3.1
	CV (%)	16.3	18.7	21.4	24.6	16.8	23.1	14.7
	Shift in mean (%)	4.6	13.8	16.8	35.8	21.5	14.8	28.9

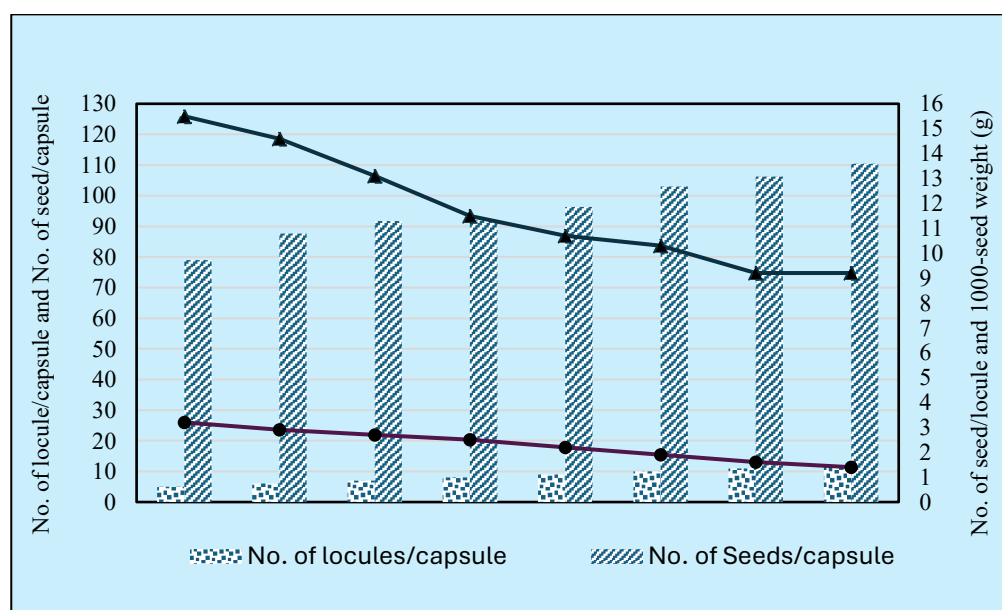
Table 2: Study on different yield attributing traits in M₃ progenies of *Nigella*

No. of locules/ capsule	No. of Seeds/ capsule	No. of Seeds/ locule	Test weight (g)	Seed wt./ capsule (g)
4	72.7	16.3	2.18	0.231
5	79.0	15.8	2.92	0.229
6	87.6	14.6	2.99	0.254
7	91.7	13.1	2.84	0.257
8	92.0	11.5	2.62	0.239
9	96.3	10.7	2.28	0.212
10	103.0	10.3	2.17	0.216
11	101.2	9.2	1.93	0.192
12	110.4	9.2	1.92	0.210
Control	68.7	16.2	2.15	0.252

Correlation studies

Seed yield is a quantitative and complex inherited trait that is highly affected by environmental conditions (Emamgholizadeh et al. 2015). Therefore, yield attributing traits can be identified through analysis of relationships among seed yield and vital attributing traits. Forty-five M₃ progenies were subjected to correlation studies which exhibited higher in test weight, a greater number of capsules/plants, more locules/capsule, a greater number of seeds/capsules as compared to parental variety. Observation of these parameters was taken from thirty plants (n=30 and p=0.05) per progeny. Number of locules/capsule of selected progenies ranged from 7-12 as compared to mean value of 5.2 in parental variety. Correlation studies revealed that number of seeds/capsule positive and significantly correlated with locule number/capsule (0.69) and seed weight/capsule (0.52) while, test weight was decreased with increasing locule numbers/capsule (-0.66) and number of

locules/capsule and seeds/locules were also found negatively associated (-0.55). Correlation studies among above traits revealed that 6-7 locules/capsules found optimum number for better yield through better test weight and optimal number of seeds/capsule. Sabaghnia (2024) reported in correlated study of 27 genotypes of *N. sativa* that traits such as the number of seeds per follicle (locule) and number of follicles per plant is positive correlated while 1000 seed weight was negatively correlated with seed yield. Gashaw (2020) reported that correlation analysis on thirty-six *N. sativa* accessions showed that seed yield had positive and significant correlation with number of effective capsules (0.88), plant height (0.72), total branches (0.71), harvesting index (0.47). Bardideh, (2013) conducted study for correlation on *N. sativa* and reported that seed yield was positive and significantly correlated with biological yield, number of capsules/plant, thousand seed weight and number of branches.

**Fig. 2** Inter relationship among No. of locule/capsule, No. of seed/capsule, No. of seed/locule and 1000-seed weight (g)

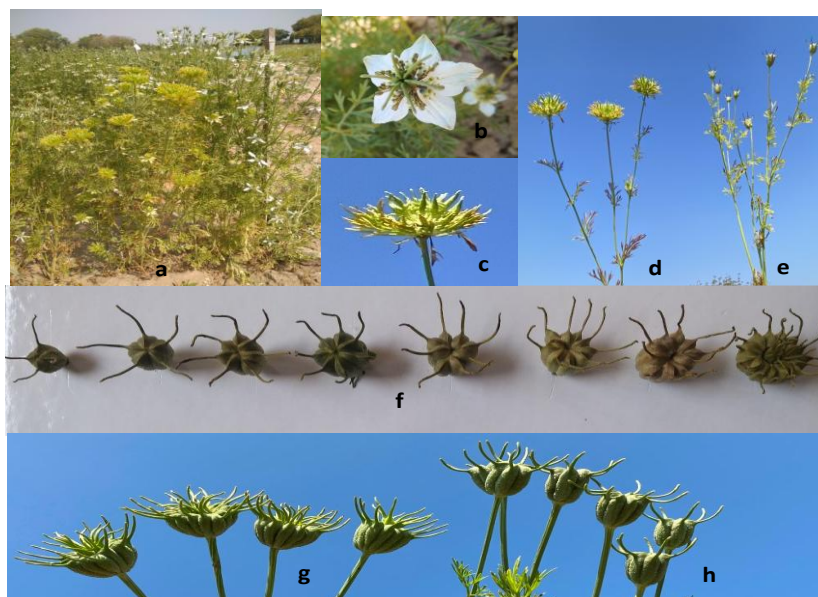


Fig. 3 Morphological variations induced through gamma irradiation observed:

- a) mutant progeny with capsule converted like a flower
- b) Normal flower
- c) mutant flower
- d) Mutant plant

- e) normal plants
- f) variation for locules/capsule
- g) mutant capsules
- h) normal capsules

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Conflict of interest

The authors declare that they have no financial, personal, professional, or institutional conflicts of interest that could have influenced this research or its reporting.

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