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Induced Morphological and Chromosomal Diversity in the Mutagenized Population of Black Cumin (*Nigella sativa* L.) Using Single and Combination Treatments of Gamma Rays and Ethyl Methane Sulfonate

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Abstract

Induced mutagenesis has successfully been proven as the best viable approach for the genetic improvement of crop species. In the present scenario of high health vulnerability, the global demand for natural medicine derived from plant species has increased tremendously. Black cumin (*Nigella sativa* L.) – an important medicinal plant species of the family *Ranunculaceae* with immense therapeutic values, was selected for the present study in order to bring genetic improvement using the technique of induced mutagenesis. Dry and healthy seeds of two varieties of black cumin were exposed to different doses of gamma rays and EMS singly and in combination. The observations were recorded on morphological, cytological and physiological parameters in M_1 and M_2 generations to evaluate the mutagenic potency and to induce the desirable genetic variability in the crop. A broad spectrum of morphological variations with different frequencies affecting different plant parts and chromosomal aberrations were screened out in both the varieties in M_1 generation. Cytological abnormalities increased with increasing the doses/concentrations of the mutagen. The results reflect an increase in the mean values for chlorophyll and carotenoid contents at the 0.1 % EMS treatment in both varieties indicating an improved photosynthetic activity in this treatment. The rest of the treatments showed a decreasing trend in relation to the controls with increasing the mutagenic doses/concentrations vis-à-vis chlorophyll and carotenoid contents in both varieties. Observations on quantitative traits including plant height, number of fertile branches, number of capsules per plant, number of seeds per capsule and 1000 seed weight (g) showed significant inter-treatment variations at different mutagenic doses. A positive correlation among various yield attributing traits was recorded in M_2 generation. The findings of the present study are encouraging, and show that significant genetic variability had been induced by the mutagens, thus the rigorous selection of the desirable mutants may result in the development of improved and high yielding mutants of *Nigella sativa* in subsequent generations.

Keywords: Mutagens, Morphological variants, Chromosomal aberrations, Quantitative traits, *Nigella sativa*, Gamma radiation.

1. Introduction

Almost two third of the world's plant species have medicinal value in one way or another. Different plant parts such as stem, bark, leaves, seeds, and fruits are used for the treatment of a wide range of cardiovascular and inflammatory diseases. *Nigella sativa,* commonly known as black cumin, is an annual diploid (2n = 2x = 12) multi-branched herb with a great medicinal and therapeutic importance (Kirtikar and Basu, 1982; Chopra *et al.*, 1982). Most evidences show that black cumin is native of the Middle East and Western Asia (Iqbal *et al.,* 2010). In India, it is mostly grown in M.P., Bihar, Assam and Punjab. The other small scale cultivated states of India are U.P., Rajasthan, West Bengal and Tamil Nadu (Malhotra and Vashishtha, 2008).

Induced mutation has become increasingly popular in recent times as an effective tool for crop improvement. The mutant varieties developed in major crops have been cultivated by farmers in large areas and have boosted the food production, thus contributing to food security (Suprasanna *et al.*, 2016). Chemical mutagens have gained immense popularity, since they are easy to use, and can induce mutation at a very high rate (Raina *et al.*, 2016). Ethyl methane sulfonate (EMS) is an alkylating agent that donates alkyl group i.e., ethyl group (CH_3-CH_2) to the guanine producing O^6-ethyl guanine which pair with thymine to eventually produce point mutations. EMS induces miss pairing and base changes due to chemical modification of nucleotides (Greene *et al.*, 2003). Gamma rays, the highly energetic ionizing radiations constitute the most efficient and effective physical mutagen. Gamma rays are known to have a higher penetration power, and hence can induce various changes at the molecular level.

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Mutation breeding, a much heralded shortcut breeding method mainly based on conventional breeding approaches, brings novel and high yielding genotypes through heritable changes (Singh *et al*., 2011; Laskar *et al.*, 2018; Ramachander *et al*., 2018; Verma *et al.*, 2018; Wani, 2018). It is generally employed to improve various agro-economically important traits such as seed yield, oil quantity and quality, etc. in different oil seed crops including *Nigella*. In the past, although different mutants in *N. sativa* have been created which include bushy and dwarf habit, feathery leaf, lax branching, early flowering and brown seed coat color (Datta *et al*., 1986), yet very limited work has been done vis-à-vis the induction of useful genetic variability which could lead to the genetic improvement of this crop. In this back drop, the present study was aimed at the generation of such micro- and macro-mutations in the agro-economic traits of *N. sativa* which could be propagated and exploited in subsequent generations for better profit. In addition, cytological abnormalities were estimated for establishing the sensitivity of the crop towards the mutagens applied.

2. Materials and Methods

2.1. Experimental Site

Aligarh, the site of present study, has a characteristic semi-arid and sub-tropical climate with hot dry summers and cold winters. The average rainfall in this district is 847.30 mm, while the average temperature is 35^{o}C and 15^{o}C during summer and winter, respectively. The soil of Aligarh is sandy loam and alkaline.

2.2. Biological Material

The experimental plant material selected for the present investigation is *N. sativa* L., commonly known as black cumin. Two varieties viz., NRCSSAN-1 and BHUVN-1 were used and the seeds were procured from National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh, India.

2.3. Experimental Procedure

Dry (moisture content 10-12%) and healthy seeds of black cumin (*N. sativa* L.) were used for the mutagenic treatments of EMS and gamma rays alone as well as in a combination. Seeds from each variety were distributed into thirteen sets of fifty seeds each for this experiment. One set of the seeds was taken as control, whereas the rest twelve sets were treated with different doses/ concentrations of mutagens i.e., four sets of seeds were irradiated with gamma rays (25Gy, 50Gy, 75Gy and 100Gy at a dose rate of 11.58 Gy/sec), four sets with different concentrations of EMS (0.1% EMS, 0.2% EMS, 0.3% EMS and 0.4% EMS) and four sets were treated with combination treatments (25Gy + 0.1% EMS, 50Gy + 0.2% EMS, 75Gy+ 0.3% EMS and 100Gy+0.4% EMS). As for the chemical treatment, pre-soaked seeds were subjected to a six-hour treatment with intermittent shaking at a room temperature of 25±2^{o}C. The gamma ray treatment was done with radioisotope ^{60}Co source at the National Botanical Research Institute, Lucknow, Uttar Pradesh, India. As for the combination treatments, the irradiated seeds were treated with EMS doses similarly as individual treatments. The treated seeds were then sown at University Agricultural Farm, Aligarh Muslim University, Aligarh in five replications of ten seeds per treatment to raise M_1 generation during the rabi season of 2015-2016.

For cytological studies, the flower buds of the treated and control plants were randomly collected from each replication separately, and the chromosome preparations were stained with 1 % acetocarmine solution and examined microscopically. Frequency of meiotic abnormalities was calculated according to Khursheed *et al.* (2015). The percentage of seed germination, pollen fertility and plant survival at maturity were calculated for each treated and control population according to Wani *et al.* (2011a). The seeds of normal-looking M_1 plants were harvested treatment wise individually and advanced for raising the M_2 generation in the plant progeny row during rabi season of 2016- 2017. Data on the morphological variations were taken throughout the season and tabulated according to the phenotypic category. Chlorophyll and carotenoid contents from fresh secondary emergent leaflets were extracted in 80 % acetone in mg.g^{-1} and estimated according to MacKinney (1941). In the M_2 generation, the breeding behavior was observed and different agronomic traits such as the number of fertile branches, the number of capsules per plant, the number of seeds per capsule and 1000 seed weight (g) were evaluated. Statistical analysis, namely mean, standard error, standard deviation, coefficient of variation (CV), analysis of variance (ANOVA), Duncan multiple range test (DMRT) and Pearson's correlation coefficient (*r*) were done using IBM SPSS 20 software to assess the induced intra- and inter population variations in these quantitative traits.

3. Results

3.1. Bio-physiological Parameters in M_1 Generation

Seed germination in the var. NRCSSAN-1 was recorded as 90.00 % in the control; however it decreased with increasing the concentrations of EMS, gamma rays and their combination. For var. NRCSSAN-1, germination was 80 % with 0.1 % EMS, while for gamma rays and combination treatments; it was 60 % with 100 Gy and 0.4 % EMS+100Gy. In the var. BHUVN-1, the seed germination was recorded as 80.00 % in the control and decreased to 50 % at 0.4 % EMS +100 Gy (Table 1). The pollen fertility decreased in a regular pattern with increasing the mutagenic concentrations. In the var. NRCSSAN-1, the pollen fertility decreased from 92.40 % in control to 77.00 % in 0.2 % EMS +50 Gy, whereas it decreased from 92.80 % in the control to 75.40 % with 0.2 % EMS +50 Gy in the var. BHUVN-1 (Table 1). The survival of plants at maturity decreased irregularly with respect to the controls of both the varieties when increasing the concentrations of the mutagens. The survival of untreated plants at maturity was 96 % and 95 % for the varieties NRCSSAN-1 and BHUVN-1 respectively, while it decreased from 92 % (0.1% EMS) to 75 % (0.4 % EMS +100 Gy) in NRCSSAN-1 and from 90 % (0.1% EMS) to 73 % (0.4 % EMS +100 Gy) in the var. BHUVN-1 (Table 1).

Table 1. Estimates of seed germination, pollen fertility, chlorophyll, and carotenoid contents in M_1 generation of *Nigella sativa*.

Treatment	Seed germination (%)		Pollen fertility (%)		Survival (%) at maturity		Chlorophyll content (mg/g)		Carotenoid content (mg/g)	
	NRCSS AN-1	BHUVN-1	NRCSS AN-1	BHUVN-1	NRCSS AN-1	BHUVN-1	NRCSS AN-1	BHUVN-1	NRCSS AN-1	BHUVN-1
Control	90	80	92.40	92.80	96	95	0.550	0.545	0.280	0.277
0.1%EMS	80	80	91.40	90.80	92	90	0.578	0.566	0.293	0.282
0.2%EMS	70	70	90.00	89.80	92	88	0.530	0.520	0.245	0.249
0.3%EMS	60	60	88.60	87.20	83	85	0.510	0.505	0.221	0.215
0.4%EMS	60	60	87.40	86.20	80	81	0.520	0.500	0.215	0.211
25 Gy	70	70	86.60	86.00	90	88	0.469	0.462	0.267	0.261
50 Gy	70	60	84.40	84.80	88	87	0.444	0.431	0.237	0.229
75 Gy	60	60	82.40	80.80	85	82	0.419	0.398	0.214	0.221
100 Gy	60	60	82.40	77.80	78	79	0.400	0.410	0.203	0.198
0.1%EMS + 25 Gy	70	70	78.60	77.00	89	85	0.433	0.351	0.219	0.208
0.2%EMS +50 Gy	60	60	77.00	75.40	86	83	0.367	0.303	0.214	0.199
0.3%EMS +75 Gy	60	60	80.60	81.40	79	75	0.315	0.287	0.192	0.186
0.4%EMS +100 Gy	60	50	77.20	76.20	75	73	0.258	0.243	0.187	0.183

3.2. Chlorophyll and Carotenoid Contents (mg/g Fresh Weight of Leaf)

The total chlorophyll and carotenoid contents in the M_1 generation decreased with increasing the concentrations of the mutagens except at 0.1 % EMS where it showed an increase over the controls in both varieties. The highest chlorophyll (0.578 mg/g and 0.566 mg/g) and carotenoid (0.293 mg.g-1 and 0.282 mg.g-1 FW) contents were noticed with the treatment of 0.1 % EMS in the varieties NRCSSAN-1 and BHUVN-1 respectively. The combination treatments showed more reduction with respect to chlorophyll and carotenoid contents as compared to the individual ones (Table 1).

3.3. Cytological Studies in M_1 Generation

The potency of mutagens is reliably estimated by cytological observations in M_1 generation. In this context, chromosomal abnormalities were studied in both varieties at different stages of meiotic division. The chromosomal variations include univalent, multivalent, laggards, bridges, stickiness, disturbed polarity, multinucleate condition and unequal separation of chromosomes at various stages. At the metaphase stage, the pollen mother cells (PMCs) with multivalent, precocious movement, stray chromosomes and chromosomal stickiness were observed in the treated population. The abnormalities increased at metaphase I/II with increasing the concentrations of the mutagens in both varieties. The induced anaphase abnormalities consisted of laggards, bridges and an unequal separation of chromosomes. The frequency of such abnormalities had increased significantly with increasing the mutagenic concentrations. The main chromosomal aberrations observed at telophase I/II were laggards, bridges, micronuclei, multinucleate condition and disturbed polarity (Plate I; Figures A-L). The maximum frequency of 22.46 % and 26.53 % chromosomal abnormalities was noticed with the treatment of 0.4 % EMS+100 Gy in the varieties NRCSSAN-1 and BHUVN-1 respectively (Tables 2 and 3).

Table 2. Frequency and spectrum of chromosomal abnormalities induced by EMS, gamma rays, and their combination in M_1 generation of *Nigella sativa* var. NRCSSAN-1.

Treatment	Total No. of PMCs observed	Metaphase-I/II					Anaphase-I/II				Telophase- I/II						
		Multivalent	Precocious movement	Stray chromosomes	Stickiness	Abnormalities (%)	Laggards	Bridges	Unequal separation	Abnormalities (%)	Laggards	Bridges	Micro nucleate	Multi nucleate	Disturbed Polarity	Abnormalities (%)	Total (%) of Abnormalities
Control	270	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1% EMS	263	1	-	-	1	0.76	-	-	-	-	-	-	1	-	-	0.38	1.14
0.2%EMS	271	2	2	-	3	2.58	1	2	1	1.47	-	-	1	1	-	0.73	4.78
0.3%EMS	230	2	1	1	2	2.60	1	-	3	1.73	-	-	1	-	2	1.30	5.63
0.4%EMS	250	2	1	1	3	2.80	1	-	4	2.00	1	-	-	2	3	2.40	7.20
25 Gy	260	1	1	-	1	1.15	-	-	-	-	-	-	1	-	1	0.76	1.91
50 Gy	264	1	1	-	1	1.13	-	1	-	0.37	-	1	-	1	-	0.75	2.25
75 Gy	255	-	3	2	3	3.13	1	1	2	1.56	-	1	1	3	-	1.96	6.65
100 Gy	276	4	3	2	4	4.71	3	4	2	3.26	2	-	2	2	2	2.89	10.86
0.1%EMS + 25 Gy	267	3	3	3	4	4.86	4	2	3	3.37	1	1	1	2	3	3.03	11.26
0.2%EMS +50 Gy	284	4	6	4	4	6.33	5	3	3	3.87	2	1	2	3	3	3.87	14.07
0.3%EMS +75 Gy	232	4	5	3	4	6.89	4	6	3	5.60	2	1	2	2	3	4.31	16.80
0.4%EMS +100Gy	267	6	7	5	6	8.98	7	6	5	6.74	2	3	5	4	4	6.74	22.46

Table 3. Frequency and spectrum of chromosomal abnormalities induced by EMS, gamma rays, and their combination in M_1 generation of *Nigella sativa* var. BHUVAN-1.

Treatment	Total No. of PMCs observed	Metaphase-I/II					Anaphase-I/II				Telophase- I/II						
		Multivalent	Precocious movement	Stray chromosomes	Stickiness	Abnormalities (%)	Laggards	Bridges	Unequal separation	Abnormalities (%)	Laggards	Bridges	Micro nucleate	Multi nucleate	Polariy	Disturbed	Abnormalities (%)	Total (%) of Abnormalities
Control	264	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1% EMS	253	1	-	1	1	1.18	-	-	-	-	-	-	1	-	-	0.39	1.57
0.2%EMS	238	2	1	-	2	2.10	1		1	0.84	-	-	1	1	-	0.84	3.78
0.3%EMS	219	2	1	1	2	2.73	1	-	3	1.82	-	-	1	-	2	1.36	5.91
0.4%EMS	217	2	2	1	2	3.22	-	2	4	2.76	1	-	-	2	3	2.76	8.74
25 Gy	260	1	-	1	1	1.15	-	1	-	0.38	-	-	-	1	1	0.76	2.29
50 Gy	255	1	1	-	1	1.17	-	1	1	0.78	-	-	1	1	1	1.17	3.12
75 Gy	245	2	2	1	2	2.85	1	1	2	1.63	-	-	1	2	2	2.04	6.52
100 Gy	227	3	2	2	3	4.40	3	3	2	3.52	2	-	1	1	2	2.64	10.56
0.1%EMS + 25 Gy	265	3	3	3	3	4.52	3	2	2	2.64	1	1	1	1	3	2.64	9.80
0.2%EMS +50 Gy	244	4	3	3	4	5.73	4	1	3	3.27	2	1	2	2	3	4.09	13.09
0.3%EMS +75 Gy	233	4	5	3	3	6.43	4	6	-	4.29	2	1	2	2	3	4.29	15.01
0.4%EMS +100Gy	211	5	4	5	7	9.95	6	7	5	8.53	2	3	3	5	4	8.05	26.53

3.4. Morphological Variations

Variations in cotyledonary and leaf morphology were observed after mutagenic treatments in both varieties. At the time of germination, a broad spectrum of cotyledonary leaf variants such as single (mono), three (tri) and four (tetra) cotyledonary leaves were observed in the treated population. Besides, variation in the number of cotyledonary leaves, change in cotyledonary leaf shape such as the needle-shaped and deformed leaves were also noticed (Plate II; Figures A-F). Other morphological variations include the increased number of leaflets (9-11) and a changed pattern of leaflets from alternate to opposite in some treatments (Plate II; Figures K-1-2).

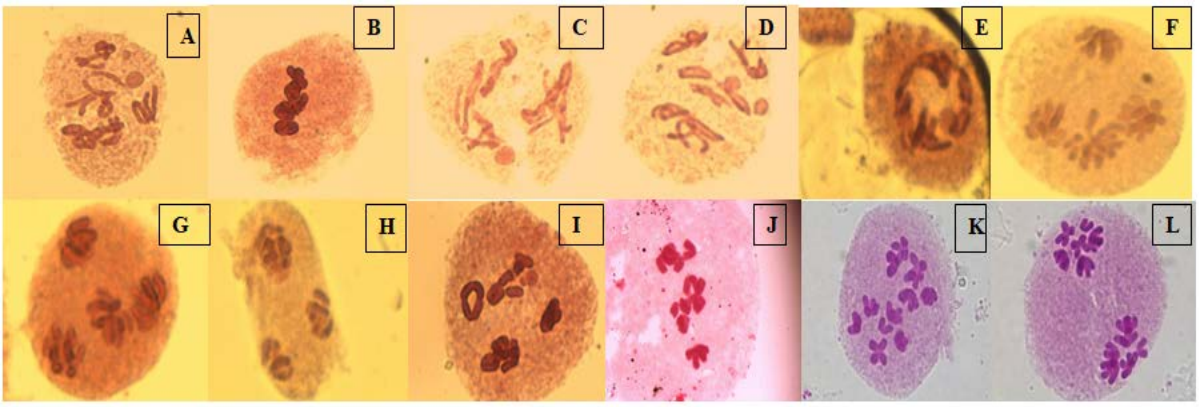

Plate I. Various chromosomal abnormalities observed in M_1 generation of *Nigella sativa* L.

A. Diakinesis showing bivalents and univalent with micronucleus; **B**. Chromosomal stickiness at metaphase- I; **C**. Disorientation of bivalents at diakinesis; **D.** Ring and rod-shaped bivalents ; **E**. Clumping of bivalents; **F**. Unequal separation at telophase- II with micronucleus; **G**. Unequal separation at anaphase- II; **H**. Tripolar telophase- II; **I**. Chromosomal stickiness at diakinesis; **J**. Chromosome clumping at diakinesis; **K**. Chromosomes moving towards poles at anaphase I (control); **L**,Telophase- I (control).

Three different types of chlorophyll variants, namley chlorina, xantha and albina were also recorded (Plate II; Figures G, I, J-2) in M_1 generation. The combined treatment of EMS and gamma rays showed a higher number of chlorophyll variants compared to the individual treatments in both varieties. The observation revealed that the leaf morphology was the most sensitive towards the applied doses of mutagens. The arrangement of petals in flowers and the number of petals varied from five in the control to six in the treated population. The other variation includes the flower which had leafy petals (Plate II; Figures L-N). Alteration in capsule size, i.e. small, bold, narrow and also the number of locules in capsule varied from five in the control to six, ten and thirteen in the treated population (Plate II; Figures O-1-4). On comparing different treatment doses, it was found that 0.4 % EMS+100 Gy generated the highest number of morphological variants followed by gamma rays and EMS in both varieties.

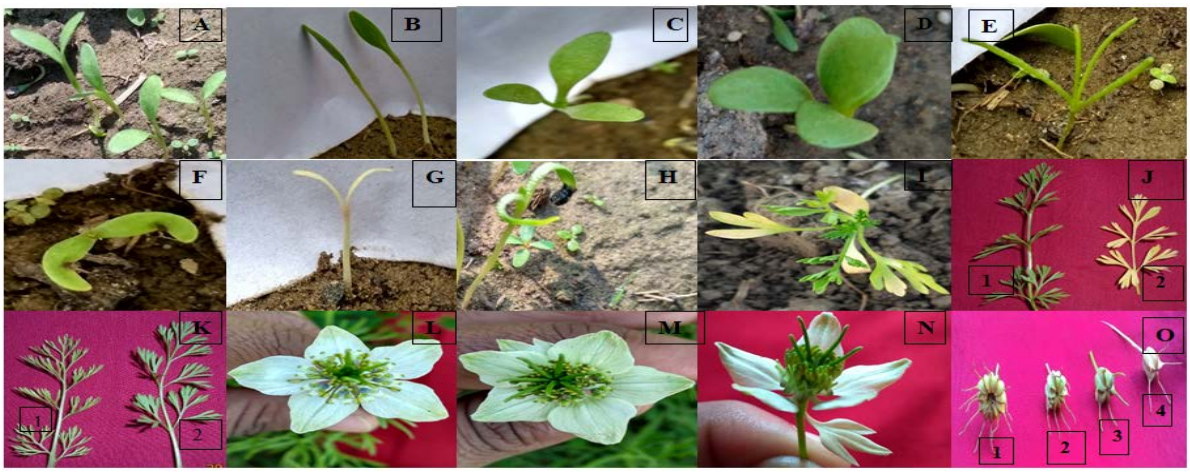

Plate II. Various morphological variants observed in M_1 generation of *Nigella sativa* L.

A. Bicotyledon plant (control); **B**. Monocotyledon ; **C**. Tricotyledon ; **D**. Tetracotyledon **; E**. Needle-shaped tetra cotyledon;**F**. Deformed cotyledon ; **G**. Albina cotyledon ; **H**. Horn-shaped cotyledon ; **I**. Xantha plant ; **J**. 1. Control plant 2. Chlorina plant; **K**. 1. Leaf with alternate leaflets (control) 2. Leaf with opposite leaflets; **L**. Flower with five petals (control); **M**. Flower with six petals; **N**. Flower with leafy petal ; **O**. Variation in locules number per capsule 1. Thirteen 2. Ten 3. Six 4. Five (control).

3.5. Quantitative Traits in M_2 Generation

The mean plant height of 60.14 cm and 59.58 cm was noticed in the controls of the varieties NRCSSAN-1 and BHUVN-1 respectively. The range of mean plant height was 56.96 cm (0.4 % EMS + 100 Gy) to 68.68 cm (25 Gy) in the var. NRCSSAN-1, while in the var. BHUVN-1 it was 54.76 cm with 0.4 % EMS + 100 Gy to 66.20 cm with 25 Gy (Table 4). The mean number of fertile branches decreased from 3.8 in control to 2.4 at 0.4 % EMS +100 Gy in the var. NRCSSAN-1, whereas in the var. BHUVN-1 it decreased from 5.80 in the control to 3.60 at 0.4 % EMS +100 Gy treatment (Table 4).

The number of capsules per plant increased from 7.8 (control) to 8.4 (0.1 % EMS) in the var. NRCSSAN-1 (Table 4), while in the rest of the treatments, it showed a decrease with respect to the control in both varieties. The number of seeds per capsule ranged from 72.40 to 78.60 in the var. NRCSSAN-1, whereas it ranged from 72.00 to 78.20 in the var. BHUVN-1 (Table 4). Mean 1000 seeds weight (g) in the control population was 2.02 g and 1.98 g in the varieties NRCSSAN-1 and BHUVN-1 respectively. In the var. NRCSSAN-1, the mean 1000 seed weight ranged from 1.62 g (0.4 % EMS+100Gy) to 2.02 g (0.1 % EMS) and in the var. BHUVN-1, the range was 1.56 g with 0.4 % EMS +100 Gy to 2.06 g with 0.1 % of EMS treatment (Table 4). Correlation studies among various pairs of yield attributing traits in M_2 generation are presented in Table 5. A positive correlation was seen among different yield contributing traits in both varieties.

4. Discussion

In the present investigation, seed germination, plant survival at maturity, and pollen fertility declined with increasing mutagenic concentrations. However, the extent of decrease was different with different mutagenic treatments as also reported earlier by different workers regarding various crop plants (Bhat *et al.*, 2006; Khan *et al.*, 2009; Jafri *et al.*, 2011; Amin *et al.*, 2015). The decrease in seed germination after mutagenic treatments has been ascribed to chromosomal aberrations, disturbed DNA and auxin synthesis and impaired cell metabolism (Kirtane and Dhumal, 2004). Progressive decrease in the rate of plant survival when increasing the doses of physical and chemical mutagens has been previously reported by Jayabalan and Rao (1987) in *Lycopersicon esculentum*, Kumar and Dubey (1998) in *Lathyrus sativus* and Amin *et al.* (2016) in *Nigella sativa*. Physiological imbalances and/or different types of chromosomal aberrations could be the main cause for the decreased plant survival (Khursheed *et al.*, 2015).

The magnitude of pollen sterility also increased with increasing the concentrations of mutagens. High pollen sterility was recorded with higher doses of individual and combination treatments. These results are in agreement with Kumar and Dubey (1998) and Jafri *et al.* (2011) who also reported a dose dependent increase in pollen sterility after mutagenic treatments. Estimation of chlorophyll content showed a wide variation in the mutagen-treated population. In this study, the total chlorophyll content decreased with increasing the mutagenic concentrations. Photosynthetic activity is greatly dependent upon chlorophyll content (Larcher, 1995). During the onset of the flowering phase, the greater content of chlorophyll which takes part in the process of organogenesis was observed (Simova-Stoilova *et al.*, 2001).

The frequency of morphological variants increased with increasing the mutagenic dose. The maximum frequency was observed with combination treatments of EMS and gamma rays. The variations in leaf morphology such as unequal notches, rudimentary and poor development of leaflets, stunted and poorly-branched plants were commonly observed in this study. Similar abnormalities have also been reported by various workers in *Lens culinaris* (Amin *et al.*, 2015), *Cicer arietinum* (Laskar *et al.*, 2015), *Choris gayana* (Krishna *et al.*, 1984), *Vicia faba* (Kumar *et al.*, 1993) and *Vigna radiata* (Wani *et al.*, 2011b). The leaf abnormalities have been mainly ascribed to chromosomal aberrations (Blixt, 1972; Grover and Virk, 1986.)

Table 4. Statistical analysis of various quantitative traits in M_2 generation of *Nigella sativa* L. varieties NRCSSAN-1 and BHUVN-1.

Treatment	Plant height Mean ± SE, CV%, shift in mean		No. of fertile branches Mean ± SE, CV%, shift in mean		No. of capsule per plant Mean ± SE, CV%, shift in mean		No. of seeds per capsule Mean ± SE, CV%, shift in mean		1000 seeds weight (gm) Mean ± SE, CV%, shift in mean	
	NRCSSAN-1	BHUVN-1	NRCSSAN-1	BHUVN-1	NRCSSAN-1	BHUVN-1	NRCSSAN-1	BHUVN-1	NRCSSAN-1	BHUVN-1
Control	60.14±1.09, 4.04, -	59.58^{bc}±1.50, 5.36, -	3.8^{a} ± 0.37, 22, -	5.8^{a}±0.48, 18.79, -	7.8^{ab} ± 0.2, 5.37,-	7.6^{a} ± 0.24, 7.19, -	78.20^{ab}±0.66, 1.89, -	76.8^{ab}±0.66 ,1.93,-	2.02^{a}± 0.03, 4.10, -	1.98^{a}± 0.03, 4.19, -
0.1% EMS	61.58^{b}±0.23, 0.83, 1.44	61.58^{ab}±0.22, 0.83, 2.0	3.6^{ab}±0.24, 15.19, -0.2	5.4^{ab}±0.24, 10.12, -0.4	8.4^{a} ± 0.24, 6.51, 0.6	7.6^{a} ± 0.24, 7.19, 0	78.60^{a}±0.24, 0.69, 0.40	78.2^{a}±0.48, 1.4, 1.4	2.02^{a} ± 0.03, 4.1, 0	2.06^{a}± 0.04, 4.32, 0.08
0.2%EMS	61.16^{b}±0.48, 1.75, 1.02	61.32^{ab}±0.68, 2.48, 1.74	3.2^{ab} ± 0.2, 13.96, -0.6	5.4^{ab}±0.24, 10.12, -0.4	7.4^{bc}± 0.24, 7.39, -0.4	7.2^{ab} ± 0.20, 6.2, -0.4,	76.20^{bcd}±0.37, 1.09,-2.0	76.4^{abc}±0.24, 0.71, -0.4	1.96^{ab}±0.04, 4.54, -0.06	1.94^{ab}±0.05, 5.87, -0.04
0.3%EMS	59.82^{b}±0.43, 1.61,-0.32	60.26^{bc}±0.47, 1.17, 0.68	3.8^{a}±0.58, 34.28, 0	5^{abc}±0.70, 31.60, -0.8	6.4^{def}±0.24, 8.54, -1.4	6.4^{cd}± 0.24, 8.54, -1.2	75.60^{cde}±0.50, 1.50,-2.6	75.2^{bcd}±0.37, 1.11, -1.6	1.82^{abc}±0.05 ,7.14, -0.2	1.8^{bc} ± 0.07, 8.77, -0.18
0.4%EMS	59.42^{ab}±0.49, 1.85,-0.72	59.20^{bc}±0.20, 0.77, -0.38	3.2^{ab}±0.37, 26.12, -0.6	4.2^{bcd}±0.37, 19.90, -1.6	6.4^{def}±0.24, 8.54, -1.4	6.6^{bcd}±0.24, 8.28, -1.0	74.80^{def}±0.37, 1.11,-3.4	74.8^{bcde}±0.2, 0.59, -2.0	1.80^{abc}±0.06, 7.83, -0.22	1.76^{cd}±0.04, 5.05, -0.22
25 Gy	68.68^{b} ± 6.46, 21.05, 8.54	66.20^{a} ± 5.44, 18.36, 6.62	3.8^{a} ± 0.37, 22, 0	5.4^{ab} ± 0.50, 21.11, -0.4	7.4^{bc} ± 0.24, 7.39, -0.4	7.2^{ab} ± 0.2, 6.2, -0.4,	77.20^{abc} ± 0.58, 1.68,-1.0	76.2^{abc} ± 0.96, 2.84, -0.6	1.86^{abc} ±0.11, 13.44, -0.16	1.78^{bc} ±0.08, 10.78, -0.20
50 Gy	59.18^{b} ± 1.73, 6.53, -0.96	58.18^{bc}± 1.71, 6.60,-1.4	3.4^{ab} ± 0.50, 33.52, -0.4	4.8^{abcd} ±0.58, 27.08, -1.0	6.6^{cde} ± 0.24, 8.28,-1.2	6.4^{cd} ± 0.24, 8.54, -1.2	74.40^{defg} ±0.40, 1.20,-3.8	74.6^{cde} ± 0.4, 1.19, -2.2	1.80^{abc} ±0.05, 6.77, -0.22	1.8^{bc}±0.07, 8.77, -0.18
75 Gy	59.44^{b}± 1.35, 5.09, -0.7	58.14^{bc} ± 1.32, 5.10,-1.44	3.6^{ab} ± 0.24, 15.19, -0.2	4.4^{bcd} ±0.24, 12.43, -1.4	6.2^{defg} ± 0.2, 7.29, -1.6	5.6^{e}± 0.24, 9.76, -2.0	75.00^{cde} ±0.54, 1.63,-3.2	74.8^{bcde} ± 0.37, 1.11, -2.0	1.76^{bc}±0.08, 10.28, -0.26	1.74^{cd} ± 0.05, 6.55,-0.24
100 Gy	59.04^{b} ± 1.30, 4.93, -1.1	57.64^{bc} ± 0.95, 3.69,-1.94	3.2^{ab} ± 0.37, 26.12, -0.6	4.2^{bcd} ± 0.37, 19.90, -1.6	5.6^{fg} ± 0.24, 9.76, -2.2	5.4^{e} ± 0.24, 10.12, -2.2	73.80^{efg} ± 1.28, 3.87,-4.4	74^{def} ± 0.77, 2.34, -2.8	1.80^{abc} ±0.05, 6.77, -0.22	1.76^{cd} ± 0.02, 3.06,-0.22
0.1%EMS + 25 Gy	58.92^{b}± 0.68, 2.59, -1.22	57.04^{bc} ± 0.84, 3.29,-2.54	3^{ab} ±0.31, 23.56, -0.8	4.4^{bcd} ±0.40, 20.31, -1.4	7^{bcd}± 0.44, 14.28, -0.8	6.8^{bc} ± 0.20, 6.57, -0.8	72.60^{fg} ± 1.02, 3.17,-5.6	73.6^{def} ± 0.87, 2.64, -3.2	1.76^{bc} ±0.08, 10.28, -0.26	1.74^{cd} ± 0.05, 6.55,-0.24
0.2%EMS +50 Gy	58.58^{b} ± 0.53, 2.02, -1.56	57.40^{bc} ±1.80, 7.02,-2.18	3.4^{ab} ± 0.24, 16.08, -0.4	4^{cd} ± 0.31, 17.67, -1.8	6.2^{defg}± 0.2, 7.2, -1.6	6^{e} ± 0.31, 11.78, -1.6	72.40^{g} ± 1.20, 3.73, -5.8	72^{f}± 1.18, 3.67,-4.8	1.72^{bc} ±0.10, 13.25, -0.3	1.66^{cde} ± 0.06, 9.09,-0.32
0.3%EMS +75 Gy	58.40^{b}± 0.88, 3.37, -1.74	56.18^{bc} ± 1.48, 5.89,-3.4	2.8^{ab} ± 0.37, 29.85, -1	3.8^{cd} ± 0.37, 22,-2.0	5.4^{g} ± 0.24, 10.12, -2.4	5.4^{e} ± 0.24, 10.12, -2.2	73.40^{efg} ± 0.50, 1.55,-4.8	73.4^{def} ± 0.24, 0.74, -3.4	1.68^{c} ±0.11, 14.76, -0.34	1.6^{de}± 0.03, 4.37, -0.38
0.4%EMS +100 Gy	56.96^{b}± 1.04, 4.11,-3.18	54.76^{c} ± 1.21, 4.94,-4.82	2.4^{b} ± 0.24, 22.86, -1.4	3.6^{d} ± 0.24, 15.19, -2.2	6^{efg} ± 0.31, 11.78, -1.8	5.6^{e} ± 0.24, 9.76, -2.0	73.40^{efg} ± 0.67, 2.06,-4.8	73^{ef} ± 0.70, 2.16, -3.8	1.62^{c} ±0.07, 10.12, -0.4	1.56^{e} ± 0.04, 5.7, -0.42

[#] Means within columns followed by the same letter is not different at the 5 % level of significance, based on the Duncan Multiple Range Test

Table 5. Correlation coefficients among various character pairs in M_2 generation of *Nigella sativa* varieties NRCSSAN-1 and BHUVN-1.

Character	NRCSSAN-1	BHUVN-1	NRCSSAN-1	BHUVN-1	NRCSSAN-1	BHUVN-1	NRCSSAN-1	BHUVN-1	NRCSSAN-1	BHUVN-1
	Plant height		No. of fertile branches		No. of capsules per plant		No. of seeds per capsule		1000 seed weight	
Plant height	1	1								
No. of fertile branches	0.157401	0.250644	1	1						
No. of capsules per plant	0.342627	0.383672	0.277462	0.569997	1	1				
No. of seeds per capsule	0.385105	0.447319	0.239124	0.467503	0.62645	0.453707	1	1		
1000 seed weight	0.301582	0.309606	0.102888	0.47832	0.609317	0.5803	0.515105	0.59463	1	1

Meiotic abnormalities have been considered to be one of the reliable indices for estimating the mutagenic sensitivity of the crop species. Structural rearrangement of chromosomes could be achieved through induced mutagenesis to create new combinations which otherwise are rarely obtained spontaneously or by conventional methods. In the present study, a broad spectrum of meiotic abnormalities including univalent, multivalent, laggards, bridges, stickiness, disturbed polarity, unequal separation of chromosomes etc. was induced by individual and simultaneous treatments of EMS and gamma rays in M_1 generation. The maximum chromosomal abnormalities were recorded with combination treatments compared to individual ones in both varieties. Similar mutagen-induced chromosomal abnormalities have been previously reported by many workers with different plant species including Kumar and Srivastava (2001) in *Plantago ovata*, Bhat *et al*. (2005) in *Vicia faba*, Jafri *et al.* (2011) and Khan *et al*. (2009) in *Cichorium intybus* and Goyal and Khan (2010) in *Vigna mungo*. The multivalent formation has been attributed to chromosomal pairing due to translocation and inversion. The occurrence of multivalent association is a common feature in the treated plants with the presence of more than two homologous chromosomes. Precocious separation occur by the effect of chemicals which break the protein moiety of the nucleoprotein backbone (Kumar and Rai, 2007; Amin *et al*., 2016). Laggards occur due to abnormal spindle formation and chromosomal breakage (Singh and Chaudhary, 2005; Khursheed *et al*., 2015).

Chromosomal bridges were observed at anaphase which seems to be the result of the non-separation of chiasma due to stickiness. According to Kumar and Singh (2003), the unequal separation of chromosomes occurs due to the random univalent movement to any one of the poles. The unequal separation, seen in the present investigation, may be due to the stickiness of chromosomes as was also reported in chickpea (Sharma and Kumar, 2004) and fenugreek (Srivastava and Kapoor, 2008). The formation of micronuclei due to lagging chromosomes has been reported by Kumar and Kumar (2000) and Ganai *et al.* (2005). During the anaphase and telophase stages, disturbed polarity occurred due to spindle disturbance. Stickiness of chromosomes was one of the most common abnormalities observed in the present investigation. Chromosomes were found clumped into one, two or many groups due to stickiness at metaphase causing a difficulty in the normal disjunction of chromosomes. These results are in agreement with those of Srivastava and Kapoor (2008) in fenugreek.

The nature and extent of genetic variability available within the species forms the basis for an effective selection for agro-economic traits under improvement. The mutagenic effectiveness of various doses of EMS and gamma rays alone and in combination was investigated concerning various quantitative traits, such as plant height (cm), number of fertile branches per plant, number of capsules per plant, number of seeds per capsules and 1000-seed weight (g) in M_2 generation. The assessment of mean, standard deviation (SD) and coefficient of variation (CV) in the control and treated population indicates that mutagenic treatments had induced a wider magnitude of variability for all these traits. Plant height had increased with the lower concentrations of EMS and gamma rays. Similar results were reported by Raina *et al.* (2017) in chickpea and Suprasanna *et al.* (2012) in vegetable crops. The increase in plant height at lower doses may be attributed to the induction of chromosomal abnormalities.

The characters like the number of capsules per plant, seeds per capsule and 1000-seed weight (g) showed a slight increase in the mean values at the lowest concentration of EMS (0.1 %), while higher concentrations of the single and combination treatments showed an inhibitory effect regarding these traits. The current results are consistent with the earlier reports of Tantray *et al.* (2017) and Khursheed *et al.* (2017). Positive correlations were observed among different character pairs controlling the yield. The possible cause of such positive associations could be ascribed to gene mutations.

5. Conclusion

It may be concluded from the present study that ample genetic variability had been induced by using lower doses of EMS and gamma rays alone or in combination. The mutagenic potency of such mutagens could be exploited further by carrying out extensive research in *N. sativa*- an economically important medicinal plant for a safer future.

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