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Research Article

STUDIES ON THE EFFECTS OF GAMMA RAYS ON *NIGELLA SATIVA* L. IN M_1 GENERATION

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ABSTRACT

In the present situation of variable climatic conditions, global warming and sky-high populations, sustainable boost in the agricultural productivity is the utmost priority. View this point, induced mutation is the best approach to create the new genetic variability. The present study has been conducted to study the effects of gamma rays on *Nigella sativa* L. var. NRCSSAN-1. The seeds of *Nigella sativa* were mutagenized with 25, 50, 75 and 100 Gy radiation of gamma rays to develop a viable mutant. Different qualitative and quantitative traits of mutant plant were screened time to time and compared with control (untreated/normal plant). A macro mutations were induced affecting all plant parts and different morphological variants were screened and isolated on the basis of economic importance. The different morphological parameters such as plant height, number of fertile branches per plant and number of capsules per plant, number of locules per capsule, number of seeds per capsule and 1000 seeds weight were increasing with decreasing the doses. The different morphological traits having positive and strong correlation, will definitely be helpful in selection of improved mutants in subsequent generations. Cytological abnormalities were increasing with increasing the doses. The most abnormalities were seen in 100 Gy and lowest in 25 Gy. It is concluded that gamma rays at its optimal doses (25 Gy and 50 Gy) are ameliorative for medicinal herb- *Nigella sativa*. L.

Key words: induced mutation, genetic variability, gamma rays, *Nigella sativa*

INTRODUCTION

Plants have been used for medicinal purposes long before prehistoric period. Ancient Unani manuscripts Egyptian papyrus and Chinese writings described the use of herbs. Evidence exist that Unani Hakims, Indian Vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Indigenous cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically. *Nigella* (*Nigella sativa* L.) is an annual herbaceous plant belonging to the family Ranuculaceae. It attains 30-50 cm length and also the plant is multi-branched and their number varies from plant to plant. Plant is delicate and with shallow delicate tap root system. Stem is erect, light green and irregular but anatomically solid. Leaf alternately arranged, leaf phylotaxy 1-2, broad leaf pinnae and the number of pinnae per rachis 5-6. The color of leaf is green and surface with little feathery. Mature seeds are consumed for edible as spice and medical purposes. *Nigella sativa* is highly used in traditional system of medicine for diabetes, asthma, bronchitis, headache, paralysis, hemiplegia, infection, inflammation and in gastro intestinal problems such as dyspepsia, diarrhea, dysentery and flatulence[1]. *Nigella* seeds are used as carminatives, diuretics, and for delayed menses and lactation, while their oil has protective action against histamine induced bronchospasm, cough and bronchal asthma In Egyptian folk medicine[2]. *Nigella* seeds carry moisture content ranged from 5.52 to 7.43%, crude protein from 20 to 27%, ash from 3.77 to 4.92%, ether-extractable lipid from 34.49 to 38.72% and carbohydrates from 23.5 to 33.2%[3]. Fixed oil of nigella seeds is rich in linoleic, oleic and plamitic acids[4]. Several authors have investigated the essential oil of nigella seeds and isolated and identified active constituents that have beneficial clinical effects[5]. Genetic variation is the currency of any crop improvement experiment and it is the long history of conventional crop breeding that causes the genetic bottlenecks which affects yield and quality. *Nigella* being a self-pollinated diploid ($2n = 12$)[6]. Mutation breeding is the only feasible and sustainable technique to broaden their narrowing genetic bases to create a gene pool of numerous desirable traits of economic importance. Physical mutagenesis is a coherent tool used in mutation breeding program for creating new. The range of induce mutation by different mutagenic doses varies according to the genotype used and the trait targeted. This investigation was carried out with some objectives of inducing genetic variability in morphological and yield contributing traits in *Nigella sativa* L. (var. NRCSSA1) using physical mutagen gamma rays.

METHODS AND MATERIALS

Genetic variability was induced in Nigella sativa L. (var. NRCSSAN-1) genotype using physical mutagen (gamma rays), 'considered to widen genetic variability for its overall genetic improvement (yield and nutrition) into an elite variety. The healthy and viable seeds (moisture 7 %) were irradiated with 25, 50, 75 and 100 Gy of gamma rays with a radioisotope 60Co, Cobalt-60, source at the National Botanical Research Institute, Lucknow, Uttar Pradesh, India. Initially, an experiment was conducted to determine

the lethal dose (LD 50) and suitable concentrations of the mutagens. The seeds were sown in the pots as well as agricultural fields of Aligarh Muslim University, Aligarh, India in mid-October 2015. The experiment was designed in triplicate in three rows for each treatment following a complete randomized block design. Breeding behavior was observed and different agronomic traits viz., plant height, number of primary branches/plant, capsule/plant, seeds/capsule, seeds/plant, 1000 grain weight, and yield/plant were evaluated. To determine pollen fertility (%), the pollen grains from freshly dehisced anthers of treated populations and control populations were fixed in Carnoy's fluid (absolute alcohol: chloroform: acetic acid, 6:3:1 v/v) for 24 h after which they were stained with 1% acetocarmine through squashed technique and five slides per treatment were observed. The pollen grains stained as uniform deep red colors were counted as fertile and others as sterile. For cytological examination, appropriate sized flower buds from control and treated plant were fixed separately in Carnoy's fluid for 24 hours and preserved in 70% alcohol. Anthers were compacted in 1% propionocarmine and permanent slides were made through an alcohol (NBA series). Different meiotic stages were recorded through photographs, using a high resolution (Dsx 100 Olympus) Microscope. The treated as well as control populations were carefully screened for morphological mutations throughout the growth period in both the generations. Statistical analysis, namely, Mean ($\bar{X}$), Standard error (SE), Standard deviation (SD), Coefficient of variation (CV %), Least significance difference (LSD), and Pearson's correlation coefficient (r), were done using R 3.1.0 and IBM SPSS statistics 20 to assess the intra- and inter-population (mutagen) variations in different quantitative traits.

RESULT

Analyzing the effects of different concentrations (25, 50, 75 and 100) of gamma rays on various morphological, qualitative and quantitative traits.

Qualitative and quantitative traits: Treated plants took 7-9 days for germination, 114 days for flower initiation, 130 days for reaching to 50% flowering and 150 days for maturity which is comparable with all these quantitative traits (**Table1**) in control plant as 8-10 days for germination, 125 days for flower initiation, 147 days for 50% flowering and 160 days for maturity respectively. Pollen fertility decreased from control to treated (94% - 82.20). The 25 Gy treated plant and 50 Gy treated plant were also found superior over control on an account of Qualitative traits. The correlation among different qualitative and quantitative traits are strongly positive except between pollen fertility and locules per capsule (**Table 4**).

Morphological parameters: Different morphological parameters were contrasted between control and treated plants (**Table 2**). The treated (25 Gy and 50 Gy) plant was taller increasing the height to 69.30 and 65.98 cm respectively compare to 61.10 cm in control. Number of fertile branches per plant was 6.20 in 25 Gy while it is decreased to 4.60 in control plant. Number of locules per capsules were also increased in treated plants as compare to control. The 25 Gy plant was vigorous bearing 9.40 capsules per plant which is significantly comparable with that of control (only 7.20 capsules/plant). Increased number of seeds per capsule (57.20 and 54.20) were observed in 25 Gy and 50 Gy respectively against 49.60 in control plant. The 1000 seeds weight was also markedly increased from 2.02g (control) to 2.42 g in 25 Gy.

Meiotic studies: Different types of chromosomal aberrations (**Table 3**) viz. multivalents, stickiness, precocious separation, stray bivalent (at metaphase I/II), bridges, laggards and unequal separation, cytomixis, disturbed polarity, micronuclei and multinucleate condition (at telophase I/II) as observed in the present investigation.

Table 1: Salient qualitative and quantitative characters to differentiate control and mutant

Quantitative traits			
S. No.	Traits	Control	Mutant
1	Plant Habit	Erect	Erect
2	Growth	Normal	Vigorous
3	Flower	Pentapetalous	Hexapetalous, and overlapping petalous
4	Flower Color	White	Yellowish White and bluish white
5	Capsule	Pentalocular	Hexa, septa, octa and more locular
6	Seed Size	Normal	Big,
7	Seed Color	Black	Black
Qualitative traits			
8	Days to germinate	8-10	7-9
9	Days to flower initiation	125	114
10	Days to 50% flowering	147	130
11	Days to maturity	160	150

Table 2: **Statistical analysis of comparative effect of gamma rays on various qualitative and quantitative characters in M_1 generation of *N. sativa* var. NRCSSAN-1**

Treatment	Plant height Mean ± SE, CV%, shift in mean	No. of fertile branches mean ± SE, CV%, shift in mean	No. of capsules per plant Mean ± SE, CV%, shift in mean	No. of locules per capsule 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	Pollen fertility Mean ± SE, CV%, shift in mean
Control	61.10^{c}, ± 0.688, 3, -	4.60^{b}, ± 0.509, 25, -	7.20^{b}, ± 0.374, 12, -	5.00^{d}, ± 0.000, 0, -	49.60^{c}, ± 0.871, 4, -	2.02^{b}, ± 0.058, 6, -	94.00^{a}, ± 0.707, 2, -
25 Gy	69.30^{a}, ± 0.700, 2, +8.2	6.20^{a}, ± 0.374, 13, +1.6	9.40^{a}, ± 0.245, 6, +2.2	8.20^{a}, ± 0.374, 10, +3.2	57.20^{a}, ± 0.583, 2,+7.6	2.42^{a}, ± 0.037, 3, +0.4	89.20^{b}, ± 0.800, 2, -4.8
50 Gy	65.98^{b}, ± 0.778, 3,+4.88	4.20^{bc}, ± 0.374, 20, -0.4	6.60^{bc}, ± 0.244, 8, -0.6	6.80^{b}, ± 0.374, 12, +1.8	54.20^{b}, ± 0.374, 2,+4.6	1.78^{c}, ± 0.037, 5, -0.24	85.00^{c}, ± 0.447, 1, -9
75 Gy	59.80^{cd}, ± 1.236, 5, -1.3	3.40^{c}, ± 0.244, 16, -1.2	6.20^{cd}, ± 0.200, 7, -1	6.00^{bc}, ± 0.316, 12, +1	46.20^{d}, ± 0.583, 3, -3.4	1.60^{d}, ± 0.031, 4, -0.42	82.20^{d}, ± 0.374, 1, -11.8
100 Gy	57.82^{d}, ± 0.877, 3, -3.82	3.20^{c}, ± 0.200, 14, -1.4	5.60^{d}, ±0.244, 10, -1.6	5.80^{cd}, ± 0.374, 14, +0.8	44.60^{d}, ± 0.678, 3, -5	1.34^{e}, ± 0.024, 4, -0.68	82.20^{d}, ± 0.860, 2, -11.8

"#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 3: Frequency of chromosomal abnormalities induced by gamma rays in *Nigella sativa* L. (M_1 generation) in NRCSSAN-1 variety

Treatment	Total No. of PMCs Observed.	Metaphase-I/II					Anaphase-I/II				Telophase- I/II							
		Multivalent	Precocious Movement	Stray chromosomes	Stickiness	% of Abnormalities PMCs	Laggards	Bridges	Unequal Sep.	% of Abnormalities. PMCs	Laggards	Bridges	Micro nucleate	Multi nucleate	Disturbed Polarity	% of Abnormality PMCs	Total No. of Abnormalities PMCs Observed	Total % Of Abnormalities. PMCs Observed
CONTROL	270	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25 Gy	260	1	1	-	1	1.15	-	-	-	-	-	-	1	-	1	0.76	5	1.92
50 Gy	264	1	1	-	1	1.13	-	1	-	0.38	-	1	-	1	-	0.75	6	2.27
75 Gy	255	-	3	2	3	3.13	1	1	2	1.56	-	1	1	3	-	1.96	17	6.66
100 Gy	276	4	3	2	4	4.71	3	4	2	3.26	2	-	2	2	2	2.89	30	10.86

Table 4: Correlation among the qualitative and quantitative traits

	Plant height	Fertile branch	Capsule/plant	Locules/capsule	Seeds/capsule	1000 seeds wt.	Pollen fertility
Plant height	1						
Fertile branch	0.72368	1					
Capsule/plant	0.71141	0.768906	1				
Locules/capsule	0.678458	0.421765	0.560702	1			
Seeds/capsule	0.904888	0.68354	0.730948	0.643874	1		
1000 seeds wt.	0.732949	0.794629	0.86264	0.505723	0.790329	1	
Pollen fertility	0.355281	0.570952	0.514758	-0.09854	0.429452	0.67986	1

Figure 1: Morphological variations between control and treatment of *Nigella sativa* L
a control plant and b mutant plant; 2. a, b, d, e, f and g mutant capsules and c control capsule; 3. a control flower b mutant flowers; 4. a control seeds and b mutant seeds; 5. a control leaf and b, c, d, e and f mutant leaves.

DISCUSSION

Chemical and physical mutagens have been used since time for the induction of mutants. Induced mutations in plants on account of mutagenesis have been studied by various workers [7-8]. But Limited work has been done on *Nigella sativa* for evaluation of useful mutants as a result of physical mutagenesis. The present investigation proved fruitful for the induction of viable early maturing mutants on screening the M_1 generation which clearly indicated the potential of gamma rays in *Nigella sativa*. Stimulatory effect of gamma rays on different qualitative and quantitative traits has also been suggested in *Vicea faba*[9]. In present work also gamma rays has shown its ameliorative action for modulating useful drug plant with meiotic stability. Such mutant type with normal meiosis is quite similar with that of early flowering mutant[10]. Mutant evaluated due to stimulatory effect of gamma rays showed increased height with higher number of branches. The probable cause is the loss of apical dominance which results in lateral distribution of growth hormone and hence the increased branching. Mutant with increased height and branches was also obtained in lentil due to mutagenic effect of MMS[11]. Total yield per plant was positive corelate with number of capsules per plant because whenever the number of capsules per plant increased, total number of seeds increased and hence the yield also increased. It was also reported that treated kalonji with higher yield has also been evaluated[12]. Moreover, the treated populations were hexapetalous petals instead of five (in control plant) which is the index of increased number of seeds and better yield. The similar character was also noticed in *Nigella sativa* induced by gamma rays[13]. Apart from yield, big seeds size also comprised higher quantity of important chemical constituents which are gift for pharmacological domain and enhanced level of nutritive oil. Usually induced mutants exhibit different types of chromosomal aberration due to toxic effect of mutagens. In such cases the mutagens exhibit the stimulatory effect on plant morphology with or without disturbing the cell cycle. Similar is the case of our results for normal and abnormal meiosis exhibited in mutant plants. The present study is highly significant having practical utility for plant breeders as it is early matured with improved qualitative traits coupled with increased height, number of branches, yield and other yield attributing traits. Similar findings have also been observed[14]. The mutant is also meiotically stable or unstable which can be directly linked with greater pollen fertility. It was reported that 98.07% pollen fertility in early mutant of *Nigella*[15]. Therefore this mutant type is safe, could be used as spice or medicine.

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