International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:113-122
UGC Approved Journal No 48951

Original Article
Open Access

Induction of Mutation by Gamma Irradiation in *Brassica compestris L.*

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Manuscript details:

Available online on http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Awaley Mundeep G (2018) Induction of Mutation by Gamma Irradiation in *Brassica compestris L*, *Int. J. of. Life Sciences*, Special Issue, A12: 113-122.

ABSTRACT

Pure line seeds of local variety of *Brassica compestris L*. were used in the present study. The certified, healthy and dry seeds (10% moisture content) of these variety were procured from Krishi Viggyan Kendra, Nagpur. This variety well adapted to the agro climatic conditions. The seed of mustard were treated w ith different doses/treatment of physical mutagens. The physical mutagens used were gamma rays. Uniform healthy dry seeds (10% moisture content) of the mustard were exposed to different doses of gamma rays (10GY, 20GY, 30GY, 40GY, 50GY, 60GY, 70GY, 80GY, 90GY and 100GY) with a dose rate of 20Kr/20min. from 60 cobalt source at the Deparment of chemistry, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. The mutagenic effect studied on M1 parameters included seed germination, Seedling height, plant survival, and various Quantitative traits. Seed germination, seedling growth, plant survival Increased with an increase in mutagenic treatment. Gamma rays proved to be most effective in causing maximum biological damage. Studies on various quantitative parameters showed the inhibitory effect of Lower treatments and stimulatory effect of Higher or intermediate treatments in M1 generation. The mean values for various quantitative traits Increased at higher treatments, but Inhibitory effects were noticed at some lower treatments. A significant amount of variability was induced in the treated populations as compared to Control. 70 Gy, 90Gy and 100 Gy treatments of gamma irradiation was found to be most effective.

Key words: *Brassica Compenstris L., Gamma rays, Physical mutagen, Quantitative trait.*

INTRODUCTION

The BRASSICACEAE or Cruciferae (also known as Mustered Family) is a large angiosperm (Flowering Plant) dicot family of Plant kingdom which belongs to the order Brassicales and has been divided into 10-19 tribes with a total of 338- 360 genera and 3,709 species.

The Brassicaceae are easily recognized by having unique flowers with four petals, forming a cross or some time reduced or lacking; six stamens, the outer being shorter than the inner four (however sometimes only two or four stamens are present) and capsule (having two valves capsule with a septum dividing it include two chambers). The plant family Brassicaceae includes several plant species of great scientific, economic and agronomic importance including model species (*Arabidopsis* and *Brassica*), developing model generic system (*Boechera, Brassica* and *Cardamine*), as well as many widely cultivated species.

The genus is native in the wild in western Europe, the Mediterranean and temperate regions of Asia and many wild species grow as weeds, especially in North America, South America, and Australia.

A dislike for cabbage or broccoli can result from the fact that these plants contain a compound similar to phenylthiocarbamide (PTC), which is bitter or tasteless to some people depending on their 'taste buds'.

The main objectives of the present study are:

1. To study the effect of different treatments on various biological parameters.
2. To investigate the chromosome behavior of treated populations with respect to controls.
3. To quantify the magnitude of the genetic variability induced in various quantitative traits.
4. To isolate promising mutants based on changes in phenotypic traits.

The present study was conducted in the Experimental Field of Botany Department, of Bhawabhuti Mahavidyalaya, Amgaon. Dry seeds of *Brassica comprestris L.* were irradiated with different doses of gamma rays (00, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Krad) form a ^{60}Co gamma chamber at RTM University Nagpur Chemistry Department. Irradiated seeds along with control were sown in earthen pots of equal size. The pots were maintained in the field in a Complete Randomized Design (CRD), each treatment being replicated four times. Growth parameters such as days to germination, days to completion of germination, germination percentage, survival percentage, shoot length, root length, number of leaves, number of branches were recorded at different periods after sowing was done. Collected data was subjected to Analysis of Variance technique (Fisher, 1985) and LSD test at 5% probability (Steel & Torrie, 1980).

Gamma radiation, also known as gamma rays, and denoted by the Greek letter γ, refers to electromagnetic radiation of an extremely high frequency and are therefore high-energy photons. Gamma rays are ionizing radiation, and are thus biologically hazardous. They are classically produced by the decay of atomic nuclei as they transition from a high energy state to a lower state known as gamma decay, but may also be produced by other processes. Paul Villard, a French chemist and physicist, discovered gamma radiation in 1900, while studying radiation emitted from radium. Villard's radiation was named "gamma rays" by Ernest Rutherford in 1903.

Natural sources of gamma rays on Earth include gamma decay from naturally occurring radioisotopes, and secondary radiation from atmospheric interactions with cosmic ray particles. Rare terrestrial natural sources produce gamma rays that are not of a nuclear origin, such as lightning strikes and terrestrial gamma-ray flashes. Additionally, gamma rays are produced by a number of astronomical processes in which very high-energy electrons are produced, that in turn cause secondary gamma rays. However, a large fraction of such astronomical gamma rays are screened by Earth's atmosphere and can only be detected by spacecraft.

Gamma rays typically have frequencies above 10 exahertz (or >10^{19} Hz), and therefore have energies above 100 keV and wavelengths less than 10 pico meters (10^{-12} meter), which is less than the diameter of an atom. However, this is not a hard and fast definition, but rather only a rule-of-thumb description for natural processes. Electromagnetic radiation from radioactive decay of atomic nuclei is referred to as "gamma rays" no matter its energy, so that there is no lower limit to gamma energy derived from radioactive decay. This radiation commonly has energy of a few hundred keV, and almost always less than 10 MeV. In astronomy, gamma rays are defined by their energy, and no production process needs to be specified. The energies of gamma rays from astronomical sources range to over 10 TeV, an energy far too large to result from radioac-tive decay. A notable example is extremely powerful bursts of high-energy radiation referred to as long duration gamma-ray bursts, of energies higher than can be produced by radioactive decay. These bursts of gamma rays, thought to be due to the collapse of stars called hypernovae, are the most powerful events so far discovered in the cosmos.

Mustard (*Brasicca comprestris L.*) commonly known as Mohari (Rahi) is a well known economic herb of the family Cruciferae. The young plants serve as vegetable for human consumption seeds as a spice or as herbal medicine (Petropoulos, 2002). Physical and chemical mutagens induce physiological damages (injury), gene mutations (point mutations) and chromosomal aberrations in the biological material in M1 generation (Gaul, 1970). Gamma rays, an energetic form of electromagnetic radiations are known to be the most popular mutagens for their simple application, good penetration, reproducibility, high mutation frequency and less disposal problems (Chahal and Ghosal, 2002). Ethyl methane sulphonate (EMS), a chemical mutagen of the alkylating group has been reported to be the most effective and powerful mutagen and usually causes high frequency of gene mutations and low frequency of chromosome aberrations in plants (Van Harten, 1998; Khatri *et al*., 2005). Sodium azide (NaN3) is the least dangerous and the most efficient mutagen and has been reported to be mutagenic in several crop species (Adamu and Aliyu, 2007; Mostafa, 2011). The mutagenicity of sodium azide is arbitrated through the formation of an organic metabolite which enters the nucleus, interacts with DNA and generates point mutations in the genome. According to Nilan *et al* (1977), SA is relatively safe to handle, inexpensive and noncarcinogenic as compared to other mutagens. In mutation breeding studies, it is important to determine a suitable dose/ concentration of mutagen for a crop plant which can be employed for inducing maximum variability through point mutations. Seed germination, seedling growth, pollen sterility and chromosomal aberration are the commonly used criteria for studying radio-sensitivity in plants (Kon *et al*., 2007; Lal *et al*., 2009; Sangle *et al*., 2011; Sheikh *et al*., 2012). The aim of present study was to determine the response of mustard seeds to gamma rays based on germination and survival percentage, root-shoot length, and pollen fertility with the main aim of identifying appropriate dose/conc.

Plant Morphology is considered to be an important tool for isolation of desirable Mutants. Several induced Morphological Mutations have been reported in literature showing alterations in the Morphology of various plant parts. Raw and Jama (1976) Subjected the seed of Black Gram (phaseolus Mungo) to X-rays & EMS treatments with the objective for obtaining some promising mutants. The induced leaf mutants scored comprised of Crinkled leaf and waxy leaf narrow leaf and unifoliate mutants. Crinkled leaf and waxy leaf mutants had normal fertility and vitality and whereas the narrow leaf mutant was partially sterile and the unifoliate and extreme dwarf mutant was also isolated which was completely sterile. Chandra and Tewari (1978) in bean (Phaseolus aureus) var. Pusa Baisakhi observed that increasing doses of gamma rays and neutons caused a gradual reduction in germination of seeds. Irradiation caused the appearance of leaf abnormallities including unifoliate, bifoliate, trifoliate, tetrafoliate and pentafoliate characters. Under the influence of neutrons both tetra and pentafoliate leaves were observed on the same plant of cv. S-8 apparently associated with enhanced luxuriance of plants which resulted in enhanced pod formation. Moh (1972) induced variations in seed coat colour of some black bean (*Phaseolus vulgaris*) varieties of Latin America. Though the varieties under improvement were disease resistant and good yielding yet were considered inferior because of their seed coat colour. The seeds were treated with EMS and gamma rays and a special screening technique was employed in which seed coat colour mutants were correlated with green hypocotyls colour, for isolation of the potential mutants at a very early stage of seeding development. Mutagenesis resulted in inducing some seed coat colour mutants who varied from white, yellow to various degrees of brown and their seed coad colour was associated with a change in hypocotyl colour from red to green. All these mutnts were bearing white flowers instead of red in the parents but their morphology, growth habit and disease resistance wre similar to that of the parents. Further studies revealed that these induced characters were recessive and their inheritance followed a simple Mendalian manner. Kaul and Chaudhary (1972) conducted mutagenic studies in Atropa belladonna after exposing its seeds to different doses of gamma rays. Studies were aimed at assessing the variability in polygenic character released in M1 generations of belladonna. A higher variability was noted in than in M1. After observing a greater variability for tiller number and alkaloid content than that for plant height and leaf length, it was inferred that different characters may respond differently to different mutagenic treatments.

Mouli and Patil (1976) subjected peanuts (*Arachis hypogea*) to gamma irradiation. They isolated a suppressed branched mutant with larger leaves, altered flowering pattern, reduced shelling, smaller kernels and branch length as compared to normal in the autumn and spring s growing seasons respectively. An extremely poor pod shelling was observed in autumn grown plants as compared to spring grown ones. Narsinghani and kumar (1976) in a mutations breeding programme subjected the seed of Cowpea (*Vigna Sinensis L.*) to EMS and MMS treatments. In M1 generations, reduction in survival percentage, mean pod number, seed yield per plant and average pollen fertility was observed. Gamma ray induced morphological mutationhave also been 27 reported by Morishita (2001) in Buckwheat and by Tah (2006) in Mutabean. Kumar *et al*. (2003) reported several viable mutants induced by gamma rays in Lima bean (*Phaseolus Lunatus L.*) which included earliness, erect plants, profuse flowering and high yielding mutants. Wani (2011) reported a series of morphological mutants in chickpea isolated in separate and combined treatments of gamma rays and EMS. The Various types of mutants reported included plant height, leaf, pod and seed mutants. Combination treatments in general were found more effective and efficient in inducing various types of morphological mutants.

METHODOLOGY

Variety used: Pure line seeds of local variety of *Brassica compestris L*. were used in the present study. The certified, healthy and dry seeds (10% moisture content) of thise variety were procured from Krishi Viggyan Kendra, Nagpur. This variety well adapted to the agro climatic conditions.

Mutagens used: The seed of mustard were treated with different doses/treatment of physical mutagens. The physical mutagens used were gamma rays.

Gamma rays: Uniform healthy dry seeds (10% moisture content) of the mustard were exposed to different doses of gamma rays (10GY, 20GY, 30GY, 40GY, 50GY, 60GY, 70GY, 80GY, 90GY and 100GY) with a dose rate of 20Kr/20min. from 60 cobalt source at the Deparment of chemistry, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur.

Method of treatment with physical Mutagen: Prior to the mutagenic treatment, the seeds were choose and passes through the gamma rays in different doses of Kr.

Sample size: A set of 150 seeds were chosen for each dose / treatment including the control. Out of these 150 seeds, 10 seeds for each treatment and control were shown in the field for morphological and cytological studies, Whereas the remaining set of seeds was allowed to germinate on a moist cotton in petriplates for measuring Root- shoot length.

Table 1: Details of Mutagenic Treatment given to mustard seeds

Mutagen used	Dose / Concentration	Duration of presoaking (Hrs.)	Duration of Treatment (Hrs)
Control	DDW	12.0	-
Gamma rays (GY)	10	-	-
	20	-	-
	30	-	-
	40	-	-
	50	-	-
	60	-	-
	70	-	-
	80	-	-
	90	-	-
	100	-	-

Sowing of seeds in the field: Nursery beds were prepared for sowing seeds and raising M1 generation. In January, 2015, the treated as well as un treated (control) seeds were shown in three replicates in a complete Randomized Block Design (CRBD) at the Bhawabhuti Mahavidyalaya, Amgaon. The distance between the seeds along a row was kept 5cm whereas row to row distance was maintained at 10cm in each experimental plot in a replication.

Mechanism of action of physical mutagens

a) Physical mutagens: Gamma rays are the most energetic form of electromagnetic radiation, possessing the energy level from 10 kilo electron volts (kev) to several hundred kev, and they aare considered the most penetrating in comparison to other radiation such as alpha and beta rays (Kovacs and Keresztes, 2002).

Gamma rays typically have frequencies above 10 exahe-rtz (or >10^{19} Hz), and therefore have energies above 100 keV and wavelengths less than 10 picometers (10^{-12} meter), which is less than the diameter of an atom. However, this is not a hard and fast definition, but rather only a rule-of-thumb description for natural processes. Electromagnetic radiation from radioactive decay of atomic nuclei is referred to as "gamma rays" no matter its energy, so that there is no lower limit to gamma energy derived from radioactive decay. This radiation commonly has energy of a few hundred keV, and almost always less than 10 MeV. In astronomy, gamma rays are defined by their energy, and no production process needs to be specified. The energies of gamma rays from astronomical sources range to over 10 TeV, an energy far too large to result from radioactive decay. A notable example is extremely powerful bursts of high-energy radiation referred to as long duration gamma-ray bursts, of energies higher than can be produced by radioactive decay. These bursts of gamma rays, thought to be due to the collapse of stars called hypernovae, are the most powerful events so far discovered in the cosmos.

The first gamma ray source to be discovered historically was the radioactive decay process called gamma decay. In this type of decay, an excited nucleus emits a gamma ray almost immediately upon formation (it is now under -stood that a nuclear isomeric transition, however, can produce inhibited gamma decay with a measurable and much longer half-life). Paul Villard, a French chemist and physicist, discovered gamma radiation in 1900, while studying radiation emitted from radium. Villard knew that his described radiation was more powerful than previously described types of rays from radium, which included beta rays, first noted as "radioactivity" by Henri (1896), and alpha rays, discovered as a less penetrating form of radiation by Rutherford, in 1899. However, Villard did not consider naming them as a different fundamental type. Villard's radiation was recognized as being of a type fundamentally different from previously named rays, by Ernest Rutherford, who in 1903 named Villard's rays "gamma rays" by analogy with the beta and alpha rays that Rutherford had differentiated in 1899. The "rays" emitted by radioactive elements were named in order of their power to penetrate various materials, using the first three letters of the Greek alphabet: alpha rays as the least penetrating, followed by beta rays, followed by gamma rays as the most penetrating. Rutherford also noted that gamma rays were not deflected (or at least, not easily deflected) by a magnetic field, another property making them unlike alpha and beta rays.

Gamma rays were first thought to be particles with mass, like alpha and beta rays. Rutherford initially believed they might be extremely fast beta particles, but their failure to be deflected by a magnetic field indicated they had no charge. In 1914, gamma rays were observed to be reflected from crystal surfaces, proving they were electr-omagnetic radiation. Rutherford and his coworker Edward Andrade measured the wavelengths of gamma rays from radium, and found that they were similar to X-rays but with shorter wavelengths and (thus) higher frequency. This was eventually recognized as giving them also more energy per photon, as soon as the latter term became generally accepted. A gamma decay was then understood to usually emit a single gamma photon.

RESULT

Evaluation of M1 generation

Seed germination: The data on seed germination was recorded right from the emergence of first shoot in each treatment including control. After recording the data, percentage of seed germination was calculated by using the formula.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100$$

Seedling height (cm): Seedling height was estimated on 10 th day of germination by measuring root and shoot length of randomly selected seedling from each treatment as well as control. Seedling injury as measured by the reduction in root and shoot length and calculate in terms of percentage of root and shoot injury.

$$\text{Percent injury} = \frac{\text{Control- Treated}}{\text{Control}} \times 100$$

Plant survival: The surviving plants in different treatments were counted at the time of maturity and the survival percentage and percent lethality were calculated by the following formula.

$$\text{Survival (\%)} = \frac{\text{No. of plants at maturity}}{\text{No. of seeds germinated}} \times 100$$

$$\text{Lethality (\%)} = \frac{\text{Control- Treated}}{\text{Control}} \times 100$$

Quantitative characters of M1 generation: The following morphological parameters were recorded in M1 generation .

1. Plant height (cm): The height of 15 randomly selected plants was measured from the point above the ground to the tip of the main axis of the plant.

2. Number of pods per plant: Total number of pods per plant for a selected number of 15 plants from each concentration including control was recorded.

3. Length of pods per plant (cm): The length of five pods per plant from 15 randomly selected plants in each treatment including control was recorded.

4. Number of seeds per pod: Five pods per plant from 15 randomly selected plants in each treatment were used to calculate the mean seeds per pod.

5. Seed yield per plant: Randomly selected 15plants per treatment were used for calculating the mean seed yield per plant.

Figure 1: Seed germination of Control & Different   Doses of Gamma rays

Figure 1 Sowing of Seeds in the Seed Bed

Figure 3 Sowing of Seeds in the Seed Bed

Figure 4 Seed Germination and Seedling Height

Figure 5 Seed Germination and Seedling Height

Control **Treated** **Control** **Treated**

STUDIES IN M1 GENERATION:

The mutagenic effects of gamma rays were studied on seed germination, seedling height, plant survival, various quantitative characters in M1 generation of *Brassica compestris L.*

Seed germination:

Germination percentage was found to be significantly reduced in all the mutagenic treatments Except 50 Gy. The maximum inhibition in germination was recorded at 80 Gy treatments. Seed germination was about 100% in control. In gamma rays it ranged from 90% (10Gy) to 60% (80Gy). The percentage of seed germination was found 100% in 50Gy treatment of gamma irradiation.

Plant survival:

Plant survival was higher in control (95%) than in all the ten mutagenic treatments (Table 3). Plant survival tended to decrease with the increase in the dose/concentration of mutagens are follows:

The plant survival was 55.22, 33.33, 87.5, 22.22, 90.00, 44.44, 87.50, 99.00, 88.88, and 85.71 percent at 10Gy, 20 Gy, 30 Gy, 40Gy, 50 Gy, 60 Gy, 70 Gy, 80 Gy,90 Gy, and 100 Gy treatment of Gamma irradiation respectively against 95 % in control. The maximum plant survival was found in 50Gy and 80Gy treatment of gamma irradiation.

Table 2: Seed germination

Treatment	No. of seeds sowing	No. of seeds germinate
Control	10	10
Gamma rays		
10	10	09
20	10	09
30	10	08
40	10	09
50	10	10
60	10	09
70	10	08
80	10	06
90	10	09
100	10	07

Table 3: Effect of gamma rays survival and pollen fertility in M1 generation in *Brassica compestris L.*

Treatments	Germination (%)	Plant survival (%)	Lethality (L)
Control	100	95.00	60.00
Gamma rays (Gy)			
10	90	55.55	44.45
20	90	33.33	66.67
30	80	87.5	12.5
40	90	22.22	77.78
50	100	90.00	10
60	90	44.44	55.56
70	80	87.50	12.5
80	60	90.00	10
90	90	88.88	11.12
100	70	85.71	14.29
Mean	**85.45**	**67.96**	**33.17**

Table-4 Effect of various doses (Kr) of gamma irradiation on shoot length (cm), root length(cm), number of branches/plant, number of pods

Doses /Treatment	**Shoot length** (cm)	**Root length** (cm)	**No. of branches/ plant**	**No. of pods/ plant**
(Control) 00	17	7	2	-
Gamma rays - 10	38	18	3	-
20	17	9	1	-
30	26	10	3	8
40	23	20	2	12
50	75	10	4	10
60	39.5	11	5	14
70	84	17	8	17
80	51.5	13	5	18
90	61.5	18	4	10
100	91	13	6	30

Seedling height (Shoot length): Data recorded on seedling height measured in terms of root + shoot length is presented in Table 4. It is evident from that seedling height Increases with an increase in dose / concentration of mutagens. The shoot length was 38 cm, 17cm, 26cm, 23cm, 75cm, 39.5cm, 84cm, 51.5cm, 61.5cm and 91cm at 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy, 60 Gy, 70 Gy, 80 Gy, 90 Gy, and 100 Gy treatment of gamma irradiation respectively against 17cm in control. The maximum Shoot length was found in 100Gy treatment of Gamma irradiation.

Seedling height (root length): The root length was 18 cm, 9cm, 10cm, 20cm, 10cm, 11cm, 17cm, 13cm, 18cm and 13cm at 10Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy, 60 Gy, 70 Gy, 80 Gy, 90 Gy, and 100 Gy treatment of gamma irradiation respectively against 7cm in control. The maximum root length was found in 40Gy treatment of Gamma irradiation. Mean squares form Table-4 that the differences for various treatment of gamma irradiation were not able to reach the level of significance. Increase in higher germination percentage at higher doses might be due to their stimulating effects on activating RNA synthesis or protein synthesis (Kuzin *et al*., 1975, 1976) or it could be due to the elimination of germinating bacterial populations, their spores and mould fungi (Gruner *et al*., 1992)

Higher exposures of gamma rays may cause injury in seeds (Mehetre *et al*., 1994) and usually shows inhibitory effects on seeds of angiosperms and gymnosperms (Akhaury and Singh 1993; Thapa, 1999). These results are in line with Sahrif *et al*., (2000), Din *et al*., (2003) and Kon *et al*., (2007).

Shoot and root length: Effect of gamma rays on shoot and root length in this study was adverse and inhibitory as is evident from data in Table-4 Seeds exposed to higher doses produced Tall plants with long roots. This effect of gamma rays on shoot and root length of plants was more pronounced at 70 Kr to 100 Kr (Table-4). Shakoor *et al*., (1978) and Khalil *et al*., (1986) attributed decreased shoot and root lengths at higher doses of gamma rays to reduced mitotic activity in meristematic tissues and reduced moisture contents in seeds respectively. Decrease in shoot and root lengths of a number of crops has been reported by Thimmaiah *et al*., (1998), Muhammad and Afsari, (2001), Al-Salhi *et al*., (2004), Token *et al*., (2005), Kon *et al*., (2007).

Number of Pods and number of branches: Both number of branches and number of pods were highly significantly increased by radiation doses (Table-4). LSD values showed that maximum means were observed 100 Kr (control) and minimum values for number of branches and number of leaves were recorded at control (Table-3).

CONCLUSIONS

The present investigation was conducted to study the mutagenic effect of gamma rays, in the local variety of Mustard. The main objective of the study was to induce the genetic variability in quantitative traits and to isolate the promising mutants associated with increase in yield potential of the crop. The significant findings are summarized as follows:

The mutagenic effect studied on M1 parameters included seed germination, Seedling height, plant survival, and various Quantitative traits.

a) Seed germination, seedling growth, plant survival Increased with an increase in mutagenic treatment.

c) Gamma rays proved to be most effective in causing maximum biological damage.

d) Studies on various quantitative parameters showed the inhibitory effect of Lower treatments and stimulatory effect of Higher or intermediate treatments in M1 generation.

e) The mean values for various quantitative traits Increased at higher treatments, but Inhibitory effects were noticed at some lower treatments.

f) A significant amount of variability was induced in the treated populations as compared to Control.

h) 70 Gy, 90Gy and 100 Gy treatments of gamma irradiation was found to be most effective.

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