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Effect of gamma irradiation on Growth responses and Cytogenetical characterization in *Eclipta alba* (L.) Hassk.

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ABSTRACT
The current investigation engrossed on the role of genetic makeup, morphogenetic markers and biochemical attributes of Bhringraj (Eclipta alba) under the influence of gamma irradiation. The genotype was subjected to physical mutagenesis to induce the variability in morphogenetic, cytogenetic and biochemical characteristics. The seeds of Bhringraj were irradiated at different doses of gamma rays viz., 100, 200 and 300 Gy, and the impact on cytomorphological and biochemical aspects were analysed.The plant varied in sensitivity to gamma radiations, as observed germination, survival, plant height, days to 50% flowering, number of primary branches, cytological and biochemical aspects. The lower dose of gamma radiation shows stimulatory effect whereas higher dose of gamma radiation shows inhibitory effects. The lower doses of gamma radiation induce chromosomal anomalies such as stickiness, bridges and precocious etc. which are responsible to create variations. The abnormalities may also affect the pollen fertility at the higher dose of radiation. The result suggests the enhancement of proline content which is dose dependent on gamma radiation. The gamma radiation increases the genetic variation in plants. At higher doses radiation has negative impact on several parameters but at the lower doses, gamma ray treatment resulted a wide range of morphological and chromosomal abnormalities which may be useful in inducing beneficial traits in Bhringraj.
Keywords: *Mutagenesis, Gamma ray, Abnormalities, Traits, Variants.*

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INTRODUCTION

Mutagenesis is the process by which sudden heritable changes occur in the genetic information of an organism not caused by segregation or genetic recombination but induced by chemical, physical or biological agents [28]. Mutation breeding employs three types of mutagenesis. Among different types of induced mutagenesis techniques the physical mutagen is the most efficient tool to create genetic diversity. In the category of physical mutagens, gamma rays are the most commonly used mutagen in plant improvement programmes.

Gamma radiation can affect the atoms to form free radicals at cellular levels, and these radicals may damage or alter the physiology and metabolic pathway of plant depending on the irradiation levels [5]. These effects outwardly show variation in the morphology of plant by inward actions such as alteration of photosynthesis and formation of Reactive Oxygen Species (ROS) [14], [6] and [19]. Gamma rays also affect the meiotic stages of the plant and are the best way to prove the mechanism of inheritance. Gamma radiation impinge with dividing cells, resulting in cytogenetical aberrations and a decrease in frequency of dividing cells, which is ultimately reflected in limited seedling growth and other deformities and aberrations [1].

In the present time, the pharmaceutical industry as well as health sector has become more conscious in utilizing indigenous herbal flora for their remedial properties and this has formed the stepping stone for the era of "Herbal renaissance". Recent reports of World Health Organization (WHO), state that 80% of the world populations worldwide rely on herbal medicines for their basic health needs. Medicinal herbal flora is a boon for any nation due to numerous curative properties they augment against diverse ailments. India which is among the twelve mega diversity centres, has immense biotic wealth marked by remarkable ecosystem, species and genetic diversity. The luxuriantly diversified indigenous flora comprises of numerous plants with an ample pharmacological properties. Due to the medicinal

bioformulations extracted from these plants, they contribute a lot for health management practices. These plants participate as an important ingredients of our conventional folklore medicines, which are cost effective, with no significant side effects and are easily accessible. Phytoconstituents yielded from herbal plants are the mainstay in the preparation of Ayurvedic, Unani and other native prescriptions. One such plant with magnificent pharmaceutical and therapeutic value is *Eclipta alba* (L.) Hassk. The plant *Eclipta alba* (L.) Hassk. (2n=22), commonly known as 'Bhringraj', is one of the most valuable medicinal plant, belonging to Asteraceae family. In Ayurveda, *Eclipta*is a plant blessed with best curative properties for the treatment of liver ailments such as cirrhosis, infective hepatitis and other conditions involving hepatic enlargement [7]. The plant can be used to cure many severe diseases such as epilepsy, diabetes and hepatic cancer. The splendid therapeutic value of the plant is also discussed in the ancient ayurvedic literature like Charaksamhita and Sushrutsamhita. There is accelerating demand of natural products which have lead to the exhaustive use of the herb. This upsurge in demand can only be accomplished through formulation of replenishing strategies. With this aim, induced mutagenesis technique is evolved and conceived, by which a sincere attempt to increase the productivity may be planned out. The technology of induced mutagenesis is applied on the plant research work. Induced mutagenesis is well adapted and high throughput technique for accomplishing quantitative and qualitative trait improvement. It provides unique opportunities to create new traits. Hence, keeping all these points in mind, the present study was undertaken to bridge the knowledge gap on the effect of gamma irradiation at different morphological, cytological and biochemical aspects of the Bhringraj.

MATERIAL AND METHODS

Seed Procurement: Seeds of *Eclipta alba* were collected from CCRAS (Central Council For Research in Ayurvedic Sciences), Jhansi, Uttar Pradesh, India.

Gamma Radiation: Fresh seeds of *Eclipta alba* were treated with Gamma rays at doses 100 Gy, 200 Gy and 300 Gy, respectively through a ^{60}Co source at National Botanical Research Institute, Lucknow, India. Immediately after irradiation, seeds were soaked in distilled water for 1hour along with one set of control and sown in triplicates to raise population for meiotic study.

Agro-climatic conditions of experimental site: The present experiment has been performed in the area of Roxburgh Botanical Garden, Department of Botany, University of Allahabad , Prayagraj, U.P., India, during Kharif season. The meticulous experimental location is 25^{0}27´43.01´´N, 81^{0}51´10.42´´E. Prayagraj is situated 98 m above mean sea level. Prayagraj is in the subtropical climatic zone; the average rainfall is 1027mm and relative humidity is 59%.

Meiotic preparation: Suitable sized young capitula were collected during day time and fixed in Carnoy's fixative (3:1, absolute alcohol: glacial acetic acid v/v) for 24 hrs and preserved in 70 percent alcohol at room temperature i.e. 25±2 ºC. The anthers were squashed in 2% aceto-carmine stain, and the slides were covered with cover slips following gentle tapping. Slides were observed under optical microscope and photomicrographs were captured by Olympus PCTV Vision Software. Following formulae used for calculation of abnormality percentage –

Total Abnormality percentage (TAB) % = No. of Abnormal Pollen mother cell (PMC)/Total no. of Pollen mother cell observed *100

Pollen fertility: Mature capitula were collected and anthers having pollen grains were dusted over glass slide and stained with acetocarmine. Then mounted with glycerin and observed under optical microscope. Frequency of fertile and sterile pollen grains were enumerated to estimate pollen fertility.

Estimation of morphological characteristics : The morphological parameters were taken such as germination (7 days), survival percentage (14 days), plant height (cm), Days to 50% Flowering and Number of primary branches for analyzing the impact of Gamma radiation on Bhringraj.

Estimation of Proline content : The proline content was quantified by the process reported by Bates et al. (1973). 250 mg sample of leaves of the plant material was homogenized in 10 ml of aqueous solution of 3% sulfosalicylic acid to prepare crude extract and centrifuged at 3000 rpm for 10 minutes. This supernatant was allowed to react with 2ml acid ninhydrin and 2 ml of 3% glacial acetic acid in a test tube. The mixture was kept in water bath at 100^{0}C for 1 hour and for the termination of reaction, the test tube was placed in an ice bath. The reaction mixture was transferred to a separating funnel and extracted with 4.0 ml of toluene and then vortexed for 10-15 second. The toluene layer containing organic and inorganic phase was separated, warmed at room temperature and the absorbance of the organic toluene phase containing the chromophore was recorded at 520 nm in a spectrophotometer (UV Visible

Spectrophotometer 118, Systronic) using toluene as blank. The concentration of proline in a plant tissue was determined from a standard curve and calculated on the basis of Fresh Weight (FW).

Statistical analysis: Each treatment was analysed in three replications at the 7th day after sowing. The replicate samples were analysed and standard deviations were worked out and expressed. One way Analysis of variance (ANOVA) and Statistical analysis were performed using the SPSS 16.0 software and Duncan's Multiple Range Test (DMRT, P< 0.05) was conducted for mean separation and the data was subjected to analysis of variance.

RESULT

Influence of Gamma ray on morphological aspects:

Germination percentage(%) under gamma ray treatment:

Germination percentage decreased with increasing doses of gamma rays treatment in which 300 Gy doses shows a maximum reduction of germination percentage i.e.65.0±2.70^{c} as compared to control set i.e.(97.0±0.95^{a}) (Figure 1A)

Survival percentage(%) under gamma ray treatment:

The survival percentage was found to be decreased in irradiated sets as compared to untreated sets. Control exhibits survival percentage i.e. 97.0±1.28^{a} and it was reduced to 51.0±2.34^{d} at highest gamma rays dose (300 Gy). The reduction in survival percentage is dose dependent i.e. along with increasing doses there was a gradual decrease in survivability (Figure 2B). About 50 % of plants survived at 300 Gy indicating LD_{50} of gamma rays in Bhringraj.

Growth parameters under gamma ray treatment:

Plant height:

Plant height trend divulges with noteworthy incentive at lowest dose (100 Gy) over control however besides this a continual reduction was recorded in the plant height tendency. The mean value of plant height in control was 40.2 cm while at 100 Gy it was recorded as 48.3 cm (Figure 1C). Extreme reduction in plant height was recorded at highest gamma ray dose (300 Gy).

Days to 50% flowering under gamma ray treatment:

In Bhringraj there was slight increase in the mean value for days to 50% flowering in radiation dose equated to that of control. The highest mean value for days to 50% flowering was found at 300 Gy (62 days). There was gradual increase in the mean value of days to 50% flowering along with increasing dose of gamma rays. At 100, 200 and 300 Gy the mean value were found to be 54, 57 and 62 days respectively, which is greater in comparison to control (Figure 1D).

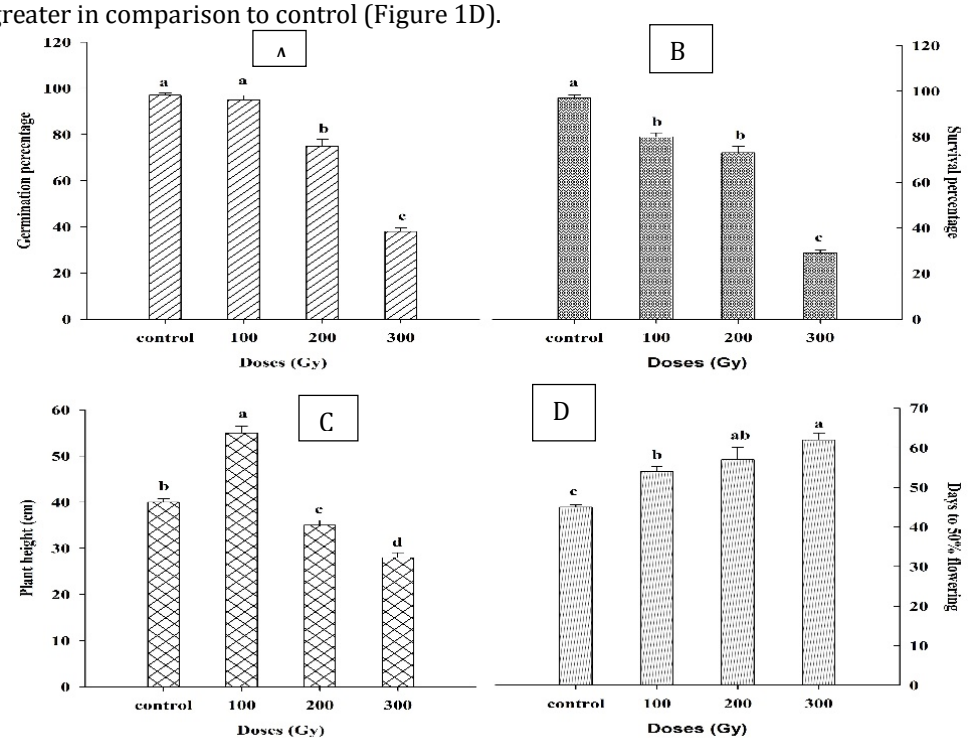

FIGURE1: Effect of Gamma ray treatment on *Eclipta alba* (L.) Hassk. On morphological parameters (A) Germination percentage, (B) Survival percentage, (C) Plant height, (D) Days to 50% Flowering

***Influence of number of primary branches under gamma ray treatment*:**
At the 100 Gy, the mean value of number of primary branches per plant was maximum (14) as compared to control plant but in case of 200 Gy and 300 Gy, the no. of primary branches progressively declined from the control recorded to be 9,7 respectively (Figure 2 A).

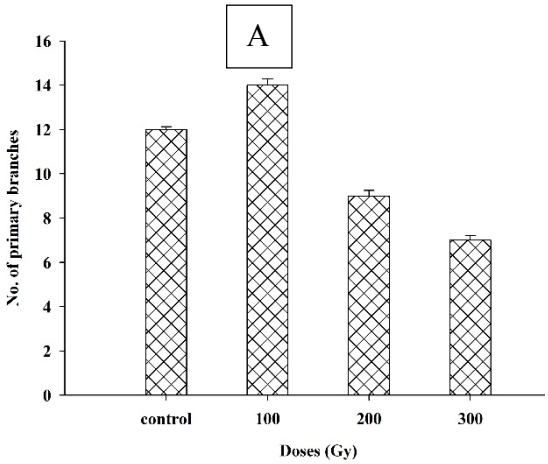

FIGURE2: Effect of Gamma ray treatment on *Eclipta alba* (L.) Hassk. On morphological parameters (A) No. primary branches

***Morphological variants induced by gamma radiation*:**
Gamma radiation induces abnormalities in the leaves of the Bhringraj, *viz.* bifurcated, spatulate and obovate shaped leaf (Figure 3 B,C,D). These abnormalities show a clear deviation from the normal lanceolate leaf (Figure 3A). It also causes abnormalities in branching pattern. At 300 Gy dose, multiple branching which is distinct to normal branch pattern, was observed (Figure 3E).

FIGURE 3: Morphological Variants/Mutants A. Control leaf of *Ecliptaalba* B. Bifurcation of leaf C. Spatulate leaf D. Obovate shape leaf E. Multiple branching pattern, Control (Left).

***Cytogenetical activities under gamma ray treatment*:**
Meiosis was completely normal in control plant with 11 bivalents (2n = 22) at metaphase I stage (Figure 4) and chromosomes were seen clearly separating at anaphase stages (Figure 4B) without any abnormality. At metaphase I, the treated material showed stickiness. The percentage of cells showing stickiness increased as the dose of radiation increased (Table1). At the lower dose, stickiness percentage was recorded as 1.49±0.11 which increased upto 4.21±0.57 at the maximum dose of radiation i.e.300 Gy. At anaphase, laggards, bridge formation and asynchronous condition *etc.* were observed at higher doses of radiation that is 200 Gy and 300 Gy. Occurrence of cells having laggards and bridges have an increasing trend with increasing dose of radiation intensity. Thus, in the present study, the total abnormality percentage showed an increasing trend with the increasing irradiation doses. The highest Total abnormality percentage was recorded at 300 Gy i.e. 19.04±1.52 which is much greater than lower doses.

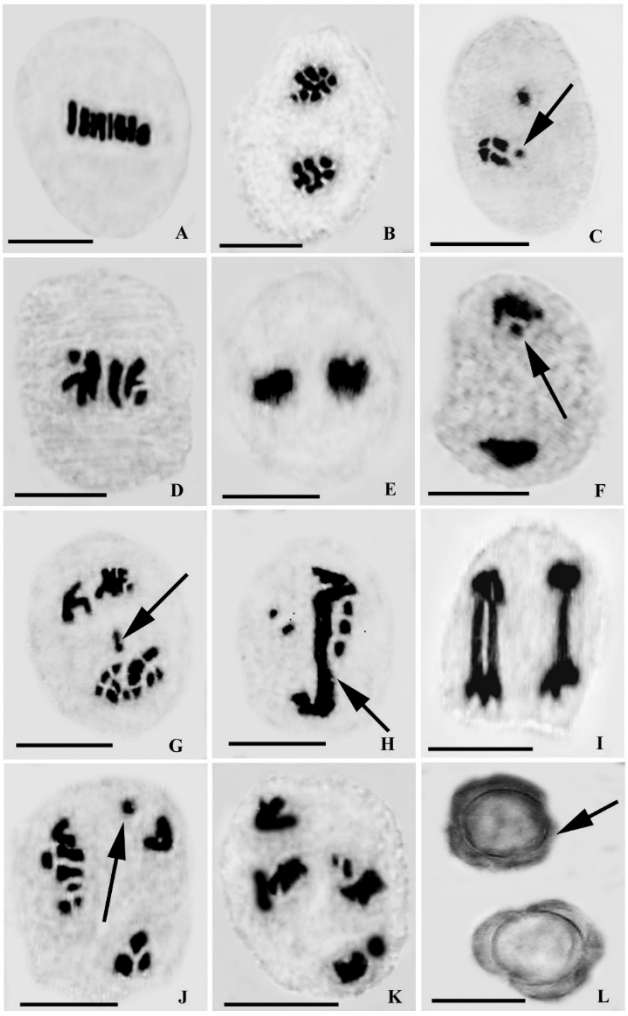

FIGURE.4: Chromosomal anomalies induced by Gamma ray irradiation: A Normal Metaphase I (n=11) B. Normal Anaphase C. Precocious chromosome at Metaphase with stickiness D. Multivalents E. Stickiness at Metaphase II F. Stickiness at Anaphase I with laggard G. Unoriented AnaphaseI with laggard H. Sticky bridge with laggards I. Double bridge formation at Sticky Anaphase II J. Tripolarity with one laggard K. Disturbed polarity L. Fertile(Arrow) and Sterile Pollen Scale bar- 10.73µm.

Pollen fertility under Gamma ray treatment:
Pollen fertility rate was also studied and found to have an inverse relationship with increasing doses of gamma irradiation. In control set it was found to be 93.5±1.94 whereas along with the increasing doses of gamma radiation, the rate of pollen fertility was found to be decreased, maximum reduction was noted at 300 Gy i.e. 65.0±1.95 (Table 1).

Proline content under gamma ray treatment:

A gradual increase of mean performance was noticed in proline content for all the mutagenic doses when compared with control. Among them, maximum content was observed at 300 Gy i.e. 28.30±0.70[a] µmole/gm as compared to control (Figure 2B).

Table 1 – Cytological abnormalities and pollen fertility(%) induced by Gamma irradiation in *Eclipta alba* (L.) Hassk.

Dose Gy	No. of PMC's observed	Metaphasic Abnormalities (%)					Anaphasic Abnormalities (%)					Pollen Fertility%	Oth. (%)	T.Ab. (%)
		Sc	Pm	St	Un	Mv	Asy	Br	lg	Un	St			
Ct	390	-	-	-	-	-	-	-	-	-	-	93.5±1.94a	-	-
100	374	0.53±0.16	0.44±0.09	1.49±0.11	0.35±0.08	0.17±0.08	0.79±0.25	-	-	0.44±0.09	1.15±0.06	89.4±2.16a	0.44±0.09	5.85±0.17
200	359	1.42±0.20	1.21±0.27	2.31±0.48	1.02±0.09	0.64±0.74	0.92±0.06	0.18±0.09	0.54±0.14	0.92±0.09	1.27±0.50	80.80±2.23b	0.72±0.22	11.21±0.49
300	344	1.44±0.11	1.80±0.45	4.21±0.57	1.33±0.21	0.67±0.75	1.15±0.12	2.12±0.21	1.43±0.24	0.96±0.06	2.92±0.39	65.0±1.95c	0.96±0.25	19.04±1.52

Where,

Abbreviations: Sc- Scattering; **Pm-** Precocious movement of chromosomes**; St-** Stickiness**; Mv-** Multivalent; **Lg-** Laggard; **Un-** Unorientaion; **Asy-** Asynchronous division; **Br**- Bridge formation of chromosome **; Oth-** Others;**T.Ab. –** Total abnormalities

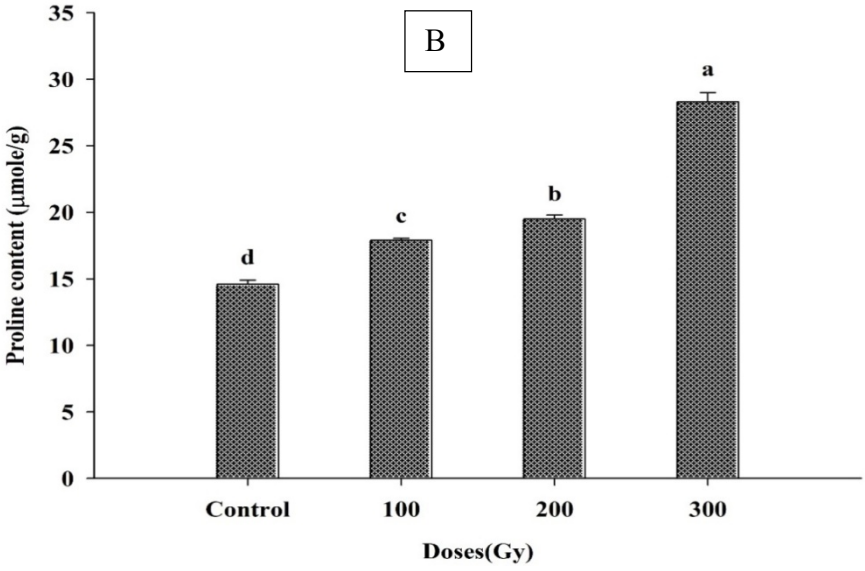

FIGURE 2: Effect of Gamma ray treatment on *Eclipta alba* (L.) Hassk. Biochemical content (B) Proline content.

DISCUSSION

It is known that gamma radiation is tremendously used as physical mutagen in mutation breeding and found to be very applicable for persuading disparity in plant system. This consequence can occur both impulsively by nature and artificially by mutagens. Present study divulges the gamma radiation effects *i.e.* plant's different growth parameters as morphological traits, cytogenetical characteristics and biochemical constituents. The study revealed that gamma radiation affects germination, survivability of the plant and induces morphological and chromosomal peculiarities. Exposure of gamma radiation significantly reduces germination and survivability of plant which might be due to the mutagenic effects. The stimulus of irradiation was dose dependent and the reduction of germination and survival was more at the higher dose of gamma radiation. The frequency of chromosomal damages also increased with increase in the radiation doses (Table 1) which may suppress the germination and plant survival [16], [20] . Irradiation of the seeds with gamma rays, in particular acts upon the activities of vital metabolic enzymes and protein synthesis [9],[17] leading to disorder and inhibition of germination and growth cascade [21] . Striking effects of mutagenic treatment of gamma rays causes inhibition of cell division in meristematic zone of growing parts which might be a reason behind reduction in germination and survivability percentage of Bhringraj. Moreover, suppression of auxin biosynthesis and changes in ascorbic content may also cause inhibition of germination and developmental cascade [29].

Other morphological aspects such as plant height, days to 50% flowering and number of primary branches were also affected by the radiation treatment. The present investigation revealed two antagonistic arrays namely radio stimulation and radio inhibition. From the data obtained (Figure 1) it is very much clear that gamma rays had extremely noteworthy influence on plant height and number of primary branches. There was an increment noted at lower doses than control plants. Lower doses of gamma rays were more positively effective on subsequent growth of plant [30]. Plant height and number of primary branches at the 100 Gy slightly increased from the control which is the stimulatory effect of lower doses of gamma radiation. Wi *et al*. [33] noted that there is a hypothesis that the lower dose irradiation will induce the growth by changing the hormone signaling crosstalk in plant cell or by increasing the antioxidant capacity of the cells to easily overcome daily stress factors such as fluctuation of light intensity and temperature in the growth conditions. This exhaustive study confirmed that the lower dose of radiation induces some beneficial traits in of Bhringraj which is termed as radiation hormesis. Hormesis in general divulges as excitation on small doses by any agent in any system [34]. In contrast, the high dose irradiation that caused growth inhibition has been ascribed to the cell cycle arrest at G2/M phase or various damages in the entire genome [26]. Figure 3, showed that there was abnormal phenotypes observed by gamma rays treatment. These variants were found at high frequency of radiation *i.e*. 300 Gy. At this dose, the branching pattern was altered from the normal pattern which might be due to the high amount of auxin and cytokinin which induces multiple branching [22]. The same hormonal balance may also be caused due to gamma irradiation. Another reason for multiple branching is the inhibition of apical dominance which results in lateral distribution of growth hormones [29]. One possible reason behind increased branching could also be the retrotransposons in plants which are likely to be exposed to relatively weak radiation and may lose their jumping function and it seems that plants have adaptive strategies to overcome the harmful effects caused by the radiation [12] and [25]. Different leaf variants were also found at the higher dose of radiation which might be a result of the chromosomal disturbance and irregularities found at the hormonal level which cause the different type of shapes and deviations in the margins of the leaf [24].

Another radiogenic trait i.e. days to 50% flowering is affected by the treatment. The flowering was delayed due to the biological damage. At higher doses, delayed flowering might be due to interrupted biochemical pathways including phytohormones which are active participants of floral induction pathway. Misra*et al*. [23] and Kim *et al*. [15] reported that there was less or delayed flowering observed at higher doses because of changes in metabolic activities, flowering physiology and negative response of plant hormones. These evidences support present investigation i.e. delay in flowering might be a reason of radiation block in floral hormones.

Cytogenetical study deciphered the specific and various types of meiotic anomalies recorded at different doses of gamma ray treatment i.e. stickiness, scattering, precocious, laggard, bridge, *etc.* (Figure 4). Stickiness is predominant abnormality which is due to the depolymerization of nucleic acid caused by mutagenic treatment, and it may also be caused by the dysfunctioning of histone protein [10] (Figure 3E). The formation of multivalents may also be attributed to the abnormal pairing and non-disjunction of bivalents (Figure D) [32]. Jafari *et al.* [11] proposed that precocious movement of chromosome was possibly produced by spindle dysfunction (Figure 3C). According to Khan *et al*. [12], precocious movement of univalents or bivalents towards poles due to unequal distribution of chromosome or loss of

a complete set of chromosomes called as stray chromosome. Laggard formation (Figure 3F,G) is due to delayed terminalisation, chromosomal stickiness or failure of chromosomal movement [27]. The bridge formation reported is caused by defective functioning of target proteins due to gene mutation or direct action of mutagens on proteins that create the disturbance during chromosome separation [18] and [3] (Figure 4H,I). The pollen fertility in comparison to control, got decreased at successive doses of gamma radiation because of increased chromosomal aberrations. It causes the delayed flowering which increases the sterility of the plant. The variations induced by the gamma radiation may be helpful in the creation of new genotype.

The accumulation of proline content at all the mutagenic doses depicts that it functions as osmolyte under abiotic stress as protective mechanism. Our present result was supported by Esfandiari *et al.* [8] that testified that the increase in proline content observed in irradiated plants due to the protective procedure in the synthesis of osmolytes was crucial to plant growth. Proline content is involved in various functions such as preserving enzyme structure and activities; it decreases enzyme denaturation caused by environmental stresses [2]. An increment in proline content is helpful to protect plant against the stress and shows promising aspects in creating mutants (Figure 2B).

CONCLUSION

The outcome of the experiments indicate that higher doses of gamma radiation show bionegative effects but the lower doses found to cause biopositive effects on plant which may be used for the enhancement of qualitative and quantitative traits of Bhringraj to upsurge the demand of this medicinal plant. Gamma rays shows the stimulatory effect at the lower doses which influences the genetic variation in plant and helpful in creating new traits. These findings in future may revolutionise the pharmaceutical industries and may be proved to be panacea for mitigating various ailments of human beings.

DECLARATION OF INTEREST

The authors report no conflict of interest.

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