Cytogenetic effects of gamma radiation-induced seed extracts of *Panicum miliaceum* L. on the somatic chromosomes of *Allium cepa* L.

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Abstract

Studies on the interaction between biological systems and Gamma radiation have been used as an efficient approach for determining both its beneficial and damaging effects. The present study was carried out to assess the induced Gamma irradiation effects on cytogenetic parameters in proso millet (Panicum miliaceum L.) variety CO(PV) 5 by exposing the seeds to Gamma source - Cobalt-60 (^{60}Co) at doses ranging from 50, 100, 150 and 200 Gy.

The results of cytological studies with Allium cepa bioassay revealed a wide range of chromosomal aberrations viz. stickiness, bridges, laggards, fragments, nuclear lesions, binucleated cells, precession, promiscuous chromosomes, disoriented oblique spindles and chromosome degeneration. The study concluded that the results were concentration-dependent, time-dependent and Gamma-dose-dependent.

Keywords: *Allium cepa* bioassay, Cytogenetic parameters, Cytotoxicity, Gamma source, Induced gamma radiation.

Introduction

Induced mutagenesis serves as a unique approach, making it one of the most efficient tools in plant genetics in the areas of genetic variability, plant growth, functional genomics, crop breeding and improvement and key regulatory gene identification for economically important characters or traits[8,18,27,34]. The application of mutation techniques has been known to induce genetic variability and plays a significant role in plant breeding and genetic studies[33].

Ionizing radiation alters or modifies physical, chemical and biological properties of any substance or matter and has enormous beneficial applications in the fields of medicine, pharmaceuticals, agriculture, food technology and research. It has harmful or hazardous mutagenic effects on exposure results in somatic and genetic damage[9].

Gamma radiation has been reported as an environmentally important ionizing radiation with very short wavelength (~10^{-3} to 1 Å), high frequency and high-energy photons[39]. Photons are highly penetrating and originate from the settling process of an excited nucleus of a radionuclide after radioactive decay[7,10]. The interaction of γ-rays with biological matter results in ionization and excitation of macromolecules leading to DNA breaks (single or double strand) or production of reactive oxygen species (ROS)[37]. They are widely used for creating genetic variability by inducing mutation as they cause damage in biomolecules (DNA, proteins and lipids) and induce pernicious effects on growth, morphology and reproduction[20]. Known for a wide range of applications, Gamma radiation has also been reported to cause useful and harmful cytogenetic effects on a wide range of plant species[19,35,36].

Panicum miliaceum L. (proso millet) of family Poaceae (according to Bentham and Hooker classification) is a warm-season annual herbaceous cultivated crop which is well adapted to many soil and climatic conditions due to its high water-use efficiency[1,4,31]. It is a tetraploid millet (2n=4x=36) with a high content of calcium, dietary fibre, iron, magnesium, potassium, phosphorous, protein, zinc, B-complex vitamins, minerals and sulphur-containing essential amino acids[16,29]. Epidemiological studies reveal that increased consumption of proso millet and its products were associated with significant functional components and health benefits including reduced risk of chronic diseases such as elevated serum cholesterol[25], cardiovascular disease[22], type II diabetes[11] and liver injury[26], with special reference to anti-cancer, antioxidant and antiproliferative effects[6,28,42].

Proso millet and its functional products serve as healthy food in the daily diet and are better substitutes for wheat and rice due to its nutritional and biological significance[30,32]. Therefore, the present study focuses on the effects of induced Gamma radiation in proso millet on cytogenetic effects of Gamma-irradiated proso millet seed extracts on the somatic chromosomes of *A. cepa*.

Material and Methods

Plant Material: The seeds of *Panicum miliaceum* Linn. (panivaragu / proso millet) variety CO (PV) 5 were obtained from Centre of Excellence in Millets (CEM), Tamil Nadu Agricultural University (TNAU), Athiyandal, Tamil Nadu, India.

Gamma irradiation: Irradiation of proso millet seeds was performed with Gamma chamber 5000 using a Cobalt-60 (^{60}Co) Gamma source in ambient conditions at the Indian Council of Agricultural Research-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru, India. The exposure doses were 50, 100, 150 and 200 Gy whereas non-irradiated seeds served as control.

Mitotic Preparation: The somatic chromosome studies with *A. cepa* root tips were done by the Haematoxylin squash method[24]. The root tips were treated with the aqueous extracts of proso millet seed powder of respective irradiation dosages (50, 100, 150 and 200 Gy) and untreated tips were taken as control. The onion root tips were hydrolysed with hydrochloric acid (1 N) for one minute and then washed in a series of distilled water. The hydrolysed tips were mordanted with 4% iron alum for 15-20 minutes. These mordanted root tips were washed and stained using commercially available haematoxylin stain for 25-30 minutes, followed by glacial acetic acid treatment for 1-2 minutes. The root tips were mounted on a clean glass slide and a cover slip was placed on top of it and gently tapped for equal spreading of cells.

The slides were viewed under the microscope to study cytological parameters like mitotic index (MI) and screening of chromosomal aberrations (CAs) from the observed mitotic stages. Approximately, 300 cells per treatment and control were analysed to score the frequency of mitotic index and chromosomal aberrations. Photomicrographs of some selected representative stages from different concentration treatments were taken. Cytological parameters were calculated by using the following formula[12]:

- Mitotic Index (MI) = Number of dividing cells/Total number of cells observed) × 100
- Aberration Percentage = Number of aberrated cells/ (Total number of cells) × 100

Results and Discussion

The present study showed that mitotic phases in control sets were normal with no chromosomal irregularities. Results of *A. cepa* bioassay showed different types of chromosomal aberrations subjected to different aqueous extract concentrations (1%, 3% and 5%) of Gamma irradiated (50 Gy, 100 Gy, 150 Gy and 200 Gy) seeds of *P. miliaceum*, at time intervals of 12, 18 and 24 hours.

In the present investigation, root tips treated with aqueous seed extracts were analysed to find out different types of chromosomal abnormalities as an outcome of mitotic inhibition such as chromosomal bridges, fragments, laggards, nuclear lesions, stickiness, precession, irregular chromosomal clumping, disoriented oblique spindle, chromosome degeneration, binucleated cells and elongated nucleus at mitosis in *A. cepa* (Figure 1).

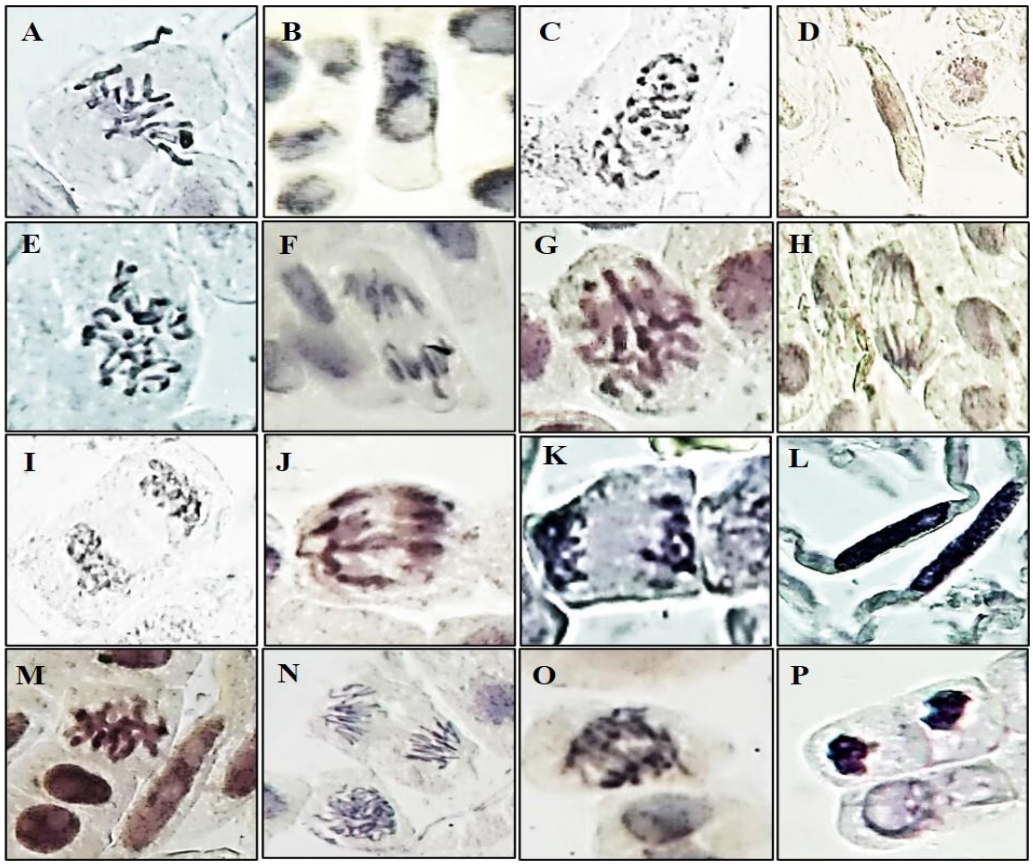

Figure 1: Mitotic chromosomal aberrations observed in *Allium cepa* root tip cells using aqueous extract of *Panicum miliaceum*.
(A) Irregular arrangement of chromosomes, (B) Nuclear lesion, (C) Chromosomal fragments, (D) Ghost cell, (E) Dicentric chromosomes, (F) Precession, (G) Scattered and condensed chromosomes, (H) Laggard, (I) Degenerating chromosomes at late anaphase, (J) Chromosomal bridges, (K) Promiscuous chromosome, (L) Linear cell and nucleus, (M) Elongated cell and nucleus, hyperchromasia, (N) Vagrant chromosomes at anaphase, (O) Disoriented oblique spindle, chromosome stickiness and bridges, (P) Irregular clumping of chromosomes.

The occurrence of chromosomal aberrations varied within the treatments with an increase in Gamma irradiation dosage and time interval. The chromosomal aberrations were observed to be high in 5% concentration followed by 3% and 1% concentrations. Exposure of cells to radiation or carcinogen leads to DNA breaks and the broken ends may rejoin in different patterns from their original arrangement which may be visualised at mitosis when cells divide, hence, this assay has been extensively utilized for many years to cause mutations and chromosomal damage for experimental purpose[2]. Like the aberrations observed in this study, a broad range of chromosomal abnormalities induced by ionizing radiation such as dicentric, tricentric, acentric chromosomes, ring chromosomes, deletion, laggards, chromosome bridges, chromosome fragmentation and micronuclei formation have been reported[3,21,23,41].

These aberrations occur because cells' nuclear material, especially DNA, is susceptible to radiation damage and free radicals produced through the radiolysis of water[40] also revealed that an increase in the concentration of aqueous seed extracts aggravated the occurrence of abnormalities in the somatic cells of onion root tips. This assessment shows an inverse relationship between the mitotic index and chromosomal aberrations. The mitotic index and aberration percentage of 1%, 3% and 5% aqueous seed extracts of Gamma-irradiated and non-irradiated *P. miliaceum* seeds on root tips of *A. cepa* at different time intervals were tabulated (Tables 1 and 2) respectively.

The mitotic index at 12, 18 and 24 hours was significantly higher in onion root tip cells treated with 1%, 3% and 5% control (non-irradiated *P. miliaceum* seed extract) compared to cells treated with 1%, 3% and 5% concentrations of other treatments (50 Gy, 100 Gy, 150 Gy and 200 Gy). The highest mitotic index was observed in onion root tip cells treated with 1% control at 12 hours, while the lowest mitotic index was observed in onion root tip cells treated with the 5% concentration of 200 Gy irradiated *P. miliaceum* seed extract at 24 hours. Figure 2 demonstrates that the mitotic index of each treatment showed a gradual decrease as the concentration of the seed extracts increased.

The highest aberration percentage was observed in the onion root tip cells treated with 1%, 3% and 5% concentrations of 200 Gy Gamma-irradiated *P. miliaceum* seed extract compared to the same concentrations of other treatments (50 Gy, 100 Gy and 150 Gy) at 12, 18 and 24 hours. The highest aberration percentage was observed in the 5% concentration of *P. miliaceum* seed extract irradiated with 200 Gy at 24 hours.

Conversely, the lowest aberration percentage was observed in the 1% concentration of *P. miliaceum* seed extract irradiated with 50 Gy at 12 and 18 hours. In contrast with the results of the mitotic index, with an increase in the concentration of seed extracts, there was a substantial increase in the aberration percentage in all the Gamma-irradiated treatments, as seen in figure 3. Thus, the increased aberration percentage with reduced mitotic index due to the inhibition of DNA synthesis at S-phase could be interpreted as cellular lethality induced by gamma radiation[17,38]. With a rise in aberrated cells and higher doses of Gamma irradiation and concentrations of seed extract, the reduction in mitotic indices compared to the control indicates a mitodepressive effect on cell division.

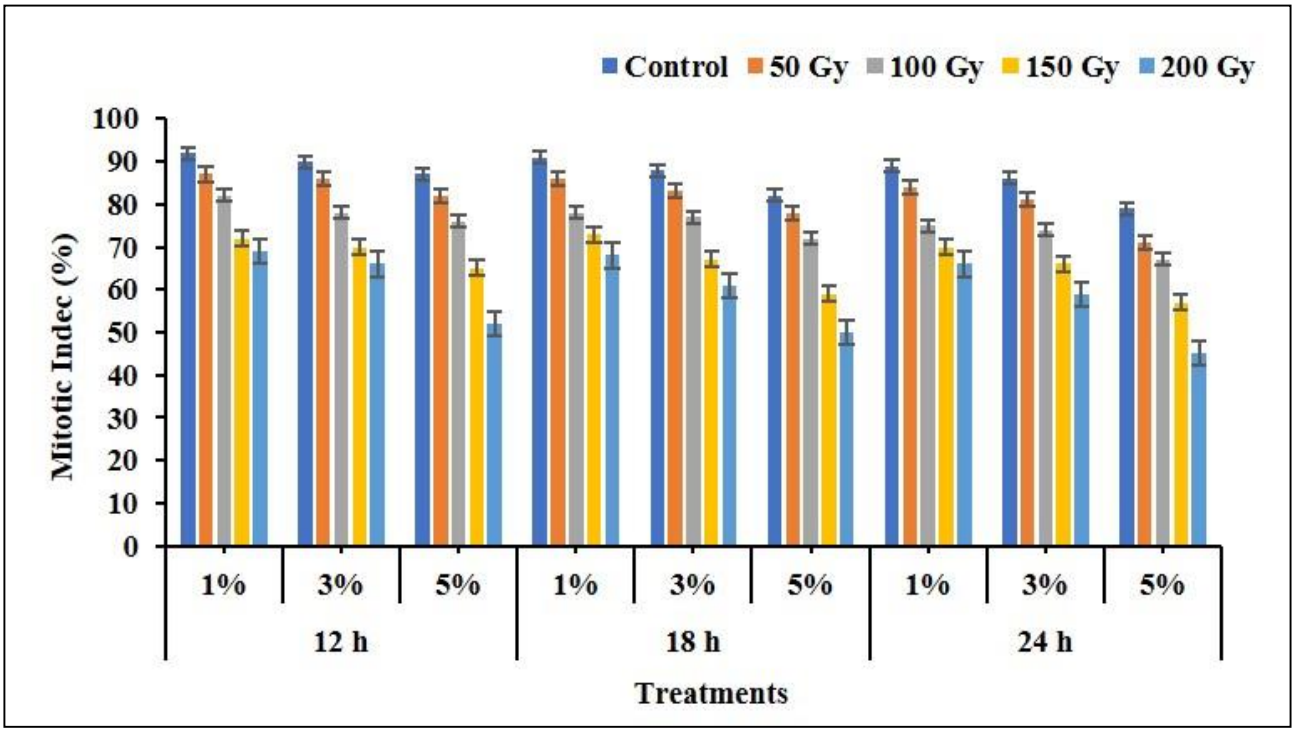

Figure 2: Effect of gamma irradiation in aqueous seed extracts of *Panicum miliaceum* on mitotic index of *Allium cepa* root cells at different time intervals.

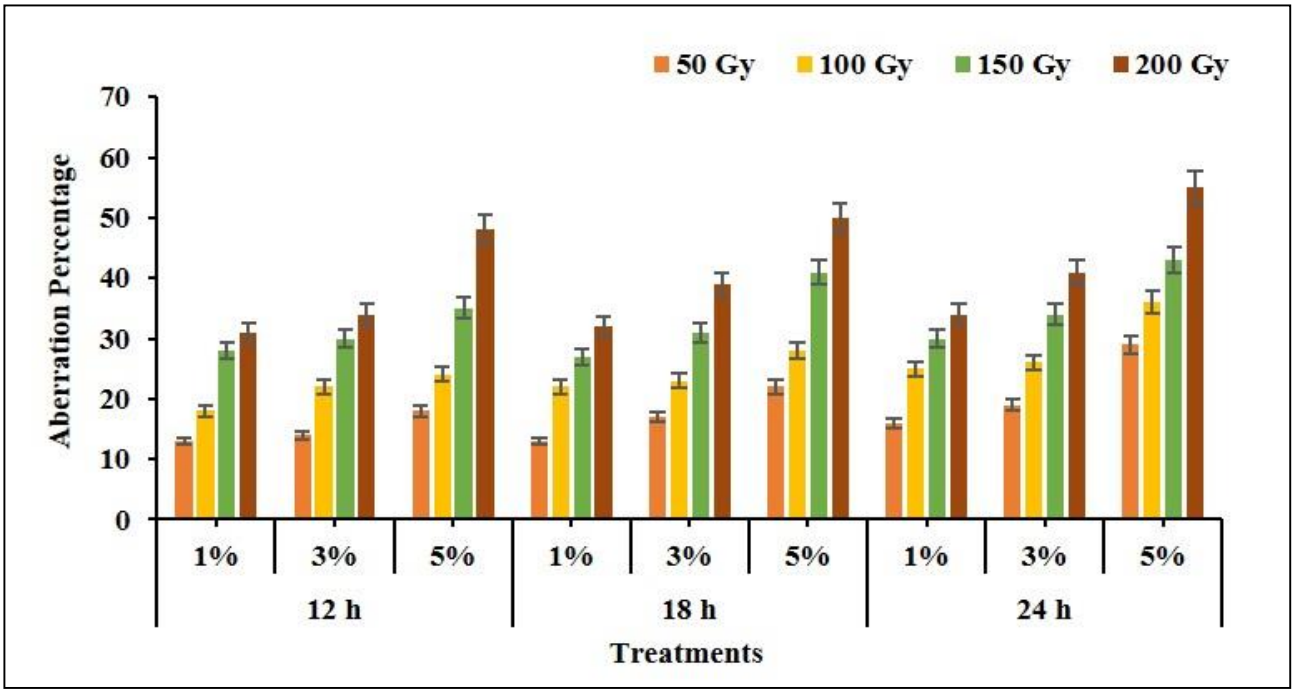

Figure 3: Effect of Gamma irradiation in aqueous seed extracts of *Panicum miliaceum* on aberration percentage of *Allium cepa* root cells at different time intervals

Table 1
Effect of Gamma irradiation in aqueous seed extracts of *Panicum miliaceum* on mitotic index of *Allium cepa* root cells at different time intervals.

Time Interval	Concentration of extract	Radiation Dosage (Gy)				
		Control	50	100	150	200
12 h	1%	92	87	82	72	69
	3%	90	86	78	70	66
	5%	87	82	76	65	52
18 h	1%	91	86	78	73	68
	3%	88	83	77	67	61
	5%	82	78	72	59	50
24 h	1%	89	84	75	70	66
	3%	86	81	74	66	59
	5%	79	71	67	57	45

Table 2
Effect of Gamma irradiation in aqueous seed extracts of *Panicum miliaceum* on aberration percentage of *Allium cepa* root cells at different time intervals.

Time Interval	Concentration of extract	Radiation Dosage (Gy)			
		50	100	150	200
12 h	1%	13	18	28	31
	3%	14	22	30	34
	5%	18	24	35	48
18 h	1%	13	22	27	32
	3%	17	23	31	39
	5%	22	28	41	50
24 h	1%	16	25	30	34
	3%	19	26	34	41
	5%	29	36	43	55

In an experimental study on the induction of cytogenetic effects by acute γ- irradiation in seeds of crops, a significant increase in the occurrence rate of aberrant cells in the root meristem was observed at comparable doses and an insignificant declination of mitotic activity was reported with an increase in the dose absorbed in rye[13-15]. Thus, the

chromosomal aberrations, mitotic indices and aberration percentage unequivocally showed dependence on concentration, time and gamma dose.

Conclusion

The outcome of the present research reveals that different doses of Gamma radiation have diverse effects. The occurrence of chromosome abnormalities reflected complex cellular responses triggered by Gamma radiation exposure which may interfere with the normal growth of plants. The research showed that exposure to Gamma radiation had harmful effects on cytogenetic parameters. The 200 Gy dosage was found to be lethal as it resulted in the reduction of the mitotic index and a drastic increase of aberration percentage with various chromosomal abnormalities.

This implies that the radiation had damaging effects on the genetic material within the cells, potentially leading to mutations or other genetic abnormalities. Thus, the observed chromosomal abnormalities in this study were evident to prove that gamma radiation has cytotoxic effects on somatic chromosomes of *A. cepa* treated with Gamma-irradiated *P. miliaceum* seed extracts.

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