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ORIGINAL ARTICLE

Comparative Cytogenetic and Biochemical Effects of Gamma Radiation on Two Accessions of *Setaria italica* (L.) Beauv.

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ABSTRACT
Physical mutagens have been an important tool in mutation breeding programmes. Mitotic studies are used to analyse the tolerance and determination of optimum dose which may have potential to induce beneficial traits. Since, actively dividing cells show more susceptibility towards mutagens so Active Mitotic Index (AMI) and cytological abnormality may be an important parameter for assessment of effectively and potential of a mutagen as well as the susceptibility of varieties. In the present study, seeds of two accessions of Setaria italica (L.) Beauv. were irradiated at four doses of gamma rays viz. 100Gy, 200Gy, 300Gy and 400Gy by a ^{60}Co source. Germination percentage, survival percentage, AMI, cytological abnormalities and pigment content were evaluated. On the basis of comparative study, it was found that scattering, precocious movement, stickiness, unorientation, loop formation, bridges and forward movement were common abnormalities and stickiness was most prevalent. Germination, survival, AMI and pigment content were also affected with increasing doses of mutagens. The study inferred that accession I is more sensitive to physical mutagen so it may be useful in development of a potential hybrid using mutation breeding.
Keywords: *Active Mitotic Index, Cytological abnormalities, Setaria italica, Physical mutagen, Pigment content*

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INTRODUCTION

Setaria italica (L) Beauv., commonly known as foxtail millet, is one of the oldest cultivated agronomically important climate resilient small seeded grass. It is a versatile crop known for its adaptability to adverse climatic conditions, poorly nourished soil, minimal requirement of inputs and highly nutritious seeds. Taxonomically, *Setaria* belongs to sub-family Panicoideae of family Poaceae, in the commelinid clade of monocots [22].

Millets are considered nutritionally superior over other cereals since they are rich in minerals, vitamins, crude edible fibres and are gluten-free [13]. Moreover, due to low Glycemic Index (GI) millets are slowly digested, absorbed and metabolised resulting in a lower and slower rise in blood glucose [19]. In the present scenario of climate change, climate resilient crops are of great importance. Due to nutritional superiority and high adaptability, millets are considered to be the 'crop of future' and 2023 will be celebrated as International Year of Millets. Hence, there is need to develop improved varieties with beneficial traits.

Mutation breeding is a crop improvement strategy based on use of mutagens with an aim to increase genetic variation in a crop species [1]. Gamma radiation is most common physical mutagen used for inducing mutation by creating genetic variability [16]. Established genetic variability is used to select genotypes with certain specific traits which may be of great economic importance. The basic idea behind the use of a physical mutagen is its action on several sites of genetic material. Besides it also damages cell either by producing free radicals or directly affecting several cellular parts [12].

Pretreatment of seeds with different conditions or chemicals may affect the whole morphogenesis process leading to aberrations at both morphological as well as genetic levels. Since germination is the first event in developmental cycle, it may be used for the preliminary detection of any effect of radiations

or chemicals [4]. So it may be utilized as a system to study the effectiveness of mutagen and its tolerance in plant. Germination percentage and survival of any variety is an important indicator to determine the lethality and effectively of any mutagen. It may help to decide the doses of any mutagen which may be tolerated by the plant [25]. Mitotic study helps in deciding the potential doses of mutagen for inducing genetic variability and identification of suitable genotype. Photosynthetic pigment study is an important parameter in the study of response of plant to mutagens and its lethal doses. Comparative study of two accessions may help to decide the adaptability and resilience of the plant. The present study was done with an aim to find out suitable variety of *Setaria italica* (L.) Beauv. for mutation breeding and consequences of gamma irradiation in plants.

MATERIAL AND METHODS

Procurement of seeds: Pure inbred seeds of *Setaria italica* (L.) Beauv. were procured from NBPGR, Akola centre, India. Two accessions were used for present study, accession I and accession II.

Germination and Survival: Procured seeds were irradiated at different doses of gamma rays *viz*. 100 Gy, 200 Gy, 300 Gy and 400 Gy through ^{60}Co source at NBRI, Lucknow (India). Non irradiated seeds acting as control and irradiated seeds of different doses were pre-soaked for 5 hours. Moist filter paper was used on Petridishes to place the pre-soaked seeds inside the germinator (Metrix Scientific, New Delhi) at 28±2^{o}C for germination. Some seeds were sown in garden for the study of survival ability, biochemical estimations and other effects. After 72 hours, germination percentage was calculated for control and all the doses of both accessions. Survival percentage was calculated after 15 days of germination.

Mitotic study: After 48 hours, germinated seeds were fixed in carnoy's fixative (1:3, Glacial Acetic Acid and Absolute alcohol) for 24 hours and then preserved in 70% alcohol at 4^{o}C. The experiments were performed in triplicates. For mitotic chromosomal study, fixed root tips were hydrolysed in 1N HCl for 30 seconds at 60±2^{o}C on water bath. After hydrolysis, root tips were washed under running tap water and dried using blotting paper. Chromosome squash technique was used for mitotic slide preparation using 2% acetocarmine stain. For each preparation 10 slides were prepared with analysis of 10 microscopic fields in each slide for scoring Active Mitotic Index (AMI %) and Total Abnormality Percentage (TAB %). Slides were observed at 40 X magnification and photographs were captured in Nikon phase contrast microscope using PCTV software.

Formulae used*:*

Active Mitotic Index= (Total number of dividing cells/Total no. of cells observed) * 100
Total Abnormality Percentage= Total number of abnormal cells/Total no. of cells observed *100
Germination percentage= (Number of germinated seeds/Total no. of seeds) * 100
Survival percentage= (Number of surviving plants at 15 days after germination/No. of seeds sown) * 100

Statistical analysis: Statistical analysis was performed using SPSS 16.0 software. Data for each treatment were taken in triplicate and one independent variable was used. Further analysis was done using one way analysis of variance (ANOVA). The Duncan's multiple range test (DMRT, $P<0.05$) was performed for mean and the graph was prepared using Sigma plot 10.0 software. Similar statistical analysis was done for photosynthetic pigment analysis.

Biochemical study: Biochemical study was based on photosynthetic pigment estimation. Photosynthetic pigment was estimated using Lichtenthaler's method [17]. 20 mg of fresh leaves were crushed in 5 ml of 80% acetone. The experiment was conducted in triplicate to reduce the probability of errors. The supernatant was filtered and OD was recorded at 646 nm, 663 nm and 470 nm using Spectrophotometer to find out the content of chlorophyll *a*, chlorophyll *b* and carotenoids respectively.

RESULTS

Germination percentage was found to be significantly affected by gamma irradiation. In the control, germination percentage was recorded as 100 % in accession I and 95% in accession II. Germination percentage was found to be decreased with the increasing doses of gamma rays(Fig. 1). Germination percentage in both accession I and II was lowest at the highest dose of 400 Gy gamma radiation. In accession I, at 400 Gy germination percentage reduced from 100 % to 85 % where as in accession II, at highest dose of 400 Gy, germination percentage reduced from 95% to 75%.

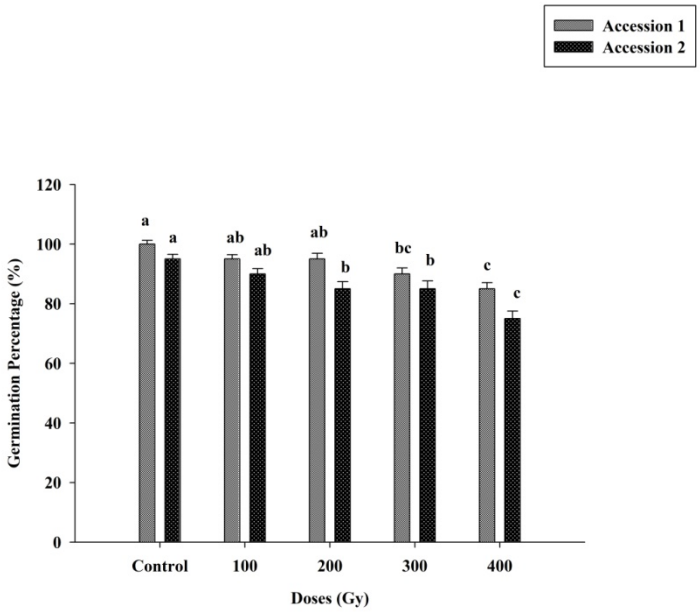

Figure 1: Graph showing effect of Gamma radiation on Germination in two Accessions of *Setaria italica* (L.) Beauv

Survival percentage uniformly decreased with the increase in the doses of gamma irradiation in both accessions (Fig. 2). In the control survival percentage was recorded as 96% in accession I and 85% in accession II. In accession I, at 400 Gy, survival percentage reduced from 96 % to 65 % where as in accession II, at highest dose of 400 Gy, survival percentage reduced from 85% to 40%.

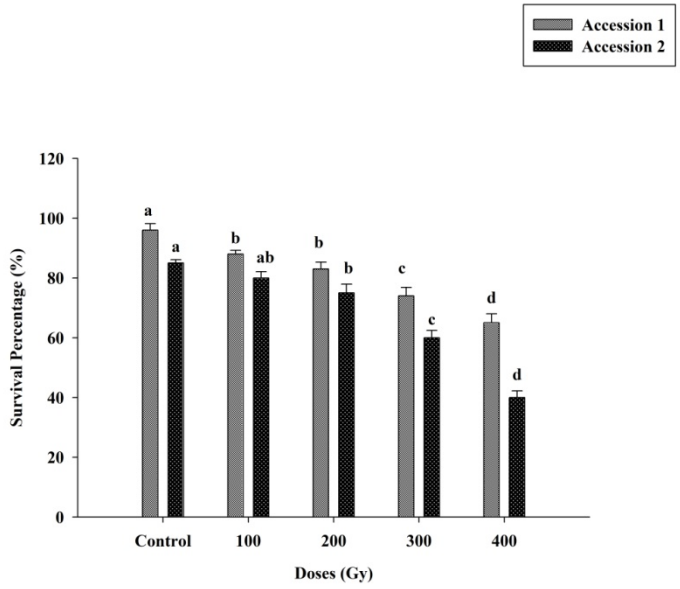

Figure 2: Graph showing effect of Gamma radiation on Survival in two Accessions of *Setaria italica* (L.) Beauv

Photosynthetic pigment analysis includes chlorophyll *a*, chlorophyll *b* and carotenoid content under different mutagenic treatments in both accessions. Chlorophyll *a*, chlorophyll *b* and carotenoid content was recorded to be increased in initial 100 Gy dose of gamma irradiation in both accessions. On higher doses all the photosynthetic pigments uniformly decreased with increase in mutagenic doses (Fig. 3).

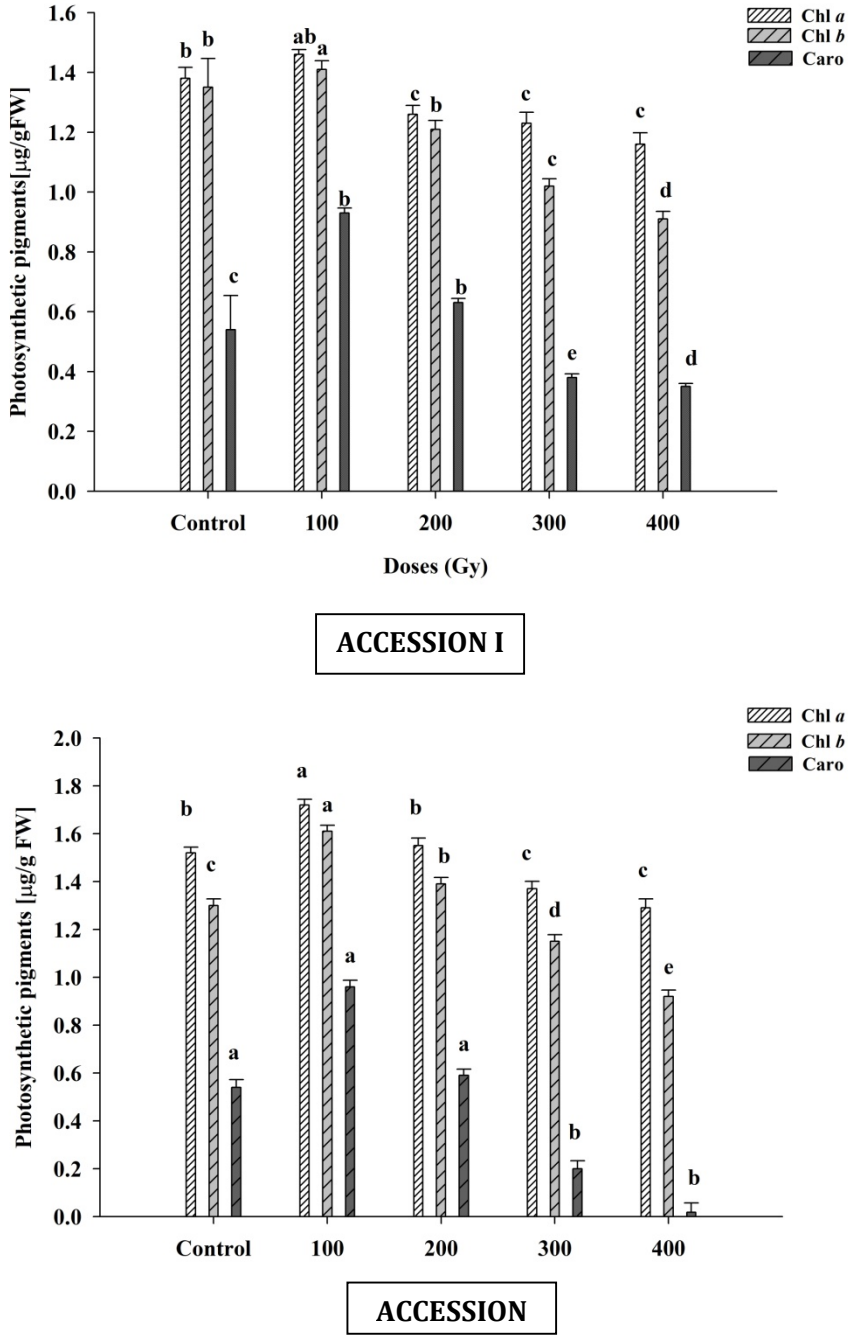

Figure 3: Graph showing effect of Gamma radiation on photosynthetic pigments in two Accessions of *Setaria italica* (L.) Beauv

In the present cytological study it was found that mitotic phases of control sets of both the accessions were found to be normal with 18 chromosomes at metaphase and 18:18 separation at anaphase without any anomaly in chromosomes. The AMI of control set was calculated as 11.62 ± 0.33^{a} and 9.80 ± 0.32^{a} in accession I and accession II, respectively (Table 1). AMI was found to be uniformly decreased with increase in dose of radiation. In both accessions, AMI and TAB are inversely proportional i.e. with increase in mutagenic doses, AMI decreases and TAB increases. As a result of mutagenic treatment different types of chromosomal abnormalities are induced which include scattering, precocious, bridge, unorientation, loop formation, stickiness, forward movement, laggards, *etc.* (Figure 4). Stickiness is the most common abnormality.

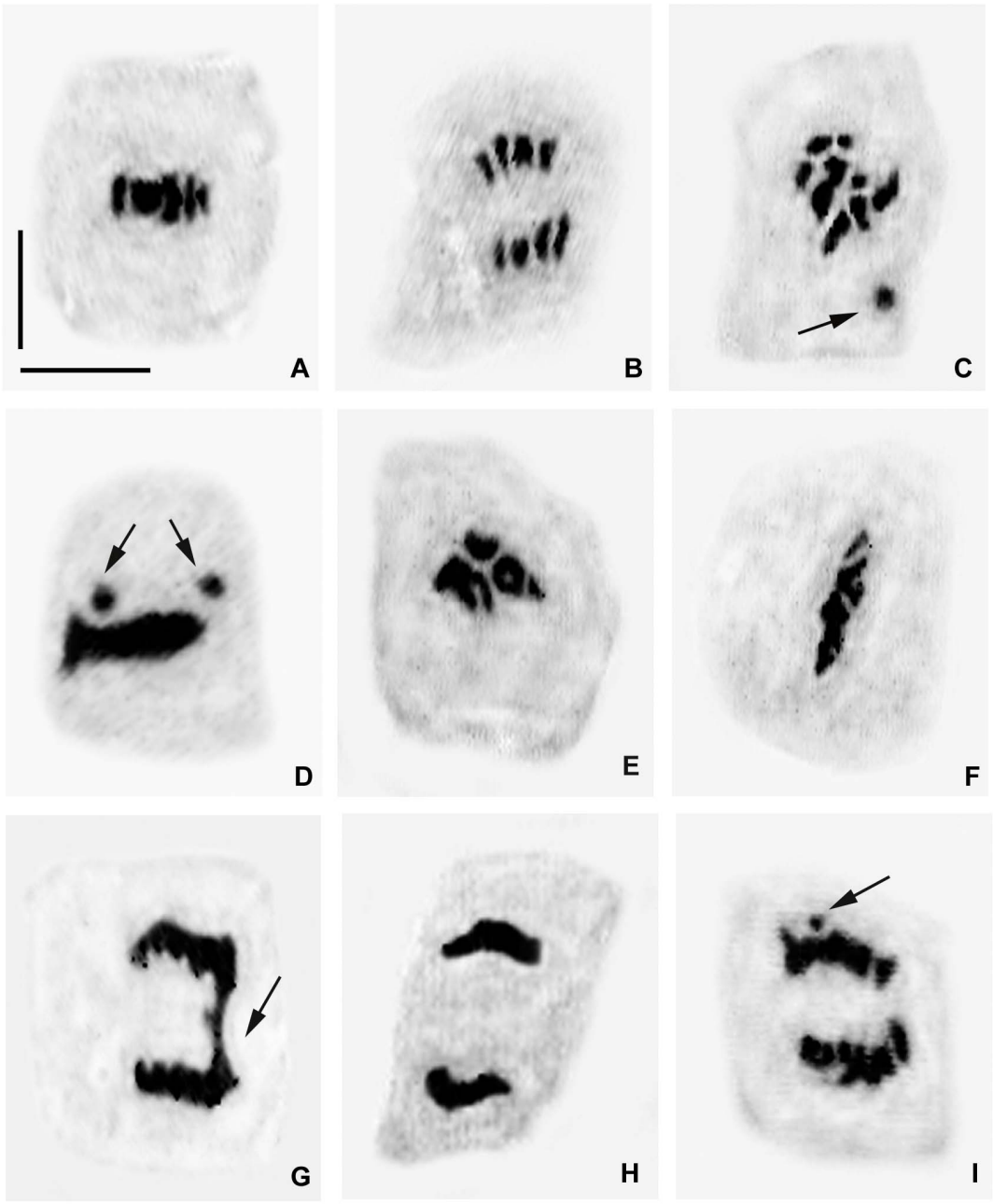

Figure 4: Cytological plate

Legend of figures- **A:** Normal Metaphase (2n=18); **B:** Normal Anaphase **C:** Scattering with Precocious at Metaphase; **D**: Precocious movement at metaphase; **E**: Loop formation at metaphase F: Unorientation at metaphase; **G**: Bridge at Anaphase; **H:** Sticky Anaphase; **I-** Forward at Anaphase.
Scale bar: Length- 10.81 µm Breadth-6.66 µm

Table 1: Effect of ionizing Gamma rays on mitotic activity and chromosomal morphology in *Setaria italica* (L.) Beauv.

Treatment	AMI%	Metaphasic Abnormalities (%)				Anaphasic Abnormalities (%)				Oth.	T.AB(%)
		Sc	St	Pm	Un	Br	St	Lg	Fm		
Accession I											
Control	11.62±0.33[a]	-	-	-	-	-	-	-	-	-	
100Gy	10.17±0.32[a]	0.35±0.008	0.70±0.19	0.35±0.005	0.35±0.08	-	0.47±0.12	-	0.22±0.11	0.35±0.01	2.81±0.17
200Gy	9.01±0.32[b]	0.49±0.12	0.94±0.30	0.48±0.10	0.36±0.01	-	0.48±0.10	-	0.36±0.22	0.11±0.11	4.08±0.07
300Gy	7.72±0.21[c]	0.50±0.34	1.09±0.17	0.51±0.10	0.37±0.22	0.23±0.20	0.52±0.15	0.25±0.12	0.39±0.24	0.38±0.22	4.78±0.04
400GY	6.60±0.31[d]	0.71±0.20	1.29±0.13	0.73±0.23	0.47±0.10	0.91±0.16	0.72±0.22	0.60±0.10	0.63±0.27	0.52±0.15	5.01±0.35
Accession II											
Control	9.80±0.32[a]	-	--	-	-	-	-	-	-	-	-
100Gy	8.20±0.29[b]	0.34±0.20	0.56±0.10	0.37±0.01	0.22±0.11	-	0.33±0.07	-	0.22±0.11	0.45±0.12	1.07±0.50
200Gy	7.10±0.29[c]	0.49±0.12	0.57±0.10	0.44±0.10	0.33±0.07	-	0.35±0.09	0.11±0.11	0.24±0.12	0.12±0.12	2.80±0.17
300Gy	6.50±0.33[c]	0.53±0.36	0.86±0.12	0.53±0.11	0.37±0.10	-	0.37±0.01	0.49±0.10	0.42±0.25	0.26±0.23	3.98±0.36
400Gy	5.08±0.27[d]	0.57±0.010	1.36±0.14	0.59±0.12	0.39±0.23	0.24±0.12	0.55±0.16	0.26±0.13	0.51±0.34	0.55±0.16	4.54±0.26

Abbreviations: Sc- Scattering; Pm- Precocious at metaphase; St- Stickiness; Br- Bridge formation; Lg- Laggard; Un- Unorientation; Fm- Forward movement; Oth- Others; TAB –Total abnormality percentage.

*Different letters in the superscript denote significant differences between means ($p < 0.05$) by DMRT (one way ANOVA)

DISCUSSION

Gamma ray, an efficient physical mutagen, is an ionizing radiation with short wavelength and high penetration power. Analysis of mitosis is considered as an important index to evaluate the potency of any mutagen. Comparative study of two accessions is an important aspect to select the variety for future experiments as per requirements. Different accessions differ in their genotype and respond differently to physical and chemical factors.

Seed germination is the preliminary stage in the life cycle of a plant. At this stage early responses of any physical or chemical factors may be detected. Germination percentage is one such factor which helps in assessment of effective doses of any mutagen [20]. It was found that in both accessions, germination percentage uniformly decreased with the increase in dose of the gamma radiation. Many studies in other plants have shown similar results [10]. In seeds irradiated with higher doses of radiation there is damage of DNA structure and synthesis causing chromosomal abnormality and delayed mitosis ultimately affecting the metabolic pathways and growth [9]. Lower germination percentage of control in accession II may be attributed to suitability of the genotype to the environmental conditions.

Mitotic study is an important tool to determine irradiation dosage that induces desired genetic variation without compromising the fertility. A range of doses indicate differential mutational genetic load showing the effectiveness of mutagens. Potency of any mutagen may be measured by the mutation frequency [11]. Active mitotic index is a measure of actively dividing cells and is an important indicator to determine the rate of root growth [18]. Active mitotic index and total abnormality percentage are directly related to inhibition of normal cell division. Lowering of AMI with increasing dose of mutagen may be attributed to inhibition of DNA synthesis at S-phase [23].

Stickiness, the most prevalent abnormality found in the study, may result from gamma induced nucleic acid depolymerisation or nucleoprotein dissociation with altered organization pattern [5,24]. In some cases, chromosomal breakage may be a reason for stickiness [7,8]. Precocious movement of chromosomes was found at the metaphase. This anomaly is caused by abnormal chromosome pairing either due to stickiness or defective spindle activity [21, 15].

Bridges were reported in the anaphase which may be due to stickiness leading to inability to separate at anaphase and breakage and reunion of chromosomes forming bridges [2, 15]. Laggards may be a result of defective spindle functioning or breakage of chromosomal bridges. Abnormal spindle functioning and stickiness restricts proper chromosomal movements resulting into laggards [14, 21]. These chromosomal anomalies may affect the cell division, genotype and commercially important traits.

Chlorophyll *a* and chlorophyll *b* content of plant has direct correlation with the photosynthetic efficiency whereas carotenoids have main role in protection against excess light energy. Photosynthetic pigment analysis inferred that overall in control as well as in treated plants of Accession I has more photosynthetic pigments in comparison to Accession II which indicates the photosynthetic efficiency of plants. Gamma rays, being ionizing radiations, cause formation of free radicals depending upon the radiation level. At 100 Gy dose of gamma irradiation chlorophyll *a*, chlorophyll *b* and carotenoid content increased in both accessions which indicates that lower dose of gamma irradiation has favourable effects on the plant. It is considered to be an effect of activated enzyme system [6]. At higher doses pigment content was reduced as gamma radiation causes damage of thylakoid, disorganized pattern of grana and stroma thylakoid and altered photosynthesis [3].

On the basis of findings of present study, it is evident that ionizing radiations are cytotoxic leading to several cytological irregularities. Gamma rays are potential mutagen which may damage DNA and affect cellular division leading to changed agronomic traits. Results inferred that accession I has more germination and survival percentage in comparison to accession II, so accession I is probably more suitable and genotype is favourable to the climatic conditions. Moreover, active mitotic index (AMI) of accession I is more than accession II which is a sign of metabolically active genotype. Total abnormality percentage of accession I is also more than accession II with increasing doses of mutagens. Therefore, we conclude that accession I is more sensitive to mutagens so it may be useful in development of a potential hybrid using mutation breeding. Biochemical study shows that accession I has higher content of chlorophyll *a*, chlorophyll *b* and carotenoids in comparison to accession II. Moreover, accession I has more chlorophyll *a* and chlorophyll *b* content in comparison to accession II which indicates better photosynthetic activity of accession I. At lower doses, increase in photosynthetic pigment indicates positive effects on plants of both accessions. This study may help in assessing the doses of gamma radiations which may be hazardous and its different consequences in foxtail millet.

DECLARATION OF INTEREST

The authors report no conflict of interest.

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