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IMPACT OF GAMMA-IRRADIATION ON THE MORPHOLOGY AND MITOSIS IN CLUSTER BEAN [*Cyamopsis tetragonoloba* (L.) Taub.]

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ABSTRACT

Induced mutagenesis is an important tool for crop improvement and gamma (γ) ray is one of the effective physical ionizing mutagens. The present pilot study was aimed to assess the effect of γ-irradiation on mitotic cells in cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.). The seeds of cluster bean were treated with different doses of γ rays *viz.*, 0, 100, 200, 300, 400 and 500 Gy to analyze the effect of γ-rays on meristematic root tips. Active mitotic index and total abnormality percentage were assessed. Increasing dose of γ rays negatively influenced AMI. Higher doses severely reduced germination and seedling height, although the initial dose of 100 Gy γ-irradiation significantly enhanced them as compared to the control. The irradiated seeds showed numerous chromosomal variations such as scattering, c-metaphase, bridge formation, etc. The evidences revealed that γ-rays have mutagenic effect at mitotic level and induced decline in mitotic activity and influenced the entity of chromosomal morphology. The study also revealed that lower doses of γ-irradiation can be effective in agricultural endeavours.

Keywords: Active mitotic index, *Cyamopsis tetragonoloba*, gamma rays, mutagen, TAB

INTRODUCTION

Induced mutagenesis augmented with mutation breeding is a decent strategy to improve the qualitative and quantitative traits in crops. Mutation breeding involves treating plant propagating materials with different chemicals or radiating agents to create variants/mutants with desirable characters. Although many studies for crop improvement through mutation breeding have been conducted in many field crops (Arora and Pahuja 2008; Mahla *et al.*, 2010; Mahla *et al.*, 2018), yet its use in guar (cluster bean) lacks systematic approach. The richness of genetic diversity hoarded in the germplasm adjudicates the success of any crop improvement program. Kumar *et al.* (2017) reported the existence of poor genetic diversity in cluster bean on the basis of DNA markers. The plant is strictly self-pollinated crop and endeavours for complete exploitation of available diversity through artificial hybridization is a tedious task due to the small size of flower and reduced seed setting in manually hybridized buds. Therefore, the most feasible approach for genetic variations can be artificial induction of mutations. Radiation-induced mutation is apparently the most feasible mean to improve mutant varieties in less time and create genetic variability. These mutagens may unleash the genes from their linkage barriers and produce many new promising traits for improvement of crop plants (Shah *et al.*, 2008). The genetic variability facilitates the selection of new genotypes with enhanced traits such as precocity, salinity tolerance, grain yield, quality, etc. by plant breeders (Ashraf

et al., 2003). Assessment of somatic cells, as part of preliminary screening, may provide first-hand information about the effect of mutagens on genetic makeup. Any positive or negative changes in mitotic index are stable indicators for monitoring the environmental constraints (Rizk *et al.,* 2015). The growth of root tips is a vital process in seed germination, and a stepping event for plants life cycle. Its cognitive response to environmental situation is, therefore, important to study the impact on morphology of chromosomal entity.

Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub] is a valuable legume forming an important component of farming system in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation with it (Timko and Singh, 2008). Cluster bean plays multitudinous role in human and animal nutrition but its seed having gelling-agents (guar gum) have become the centre of attention in present scenario. It is an important cash crop of Indian economy and its demand is rising rapidly due to its industrial use. The plant has narrow genetic base, therefore efforts for exploring its genetic variability is the need of hour. The present study was aimed to study the cytological aspects of certain morphological parameters *viz*., seed germination, plant height, etc. in cluster bean as influenced by γ-radiations**.**

MATERIALS AND METHODS

The seeds of cluster beans var. 'RGC936' were obtained from Central Arid Zone Research Institute, Jodhpur, Rajasthan, India. Fresh seeds of cluster beans were arranged in plastic packets and distributed into six sets. The seeds, in sets, were separately irradiated in Floriculture Laboratory, National Botanical Research Institute, Lucknow (India) with γ-rays from a ^{60}CO source (Gamma irradiation chamber, model GC-12000) at the rate of 3 sec per Gy for dose of 0, 100, 200, 300, 400 and 500 Gy. For mitotic study, the irradiated seeds were first pre-soaked in distilled water for 5 h and then placed in sterilized petri-plates on moistened filter paper beds. Seeds were kept in seed germinator (PLT-147; model SGSC-1) at 25±2ºC for 3 days for germination. One set of unirradiated seeds was maintained as control. After germination, the root tips were fixed in Carnoy's fixative (1: 3 parts of glacial acetic acid and absolute alcohol). After 24 h, the fixed root tips were transferred to 70% alcohol and stored at 4ºC until use. This experiment was conducted in a completely randomized design and each treatment replicated three times. Fixed root tips were hydrolyzed in 1N HCl for 30 sec at 55±2ºC. The hydrolyzed root tips were washed with tap water to remove excessive HCl and dried with blotting paper. These root tips were stained into 2% acetocarmine stain. Mitotic slides were prepared by using chromosome squash technique from the excised darkly stained root tip (Dille *et al.,* 1986; Mirzaghaderi, 2010). For each dose, five slides were prepared and ten microscopic fields analyzed. Suitable stages were photographed under a Nikon phase contrast research microscope (Nikon Eclipse, E200, Japan).

The parameters such as mitotic indices, abnormalities and germination (taken after 3 days of incubation) were calculated by applying the following formula:

$$\text{Germination percentage} = \frac{\text{Total number of seeds germinated} \times 100}{\text{Total number of seeds sown}}$$

$$\text{Active mitotic index (AMI \%)} = \frac{\text{Total number of dividing cells x 100}}{\text{Total number of observed cells}}$$

$$\text{Total abnormality percentage (TAB \%)} = \frac{\text{Total number of abnormal cells} \times 100}{\text{Total number of observed cells}}$$

The data was statistically analyzed by one-way analysis of variance (ANOVA) using SPSS 16.0 software. Duncan's multiple range test (DMRT, $P < 0.05$) was performed to compare the mean and standard error.

RESULTS AND DISCUSSION

Impacts of γ-irradiation on seed germination and plant height

γ-irradiation variably affected the seed germination in cluster bean depending upon the dose (Fig. 1A). In control and 100 Gy dose sets the seed germination rate was 88.7 and 90.5%, respectively, while in 500 Gy set the germination rate was reduced to 46.7%. The results are in agreement with Kudr and Heger (2015) and suggest that mutagen promotes physiological retardness by inducing variation at gene and chromosomal levels. Ionizing ray could cause broad spectrum nucleotide damage which consequently affects sequential cues and the errors in nucleic acid may lead to mitotic cell arrest thereby causing sharp decline in seed germination at higher doses (Broertjes and Van harten, 1988). In present study, the plant height also exhibited similar trend as that of seed germination with significant stimulation at lowest dose of 100 Gy over control, but showed steady reduction with subsequent higher doses (Fig 1B). Mean plant height in control set was 89.3 cm and in 100 Gy irradiated set 91.1 cm which then declined to 53.2 cm in 500 Gy set. The results are in line with previous reports of Songsri *et al.* (2011) and Nayak *et al.* (2015). Stimulation in germination at lower doses is in agreement with Nyembo *et al.* (2018) which shows hormesis at lowest dose. The stimulatory effect on plant height at lower doses may be attributed to the activated protein or RNA synthesis, immediately after exposure (Abdel-Hady *et al.*, 2008). It appears that lower doses trigger intrinsic extracellular ATP synthesis which increases reactive oxygen species (ROS), thereby leading to elevation in antioxidants profile after γ-irradiation (Kojima *et al.*, 2011; Mohajer, 2014).

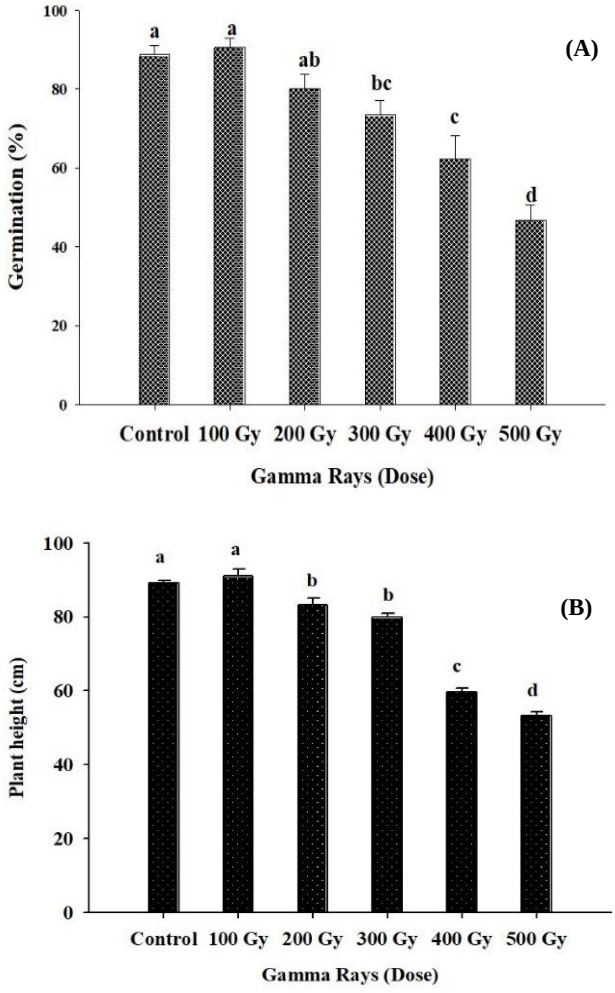

Fig. 1: Effect of γ-radiation on seed germination (%) and plant height in cluster bean

Impact on mitotic cycle and chromosomal morphology

The cytological studies of γ-irradiated cells revealed a noteworthy impact on root meristems of cluster bean. The diploid chromosome complement in cluster bean is $2n = 14$. The computed AMI for varying γ-irradiation doses exhibited an inverse relation between respective doses and AMI (Fig. 2). The

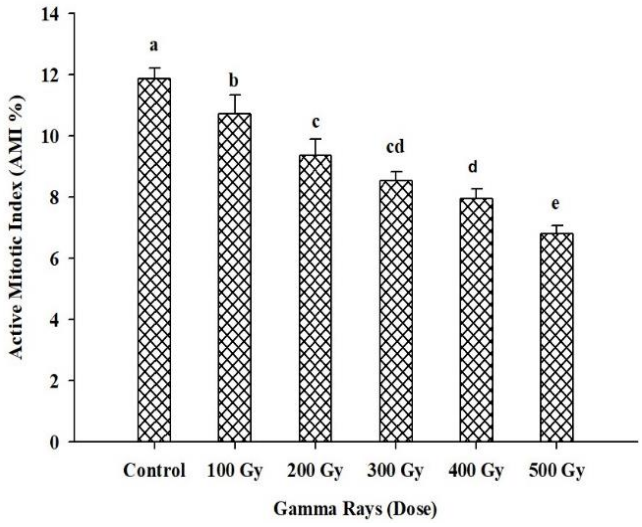

Fig. 2: Effect of γ-radiation on AMI (%) in root meristematic cells of cluster bean

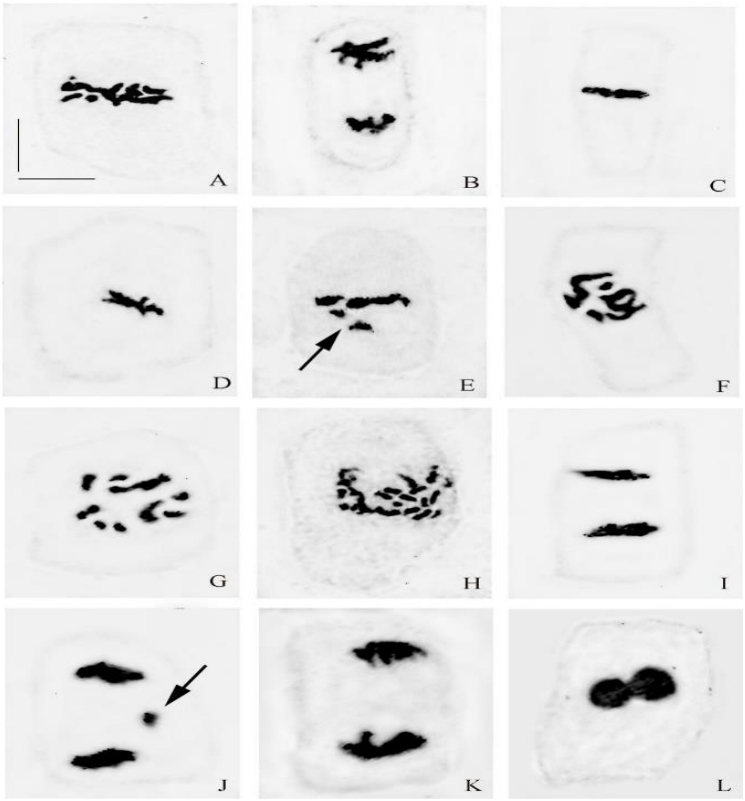

Fig. 3: Cytological anomalies induced by γ-radiation in cluster bean: A - Normal metaphase (2n = 14), **B** - Normal anaphase (14:14 separations), **C** - Stickiness at metaphase, **D** -Unorientation at metaphase, **E** - One precocious chromosome at metaphase, **F** - Loop formation at metaphase, **G** - Scattering at metaphase, **H** - Fragmentation at metaphase, **I** - Stickiness at anaphase, **J** - Laggard formation at anaphase, **K** - Unorientation at anaphase, **L** - Telophase with nuclear budding [Scale bar - length = 6.30 µm; breadth = 5.4 µm]

evaluated AMI in control was 11.8 which decreased as the dose of radiation increased with AMI of 10.7% in 100 Gy dose and 7.9% in 500 Gy. Higher dose of ionization introduces cellular lesions and offers several remunerative properties. Mashev *et al*. (1995) implied that higher radiation doses could be effective to develop more protein and essential amino acid-rich plants. The dose of γ-radiation is mito-inhibitory specifically at higher doses *i.e.* > 200 Gy as was evident from the reduction in AMI after 100 Gy dose. Mito-inhibitory effect of γ-rays have also been reported by Borzouei *et al.* (2010) and Ahirwar (2015). The reduced AMI may be the outcome of breakdown of plant self-protection system and further inhibition of cell DNA replication, transcription and protein synthesis (Liu *et al.*, 2015) or due to inhibition of DNA synthesis at S-phase that most probably happened due to decreasing ATP level and the pressure from the functioning of the energy production center (Sudhakar *et al.*, 2001; Liu *et al.*, 2015).

Cytological study showed that mitosis was normal in control set (Fig. 3A, B). Fig 3A shows normal metaphase with 14 chromosomes at equatorial plate while Fig. 3B depicts normal anaphase with 14:14 pole word separation. However, chromosomal entity was also affected by γ-rays. A concordant increase in TAB% was noticed with increase in γ-irradiation dose (Table 1). TAB% was further divided based on their occurrence at metaphase and anaphase and ascribed as metaphasic abnormalities (Fig. 4A) and anaphasic abnormalities (Fig. 4B). Chromosomal aberrations such as stickiness of metaphase

Table 1: Effect of ionizing γ-rays on mitotic activity and chromosomal morphology in cluster bean

Treat-Ment	No. of obs. Cell	Metaphasic abnormalities (%) (mean ± SE)					Anaphasic abnormalities (%) (mean ± SE)				Mn (mean ± SE)	Oth (mean ± SE)	TAB (%) (mean ± SE)
		Sc	St	Pr	Uno	Cm	St	Br	Lg	Uno			
0 (control)	295	-	-	-	-	-	-	-	-	-	-	-	-
100 Gy	285	0.22±0.11	0.46±0.10	-	0.35±0.21	-	0.11±0.12	-	-	-	-	0.35±0.07	0.89±0.11
200 Gy	277	0.36±0.09	0.48±0.12	0.12±0.13	0.23±0.11	0.35±0.19	0.36±0.01	-	-	0.37±0.22	-	0.24±0.12	1.64±0.03
300 Gy	289	0.46±0.10	0.68±0.18	0.57±0.10	0.46±0.13	0.34±0.09	0.35±0.19	-	0.35±0.01	0.46±0.10	-	0.46±0.13	2.57±0.05
400 Gy	290	0.45±0.12	0.80±0.11	0.57±0.12	0.35±0.01	0.34±0.01	0.57±0.12	0.46±0.12	0.46±0.12	0.23±0.11	0.34±0.11	0.34±0.10	3.93±0.09
500 Gy	295	0.55±0.10	0.89±0.15	-	0.46±0.12	0.33±0.11	0.91±0.14	0.44±0.09	0.67±0.02	0.55±0.10	0.33±0..01	0.33±0.19	5.09±0.15

Abbreviations: Sc - Scattering; Pr - Precocious movement of chromosomes; St - Stickiness; Br - Bridge formation; Lg - Laggard; Un - Unorientation; Cm - C-mitosis; Mn - Micronuclei; Oth – Other abnormalities; TAB – Total abnormalities
*Different letters in the superscript denote significant differences between their means ($p < 0.05$) by DMRT

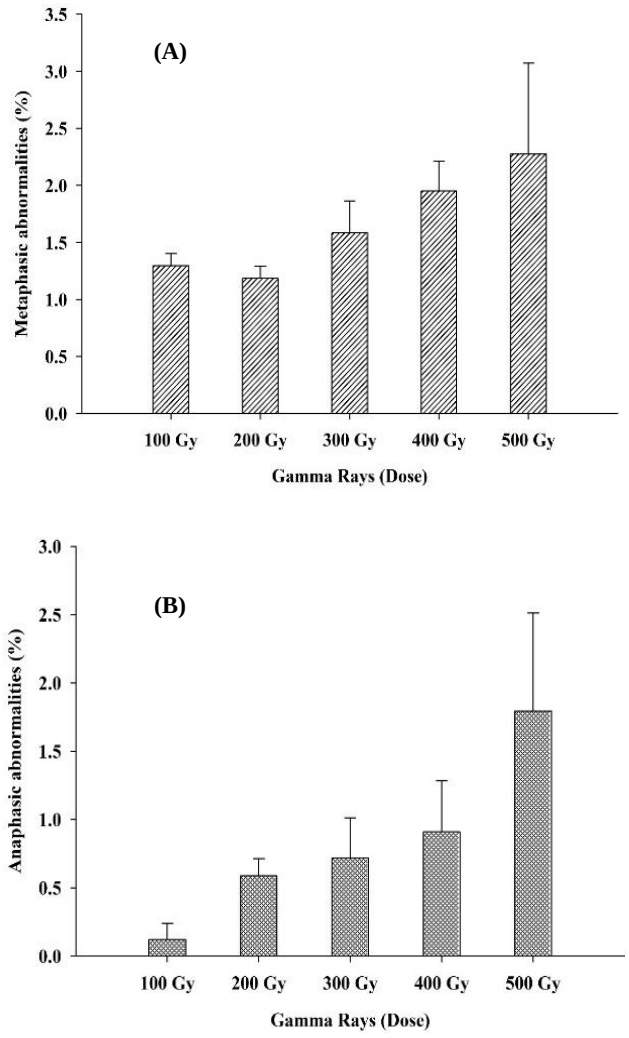

Fig. 4: Effect of Gamma radiation on metaphasic (A) and anaphasic (B) abnormalities in the root meristematic cells of cluster bean

and anaphase (Fig. 3C, I), scattering (Fig. 3G), precocious movement of chromo-some (Fig. 3E), un-orientation at metaphase (Fig. 3D) and anaphase (Fig. 3K), micronuclei, loop formation, bridge formation, laggard movement at anaphase (Fig. 3J) were profoundly observed. However, stickiness was the most predominant abnormality encountered and its frequency had a consistent increment with co-linear increment in dose of γ-radiation. Stickiness due to γ-irradiation may result from defective functioning of one or two types of specific non-histone proteins involved in chromosome organization that are needed for chromatid separation and segregation (Basi *et al.*, 2006; Datta *et al.*, 2011) and ultimately to the inactivation of DNA replication, increased chromosomal contraction and condensation or nucleoproteins and cell death (Han *et al.*, 2007).

Chromosome fragments usually originate due to DNA damage such as double strand breaks which is mispaired resulting in asymmetrical chromosome or chromosomal fragmentation. The loop formation might have originated due to the failure of kinetochores to attach with spindles and lead to the joining of ends thus forming loops (Kumar and Pandey, 2017). Lagging chromosomes may be explained on the basis of abnormal spindle formation and dysfunctioned chromatid movement. This defect originates when single chromatids

fail to segregate because the kinetochore is attached to spindle microtubules that are oriented toward opposite spindle poles (Thompson and Compton, 2008). This condition is ascribed as merotely which is only possible in eukaryotic cells where single kinetochores bind multiple microtubules and is usually disfavoured by the back-to-back geometry of sister kinetochores (Loncarek *et al.*, 2007; Paul *et al.*, 2009). Micronuclei are chromatin-containing structures found in the cytoplasm of cells and are surrounded by a membrane that has no detectable link to the cell nucleus (Schiffmann and De Boni, 1991). It can be interpreted as an indicator of clastogenic and aneugenic effects (Çavas and Ergene-Gözükara, 2005). Induced mutagenesis is recognized as one of the most important technology for the development of new varieties through genetic diversification. Genetic variations induced may both be positive or negative. While deleterious or neutral mutations that form a part of the mutations that occur in natural process may disappear in evolutionary process. The protected mutants may have desirable agricultural characteristics or easily adapt to changing environmental conditions (Jiang and Ramchandran, 2010).

Conclusion: On the basis of present investigation it may be concluded that the γ- irradiation exposure for mutagenesis is a significant technique at minimal doses. Hence the lower dose of γ-rays could be utilized for induction of genetic variability in cluster bean. Genetic variability as a result of induced mutation through various mutagens has contributed to modern plant breeding. Elevation in the plant height is an important achievement that opens several possibilities.

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Authors contribution: Conceptualization of research (G. Kumar, Radha Mishra); designing of the experiments (Radha Mishra and Shefali Singh); contribution of experimental materials (CAZRI, Jodhpur, Rajasthan, India); execution of field/lab experiments and data collection (G. Kumar, Radha Mishra and Shefali Singh); analysis of data and interpretation (Shefali Singh, Radha Mishra); preparation of the manuscript (Radha Mishra and Shefali Singh).

Declaration: The authors declare no conflict of interest.

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