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Comparative Radiocytological effect of X-Ray and Laser Beam on the Chromosomes of *Vicia species*

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A B S T R A C T

Higher plants have been used extensively to determine the cytotoxicity and genotoxicity of different type of mutagens along the years. The clastogenic effects of two physical mutagens viz. X-rays [260 and 520 mAs] and laser beam [Wavelength- 632.8nm; 1 minute and 2 minutes] were investigated on the root tip of *Vicia species*. For determining the radiation stress and self-protecting system, cytological studies were calculated: Mitotic Index (MI%) and Total Abnormality Percentage (Abn%). Mutagenic parameters were percentage of dividing cells which showed a linear relationship between the dose absorbed and chromosomal anomalies. Bridges and fragments were the pronounced abnormalities noticed in X-ray treated seeds whereas stickiness, were dominant in laser beam treatment, with some phenomenon like prolonged nucleus, unequal separation was found stage specific. It was found that X-ray > Laser Beam deleterious showing a marked mito-depressive effect on mitosis of the *Vicia species* cells.

***Key words*:** Ionizing, Non-ionizing, *Vicia faba* L., *Vicia sativa* L.

For commercial importance, mutagens (either physical or chemical) are widely used to create variations in plant species for widening the gene pool and/or inducing gene mutation [1]. But most of the mutations are lethal or semi-lethal and do not have any practical importance possibly due to doses observed or mutagens employed and that's why selection of efficient mutagen and treatment requires a prior condition, as mutagens are the potent tools that brings up direct improvement and certain qualitative and quantitative changes in crop plants [29]. Radiations are known to induce changes in the molecular organization of chromosomes manifested as gene mutations, chromosomal aberrations or alterations in the physiological activity of the cell [3]. The manner in which the yield of structural changes increases with increase of the dose of radiation has been extensively studied and the results of these studies form the main basis on which theories of induction of these changes are built [4]. Ionizing radiation (IR) has enough energy to break chemical bonds and known to cause cancer. However, because non-ionizing (NIR) lacks this energy, it was assumed that these lower frequencies cannot be carcinogenic. This concept is based on a flawed assumption. Non-ionizing can and does cause cancer not by increasing the production of free radicals but by interfering with the repair mechanisms that neutralize free-radicals. While the mechanisms differ, the consequences of both IR & NIR are the same-oxidative stress resulting in cellular damage including cancer [14]. X-rays and Laser Beam have been found to be very useful both for surgical and medicinal purposes. The effects of irradiation on the chromosomes were observed by using X-rays on the inflorescence [6], X-rays and UV irradiations on the pollen of tomato [7]. Some studies of X-rays and Laser beams have been reported on the seeds of wheat and comparative effect in *Lathyrus sativus* L. [8].

Cytological analysis is one of the most dependable indices to estimate the potency of mutagen [2]. Present investigation documents comparative mutagenic effectiveness of X-ray and laser beam with different parameters such as 260 and 520 milliampere-second (mAs) with 90 Kilo Volt (K.V.) by X-ray machine and laser wavelength of 632.8 nm with Power-75mW on *Vicia species'* chromosome biology. In India, *Vicia faba L.* (Bakhla; Faba beans) and *Vicia sativa* L. [Jhilo Sag (Santhal), Jhilo arxa (Oraon), Common Vetch] are good source of protein and carbohydrates for tribal of Jharkhand. These are one of the important pulse crops and chiefly consumed as vegetable. Its grains are rich source of high-quality proteins. Faba bean can fix up to 219 kg N ha^{-1} $year^{-1}$ under optimum conditions [09], and thus, it helps in maintain soil fertility. Many studies investigated the abnormalities caused by radiations (X-ray and gamma) on faba beans. However, to the best of our knowledge, studies on chromosomal abnormalities of both the species are

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limited. This study is to evaluate and compare the radiations and chromosomal aberrations in two of the species of *Vicia*.

MATERIALS AND METHODS

Two lines of action was taken for each treatment for observing the clastogenic and aneugenic effects of physical mutagen on chromosomes of both species of *Vicia.* The experiment was conducted with the healthy seeds of *Vicia faba L* and *Vicia sativa* L. which were procured from ICAR, Plandu, Ranchi and G. B. Pant University of Agriculture and Technology, Pantnagar, respectively. Seeds were overnight soaked in normal water. The experiment was conducted with the healthy seeds of *Vicia species* which were irradiated separately with two physical mutagens viz. X-rays and Laser Beam. Packets of 50 seeds were exposed to X- ray irradiated from the controlled source (90 KV at the distance of 10'') at Shanti Digital X-ray and 3D-4D Ultrasound, Lalpur, Ranchi. Selected doses for experiments were- 260 mAs and 520 mAs (For laser beam treatment seed packets were irradiated at the wavelength of 632.8 nm with Helium Neon Laser (Power-75mW at the distance 1mW cm^{-2}) in Physics Department, BIT Mesra, Ranchi. For mitotic studies irradiated seeds were kept for germination on moist filter paper in the Petri dishes separately. Root tips (1-2 cm) were fixed in 1:3 acetoalcohol (i.e., Carnoy's fixative) and then transferred to 70% alcohol for preservation and storage. Squash technique and 2% acetocarmine (as a stain) were used for cytological preparation. Photographs are taken by MAGNUS microscope.

RESULTS AND DISCUSSION

The whole experiment has been performed in five replicates and data in (Table 1-2) are calculated by following parameters:

$$\text{Mitotic Index (M.I)} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells observed}} \times 100$$

Actively dividing cells are the cells of metaphase and anaphase stage:

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

The result of work showed that exposure to radiations caused a reduction in mitotic index on both of the *Vicia* species' meristematic cells. The dose/duration is inversely proportional to Mitotic index and directly to Abnormality % which illustrates the cytotoxic potential of both the radiations (Table 1-2). Both the radiations are effective to induce aberrations but as the direct effect of ionizing radiation, the damages are more prominent in X-rays as compared to Laser beam. The surface area of seeds also matter over here during exposure to the radiations as *Vicia sativa* L. seeds are comparatively smaller in size thus showed slightly higher MI% and less abnormality % in comparison to *Vicia faba* L. seeds which is larger in size (Table 1-2).

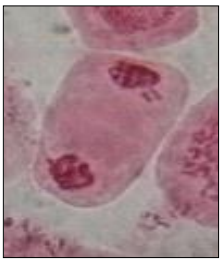

Fig 1 Telophase with two fragments at one pole

Fig 2 Clumped chromosome with U-shaped acentric chromatid

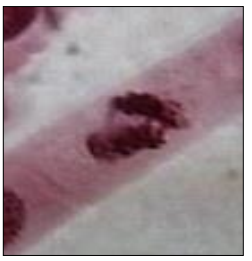

Fig 3 Clumped chromosome in anaphse with bridge

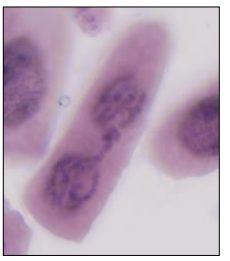

Fig 4 Telophase bridge

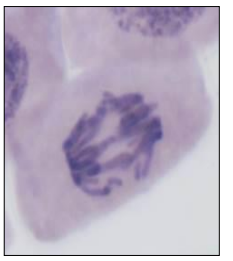

Fig 5 Multiple anaphase bridge

Fig 6 Laggards

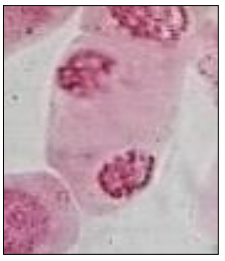

Fig 7 Diagonal orientation at telophase

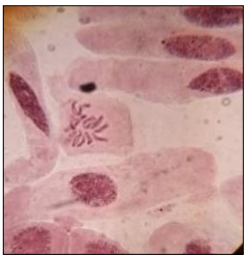

Fig 8 Prolonged necleus

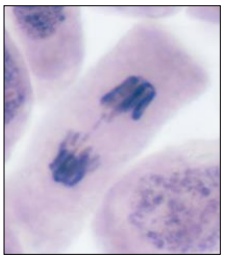

Fig 9bTelophase bridge

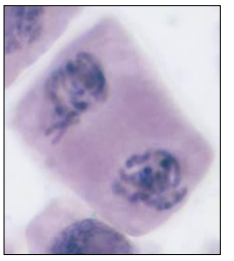

Fig 10 Non disjunction as a whole chromosome moves toward one pole

Mutagens used in the present investigation proved to be cytotoxic and were able to induce various clastogenic (structural), aneugenic (numerical) and non-clastogenic (physiological) chromosomal aberrations with varying frequencies. The clastogenic abnormalities recorded were chromosome fragments, laggards, single and multiple bridges at metaphases, anaphases and telophases. The aneugenic abnormalities recorded with proper non-disjunction as complete chromosome remains at one pole. The major non-clastogenic aberrations observed were stickiness and clumping of chromosomes, desynchronized metaphase, disoriented chromosomes at metaphase, anaphase, telophase, etc. Frequency of chromosomal aberrations was found to be colinearly increased with the

increment in dose/duration of both mutagens. The root tip cells of the control seeds were devoid of any chromosomal abnormalities, whereas the exposure of seeds to irradiation exhibited deleterious effects on the structural integrity of mitotic chromosomes and the frequency of chromosomal aberrations were found to be enhanced with the exposure rate of irradiation to seeds. Various clastogenic changes in the chromosomes, such as bridges [single (Fig 9 & 4), double (Fig 3) and multiple (Fig 5)], fragments (Fig 1), laggards (Fig 6) as well as micronuclei formation. In aneugenic changes due to precocious movement of one complete chromosome with two chromatids at one pole (Fig 10) and the non-clastogenic changes in the form of stickiness and clumping of chromosomes at metaphase, anaphase (Fig 3) and telophase were recorded from the both of mutagens the dose/duration of all the mutagens and found to be enhanced with the increase in dose/duration dependent manner.

Table 1 Effect of different doses of physical mutagens on mitotic abnormalities in root tip cells of *Vicia faba* L.

Physical mutagens	No. of cells scored	No. of cells in division	MI%	Frequency %										
				Met	Ana + Telo	Br	Fr	Lg	MN	Sc	Cl	PM	MNU	PN	Abn%
Control	2989	786	26.27±0.68	12.75	16.04	-	-	-	-	-	-	-	-	-	-
X-Rays 260 mAs	2858	660	23.11±0.21	11.50	15.18	0.73	1.11	0.91	0.50	1.37	0.61	0.37	-	-	5.84±0.21
520 mAs	2942	542	18.40±0.37	11.34	14.79	1.24	1.51	0.78	0.92	1.47	1.01	0.74	-	-	7.70±1.52
Laser beam 60 seconds	3005	814	27.10±0.32	13.27	15.56	0.63	0.24	0.39	0.08	0.31	0.16	0.29	-	-	2.15±1.79
120 seconds	2852	698	24.50±0.13	11.10	16.33	0.96	0.40	0.88	0.32	0.64	0.40	0.56	-	-	4.20±0.42

Table 2 Effect of different doses of physical mutagens on mitotic abnormalities in root tip cells of *Vicia sativa* L.

Physical mutagens	No. of cells scored	No. of cells in division	MI%	Frequency %										
				Met	Ana + Telo	Br	Fr	Lg	MN	Sc	Cl	PM	MNU	PN	Abn%
Control	2712	783	28.90±0.08	11.03	16.54	-	-	-	-	-	-	-	-	-	-
X-Rays 260 mAs	2883	703	25.04±0.12	9.77	14.64	0.53	0.82	0.69	0.18	0.76	0.42	0.14	-	-	3.80±0.13
520 mAs	2734	633	23.19±0.25	8.28	12.28	0.92	1.37	1.07	0.42	1.21	0.81	0.36	-	-	6.59±0.34
Laser beam 60 seconds	2975	834	28.05±1.01	10.68	15.47	0.38	0.15	0.21	0.02	0.17	0.10	0.18	-	-	1.25±0.42
120 seconds	2877	713	24.80±2.14	9.91	14.04	1.26	0.38	0.56	0.13	0.42	0.25	0.41	-	-	3.42±0.96

Abn- Abnormality, Ana- Anaphase, Br- Bridges, Cl- Clumping of chromosomes, Fr- Fragments, Lg- Laggards, Met- Metaphase, MI-Mitotic index, MN- Micronuclei, MNU- Multinuclei, PM- Precocious movement, PN- Persistent nucleolus, Sc- Stickiness of chromosomes, Tel- Telophase

± - Standard deviation

Since the discovery by Muller [10] and Stadler [11] that X-rays induce mutation in animals and plants, a new field has been developed in experimental genetics. Chromosomal aberrations are characterized by change in either total number of chromosome or in chromosomal structure which occur as a result of exposures to physical mutagen. Changes in the mitotic activity in mitotic phase and individual cell aberrations are the key parameter to determine the chromotoxic effect. To evaluate the different chromosomal abnormalities, several types of chromosomal aberrations were considered in different stages of M phase of cell cycle (prophase, metaphase, anaphase, and telophase). The most common visible chromosomal abnormalities were disorientation, abnormal metaphase, bridges, stickiness, etc. during anaphase in both the treatments. However, the frequency of bridges and fragments was found more in higher dose of X-ray treatment and stickiness in Laser beam. Moreover, their relative frequencies were dose/duration-dependent.

The inhibition of spindle formation has also been shown to lead to severe abnormalities such as stickiness, unequal distribution, bridges, laggards, etc. [12]. Similar results were obtained while studying the effects of X-rays on *Allium* [13]. Stickiness was found to be most dominant anomaly at metaphase, chromosomal stickiness leads to inactivation of DNA replication, increased chromosomal contraction and condensation or nucleoproteins probably leading to cell death [05]. It could be due to depolymerization of nucleic acid caused by mutagenic treatments or due to partial dissociation of the

nucleoproteins and alterations in their pattern of organization [6]. It may also arise due to improper clustering of chromosomes at any phase of cell cycle, which makes the chromatids connected by sub-chromatid bridges [16]. Bridges reported might have arisen through breaks in two chromosomes followed by union of the centric fragments [17] or due the stickiness of chromosome at metaphase and their failure to separate at anaphase or due to the breakage and reunion of chromosomes which leads to loss of genetic material [18], [19]. Gaulden [20] hypothesizes that stickiness at metaphase may be due to failure of changes in non- histone chromosome proteins i.e., topoisomerase II and peripheral proteins that are integral component of chromosome whose function is necessary for separation and segregation of chromatids. Beadle [21] reported chromosome stickiness in maize for the first time and attributed such irregularity to a mutation caused by a recessive gene called sticky (st). B/F/B is Breakage-Fusion-Bridge is because of broken ends become sticky and can fuse with another broken chromosomes. This may form bridge if the two chromosomes are located at opposite pole at anaphase stage (Fig 5 & 9). The loop forming laggards at anaphase might have originated due to failure of kinetochores to attach with spindles and leading to the joining of end forming loops i.e., merotelic kinetochores. If persist until anaphase, they cause chromatids to lag behind, hindering their segregation to spindle poles (Fig 6). Laggards result from the failure of chromosome movement or acentric fragmentation or might result from late chiasma terminalization [22]. The presence of laggard is a clear indication of chromosome breakage and deletion. The formation of small fragments can be attributed to the chromosomal breakage due to the effect of X-rays (Fig 1). A chromosome break- "terminal deletion" [23] consists of a fracture of a chromosome into a centric and an acentric fragment, sister union may occur between sister chromatid breakage ends in either or both fragments to produce a dicentric chromatid and/or an acentric U-shaped fragment and ring has been reported in present study (Fig 2). The acentric fragment may form laggard or micronuclei that tends to become lost from the successive daughter nuclei through nuclease. The behaviour of laggard chromosome is characteristic in that they generally lead to micronuclei formation [24-25].

Precocious movement of chromosomes at metaphase might be formed due to malformed homology of chromosome pairing or spindle mechanism whereby one or few chromosomes' floats in the cytoplasm rather than arranged at equatorial plate (Fig 2). Disrupted spindle functioning causes precocious chromosomes. Spindle disruption also causes scattering and diagonal orientation. As a non-disjunction visible in present investigation that whole chromosome without segregation of sister chromatids moved towards one pole was found in Laser treatment (Fig 10). However, to the best of our knowledge, this type of aberration was not seen before that's why the reason cannot be concluded precisely. The reason may be spindle assembly checkpoint proteins CENP-E and BubR1, which is responsible for microtubules-kinetochore interaction getting affected due to the radiation. According to the current understanding, structural changes, induced by microtubule attachment and tension, are translated, through phosphorylation, into a biochemical signal. It has been purposed that kinesin-related protein CENP-E and the kinase BubR1 is essential for this translation [26], [27], [28].

CONCLUSION

In our present studies, Medical X-rays, that are used in diagnostic imaging and radiation therapy which is measured by a unit i.e., milliampere-second (mAs) is potent enough to cause induced mutation and have greater Abn% with increasing hits. As far Laser used by students at laboratory is also far enough to damage the meristematic cells of *Vicia species.* This data will help the breeders in future to use both of the physical mutagens wisely for mutational breeding.

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