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Comparative Effect of Ionizing and Non-Ionizing Radiations on Genetic Stability of *Vicia* species

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Abstract*: In the present study we have investigated the comparative radiotoxic effect of both Ionizing (X-rays, Gamma rays, Electron Beam) and Non-Ionizing Radiations (UV-B & Laser beam) on mitotic cells of *Vicia species.* They were exposed separately with two hits of each. For determining the radiation stress and self-protecting system, the cytological studies: Mitotic Index (MI %) and Total Abnormality Percentage (Abn%) were calculated. A wide spectrum of chromosomal aberrations was encountered in both the species but the most dominated anomaly was the stickiness of chromosomes. Some novel cytological mutants were also isolated like whole chromosome lag behind during the anaphase and non-disjunction at the telophase. The results obtained from the present experimental work will provide a wholesome difference in percentage and types of anomalies between Ionizing and Non-Ionizing Radiations effect on the two species of *Vicia.

Index Terms:* Ionizing and non-ionizing radiation, *Vicia faba L.*, *Vicia sativa L.

I. INTRODUCTION

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. Faba bean can fix up to 219 kg Nha^{-1} $year^{-1}$ under optimum conditions (Neugschwandtner et al., 2015), and thus, it helps in maintain soil fertility. The potentiality of faba bean is around 6.0-7 .0 t /ha whereas in India its average productivity is 1.5 t/ha. To make faba bean into a perfect candidate for a sustainable agriculture, crop should be beneficial both to farmers/producers and to users (human and/or animal nutrition). This goal could be achieved through the development of new high-yielding cultivars of faba bean is greatly needed to fulfil future food and feed demands. Mutant breeding has been a source for breeders as it provides the chance of obtaining some desired traits focused on developing new cultivars. Among various mutagens, induced mutagenesis i.e., irradiation is the most promising process to bring out significant genetic variance. Induced damaging of DNA by radiation (i.e., physical mutagen) comes with the killing of that damaged cell or by repairing DNA lesions; the consequences of these processes are directly linked to mutation breeding.

Medical X-rays are used in diagnostic imaging and radiation therapy which is measured by a unit i.e., milliampere-second (mAs). This quantity is proportional to the total X-ray energy produced by a given X-ray tube. The effects of irradiations on the chromosomes were earlier observed by using X-rays on the inflorescence, X-rays and UV irradiations on the pollen of tomato (D.W. Barton 1954). Some studies of effects of X-rays and Laser beams have been reported on the seeds of wheat and comparative effect in *Lathyrus sativus L.* (R. Shukla & G. Kumar 2004). One of the physical methods considered as a safe application is Laser treatment that can improve the quality and yield of crop plants (Inyushin, et al. 1981; Ivanova, 1998; Koper, 1994; Podleoeny, 2002). Gamma radiation is widely used to induce mutation in crop plants. About 40% of the faba bean cultivars developed through induced mutation are derived from

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

Abberations: Abn- Abnormality, Ana- Anaphase, Br- Bridges, Cl-Clumping of chromosomes, Fr- Fragments, Gy- Gray, Lg- Laggards, Met-Metaphase, MI-Mitotic index, MN- Micronuclei, MNU- Multinuclei, PM-

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Precocious movement, PN- Persistent nucleolus, Sc- Stickiness of chromosomes, ± - Standard deviation, Tel- Telophase.

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
Gamma Rays															
200 Gy	2772	375	13.55±0.48	9.02	10.80	1.65	2.08	1.07	0.75	1.47	1.76	0.58	1.28	-	10.70±1.12
400 Gy	2680	289	10.75±0.12	7.76	8.36	3.05	2.72	1.98	1.32	2.75	3.31	1.03	2.08	-	18.28±0.78
Electron beam															
100 Gy	2812	428	15.20±0.55	10.01	12.71	0.78	1.37	0.66	0.47	0.98	1.01	0.33	0.72	-	6.38±0.38
200 Gy	2795	355	12.73±0.24	8.68	10.42	1.48	1.87	0.79	0.81	1.31	1.54	0.63	1.51	-	10.02±1.93
UV-B															
20 MINUTES	2880	692	24.05±0.15	11.74	16.20	0.61	1.01	0.72	0.63	1.28	0.73	0.56	-	-	5.60±0.33
40 MINUTES	2842	590	21.02±0.42	11.03	14.91	1.11	1.88	1.67	1.34	2.13	1.36	1.07	0.52	-	10.94±0.15
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

gamma radiations (IAEA 2017). Electron beam mutagenesis has been effective to create mutation in azuki bean traits (Luo et al.,2012) and chromosomal aberrations such as intense stickiness, fragmentations and double minutes in *Allium cepa* (Gavrila et al., 2004). GARINIS *et al.* (2005) stated that UV-B induce damages in DNA by creating cyclobutane pyrimidine dimers and pyrimidine dimer (6-4), if not correctly repaired can lead to break and point mutations. Ultraviolet radiation (UV) classified into UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (200-280 nm). UV-B is of particular interest because this wavelength is near about 1.5% of the total spectrum but can induce a variety of damaging effects in cell. Interaction of both individual radiations with cellular water rapidly induce reactive oxygen species (ROS), hydroxyl radical (-OH), ionized water ($H2O^{+}$) as well as the reactive nitrogen species (RNS) (Reisz et al. 2014). Effect of these molecules on the cell metabolism including repression of mitosis (Singh and Singh 1983), and structural damages to DNA (deoxyribonucleic acid) such as alteration of bases and sugars, cross-link formation, single-strand breaks (SSBs) or double-strand breaks (DSBs) and DNA clustering (Duncan and Schaefer 2009; Thompson 2012). These leads various chromosomal aberrations such as deletion, duplication, bridges, laggards, etc.

Many studies investigated the abnormalities caused by radiations (X-ray and gamma) in faba beans. However, to the best of our knowledge, studies on chromosomal abnormalities of both the species are limited. This study is to evaluate and compare the radiations and chromosomal aberrations in two of the species of *Vicia*.

II. MATERIAL AND METHODS

Two lines of action was taken for each treatment for observing the clastogenic effects of physical mutagen on *Vicia species* chromosome. The experimental seeds of *Vicia faba L.*

Table III. 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
Gamma rays															
200 Gy	2586	451	17.50±0.98	7.03	9.47	0.85	1.51	0.81	0.25	0.71	0.93	0.24	0.62	-	5.95±1.14
400 Gy	2721	388	14.28±0.14	6.89	10.05	1.41	2.05	1.15	0.70	1.34	1.50	0.50	1.21	-	9.90±0.21
Electron Beam															
100 Gy	2768	568	20.55±1.98	8.18	11.75	0.54	1.51	0.27	0.21	0.48	0.63	0.79	0.88	-	5.30±0.18
200 Gy	2632	473	18.02±0.33	7.72	10.51	1.02	1.98	0.59	0.52	0.84	0.98	1.21	1.51	-	8.67±0.31
UV-B															
20 MINUTES	2744	728	26.55±0.46	10.21	14.90	0.58	0.86	0.32	0.43	0.91	0.33	0.25	-	-	3.95±1.98
40 MINUTES	2662	633	23.79±0.33	9.89	13.13	1.17	1.73	0.73	0.82	1.62	0.75	0.54	0.21	-	7.60±0.65
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

and Vicia sativa L., which were procured from ICAR, Plandu, Ranchi and G.B. PANT UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, Pantnagar, respectively. Healthy and uniform-sized seeds were selected and exposed to three different treatment modes viz., for Ionizing radiations (Table I) dry seeds, for non-ionizing radiations presoaked in distilled water for 12h (Laser beam) and sprouted seeds for the exposure of UV-B. Ionized irradiated dry seeds then soaked in distilled water for 12h and kept for germination on moist filter paper in the Petri dishes separately along with presoaked once. Root tips of 1-2 cm were fixed in 1:3 acetoalcohol (i.e., Carnoy's fixative) with few drops of ferric chloride for fixation, preserved in 70% alcohol and stained in acetocarmine. Slides were prepared using chromosome squash technique and photographs were taken. Mitotic indices (MI) and percentage abnormality (Abn%) were calculated as per the following formulae (Jabee et al. 2008, Aney et al. 2012):

$$(\text{M. I.}) = \frac{Total\ number\ of\ dividing\ cell}{Total\ number\ of\ cells\ scored} \text{ X } 100 \quad (1)$$

$$\%\ \text{ABN} = \frac{\text{TOTAL NUMBER OF ABERRANT CELLS}}{\text{TOTAL NUMBER OF CELLS IN DIVISION}} \text{X}100 \quad (2)$$

III. OBSERVATIONS

A. Effect of physical mutagens on mitotic activities.

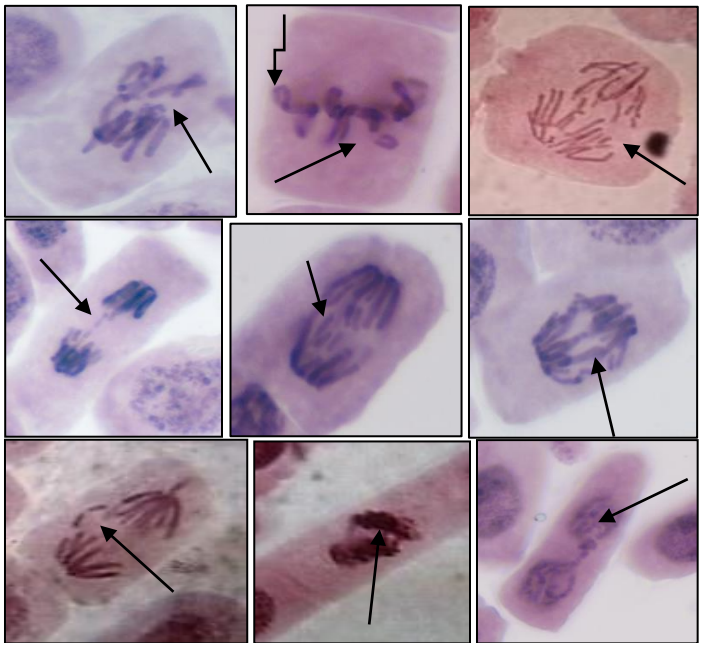
Table I: Types, doses/durations and sources of radiation.

The result of work showed that exposure to radiations (Table I) caused a reduction in mitotic index on both of the *Vicia* species' meristematic cells. The dose/duration is inversely proportional to mitotic index which illustrates the cytotoxic potential of both the radiations (Table II & III).

TYPES OF RADIATION		DOSE/ DURATION	SOURCES OF RADIATION
IONIZING RADIATIONS	X-RAYS	260 mAs	**Radiology Center-Shanti Digital X-ray and 3D-4D Ultrasound, Lalpur (Ranchi)**
		520 mAs	
	GAMMA RAYS	200 Gy	**BARC (BHABHA ATOMIC RESEARCH CENTRE), MUMBAI**
		400 Gy	
	ELECTRON BEAM	100 Gy	**BARC (BHABHA ATOMIC RESEARCH CENTRE), MUMBAI**
		200 Gy	
NON-IONIZING RADIATIONS	UV-RAYS UV-B (280-320 NM) LAMP	20 MINUTES	SCIENTIFIC STORE, RANCHI
		40 MINUTES	
	LASER BEAM HELIUM-NEON LASER (632.8NM)	60 SECONDS	**BIRLA INSTITUTE OF TECHNOLOGY, MESRA**
		120 SECONDS	

B. Effect of mutagens inducing chromosomal aberrations.

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). All 5 physical 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 (Fig.11). The major non-clastogenic aberrations observed were stickiness and clumping of chromosomes, desynchronized metaphase, disorientation at metaphase, anaphase, telophase, etc. Frequency of chromosomal aberrations was found to be colinearly increased with the increment in dose/duration of all 5 mutagens (Table II & III). 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.4 & 9), double (Fig.8) and multiple (Fig.6)], fragments (Fig.3 & 5), laggards (Fig.7 & 12) as well as micronuclei formation. In aneugenic changes due to precocious movement of one complete chromosome with two chromatids at one pole and non-clastogenic changes in the form of stickiness and clumping of chromosomes at metaphase, anaphase and telophase (Fig.8) were recorded from all the dose/duration of all the mutagens and found to be enhanced with the increase in dose/duration dependent manner. Multinucleated were reported in effect of Gamma and Electron beam radiations (Fig.15).



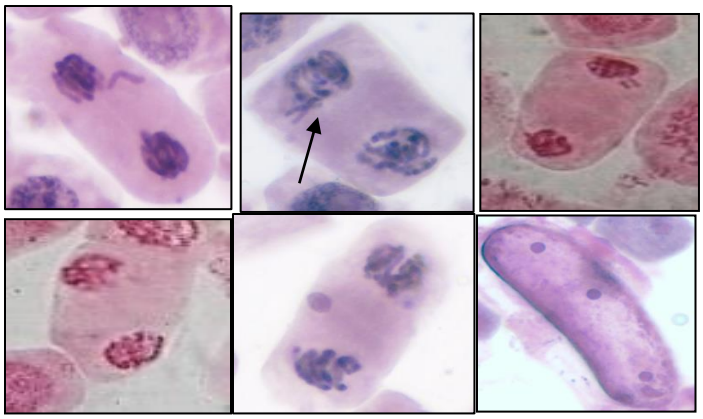


Fig. 1: Terminal Deletion in a chromosome, Fig. 2: Clumped chromosome with U-shaped acentric chromatid, Fig. 3: Scattered anaphase with fragmentation , Fig. 4 : Single bridge, Fig. 5: Fragments at anaphase, Fig. 6: Multiple bridges, Fig.7: Laggards at anaphase, Fig.8: Clumping, bridge formation and diagonal orientation at telophase, Fig. 9: Telophase bridge, Fig. 10 & Fig. 11: Non-disjunction as a whole chromosome moved towards one pole, Fig. 12: Early telophase with two laggards at one pole, Fig. 13: Diagonal orientation, Fig. 14: Persistent nucleolus, Fig.15 : Multinucleated cell.

III. DISCUSSION

Cytotoxic effects of all mutagens are evident from the cytological analysis of the data on induction of mitotic aberrant cells and the frequency of chromosomal aberrations. The dose/duration dependent increase in mitotic aberrant cells and the chromosomal aberrations revealed adverse effect of mutagens on root tip cells in both the species under investigation. Mitotic process and the chromosomal entity were found to be greatly affected by all mutagens. The enhanced frequency of mitotic aberrant cells and various chromosomal aberrations is an indication of cytotoxic effect of all the mutagens on the genome of both *Vicia species* (Table II & III). Irradiation is liable to produce a lengthening of nuclear division cycle. It has been suggested that this is due to an effect on nucleic acid cycle, resulting in a failure of conversion of ribonucleic acid to the deoxyribonucleic acid form. Ribonucleotides accumulate in the cytoplasm after irradiation (Mitchell, 1942).

Gaulden (1987) 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 (1932) reported chromosome stickiness in maize for the first time and attributed such irregularity to a mutation caused by a recessive gene called sticky (st). Recent reports on different species of the genus *Brachiaria* have been published (Mendes- Bonato et al., 2007), they suggest that chromosome stickiness may be under genetic control. It may be controlled by a single pair of genes, two pair of genes or by interaction of several genes which may be genes which may be recessive or dominant. Dowd et al. (1986) classifies stickiness from moderate to severe according to the number of chromosomes involved in the genome. TRF2 protein complex makes a capping and protect the telomere of chromosome because any change or break in the telomere induce bridge formation. 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.4 & 6). Cells at mid to late prophase at the time of irradiation will show stickiness leading to clumped metaphases and to anaphase bridges even with quite small doses (Fig 8 & 9). With large doses excessive clumping may prevent the completion of mitosis. A chromosome break- "terminal deletion" (Darlington and La Cour, 1945) 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 (Koduro and Rao, 1981; Kumar and Rai, 2006). Micronuclei also arise if laggard or non-oriented chromosomes fail to reach the poles in time to be in main telophase nucleus (Utsunomiya et al, 2002).

As a non-disjunction visible in present investigation that whole chromosome without segregation of sister chromatids moved towards one pole (Fig. 10 & 11). 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 (G.K. Chan et al., 1999; X. Yao et al., 2000; A. Abrieu et al., 2000).

Yasuhara & Oe (2011) reported severe defects in bipolar spindle formation resulting in the appearance of multinucleated cells with variable sized nuclei due to the RNAi depletion of its tobacco homologue tobacco MT binding protein 200 called TMBP200.

Both the radiations are effective to induce aberrations but as the direct effect of ionizing radiation, the damages are more prominent in X-rays, gamma and electron beam as compared to non-ionizing radiations i.e., UV-B and 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 II & III).

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