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Clastogenic and Aneugenic Responses of Supplemental UV-B Radiation on Root Meristems of Lentil

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***Abstract*: All living organisms are inseparably interdependent with natural environment and interact with each other. UV-B radiation is a part of sun's energy which due to depletion of ozone veil penetrates the earth's surface in an elevated level and disrupts the vital metabolic processes of plants and induces genetic instability, which will ultimately be translocated to various trophic levels. Considering the above forementioned risk of UV-B on plants, the present experimental work has been designed to study the effect of UV-B exposure on root meristems of Lentil (*Lens culinaris* Medik). Experiment was carried out in four sets *viz.* A, B, C and D. A,B,C sets were treated with UV-B radiations for 20min, 40min,and 60min, successively for 1 day, 2day and 3day. Set D remain unradiated being kept as control. After each irradiation, roots were cut off from seeds and fixed in Carnoy's fixative (GAA: AA, 1:3) for 24 hours then transferred in 90% alcohol. With the help of squash technique cytological slides were prepared. Various types of chromosomal anomalies were reported however the more prevalent were unorientation, precocious chromosomes, stickiness, scattering, c-mitosis, non-synchronisation, laggards, bridges etc. It was observed that the chromotoxic effect of UV-B was exaggerated as exposure duration elevated as evident in 60 minute treated sets. Hence, it was concluded that the higher intensity of UV-B exposure duration is fatal and genotoxic for plant's genetic machinery and induces genome instability.**

***Keywords*: UV-B, irradiation, chromosomal anomalies, genotoxic, *Lens culinaris* Medik.**

1. INTRODUCTION

All living organisms require energy to enable them to maintain their vital processes. Energy radiated by sun called solar energy is the primary source of energy for all living creatures. From the 20th century, understanding of the relationship between plants and incident solar rays is a major concern as it is the resource base and plays an important role as regulator and controller of growth and development. Solar radiation is the set of electromagnetic radiation emitted by sun. However, not all the radiations pass through the earth's surface as ultraviolet which are too short, are absorbed by the gases present in earth's atmosphere basically CO_2, ozone and water vapour. Due to destructive anthropogenic activities and extreme pollution, the ozone veil is being damaged and as a consequence more UV rays would reach the earth's surface. Of all the living organisms, plants are more prone and sensitive to elevated UV radiations as they are fully dependent on solar radiation for their survival. According to the wavelength, UV rays has been categorised into UV-C (200-280 nm) which is extremely harmful to organisms, but not relevant under natural conditions, UV-B (280- 320 nm) is of particular interest because this wavelength represents near about the 1.5% of the total spectrum but can induce a variety of damaging effects in plants, UV-A (320- 400 nm) represents ≈ 6.3% of incoming solar radiation and is the less hazardous part of UV radiation [1]. A small decrease in ozone level may cause a large relative increase in biologically effective UV radiation [2], [3]. UV-B can cause severe deleterious effects in biological organisms, despite representing only a small amount of total solar radiations [4], [5], [6]. UV-B can adversely react with many biological molecules including amino acids, nucleic acids, proteins, lipids and elicits stress responses at molecular, cellular and whole organism levels [7], [8]. Garinis *et. al.*, 2005 stated that UV-B can damage DNA by creating cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidine dimer, which can lead to point and break mutations if not correctly repaired. Certain responses were elicited by plants including inhibition of hypocotyls elongation and root growth [10], cotyledon opening

[11] stomatal closure [12] and anatomical changes associated with UV-B protection. In recent years, extensive researches were done by scientists to work out the plant responses against UV-B radiation. During seed germination root is the first to emerge, hence chromosomal aberrations occurred in root meristems, will lead to various impairments and abnormal growth and development.

Although numerous studies have been done with physical mutagens in diverse plant species but knowledge about activity of meristematic cells of early roots will be of supreme importance to study proper growth and developmental pattern and mutations in genotypic and phenotypic characters of plants as a consequence of UV-B radiations. As the plants are the best model system for biological studies and cheaper, easily available, hence present experimental work has been designed in aiming to determine the effects of UV-B on rate of mitotic division in root meristems of Lentil which is an important leguminous crop. Lentil (*Lens culinaris* Medik) is considered as sibling of bean and is rich source of protein, carbohydrate, and minerals. It serves as poor people's "meal" in undeveloped countries and an alternative of animal proteins in developed countries due to its low cholesterol content.

As a result of detailed literature study, this work represents a first study on this subject being an implication of UV-B on Lentil that will prove to be a light for future studies.

2. MATERIAL AND METHODOLOGY

A. Seed procurement:

Seeds of *Lens culinaris* Medik were collected from Indian Institute of Pulse Research (IIPR), Kanpur, Uttar Pradesh. Seeds were consistently selected and thorough washing was done upto 10 minutes with distilled water and surface sterilized with 0.1% $HgCl_2$.

B. Experimental Design:

Fresh seeds of Lentil were pre-soaked in distilled water for 12 hours and then kept in sterilized petriplates with wet whatmann filter paper as base for germination in seed germinator at 25±2^{o}C with humidity 60-80%. Regularly whatmann filter papers were allowed to change and sprinkle with distilled water. Four sets were prepared i.e. Set A for 20 minutes, Set B for 40 minute, Set C for 60 minute treatment and Set D as control. Nine replicates were maintained for each treatment.

C. UV-B Treatment:

Seeds with early roots of length between 5mm to 30mm of each sets A, B, C were irradiated with fluorescent UV-B (280-320 nm) lamps alongwith white light. Firstly 9 replicates of each sets were irradiated for1 day of time duration 20, 40, 60 minute. Out of which three replicates from each set were removed off from UV-B chamber for fixation and mitotic study . Then next 6 replicates of each set were again irradiated with UV-B of respective time duration on second day and three replicates were removed off. Remaining three replicate out of 9 in each sets were irradiated on third day and same process was followed for each set. Fourth set D was without UV–B treatment. The treatment was started in morning, 9am to 10 am every day.

D. Fixation:

Irradiated germinated seeds of each sets were fixed in Carnoy's fixative (glacial acetic acid : absolute alcohol, (1:3) after allowing for one hour recovery in their respective bottles along with control set. After 24 hours all of them were removed from fixative and transferred into bottles containing only 90% alcohol.

E. Mitotic preparation:

The roots of control and irradiated seedlings were excised, processed and hydrolysed in 1N HCL by adjusting water bath at 60^{0}c for 5-10 minutes so that tissues of root tips get soften. Then washed under running water to remove excess HCL and allowed to keep on blotting paper for dehydration. Dried root tips were stained with 2% acetocarmine (30 minute). By using squash technique mitotic slides were prepared and observed cells were snapped under Nikon Research Electron Microscope using Olympus PCTV Vision Software. Approx. 10 microscopic field views were recorded from each slide. Scoring was done from 3 roots of each replicate.

D. Formula used for scoring of data:

The array of mitotic indices and various abnormalities were screened out by applying below mentioned formula:

Active mitotic index (AMI) % = (Total number of dividing cells/Total number of observed cells)*100

Total abnormality percentage (TAB) % = (Total number of abnormal cells/Total number of observed cells)*100

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E. Statistical analysis:

The data obtained was analysed using statistical software, SPSS 16 and means were compared using DUNCANS multiple range test (DMRT) ($P \leq 0.05$). All the results were expressed in form of Mean ± Standard Error. The graph was plotted by using Sigmaplot 10.00 software.

3. RESULT

The data scored after UV –B treatment was documented in form of table 1 and table 2 and most frequent chromosomal abnormalities observed were shown through photo micrographs in form of cytological plates in figure 1.

Table 1: Showing the account of AMI (%) and TAB (%) induced by UV-B in root meristems of *Lens culinaris* Medik

TREATMENT (UV-B)*	DOSES (MINUTES)	AMI (%) ** (MEAN ± S.E.)	TAB (%) *** (MEAN ± S.E.)
1 DAY	CONTROL	13.10 ± 0.15^{a}	-
	20 MIN	12.54 ± 0.19^{a}	2.44 ± 0.08^{b}
	40 MIN	11.73 ± 0.15^{b}	3.51 ± 0.46^{a}
	60 MIN	9.87 ± 0.36^{c}	4.54 ± 0.21^{a}
2 DAY	CONTROL	13.10 ± 0.15^{a}	-
	20 MIN	12.08 ± 0.34^{b}	3.64 ± 0.16^{c}
	40 MIN	10.98 ± 0.16^{c}	4.80 ± 0.27^{b}
	60 MIN	8.52 ± 0.28^{d}	6.21 ± 0.15^{a}
3 DAY	CONTROL	13.10 ± 0.15^{a}	-
	20 MIN	11.39 ± 0.15^{b}	4.41 ± 0.12^{c}
	40 MIN	9.53 ± 0.59^{c}	6.13 ± 0.43^{b}
	60 MIN	7.81 ± 0.30^{d}	7.45 ± 0.33^{a}

***Abbreviations*: UV-B*- Ultraviolet B radiation, AMI (%) **- Active mitotic index, TAB (%) ***- Total abnormality percentage.
Means are followed by lowercase letter is statistically significant at $p<0.05$.

Table 2: Showing various abnormalities induced by UV-B radiations in root meristems of *Lens culinaris* Medik.

TREATMENT (UV-B)	DOSES (MINUTES)	METAPHASIC ABNORMALITY (MEAN ± S.E.)					ANAPHASIC ABNORMALITY (MEAN ± S.E.)				
		SC	CM	PR	ST	UN	BRDG	ST	UN	LG	OTH
1DAY	CONTROL	-	-	-	-	-	-	-	-	-	-
	20 MIN	0.34±0.49^{a}	0.00±0.00	0.54±0.07^{a}	0.26±0.13^{a}	0.43±0.05^{a}	0.26±0.13^{a}	0.43±0.05^{a}	0.09±0.08^{a}	0.08±0.07^{a}	0.00±0.00^{a}
	40MIN	0.54±0.14^{a}	0.21±0.11^{b}	0.63±0.05^{a}	0.23±0.11^{a}	0.54±0.14^{a}	0.09±0.09^{a}	0.63±0.05^{a}	0.54±0.14^{a}	0.09±0.09^{a}	0.00±0.00^{a}
	60MIN	0.44±0.06^{a}	0.60±0.16^{a}	0.40±0.24^{a}	0.57±0.11^{a}	0.35±0.22^{a}	0.38±0.38^{a}	0.27±0.27^{a}	0.83±0.36^{a}	0.48±0.15^{a}	0.22±0.11^{a}
2DAY	CONTROL	-	-	-	-	-	-	-	-	-	-
	20MIN	0.41±0.25^{a}	0.00±0.00	0.28±0.14^{a}	0.53±0.11^{a}	0.26±0.13^{a}	0.42±0.26^{a}	0.81±0.03^{a}	0.40±0.21^{b}	0.40±0.24^{a}	0.26±0.13^{a}
	40MIN	0.31±0.18^{a}	0.46±0.09^{a}	0.40±0.26^{a}	0.61±0.16^{a}	0.20±0.10^{a}	0.56±0.06^{a}	1.07±0.22^{a}	0.56±0.18^{b}	0.50±0.19^{a}	0.10±0.104^{a}
	60MIN	0.47±0.14^{ab}	0.57±0.11^{a}	0.45±0.06^{a}	1.02±0.13^{a}	0.38±0.22^{a}	0.35±0.03^{a}	0.80±0.03^{a}	1.46±0.17^{a}	0.47±0.14^{a}	0.22±0.11^{a}
3DAY	CONTROL	-	-	-	-	-	-	-	-	-	-
	20MIN	0. 24±0.12^{a}	0.22±0.11^{a}	0.50±0.14^{ab}	0.60±0.13^{b}	0.46±0.06^{b}	0.37±0.04^{a}	0.83±0.03^{a}	0.70±0.16^{a}	0.37±0.04^{b}	0.13±0.12^{a}
	40MIN	0.56±0.07^{a}	0.46±0.10^{a}	0.35±0.04^{b}	1.01±0.10^{ab}	0.70±0.08^{a}	0.34±0.18^{a}	1.11±0.20^{a}	0.55±0.18^{a}	0.70±0.08^{a}	0.35±0.04^{a}
	60MIN	0.60±0.12^{a}	0.23±0.12^{a}	0.72±0.07^{a}	1.28±0.26^{a}	0.82±0.03^{a}	0.58±0.33^{a}	1.36±0.28^{a}	0.92±0.27^{a}	0.46±0.06^{b}	0.50±0.15^{a}

***Abbreviations*: SC- Scattering, CM-C-mitosis, PR-Precocious movement, ST-Stickiness, UN-Unorientation, BRDG-Bridge, LG-Laggards, OTH-Other abnormalities. Means followed by lowercase letter is statiscally significant at $p<0.05$

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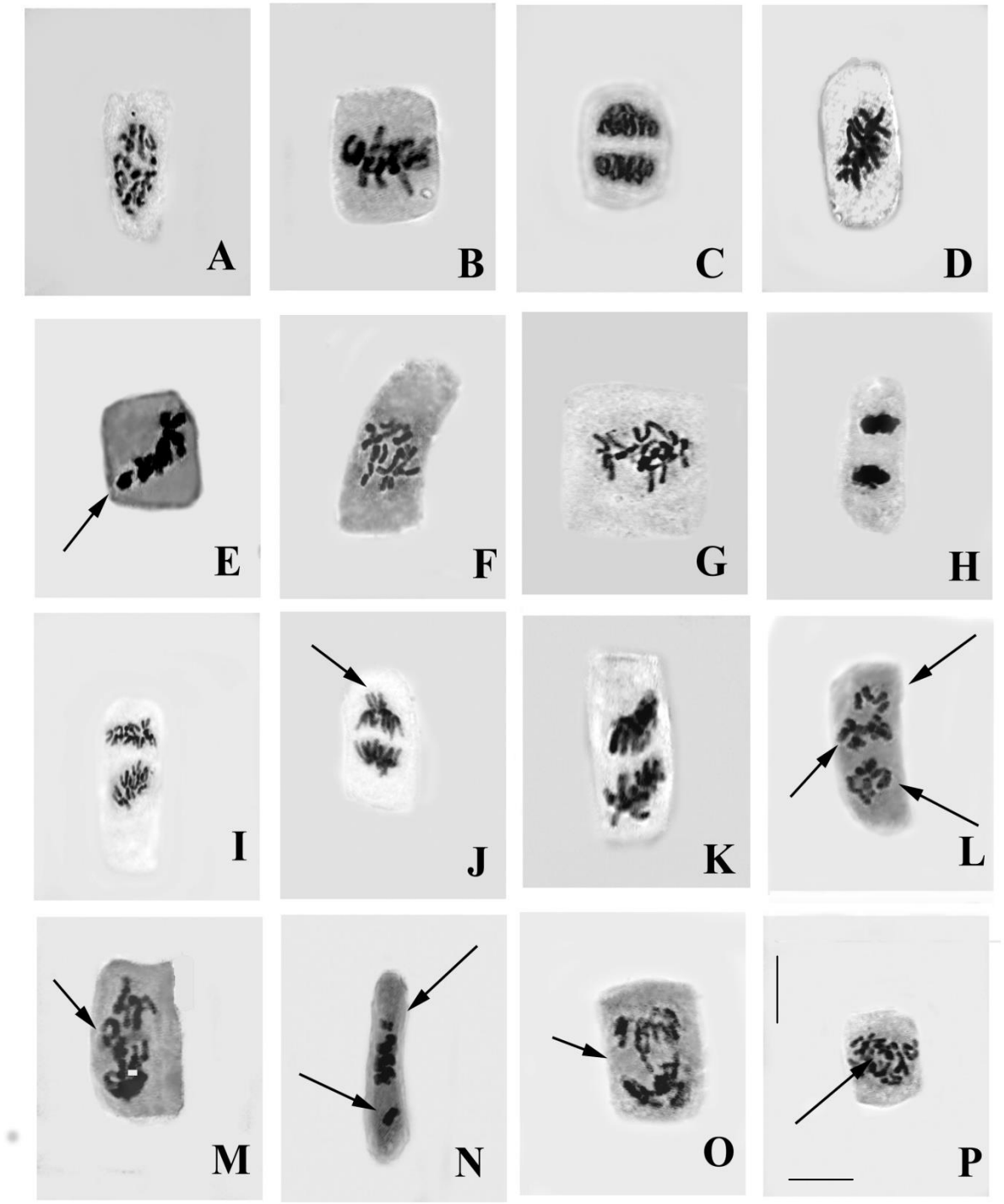

Figure 1: Showing UV-B induced chromosomal aberrations:
A: Prophase; B: Metaphase (2n = 14); C: Anaphase (14: 14); D: Unoriented metaphase;
E: Precocious movement of chromosomes with sticky metaphase; F: Scattering at metaphase;
G: C-mitosis at metaphase; H: Sticky metaphase; I: Non-synchronisation at anaphase;
J: Forward movement of chromosomes at anaphase; K: Unorientation at anaphase;
L: Segmental separation at anaphase; M: Loop forming laggard chromosome at anaphase;
N: Unequal separation at anaphase; O: Single bridge at anaphase; P: Multiple bridges at anaphase. [Scale Bar: Length = 2.16 µm, Width= 2.09 µm].

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A. Cytology of control sets:

Mitosis was studied in meristematic cells of root tips. The present study revealed normal diploid chromosome complement of *Lens culinaris* Medik (2n=14) which were aligned in form of equatorial plate at metaphase (2n =14) figure 1(B) and these were equally separated (14:14) towards each pole at anaphase figure 1 (C).The MI was found to be highest (13.10%) and no obscure chromosomal anomalies were observed.

B. Cytology of UV –B irradiated sets:

The UV-B irradiated root tips showed deviation against normal mitotic stages of cell division.

Effect on active mitotic index (AMI):

AMI is a major of cell count undergoing cellular division and governing the rate of meristematic activity. The genotoxicity level of UV-B radiation can be governed by the increase or decrease in the rate of active mitotic index. Table 1 shows the trend of progressive decline in AMI as the exposure days were increased alongwith elevated duration of UV-B. The AMI of 20 minute treated sets was recorded to be highest 2.54 $\pm 0.19^{a}$ in 1 day exposure and it seems to be mildly declined to 11.39 ± 0.15^{b}on 3 day UV-B exposure. However in 40 minute treated set a severe decline was noted from 11.73 ± 0.15^{b} to 9.53 ± 0.59^{c}. Apart from this a drastic decline in AMI was recorded in 60 minute treated sets from 9.87 ± 0.34^{b} to 7.81 ± 0.30^{d} as compared to control set. Figure 2 elucidates the pattern of gradual decrease in mitotic index on increasing the duration of supplemental UV-B irradiations. Hence it was clearly envisaged from present study that the elevated level of UV-B exposure is mitoclastic in its action and inhibits the normal mitotic cycle by reducing the rate of active mitotic index.

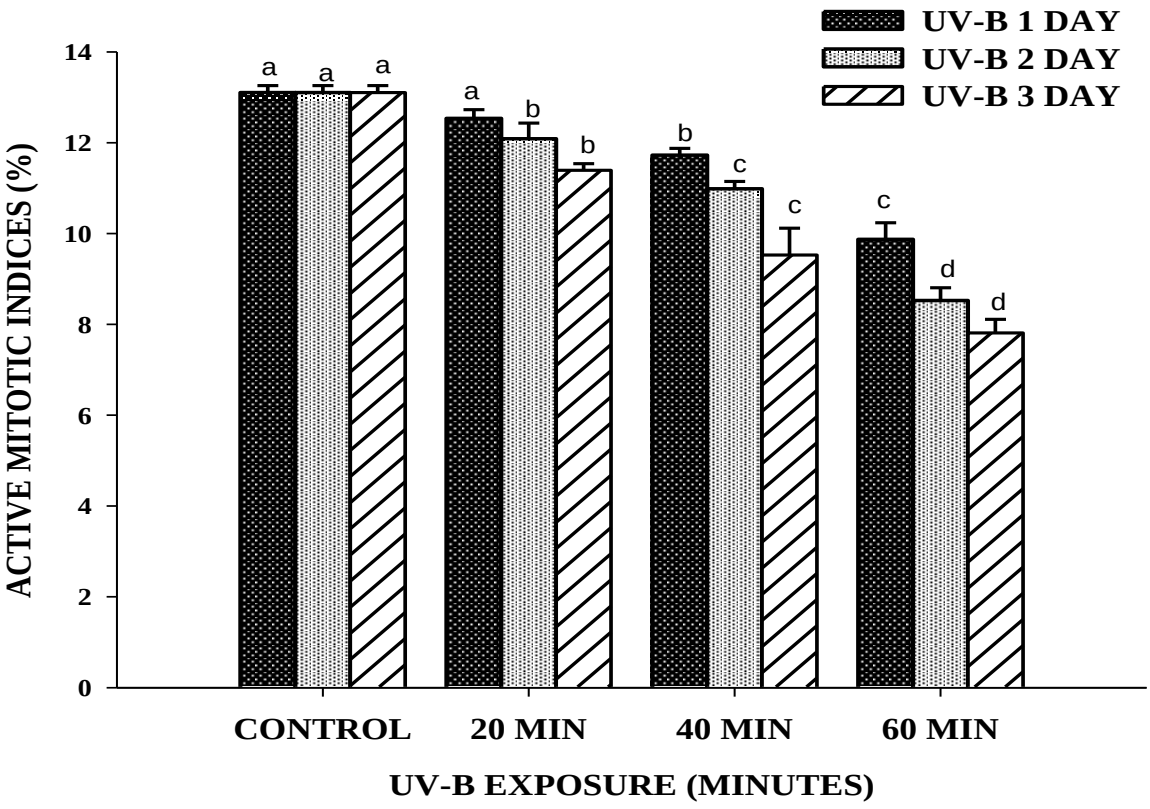

Figure 2: Supplemental effect of UV-B exposure on Active Mitotic Indices (AMI) in root meristems of *Lens culinaris* Medik exposed for 20 min., 40 min. and 60 min. Each barshown in vertical lines represents the mean of three samples assayed in triplicate. Bars showing different letters differ significantly at $P<0.05$.

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Effect of Chromosomal Organisation:

The root mitosis shows various distortions in pattern of chromosomal organisation in UV-B treated sets that leads to the induction of various types of chromosomal aberrations both clastogenic and aneugenic type. The count of total abnormal cells has been documented in form of total abnormality percentage (TAB) in table 1. The rate of TAB (%) increases from 0.44 ± 0.08^{b} (20 minutes) to 7.45 ± 0.33^{b} (60 minutes) as the exposure duration was increased. The wide ranges of chromosomal aberrations were observed such as unoriented metaphase (figure 1.D), precocious movement of chromosomes with sticky metaphase (Figure 1.E), scattered metaphase (figure 1.F), C-mitosis (figure 1.G), sticky anaphase (Figure 1.H), non-synchronisation (figure 1.I), loop formation (figure 1.M) and bridge formation (Figure1.O) etc. The most pronounced metaphasic and anaphasic abnormalities induced on1 day treatment was precocious chromosomes (0.63 ± 0.05^{a}) and on 2nd day stickiness (1.02 ± 0.13^{a}) and unorientation (1.46 ± 0.17^{a}) at 60 minutes. However, on 3rd day stickiness was found to be most dominant (1.28 ± 0.26^{a}) and (1.36 ± 0.28^{a}) at 60 minutes. Figure 3 shows the comparative trend of induced chromosomal anomalies along with increased level of UV-B exposure duration.

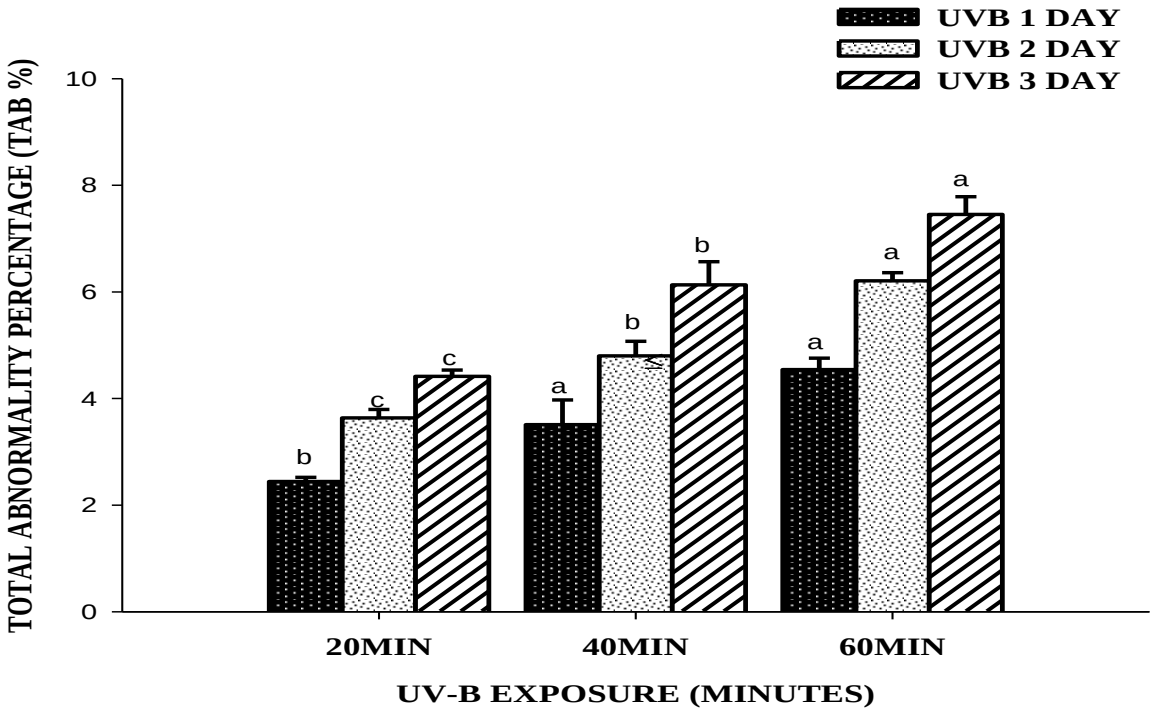

Figure 3: Comparative account of chromosomal aberrations induced by UV-B exposure in root meristemsof *Lens culinaris* Medik exposed for 20 min, 40 min, and 60 min. Each bar represents the mean of three samples assayed in triplicate and bars are shown in vertical lines significantly different at $P<0.05$.

Hence, on the account of above aforementioned observations, it was clearly manifestated that the elevated UV-B exposure will lead to induction of various chromosomal aberrations in plant cells.

4. DISCUSSION

The result of present study elucidates significant decrease in mitotic index along with dose dependent increase in percentage of chromosomal aberrations due to elevated exposure of supplemental UV-B irradiations. The studies conducted by Csilla I. Bara, (2009) and Hopkins *et al.*, (2002), were in favour of present study showing reduced mitotic indices.

The reduced AMI may occur due to breakdown of plant self protection system and further inhibition of cell DNA replication, transcription and protein synthesis [15]. Probably, more cells were arrested at interphase stage that leads to decline in cell division. The decreased ATP level and pressure exerted by energy producing centre may be the probable cause for inhibition of DNA synthesis and reduced ATP [16]. Joze Bavocon and Nada Gala (1996) were also reported

decreased mitotic activity and lesser vitality due to influence of UV-B irradiation in *Picea abies*. Wellmen (1983) stated that slowing of mitosis is a protective plant reaction, as DNA is most sensitive to UV-B during replication. The enhanced UV-B radiation doses induce different types of chromosomal aberrations characterized by change in total chromosomal number or structure. If the cells are irradiated during and after DNA synthesis (S and G2) aberrations are of chromatid type. However, if before G_1, aberrations are of chromosomal types [19].

Stickiness (Figure 1.H) was found to be most dominant chromosomal aberrations recorded in the metaphase and anaphase of mitosis. Chromosome stickiness leads to inactivation of DNA replication, increased chromosomal contraction and condensation or nucleoproteins probably leading to cell death [20].

Precocious movement of chromosomes (Figure 1.E) observed at metaphase might be formed due to early chiasma terminalisation or univalent chromosome formation at end of prophase.

Unorientation (Figure1D, K) may occur due to disruption of spindle structure or function as per UV-B radiation, leading to the spindle fibre imbalance on both sides of centromere traction power or chromosome acentric fracture cannot cause chromosome normal movement [21].

C- mitosis at metaphase was detected (Figure 1.G) firstly discovered by Levan (1938) in root tips of *Allium cepa* caused by inactivation of the spindle fibre followed by a random scattering of the chromosomes over the cell.

The loop forming laggards at anaphase (Figure 1.H) might have originated due to failure of kinetochores to attach with spindles and leading to the joining of ends forming loops. Such disorders may lead to mutations.

Formation of Chromosome Bridge was another dominant anomaly recorded at anaphase (Figure 1.O and P). According to [15], the chromosome bridge might have resulted due to enhanced activity of UV-B radiations, making chromosome breaks, then the two chromosome sides are respectively healed, producing with double centromere chromosomes i.e. “chromosome bridges”.

Haliem *et al.*, (2013) are of the opinion that the DNA damages induced by UV-B radiations might have influenced the expression of number of genes leading to alterations in proteins that control many metabolic processes like plant development, cell cycle, fertilisation and seed formation.

5. CONCLUSION

As Lentil is an important pulse commodity, hence selected to study the UV-B effect in root meristematic cells. From present study it was inferred that if plants are exposed for longer duration to direct UV-B radiations, it will affect root growth due to inhibition of cell division. Hence, this type of researches will further be reciprocated to understand the genotoxic effects of UV-B on whole plants and enhancing awareness to consumers about ill effects of UV-B poisoned plants and to devise protective measures against them.

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