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ORIGINAL ARTICLE **OPEN ACCESS**

Effect of Variable Supplemental duration of UV-B on root Meristems of *Artemisia annua* L. with special reference to Morphology and Biochemical aspects

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ABSTRACT
In the alternating environment, plants are exposed to many factors but the problem of enhanced UV-B radiation is created by the anthropogenic activities resulted in ozone layer depletion. To investigate the feasibility of UV-B radiation seeds of Artemisia annua L. were exposed to four different durations i.e. 20min, 40min, 60min and 80min along with control for determining the effectiveness of cellular behavior. Mitotic cells were found to be normal in control plants. TAB%(Total abnormality) was recorded high at higher doses of UV-B radiation i.e. UV 60min & 80min. AMI% (Active Mitotic Cells) and TAB% show inverse relationship to each other. Different chromosomal anomalies induced through UV-B rays were stickiness, scattering, laggard, unorientation and bridges etc. Major portion of chromosomal abnormalities occupied by stickiness in UV irradiated sets. Survival rate and plant height were decreased as the exposure of UV light increases. Plants are performing well to cope up with such anomalies by enhancing the proline content. The data of proline estimation depicted that proline percentage was significantly enhanced by UV-B rays. Focusing on this, the main objective of this study is to summarize the influence of variable durations of UV-B rays on the qualitative and quantitative traits of plants.
Keywords: Artemisia annua L., AMI%, Chromosomal anomalies, UV rays, TAB%.

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INTRODUCTION

Radiations from the sun are of various wavelengths; most of them are invisible to the human eye. Short wavelength radiations are considered harmful and energetic. Electromagnetic radiations are emitted from the sun, and range from infrared to ultraviolet (UV) radiations. In the earth's atmosphere, different layers of atmosphere are present to absorb or filter these radiation but mostly stratosphere absorbs most of these. UV radiations have shorter wave length ranges from 200 nm to 400 nm. Ultraviolet radiation is found in the category of non ionizing radiations and it is found in 8 % to 9 % of total radiation emitted from the sun[1]. Now a days, anthropogenic activities are the main cause of producing absorbed radiations through radioactive waste storage, nuclear radiation accidents and nuclear power production [2; 3].

UV radiation is divided into three types: UV-A, UV-B, and UV-C. UV-A radiations are the less harmful part of ultraviolet radiations. Among them UV-B are more common because UV-B levels directly depend on the ozone layer and those levels are continuously increasing due to ozone depletion [1]. For the process of photosynthesis plants need sunlight; sunlight comprises visible rays as well as ultraviolet radiations. Therefore, plants are directly exposed to the ultraviolet radiations. Plants being living organisms respond to UV radiations. UV-B is an important component of the environment acting as an ecophysiological factor with the potential to alter plant growth and photosynthesis [4]. Even a small increment in incident UV-B radiation can have significant biological effects because UV-B is readily absorbed by a number of important macromolecules such as nucleic acids, proteins, lipids and phytohormones. UV rays also damage plant processes such as physiological processes and DNA damage [5]. The Ultra violet photon has much energy to carry out a photochemical reaction by breaking down the chemical bonds [6]. UV radiations are known to cause significant damages to crop plants and the overall ecosystem, such damages include membrane disruptions, protein conformational change, effect on plant hormones and

pigments that ultimately affect the plant growth, yield, development, and numerous cellular processes such as photosynthesis and respiration [7]. The damage inflicted by UV, significantly depends on the quality and quantity of photosynthetic active radiation (PAR) which is actually the amount of solar radiation required by plants to activate photosynthesis. Earlier studies clearly demonstrate the extent of damage caused by ambient UV-B and enhanced UV-B [8;9,10] on morphological, physiological, biochemical and molecular components of crop plants. UV-B has a significant consequence on the morphology of crop plants that can affect their growth so if this radiation maintained and delivered at a proper doses then it boosts the antioxidant enzymes by protecting plants against radiation.

Information on the action of UV-B radiation as well as of endogenous or exogenous antioxidants on the meristematic cells is very limited [11;12]. The present study was planned out to study the substantial effect of UV rays on the somatic cells of *Artemisia annua* L. *Artemisia* which is commonly known as sweet wormwood, a member of Compositae (Asteraceae) family, is a sexual and diploid species with 2n=2x=18 [13]. The leafy portions of this herb contain a potentially important sesquiterpenes, lactones and anti-oxidant compounds (Flavanoides, Phenolic acids,etc.). It is used for the procurement of malaria, hemorrhage and cancer. Reason behind preferring meristematic root tips for study is that treatments can be carried out in dark to avoid photoreactivation and due to low photolyase activity root tips are very prone to UV-B radiation.

MATERIAL AND METHODS

Plant Material: Seeds of *Artemisia* of accession no. EC-415012 were collected from NBPGR, Bhowali, Nainital, Uttarakhand, India-211002.

UV-B Radiation treatment: After surface sterilization with sodium hypochlorite, the presoaked *Artemisia* seeds were allowed to germinate at 25^0 C in incubator. Germinated *Artemisia* seeds with uniform size root tips were selected. Petriplates containing germinated seeds were irradiated with UV-B rays for 20, 40 and 60 minutes, after which left undisturbed for one hour for recovery. Experiment was performed in 3 replicates along with control.

Cytological analysis: Roots of irradiated sets were fixed in carnoy's fixative (1 Glacial acetic acid: 3 Absolute alcohol) alongwith control sets. After 24 hours roots were transferred to 90 percent alcohol for preservation. Irradiated roots were hydrolyzed in 1N HCl and then washed under running water to remove additional chemical and dried on blotting paper. Roots were stained using 2 % acetocarmine , slides were prepared by squash technique and the snaps were taken under PCTV vision photography software. Experiment was performed in 3 replicates alongwith control.

Mitotic formula: Mitotic index was calculated according to Edgar [14] and Balog [15],

$$\text{Active Mitotic index (AMI) \%} = \frac{\text{Total no. of dividing cells}}{\text{Total no. of cell observed}} \times 100$$

$$\text{Total Abnormality percentage (TAB) \%} = \frac{\text{No. of Abnormal cell}}{\text{Total no. of cell observed}} \times 100$$

Morphological analysis: Morphological parameters were taken into account to study the effect of UV-B radiation.

Survivability Percentage: The data was calculated on the 14th day from the seedling emergence.

Plant height (cm) : The plant height was measured when it attained the maximum height at the maturity. These parameters were highly affected by different doses of UV-radiation.

Biochemical Analysis: Biochemical analysis was carried out on fresh leaves of plant material, which was immediately extracted and assayed.

Proline estimation: The proline content was quantified according to Bates *et al*., (1973).

Data analysis: Statistical analysis was performed using the SPSS 16.0 software. One way analysis of variance (ANOVA) and Duncan's multiple range test ($p \leq 0.05$) was performed and the graph was plotted by using Sigma plot 10.0 software.

RESULT

The UV spectral range of solar radiation is an important environmental factor, which effects the plants cytology, morphology and biochemical constituents. Numerous studies reported the deleterious effect of UV-B rays but it was found that adequate exposure time of UV-B radiation induces beneficial traits. The present experimental work scrutinized the potent affect of UV-B radiation at cytological, morphological and biochemical levels in *Artemisia* plant.

Chromosomal behavior of *Artemisia*

Cytological investigation divulged the anomalous effect of UV- B rays exposure on mitotic cells as it produced several abnormalities at different stages of cells as illustrated in Table 1. Extensive study shows

that UV-B rays raised certain modulating effect in the cell cycle progression. Mitotic chromosomal configuration was found quite normal in control *Artemisia* (2n=18) roots. With respective increase in UV-

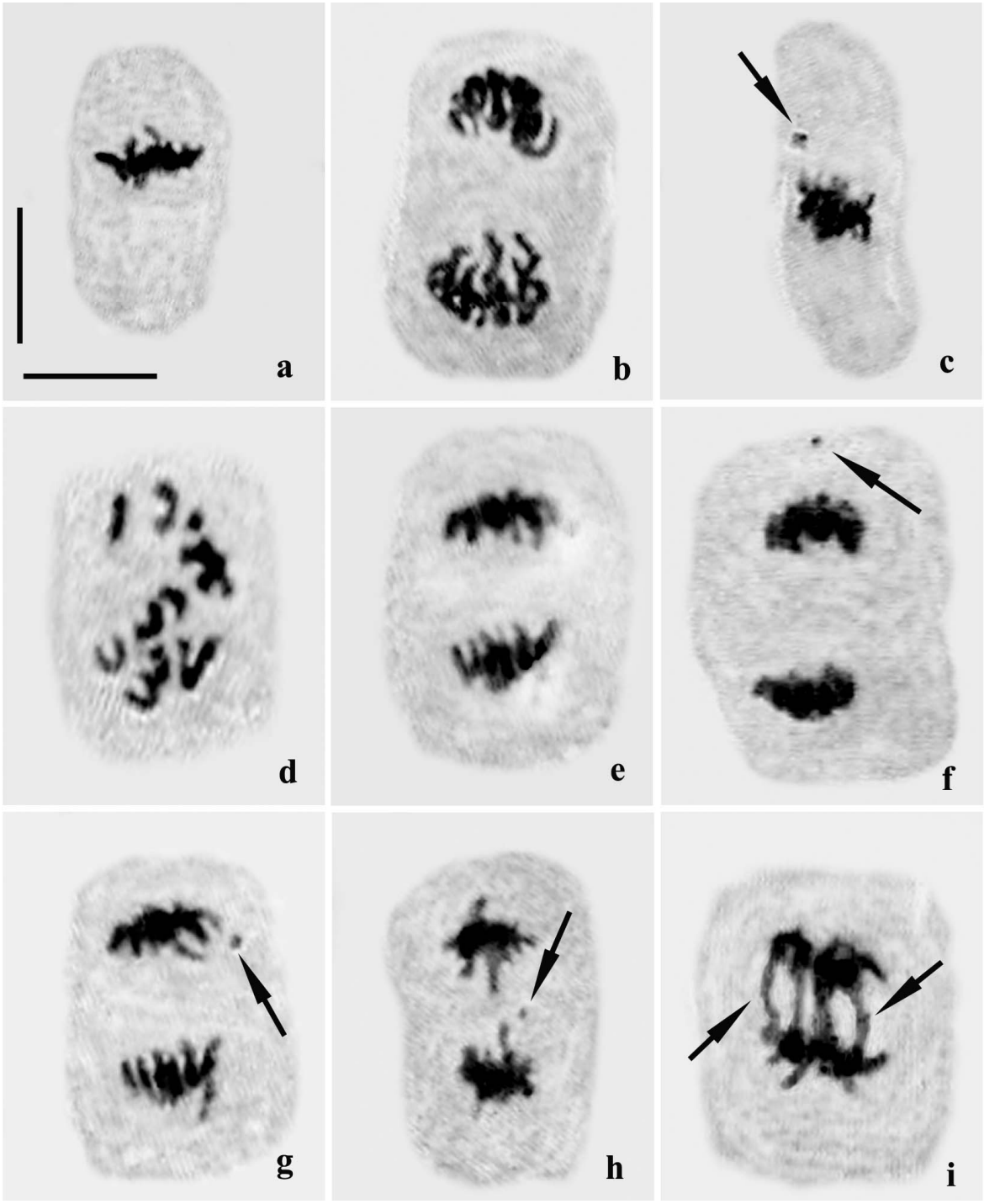

Figure 1- UV rays induced abnormalities in root meristems of *Artemisia annua* L.- Normal Metaphase (2n=18), B. Normal Anaphase (18:18 separation), c. Precocious chromosome at metaphase, d. Scattering C-metaphse e. Stickiness at anaphase f. Forward movement at Anaphase g. Laggard formation at Anaphase, h. Sticky anaphase at laggard i. Multiple bridge formation at Anaphase (Scale bar: Length-5.32µm, Width-6.12 µm)

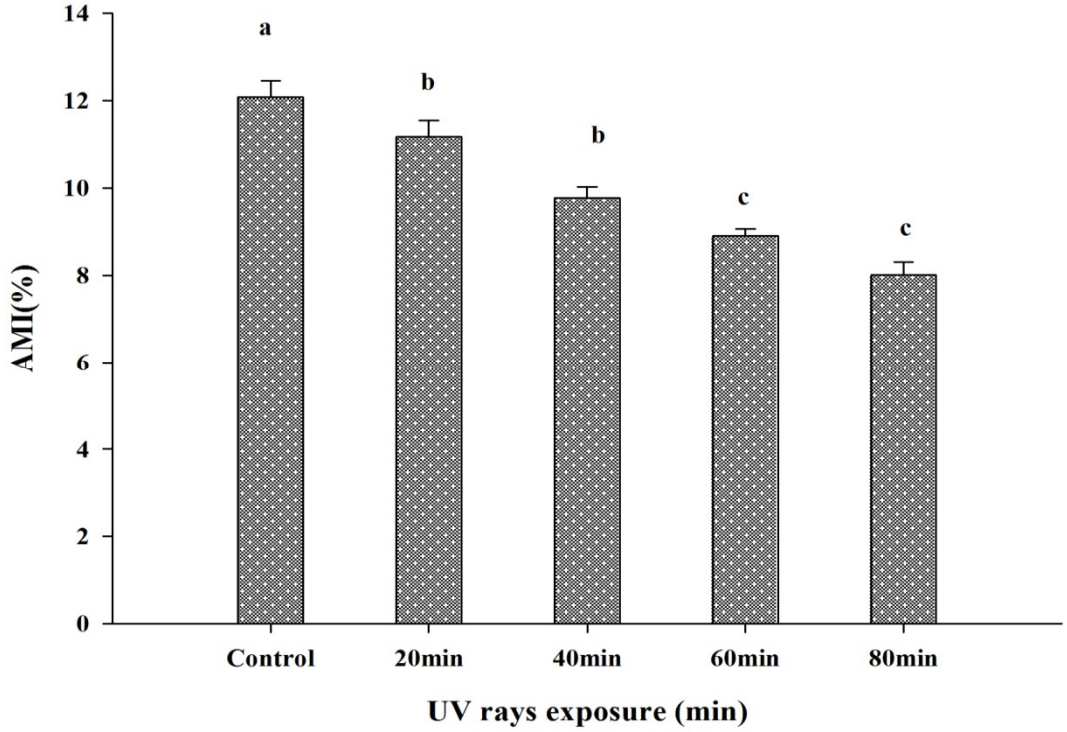

Figure 2- Effect of UV- B rays exposure on AMI% in root meristems of *Artemisia annua* L.

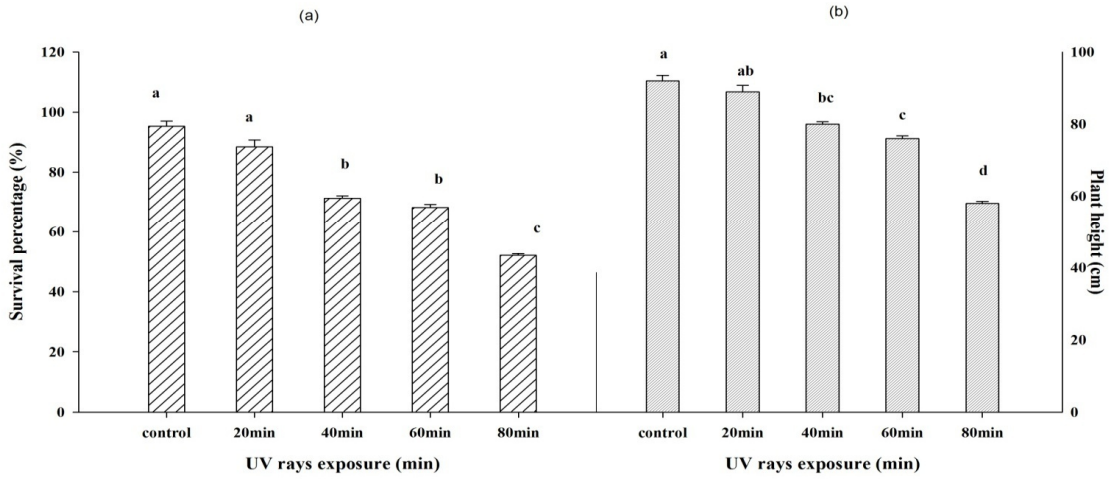

Figure 3- Effect of UV-B rays exposure on (a) Survival percentage (b) Plant height

B exposure on root meristems of *Artemisia*, AMI percentage decreases and meanwhile TAB % increases. Thus, AMI and TAB percentages show inverse relationship with each other in UV-B treated sets. The Ratio between the number of mitotic cells to the total number of examined cells (Mitotic index) is a useful measure of cellular proliferation which can be used in predicting the overall survival response to any cellular treatment [16] In control sets, highest AMI % was recorded as 12.08 ± 0.39^{a} which was reduced to 8.01 ± 0.28^{c} at UV 80 min dose (Figure 2) while UV 20 min dose depicted the lowest TAB% i.e. 3.37± 0.08, which show increment up to 5.89±0.15 in UV 80 min sets (Table 1). It was recorded that lower exposure of UV-B rays causes least chromosomal irregularity but the longer duration of UV-B rays invokes higher anomalies in addition to decrement in AMI %. Lowering of AMI% might have achieved by the inhibition of DNA synthesis at S phase that most probably happened due to decrease of ATP level and the pressure from the functioning of the energy production centre [17,18].

Morphological investigation

UV-B radiations are known to cause many anatomical and morphogenic changes in plants. UV-B treated sets were morphologically analyzed on different parameters such as survival percentage and plant height. The survival percentage and plant height of *Artemisia* plants declined with the increasing exposure of UV-B radiation. Lowest survival percentage was recorded in UV 80 min i.e., 52.03 ± 0.57^{c} followed by 68.25 ± 1.02^{b} in UV 60 min and 88.45 ± 2.29^{a} in UV 20min whereas highest percentage was 95.30 ± 1.76^{a} in control sets (Figure 3a).Maximum height (in cm) was recorded in control *i.e.* 92.01 ± 1.54^{a} followed by 89.03 ± 1.74^{ab}, 80.21 ± 0.60^{bc}, 76.01 ± 0.74^{c} and 58.12 ± 0.54^{d} in UV 20,40,60 and 80 min, respectively(Figure 3b).

Proline Estimation

The data of proline (Figure 4) estimation depicted that proline percentage was significantly influenced by UV-B rays. The percentage of proline content (µmol/mgFW) was increased maximally at UV-B 80min i.e. 6.32±0.09. Lowest value of proline content found in control plant was 3.66±0.08 which was gradually increased 3.80±0.08, 4.11±0.13 and 5.49±0.27 at UV 20min, UV 40min and UV 60min, respectively.

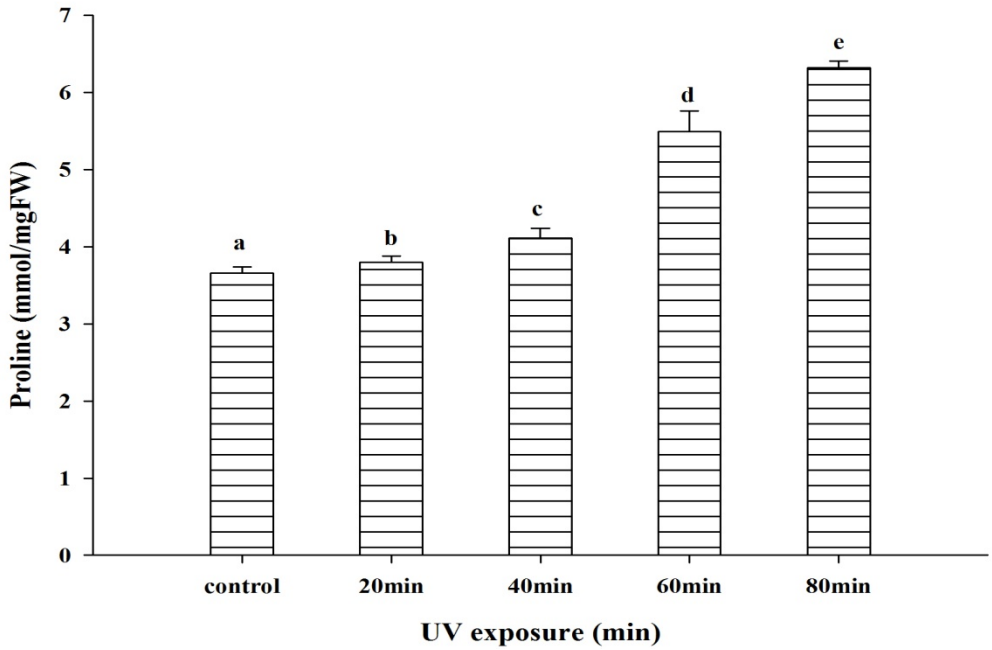

Figure 4- Effect of UV-B exposure on the proline content in *Artemisia annua* L.

DISCUSSION

Chromosomal behavior of *Artemisia*

In treated sets, various chromosomal anomalies were detected in both metaphasic and anaphasic stage *viz*., stickiness, precocious movement of chromosome, unorientation, bridges and laggard etc (Figure 1). It was observed that major portion of chromosomal abnormalities occupied by stickiness in UV irradiated sets. Stickiness (Figure e,h) could be the result of radiation action on chromosomal fibers which leads to the entanglement of chromatin threads or may be due to the radiation effect on the process of DNA depolymerization which makes the chromosome surface appear sticky [19]. Another abnormality was the unorientation of chromosome that might be due to the spindle disfunctioning which does not orient chromosome at equatorial plate. The Precocious movement (Figure 1c) of chromosome occurred due to the migration of chromosomes to the poles which can result into early chaisma terminalization at diakinesis or metaphase I [20]. Laggard formation (Figure 1g,h) is due to delayed terminalisation, chromosomal stickiness or failure of chromosomal movement [21]. Chromosome bridges (Figure 1i) are usually observed at anaphase are formed by the breakage and fusion of chromosome and chromatids, consequently increasing the risk of aneuploidy [22]. C-metaphase is may be the consequence of sudden inactivation of spindle apparatus resulted in the delay in the division of centromere.

Plants need sunlight for the process of photosynthesis; sunlight comprises ultraviolet radiations. So plants were adapted at lower exposure of UV-B rays and they can repair the minor damage caused by these rays. There are basically two mechanisms by which photorepair can occur in plants. Firstly, by the accumulation of flavonoids that are UV-absorbing compounds, and phenolic compounds in the epidermis

of leaves so that mesophyll cells can be protected and the photosynthesis process is not affected [23]. But in contrast at high dose, UV-B radiation breaks down the plant self-protection system and inhibits the action of the cell DNA replication transcription and protein synthesis [24]. Under UV-B stress conditions two adjacent pyrimidine bases form a pyrimidine dimer that affect the DNA replication and cause photodamage by the construction of the Cyclobutane Pyrimidine Dimer (CPD) and the repair mechanism was too slow that it could only repair low levels of damages and not the high concentration. These all conditions lead to higher anomalies at longer exposure of UV-B rays in plant cells.

Morphological investigation

Morphology of plants is considered to be a very effective indicator of UV-B damage. At high doses, survivability percentage was highly affected by UV-B rays. Longer duration of exposure of UV-B radiation creates the disturbance of DNA polymerase by which they are not able to read through these photoproducts, their elimination is essential for DNA replication and transcription and thus the survival of plant decreases [25]. Reduction in growth could be associated with UV-B induced inhibition in photosynthetic rate and destruction of growth promoting hormone: Indole Acetic Acid (IAA) [26]. Changes in plant height and stem diameter have been observed due to UV-B radiations. In a study on Tartary buckwheat, decrease in plant height was observed in eight populations [27]. The inhibition in growth of plants by enhanced UV-B can be related to higher production of active oxygen species, which can cause multi-targeted deleterious effect on PS II components and reduced the activity of Rubisco [28].

Proline Estimation

Proline is known to occur widely in higher plants and accumulates in large quantities to protect the plant against oxidative stress in response to UV radiations. The UV-B rays cause the over production of reactive oxygen species (ROS) which leads to the disturbance of cellular mechanism which is overcome by proline. Proline is known to be involved in alleviating cytosolic acidosis associated with several stresses [29]. Proline provides less than 5% of the total pool of free amino acids in plants under stress free condition, whereas the concentration increased up to 80% during stress [30].

CONCLUSION

From the aforesaid investigation, it can be concluded that brief exposure of UV-B rays creates lesser anomalies in mitotic cells but they proved to be genotoxic in the longer exposure. This significantly reduced mitotic activity and enhanced a wide range of chromosomal aberrations at higher doses. Thus, if UV-B rays are steadily provided to the plant, it can modulate and accelerate the plant growth because at lower dose plants can rejuvenate itself and acclimatize according to altering environment. Therefore, envisage that least amount of UV rays produces promising point mutations with less lethality in upcoming generations that could be applied in breeding programs for improvising the genetic variability in *Artemisia annua* L.

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CONFLICT OF INTEREST

There is no conflict of interest.

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