3 MATERIALS AND METHODS

Seeds of Brassica compestris and Allium cepa were soaked in distilled water in petridishes over germination paper and immediately kept under UV radiation generated with FS-40 Sunlamp (U.S.A.). The fluorescent tube was kept at a distance of 45 cm. UV-A was filtered through cellulese acetatee filter of 5 Mil thickness covered over the petridish allowing only UV-B. The distance adjusted is correspondent to UV-B irradiance at point of incidence adequate to reduce the atmospheric ozone by 50%. The entire set up also received diffused light through window but suppose to have been free from UV-B. The control was also kept side by side but was covered with additional filter of mylar D to cut off UV-B. Each petridish contained 200 seeds and kept in replication of five. After 6 hours of irradiation, every day the petridish were kept in dark. The germination percentages were recorded every four hours upto the fifth day, while root and shoot lengths were recorded after five days of germination. The temperature regium maintained here was 25°C day and 20°C night.

Twenty four hours after soaking radicles emerged enough to cut and fix. To study the mitotic index root tips were excised periodically, every four hours by randomly sampling in the replicated trials. They were fixed in Farmer's fluid. (Prepared by mixing 95% ethyl alcohol and acetic acid in proportion of 3 : 1). After 24 hours of fixation root tips

were given one or two washes of 45% acetic acid and were hydrolysed at 60°C in mixture of 1N HCl and 2% aceto orcein mixed in the proportion of 1:9. Root tips were then squashed in 1% aceto orcein. The mitotic indices were worked out by scoring the total number of cells showing division and the total number of cells observed.

The formula is as follows -

$$\frac{\text{Total no. of dividing cells}}{\text{Total no. of cells observed}} \times 100.$$

The root tips were squashed in 1% aceto orcein by using the same procedure used to observe the effect of UV-B on mitotic indices. Roots of both the seeds, are checked for chromosomal aberration with the help of microscope.

Estimation of nucleic acid content - Nucleic acid content from the roots of 5 days old seedlings was measured. Nucleic acid contents from germinating seeds of controls as well as irradiated seeds were determined by the method of Detchou and Possingham (1972). One gram of fresh tissue is homoginized in 10 ml of 5% perchloric acid in chilled morter and pestle under cold condition. After 15 minutes the homogenate was centrifuged at 2000 g for 10 minutes. The supernatent was discarded. To remove the phospholipids, the residue was then extracted four times with 10 ml of mixture of

ethanol-ether-chloroform (2:2:1) at room temperature. The final residue was dried and then hydrolyzed in 5 ml, 5% Trichloro Acetic Acid at 90^{o} for 30, minutes.

The total nucleic acid is obtained by measuring the absorbance of the hydrolysate at 268.5 nm. The total nucleic acid content was calculated by using an appropriate conversion factor. (Logan et al.1952).

Determination of mitotic cycle duration has been carried out as per the method of Van't Hoff et al.(1960), Van't Hoff and Sparrow (1963), Van't Hoff (1967) by using colchicine. The technique involves a production of a small mass of tetraploid cells by treatment of colchicine in meristems composed of diploid cells. Onion seeds, could not stand for the colchicine treatment for the unknown reason.

Here, in this experiment, the seeds of Brassica campestris were soaked in water and exposed to UV-B radiation for 6 hours and kept in natural light. On the next day, exactly after 24 hours, the seeds were treated with 0.2% colchicine solution for 6 hours and exposed to UV-B radiation simultaneously. They were then washed thoroughly in water for 15-20 minutes. From this lot, few germinating seed root tips were fixed in a fixative 'Alcohol Acetic acid' (3 parts of Absolute alcohol : 1 part of Acetic Acid). The remaining were allowed to grow under natural condition. The root tips from these were periodically sampled for every

two hours. These root tips were subsequently examined for polyploid cells under microscope.

The control was also run parallely which did not received UV-B radiation.