5.3 HIGH LET RADIATION BIOLOGY

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The experiments conducted in this field involved cell inactivation and chromosome aberration due to charged particle interaction with V79 and M5 cells by users from Presidency College, Kolkata, and studies on the germination properties, biochemical properties etc. on ion beam irradiated mustard seeds by users from MDU Rohtak. Apart from these, the this low flux beam line has been also used by the users from Bose Institute, Presidency College and SINP Kolkata for simulating the detection of cosmic particles by low cost polymer track detectors.

5.3.1 Induction of cytogenetic damage and repair in V79 cells by accelerated heavy ion

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Ionizing radiation is very efficient in inducing chromosomal aberrations which is the consequences of mis-repair of DNA double strand breaks. Radiation induced chromosome aberrations are regarded as the most sensitive biological indicator and have been used for radiation risk assessment. The classical cytogenetic assay relies on the scoring of aberrations in cells at metaphase. We have investigated different types of aberrations at different intervals with ^{16}O beam (613 keV/um) and ^{7}Li beam (60 KeV/um)in Chinese hamster lung fibroblast cells to understand the process of repair and mis-repair as well as the role played by different qualities of charged particles in inducing chromosomal aberrations. The comparison with the results of effect of sparsely ionizing radiation obtained earlier has also been carried out to get a deeper insight at cellular processes.

The radio sensitivity of Chinese hamster lung fibroblast cells has also been investigated by clonogenic cell survival assay. It has been found that cell survival depends on dose as well a radiation qualities. DNA from surviving colonies have also been isolated for mutational studies by randomly amplified polymorphic DNA(RAPD) method using markedly available RAPD primers. To investigate the level of expression of different types genes after receiving high LET radiation, RNA have been isolated at two different time intervals. High LET radiation is very much effective in inducing apoptosis (programmed cell death) in cells, for quantitative estimation of apototic cell induced after receiving high LET radiation, morphological study of cells has been carried out by fluorescence microscopy; nuclear fragmentation was also investigated by ladder formation technique in apoptotic cells.

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5.3.2 Effect of $^{7}Li^{+++}$ heavy ion irradiation on hydrogen peroxidase decomposing enzymes in leaves of *Brassica juncea*.

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Introduction:

Reactive oxygen species (superoxide radical, hydrogen peroxide, singlet oxygen, hydroxyl radicals) generation and their scavenging is a known phenomenon in the organisms under stressed conditions. Low and high LET radiations are also potential candidates for generating reactive oxygen species (ROS). In plants, UV- B exposure is known to lead to the generation of active oxygen species and eventually results in oxidative stress.

The heavy ion radiation may generate the free radicals which might be inhibitory to the biochemical processes. To mitigate and repair the damage initiated by free radicals or ROS, plants have also developed antioxidant enzymes and antioxidative molecules. Catalase and peroxidases catalyze the breakdown of H_2O_2. The organisms which are able to sustain life to over-express the antioxidative system like high level of carotenoides, ascorbic acid, glutathione and antioxidative enzymes including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APOX) and glutathione reductase (GR).

In the present study, the level of hydrogen peroxide decomposing enzymes such as peroxidase, catalase and ascorbate peroxidase were evaluated to probe the level of $^{7}Li^{+++}$ irradiation stress tolerance in a view of report of heavy ion induction in plant growth.

Material and Methods:

Irradiation Treatment and Plant Growth Conditions

Seeds of Indian mustard (Brassica juncea L cv RH-30) were irradiated with $^{7}Li^{+++}$ heavy ion (45 MeV on the sample surface) with fluences of 10^7, $5X10^7$ and 10^8 p/cm^2 using Pelletron accelerator (15 UD pelletron) at Nuclear Science Centre, New Delhi. Heavy ion irradiation effect on growth and H_2O_2-decomposing enzymes viz peroxidase (POX), catalase (CAT) and ascorbate peroxidase (APOX) was studied in

leaves of plants from irradiated seeds sown in field during winter (rabi) season at different developmental stages 30, 60 and 90 days after sowing. The seeds were sown in split-split plot of size (1 X 1 m), designed for each treatment without enriching the soil with extra nutrients.

Extraction and assay of antioxidative enzymes

For enzyme extraction, leaf tissues were homogenized in sodium phosphate buffer (0.1 M, pH 7.0 ; 1:4 w/v) containing 1 % polyvinyl pyrollidone (PVPP) followed by centrifugation at 10,000xg, for 20 min at 4°C. The supernatant obtained was used for the assay of POX and CAT. While for the extraction of APX, ascorbate (2 mM) was added to the extraction buffer (0.1 M Sodium phosphate buffer, pH 7.8, containing 1% PVPP).

POX was assayed as described by Pundir et al. [1]. For the assay, mixture of 1.8 ml sodium phosphate buffer (0.05M, pH 7.0), 0.1 ml phenol (1mg/ml), 0.1ml 4-aminophenazone (0.5mg/ml) and 0.1ml, extract was pre-incubated at 40°C for 5min. Then 1.0ml of 10mM H_2O_2 (30 % w/v) was added followed by incubation at 40°C for 10min and thereafter absorbance was recorded immediately at 520nm. The amount of H_2O_2 utilized was extrapolated from the standard curve between A_{520} and H_2O_2 concentration. One unit of enzyme activity was defined as the amount of H_2O_2 decomposed per min per mg protein.

CAT activity was assayed using the method described by Aebi [2]. The reaction mixture contained sodium phosphate buffer (0.05M, pH 7.0), 50 mmol/L^{-1} H_2O_2 and 50 µl of enzyme extract in a 3 ml volume. The activity was assayed by monitoring the decrease in absorbance at 240 nm as a consequence of H_2O_2 consumption and enzyme activity expressed as amount of H_2O_2 decomposed per min per mg of protein

APOX activity was assayed according to the Nakano and Asada [3]. Rate of ascorbate oxidation was monitored by following the decrease in absorbance at 290 for 3 min in 3.0 ml of a reaction mixture containing 2.905 ml, sodium phosphate buffer (50mM, pH 7.0), 15 µl of ascorbic acid (10 mM), and 50 µl of enzyme extract in which the reaction was triggered by the addition of 30 µl of H_2O_2 (10 mM). Correction was done for the low, non-enzymatic oxidation of ASC by H_2O_2, which was found to be 0.0123 per min. Enzyme activity was calculated by using mM extinction coefficient for ascorbic acid (2.8 $mM^{-1}cm^{-1}$) and expressed as amount of ASC oxidized per min per mg protein.

Results:

Peroxidase Activity

In M_1 generation, peroxidase activity decreased with advancement of age in the leaves of seedlings (Fig.1). A remarkable increase in peroxidase activity was registered with increase in irradiation dose at 30 and 90 day of plant. At 60^{th} day of age of plant no change in peroxidase activity at lower dose while increased at higher dose (10^8 p/cm^2)

was observed. Enzyme activity increased 3-fold at high dose (10^8 p/cm^2) at 90th day of growth of the seedlings.

Catalase Activity

A dose dependent decrease in catalase activity (5-50%) in leaves of plants from irradiated seeds was observed compared with that of control plants during different developmental stages from 30-90 days (Fig 2). Maximum decrease in activity (50%) compared with control was observed at high dose (10^8 p/cm^2) at 90th day of growth of seedlings.

Ascorbate peroxidase Activity

Ascorbate peroxidase activity decreased with advancement of the age in the leaves of seedlings (Fig 3). However, there was differential response of enzyme activity due to $^7Li^{+++}$ irradiation. At 30th day of sowing significant increase (26 and 36%) in activity was observed at both lower as well as at higher dose of irradiation, but at dose $5x10^7$ p/cm^2 of radiation activity decreased 14% compared to control (Fig 3). At 60th day of sowing APOX activity slightly decreased (7-16%) at all fluence of $^7Li^{+++}$ irradiation compared with control. Interestingly, no significant change in APOX activity was observed at 90th day, expect 16% decrease in activity observed at higher dose (10^8 p/cm^2) of irradiation.

Biomass accumulation

A general increase in biomass accumulation was observed with advancing of age and also with the increasing dose of irradiation compared to control (Fig.4). However, the increase was only 1-4% observed at 30 and 60th day of sowing. At 90th day, 3-6% increase in biomass was observed at two lower doses but 8% decrease was observed at higher dose (10^8 p/cm^2) of irradiation.

Discussion:

Heavy ion irradiation stress may be sensed by plant and transduced into resulting in alteration in activity of different classes of enzymes. Moreover, the coordinated induction of antioxidant enzymes in response to environmental stress has been also suggested in plants. However, due to heavy ion exposure there was a distinct response on the enzymes activity. The understanding of the pathways could be increased by performing more experiments for evaluating all defence system enzymes in field grown plants as well as in laboratory condition from heavy ion irradiated seeds.

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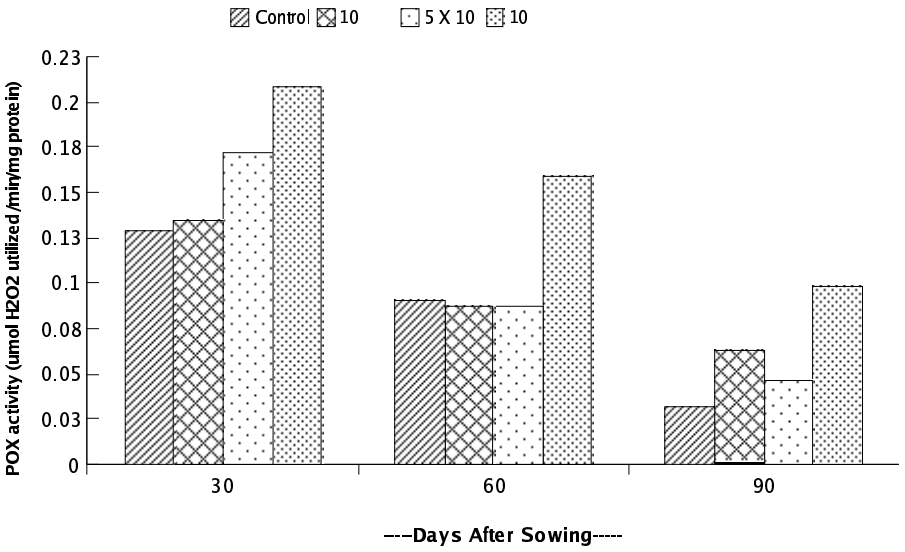

Fig. 1 : Changes in Peroxidase (POX) activity in response to $^{7}Li^{+++}$ irradiation in leaves of Indian mustard at different stages in M1 generation.

Data is the mean value (n=3) with + S.D. Asteristics indicate the significance of difference at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$(***) compared with in treatments by student's t-test.

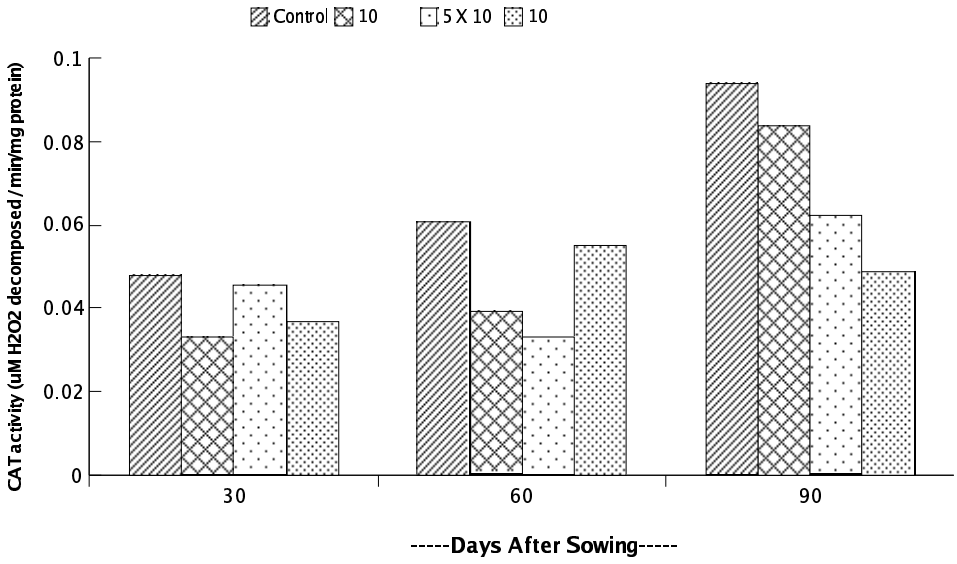

Fig. 2 : Changes in Catalase (CAT) activity in response to $^{7}Li^{+++}$ irradiation in leaves of Indian mustard,at different stages in M1 generation.

Rest legend is same as in Fig. 1.

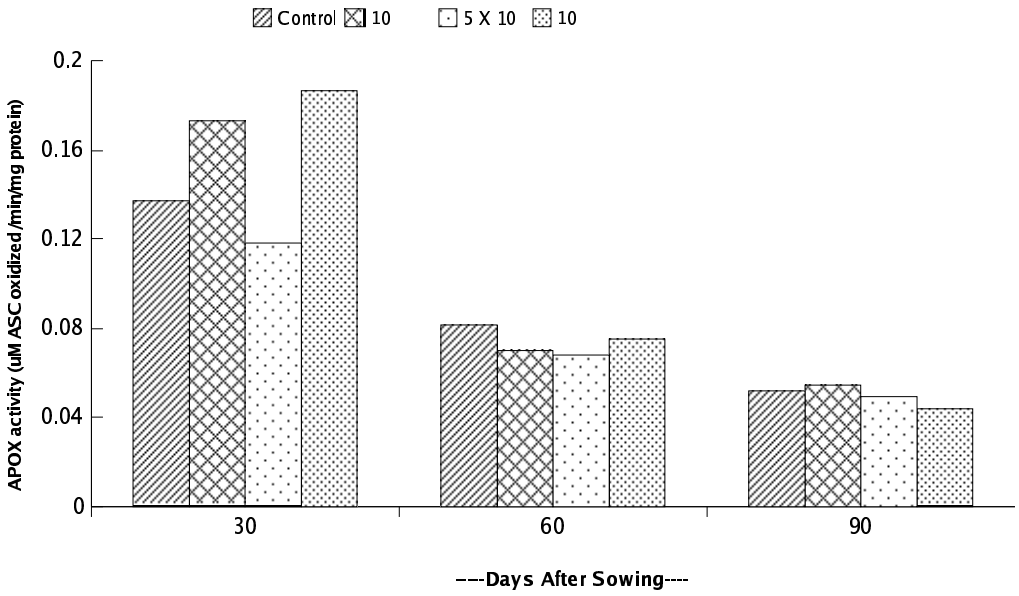

Fig. 3 : Changes in Ascorbate peroxidase (APOX) activity in response to $^{7}Li^{+++}$ irradiation in leaves of Indian mustard at different stages in M1 generation.

Rest legend is same as in Fig.1.

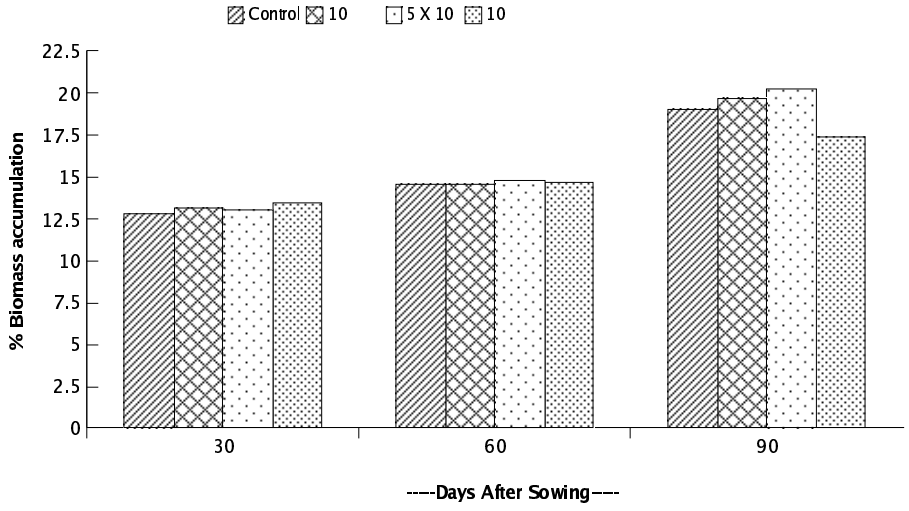

Fig. 4 : Changes in % Biomass accumulation in response to $^{7}Li^{+++}$ irradiation in leaves of Indian mustard at different stages in M1 generation.

Rest legend is same as in Fig. 1.