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# Antioxidant and radio-protection of living cells

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**Abstract** - Dry and water soaked germinated seeds of *Lens culinaris* were treated with different doses of gamma rays. Chromotoxic effects and enzyme related defence mechanism have been studied. Severity of chromotoxic effect i.e. lethal effect on cell division and chromosomal abnormalities increased with dose. Different enzymes (peroxidase and superoxide dismutase) which play great role in defence mechanism of cell, was found to be differentially sensitive to stress condition.

**Key words**: Antioxidant; chromosome; gamma ray; *Lens culinaris*; radiosensitivity.

## INTRODUCTION

Reactive oxygen species (ROS) are produced in cells under both unstressed and stressed conditions. Formation and removal of ROS remain in a balanced state under unstressed condition. For protection of cells from oxidative damage plants have well developed defence system (both enzymatic and non-enzymatic) against these ROS (NOCTOR and FOYER 1998). Whenever a plant experiences a stressed condition (biotic/abiotic) production of ROS increases many fold. Activity of the antioxidant enzymes also increases several times to scavenge the excess ROS. Oxidative stress arises from an imbalance in generation and metabolism of reactive oxygen species i.e. with more ROS being produced than are metabolized. This ROS can react with various cellular targets leading to different physiological disturbances including protein and DNA damage (HALLIWELL and GUTTERIDGE 1989).

Superoxide dismutase (SOD) antioxidant enzyme constitutes the first level of defence against ROS within plant cell. In order to scavenge the ROS, SOD produces hydrogen peroxide as reaction product. This hydrogen peroxide in turn is neutralized into $H_2O$ and $O_2$ by the activity of another antioxidant enzyme peroxidase.

The aspects antioxidants and reactive oxygen species in plants have been discussed in details at a symposium held at University of Kent, Canterbury, U.K., April 2-3, 2001 and voluminous literature have been published in a special issue of Journal of Experimental Botany, volume 53, No. 372, May 2002. However, information on relation between chromosomal disbalance and defence mechanism in a cell under gamma radiation stressed condition is scanty. Present paper reports sensitivity of living cell to stress condition (ionizing radiation - gamma radiation) in terms of changes in chromosomal behaviour, SOD and peroxidase activities.

## MATERIALS AND METHODS

Seeds of *Lens culinaris* (Fam. Leguminosae) were selected as material for the present studies. Experiments were conducted in two ways:

Dry seeds (material A) were treated with 5, 10 and 20 Krad gamma rays (Cobalt 60, dose rate 1 min 10 sec./ Krad) and then allowed to germinate after soak-

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Table 1 – Effects of gamma radiation on root tip mitosis in *Lens culinaris* (Material A)

| Treatment | Total cell examined | Dividing cell | Mitotic Index(%) | RDR (%) | Pro-phase (%) | Meta-phase (%) | Ana-phase (%) | Telo-phase (%) | Chromosomal abnormalities (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Bridge | Exclusion | Early Sepa. | Micronuclei | Cond. Chr. | Frag. Chr. | Total Aberration |
| Control | 1424 | 162 | 11.27 | - | 2.24 | 4.56 | 3.02 | 1.40 | 0.00 | - | - | - | - | - | 0.00 |
| 5 Krad | 1273 | 93 | 7.30 | - 4.47 | 1.82 | 1.88 | 3.29 | 0.31 | 0.32 | 0.15 | - | - | 0.23 | - | 0.70±0.23 |
| 10 Krad | 1625 | 102 | 6.30 | - 5.60 | 0.67 | 2.70 | 2.52 | 0.43 | 0.31 | 0.06 | 0.18 | 0.12 | 0.18 | - | 0.85±0.22 |
| 20 Krad | 2004 | 96 | 4.79 | - 7.30 | 1.67 | 0.59 | 1.04 | 1.49 | 0.15 | 0.15 | 0.30 | 0.35 | - | 0.15 | 1.10±0.23 |

ing in water. Root tips from germinating seedlings were collected for cytological analysis and also for peroxidase and superoxide dismutase (SOD) activity assay.

Water soaked germinating seeds (material B) were treated with 5, 10 and 20 Krad gamma rays and root tips were collected for cytological and antioxidant enzymes studies.

For cytological observations roots were fixed in propionic acid: absolute alcohol (1:2 v/v) for 3 hr. Root tip squash preparations were made after staining in Feulgen following usual hydrolysis method. All the slides were scored blindly. Water soaked germinating (untreated) seeds served as control.

Peroxidase activity was determined spectrophotometrically (SADASIVAM and MANICKAM 1991) by measuring the times in min required for increase in absorbance of oxidised guaiacol from 0.05 to 0.1 at 436 nm using 0.05 ml guaiacol soln. (20 mM) in 3 ml phosphate buffer (pH = 7, 0.1M), 0.03 ml $H_2O_2$ (12.3 mM) and 0.1 ml enzyme extract. Peroxidase activity was calculated as units per litre.

The enzyme activity (units) per litre = $3.18 \times 0.1 \times 1000 / 6.39 \times 1 \times \Delta t \times 0.1$

Superoxide dismutase (SOD) activity was determined by nitro blue tetrazolium (NBT) photochemical assay according to BEYER and FRIDOVICH (1987). In this method the inhibition of reduction of NBT by enzyme extract is measured spectrophotometrically at 560 nm. SOD activity was expressed in units per gram fresh weight. One unit of SOD was equal to that amount which causes a 50% decrease of the SOD-inhibitable NBT reduction.

Soluble protein content was quantified according to BRADFORD method (1976), using Bovine Serum Albumin as standard. Protein content has been expressed in mg per 100g fresh weight (mg $100g^{-1}$ FW). All the experiments were conducted in three replications and data have been presented as mean ± S.E. (standard error).

## RESULTS

Mitotic division was normal in control roots. The percentage of dividing cells (prophase, metaphase, anaphase and telophase) and total dividing cells reduced after gamma irradiation and with increase in dose. Mitotic index has been represented in the form of relative division rate (RDR) (HODA *et al.* 1991). The RDR values at each dose were negative indicating inhibition of mitotic division in both the materials (A and B). Increase in negative value of RDR was directly proportional to the severity of the mitotic inhibi-

Table 2. Effects of gamma radiation on root tip mitosis in *Lens culinaris* (Material B)

| Treatment | Total cell examined | Dividing cell | Mitotic Index(%) | RDR (%) | Pro-phase (%) | Meta-phase (%) | Ana-phase (%) | Telo-phase (%) | Chromosomal abnormalities (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Bridge | Giant cell | Sticky chr. | Clump -ing | Abn. meta | Frag. Chr. | Abn. Ana. | Total abn. |
| Control | 1424 | 162 | 11.27 | - | 2.24 | 4.56 | 3.02 | 1.40 | 0.00 | - | - | - | - | - | - | 0.00 |
| 5 Krad | 2313 | 78 | 3.37 | - 8.90 | 0.22 | 2.42 | 0.69 | 0.04 | 0.60 | - | 0.51 | 1.21 | - | 0.34 | - | 2.66±0.33 |
| 10 Krad | 2255 | 58 | 2.57 | - 9.80 | 0.35 | 1.46 | 0.41 | 0.35 | 0.67 | 2.39 | 1.11 | 0.13 | 0.18 | - | - | 4.52±0.43 |
| 20 Krad | 2146 | 24 | 1.12 | - 11.44 | - | 0.51 | 0.42 | 0.23 | 0.23 | 1.68 | 0.14 | 0.19 | 0.09 | - | 0.14 | 2.47±0.35 |

tion. Maximum mitotoxic effect was observed in material B.

A wide range of chromosomal abnormalities were detected in all the gamma ray treated seeds. Bridges, exclusion, early separation, micronuclei, condensed chromosomes and fragmented chromosomes were the chromosome abnormalities recorded in material A (Table 1). Material B showed chromosome abnormalities like cytomixis/bridges, giant cells, stickiness, clumping, abnormal metaphase, abnormal anaphase and fragmented chromosomes (Table 2, Fig. 1A-F). However, the percentage of total aberrations were higher in material B (Tables 1 and 2).

Peroxidase enzyme activity was measured in control, materials A and B. It was interesting to note that enzyme activity in roots of material A reduced and the reduction increased with the increase in gamma ray dose (Fig. 2A). Enzyme activity in roots of material B increased in lower dose (5 Krad) and then decreased with increase in dose (Fig. 2B), although activity in 20 Krad was much higher over the control.

SOD activity in roots of material A increased over the control at 5 Krad there after it decreased sharply with increase in dose (Fig. 2C). SOD activity in roots of material B slightly increased in lower dose (5 Krad) but increased abruptly in 10 Krad and then again decreased at 20 Krad which was almost 4 times more over the control (Fig. 2D).

Soluble protein content was measured in cotyledons of control seeds and material A. The protein content in control was 2185±17 mg 100 $g^{-1}$ FW. There was slight increase in protein content at 5 (2290±56 mg 100 $g^{-1}$ FW) and 10 Krad (2320±21 mg 100 $g^{-1}$ FW) and then decrease at 20 Krad (1980±25 mg 100 $g^{-1}$ FW).

Effect of gamma rays was not much prominent on protein content in roots of materials A and B . There was slight increase / decrease after irradiation over control. Protein content in control root was 351.60±2.56 mg 100 $g^{-1}$ FW, where as in roots of materials A and B after treatment with 5, 10 and 20 Krad gamma rays were 379.25±5.63, 367.20±5.89, 349.20±7.36 and 295.20±4.28, 314.40±3.83, 304.80±3.93 mg 100 $g^{-1}$ FW respectively.

## DISCUSSION

Gamma ray stressed condition was found to have lethal effect on cell division and induced

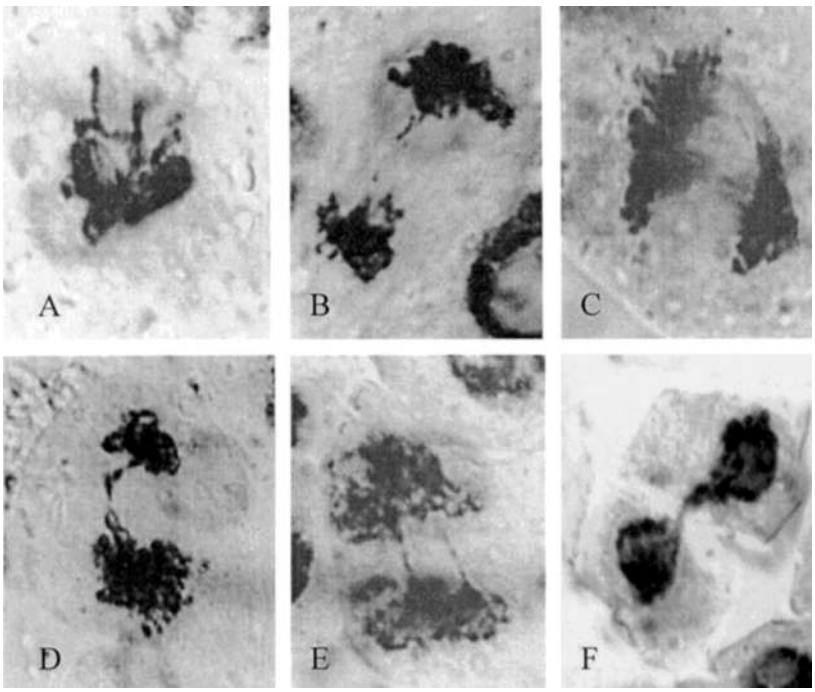


Fig. 1 – Chromosomal aberrations induced by gamma irradiation. (A) clumping; (B) late separation of chromosome; (C) abnormal anaphase; (D) and (E) single and double bridges; (F) cytomixis.

chromosomal abnormalities during root tip mitosis in both the materials A and B. Severity of chromotoxic effects increased with increased stress due to higher doses. Present correlation studies of chromosomal behavior and enzyme related defence mechanism in plants is perhaps the first report.

It was interesting to note that both the enzyme activity was increased several fold over the control in germinated seeds when treated with gamma rays (material B). After gamma treatment the level of free radical formation was high in germinating seeds in comparison to dry seeds. These free radicals interacted with each other to produce organic peroxide which were highly reactive and believed to be responsible for a good portion of biological and genetic effects of radiation (SINGH 1996).

Dry seeds were metabolically inactive and thus antioxidant enzymes failed to protect cells from free radical damage. As the half life of free radicals is few micro second, e.g. 2-4 μs for $^1O_2$ (singlet oxygen) in water (FOYER and HARBINSON 1994) they exerted their effect on chromosomes immediately which was evident after beginning of cell division.

When gamma treatments were applied in germinated seeds, antioxidant enzymes remained in active stage. To scavenge the excess free radicals they exhibited greater activity in comparison to dry seeds with increase in dose, but up to a threshold level as evident in 5 Krad (in case of peroxidase) and 10 Krad (as in case of SOD).

The aspect sensitivity/radiosensitivity of cells have already been critically reviewed (DATTA 1984). Different plants are differentially sensitive to physical and chemical stress conditions and such varietal differences in sensitivity indicate that some genotype dependent mechanism is involved in the damage or repair/protection of stress induced damage within the organism (DATTA 1992). Treatment of seeds with physical and chemical agents can induce cytological, morphological, genetical and physiological changes in cell and tissues. These in turn can result in modifications in the growth and development of roots, stems, leaves and flowers. DATTA (1988) reported that any change in the chromosomal level disturb the pathway of some chemical reactions or stress agents directly hit the biochemical pathways which in turn lead to abnormal plant growth. He also reported that different pathways

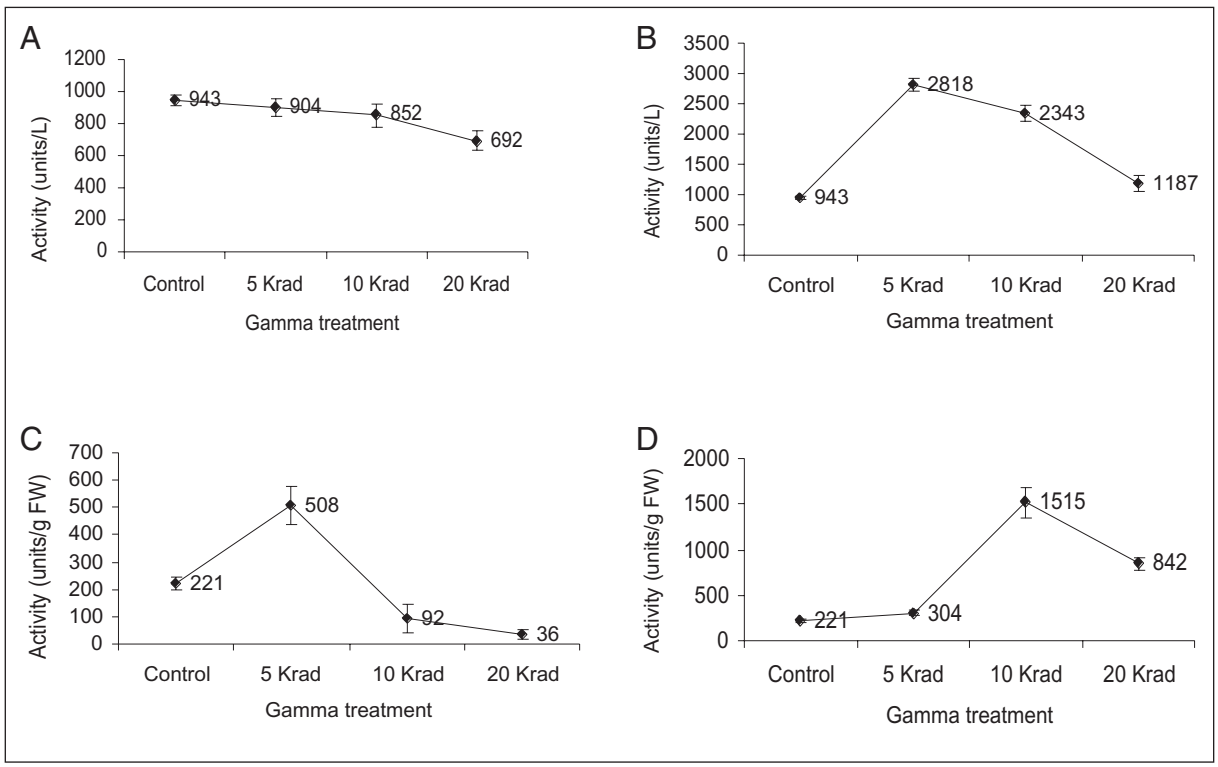


Fig. 2 – Changes in activity of peroxidase and SOD with gamma treatment. Vertical bars represent standard errors. (A) peroxidase activity in material A; (B) peroxidase activity in material B; (C) SOD activity in material A; (D) SOD activity in material B.

are differentially sensitive and the degree of sensitivity varies from material to material. It is apparent from the present experiment that different enzyme mediated defence systems against ROS are differentially sensitive to stress condition. They are either over activated due to stress or any change at chromosomal level may stimulate their activities.

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