Influence of Ionizing Radiation on Antioxidant Enzymes in Three Species of Trigonella

Muna M. Al-Rumaih and May M. Al-Rumaih
Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia

Abstract: The response of three species of Trigonella, namely, Trigonella stellata, Trigonella hamosa and Trigonella anguina to gamma irradiation stress was investigated with respect to antioxidant enzyme induction. When dry seeds were subjected to gamma rays (0, 40, 60, 80, 100 Krad) from a cobalt source $^{60}$Co at a dose rate of 233.5 rad/ min, a dose dependent increase in the activities of ascorbate peroxidase (APOX), superoxide dismutase (SOD) and glutathione reductase (GR) was observed in both shoots and roots of the studied species. On the contrary, catalase activity was repressed, particularly at the higher applied doses. Shoots were more significantly affected by irradiation than roots. The three species differed in their radio-sensitivity with respect to the characters concerned. Changes in the activity of the key antioxidant enzymes which confer tolerance to irradiation stress were discussed.

Keywords: antioxidant enzymes, ascorbate peroxidase, superoxide dismutase, glutathione reductase, catalase, irradiation, gamma, stress

INTRODUCTION

Plants often face the challenge of several environmental conditions which include such stressors as drought, salinity, pesticides, low temperature and irradiation, all of which exert adverse effects on plant growth and development [1]. Gamma irradiation was reported to induce oxidative stress with overproduction of reactive oxygen species (ROS) such as superoxide radicals (O$_2^-$), hydroxyl radicals (OH) and hydrogen peroxides (H$_2$O$_2$) [2], which react rapidly with almost all structural and functional organic molecules, including proteins, lipids and nucleic acids causing disturbance of cellular metabolism [3]. To avoid oxidative damage, plants have evolved various protective mechanisms to counteract the effects of reactive oxygen species in cellular compartments [4]. One of the protective mechanisms was the enzymatic system, which operate with the sequential and simultaneous actions of a number of enzymes including SOD, APOX and CAT [5]. SOD which occur in various cell compartments, dismute O$_2^-$ to H$_2$O$_2$ and oxygen [6]. Catalases were synthesized in a tissue specific and age dependent manner, scavenge H$_2$O$_2$ generated during photo-respiration and ô-oxidation of fatty acids [7]. Peroxidases located in the cytosole, vacuole, cell walls as well as in extra-cellular spaces use guaiacol as electron donors and utilize H$_2$O$_2$ in the oxidation of various inorganic and organic substrates [8]. The role of GR in H$_2$O$_2$ scavenging mechanism in plant cells was well established in Haliwell –Asada enzyme pathways [4].

There was a compelling evidence which show that the activities of enzymes involved in active oxygen species (AOS) scavenging were altered by several environmental stresses, including gamma irradiation. The expression patterns of GST, SOD, POX and CAT genes exhibited increased transcripts upon γ-irradiation of Nicotiana tabacum [9]. The activity and isozyme patterns of POX in Nicotiana debneyi and Nicotiana tabacum, SOD in Nicotiana debneyi, and CAT in Nicotiana tabacum increased in response to γ-irradiation treatment [10]. Chaomei and Yanlin [11] reported an increase in the activity of POX and CAT with a corresponding decline in growth of Triticum aestivum plants under higher irradiation doses (20, 40, 60, 80 Kr). Singh [12] reported induction of APOX activity in two sugar cane varieties grown under gamma irradiation. The activities of POX, CAT and SOD in radish (Raphanus sativus) leaves were enhanced by γ-irradiation (10 Gy) treatment [13]. SOD activity showed an increase in the irradiation groups (2, 4, 8, 6 Gy) of red pepper, (Capsicum annum) yeomyang variety and a decrease in joheung variety [14]. Irradiation was reported to enhance POX activity of two Phaseolus
**Materials and Methods**

Seeds of *Trigonella stellata*, *Trigonella hamosa* and *Trigonella anguina* were irradiated with different doses of gamma rays (0, 40, 60, 80, 100 Krad) from a cobalt $^{60}$Co source at a dose rate of 233.5 rad/min. Irradiated and unirradiated seeds were surface sterilized with 10% sodium hypochlorite for 10 minutes and then thoroughly rinsed in distilled water. Seeds were germinated outdoors in plastic pots filled with vermiculite and irrigated with Hoagland nutrient solution for three weeks. At the end of the experimental period, seedlings were harvested and separated into roots and shoots for analysis. Three samples of ten replicates of fresh tissue were used to determine the activities of APOX, SOD, GR and CAT.

Enzyme Extraction and assay: Enzyme extraction was carried out following the method of Costa [18]. One gram of plant tissue was homogenized with extraction buffer containing 50 mM phosphate buffer (PH 7.4), 1 mM EDTA, 1 g PVP and 0.5% (v/v) Triton X 100.

APOX activity was estimated according to the method of Nakama and Asada [19]. Enzyme activity was determined by the decrease in absorbance of ascorbate at 290 nm. Enzyme activity was expressed in enzyme units mg$^{-1}$ protein. One unit of enzyme was the amount necessary to decompose 1 µmol of substrate per minute at 25°C.

SOD activity was assayed by its ability to inhibit the photochemical reaction of nitro blue tetrazolium [20]. Fifty percent reduction in color was considered as one unit of enzyme activity. The activity of SOD was expressed in unit mg$^{-1}$ protein.

GR activity was determined as described by Fryer [21] by monitoring the glutathione-dependent antioxidation of NADPH at 340 nm. The activity was expressed in enzyme units mg$^{-1}$ protein. One unit of enzyme was the amount necessary to decompose 1 µmol NADPH per minute at 25°C.

CAT activity was determined by monitoring the disappearance of H$_2$O$_2$ at 240 nm. CAT activity was expressed as units mg$^{-1}$ protein. One unit of enzyme was the amount necessary to decompose 1 µmol of H$_2$O$_2$ per min at 25°C [22].

**Statistical analysis:** Three samples of 10 replicates were subjected to analysis of variance for split plot design using the statistical program Minitab. Means were obtained and the LSD at $P <0.01$ and $P <0.05$ was calculated to compare the significance of the difference between any two groups [23].

**Results and Discussion**

The results presented in Tables 1, 2 and 3 revealed a variable degree of stimulation in the activities of APOX, SOD and GR in shoots and roots of 21 day old seedlings of *T. stella*, *T. hamosa* and *T. anguina* developed from seeds irradiated with different doses of gamma rays. Enzyme induction was significantly and positively correlated with the dose of irradiation. A significant ($P <0.01$) increase in APOX, SOD and GR activities was observed in shoots of *T. stellata* at all exposures of gamma. Induction of APOX and GR activities in *T. stellata* roots was significant ($P <0.05$) at 40 Krad and high significant ($P <0.01$) at 60, 80 and 100 Krad (Table 1). Gamma irradiation significantly ($P <0.01$) retarded the activity of CAT in *T. stellata* shoots at all investigated gamma ray doses. CAT activity of *T. stellata* roots revealed a high significant ($P <0.01$) decrease at 60, 80 and 100 Krad doses (Table 1).

The data presented in Table 2 revealed that *T. hamosa* was less significantly affected by gamma irradiation than *T. stellata*. The activities of APOX, SOD and GR in shoots and roots of *T. hamosa* increased in a similar manner as *T. stellata* except for the lowest dose which showed a non-significant increase by irradiation treatment. CAT activity in shoots of *T. hamosa* decreased by 7%, 14%, 34% and 48% at 40, 60, 80 and 100 Krad, respectively as compared with un-irradiated control. CAT activity in roots were less significantly affected by irradiation than shoots.
Table 1: The effect of different doses of gamma radiation on APOX, SOD, GR and CAT activities in shoots and roots of *Trigonella stellata*

| Organ | Treatment | Gamma Krad | APOX | SOD | GR | CAT |
|-------|-----------|-------------|------|-----|----|-----|
| Shoots | 0 | 47.14 ± 0.59 | 109.27 ± 1.68 | 7.84 ± 0.48 | 19.07 ± 0.83 |
| | 40 | 58.93 ± 0.37** | 141.46 ± 1.89** | 9.07 ± 0.58** | 16.09 ± 0.82** |
| | 60 | 81.40 ± 0.49** | 153.73 ± 1.42** | 9.49 ± 0.40** | 12.88 ± 0.78** |
| | 80 | 93.22 ± 0.45** | 174.25 ± 1.85** | 10.80 ± 0.46** | 9.03 ± 0.79** |
| | 100 | 117.22 ± 0.43** | 248.30 ± 1.63** | 14.49 ± 0.51** | 6.21 ± 0.82** |
| LSD at 5% | 0.84 | 3.09 | 0.89 | 1.46 |
| LSD at 10% | 1.20 | 4.39 | 1.26 | 2.08 |

** and * denote significant differences between gamma irradiated plants and controls at 0.01 and 0.05% levels, respectively.

Table 2: The effect of different doses of gamma radiation on APOX, SOD, GR and CAT activities in shoots and roots of *Trigonella hamosa*

| Organ | Treatment | Gamma Krad | APOX | SOD | GR | CAT |
|-------|-----------|-------------|------|-----|----|-----|
| Shoots | 0 | 52.16 ± 1.32 | 138.81 ± 11.16 | 9.49 ± 0.90 | 26.90 ± 1.05 |
| | 40 | 53.75 ± 1.39 | 157.93 ± 11.72 | 10.71 ± 0.79 | 25.04 ± 1.04 |
| | 60 | 65.31 ± 1.56** | 208.17 ± 10.97** | 11.95 ± 0.77** | 17.69 ± 1.58** |
| | 80 | 85.62 ± 1.33** | 240.01 ± 11.38** | 13.59 ± 0.76** | 17.89 ± 1.07** |
| | 100 | 92.62 ± 1.46** | 263.96 ± 11.22** | 16.02 ± 0.73** | 13.91 ± 1.21** |
| LSD at 5% | 2.57 | 20.57 | 1.44 | 2.36 |
| LSD at 10% | 3.65 | 29.26 | 2.05 | 3.35 |

** and * denote significant differences between gamma irradiated plants and controls at 0.01 and 0.05% levels, respectively.

Table 3: The effect of different doses of gamma radiation on APOX, SOD, GR and CAT activities in shoots and roots of *Trigonella anguina*

| Organ | Treatment | Gamma Krad | APOX | SOD | GR | CAT |
|-------|-----------|-------------|------|-----|----|-----|
| Shoots | 0 | 60.58 ± 8.18 | 171.39 ± 10.21 | 12.30 ± 1.41 | 37.06 ± 3.50 |
| | 40 | 71.44 ± 9.59 | 180.13 ± 8.80 | 13.60 ± 1.46 | 35.27 ± 3.81 |
| | 60 | 75.27 ± 9.83 | 188.51 ± 8.44* | 14.71 ± 1.53 | 34.23 ± 3.51 |
| | 80 | 85.91 ± 9.08** | 205.58 ± 8.32** | 16.38 ± 1.49** | 27.88 ± 3.38** |
| | 100 | 91.48 ± 8.26** | 241.35 ± 9.25** | 18.12 ± 1.44** | 26.45 ± 3.41** |
| LSD at 5% | 16.38 | 16.41 | 2.67 | 6.40 |
| LSD at 10% | 13.51 | 24.86 | 1.68 | 2.71 |

** and * denote significant differences between gamma irradiated plants and controls at 0.01 and 0.05% levels, respectively.
Table 4: The significant levels of analysis of variance (ANOVA) for APOX, SOD, GR and CAT activities of three species of Trigonella (T. stellata, T. hamosa and T. anguina) irradiated with gamma rays

| Source of Variance | APOX | SOD | GR | CAT |
|-------------------|------|-----|----|-----|
| Dose (D)          | **   |     | ** |     |
| Species (S)       |     | ** |    |     |
| Organ (O)         | **  |     | ** | **  |
| D x S             |     | ** |    |     |
| D x O             | **  |     |    | **  |
| S x O             | **  | NS |    | **  |
| D x S x O         | NS  | NS | NS | NS  |

NS Non significant; ** Significant P <0.01

The results presented in Table 3 revealed an increase in APOX, SOD and GR activities and a decrease in CAT activity in shoots and roots of T. anguina by irradiation treatment. The changes in these measures were less significant T. anguina in comparison with T. stellata and T. hamosa respectively.

Statistical analysis of the data (Table 4) revealed a high significant variation (P < 0.01) by dose, species and organs for APOX, SOD, GR and CAT. The interaction between dose x species was high significant (P <0.01) for APOX and SOD. The interaction of dose x organ was high significant (P < 0.01) for all investigated enzymes (APOX, SOD, GR and CAT). The interaction effect of species x organ was high significant (P <0.01) for APOX and CAT and the interaction between dose x species x organ was high significant for APOX and SOD.

Several reports with other plants provided evidence of enhanced activities of APOX, SOD and GR by gamma irradiation treatment. Gamma irradiation was shown to induce oxidative stress with overproduction of reactive oxygen species. Generation of ROS, particularly H$_2$O$_2$ had been proposed to be part of the signaling cascades that lead to protection from stresses. Environmental stresses were shown to upset the balance between the production of ROS and quenching activity of antioxidants. Induction of antioxidant enzyme activities was reported to be a general strategy adopted by plants to overcome oxidative stresses.

Involvement of APOX, CAT, SOD and GR enzymes in maintaining the overall defense mechanisms against the effect of irradiation was reported. Blokhina attributed induction of POD, SOD and GR activities to enhanced production of toxic ROS levels in living organisms under stress. CAT, in cooperation with APOX and other enzymes were shown to destroy the H$_2$O$_2$ produced by SOD and other reactions. Allen associated the oxidative bursts as well as dramatic changes in the activities of various antioxidant defenses with the alteration in gene expression in a variety of tissues from phylogenetically diverse organisms. Cho confirmed this finding indicating increased transcript levels of the genes controlling the biosynthesis of GST, SOD, POX and CAT enzymes upon irradiation of Nicotiana tabacum seeds. This over expression probably occur by an efficient regulatory mechanism, adjusting when necessary enzyme expression by positive regulation of the corresponding genes to provide cells with resistance.

The results presented in Tables 1, 2 and 3 revealed induction of APOX activities concomitant with suppression of CAT activities in shoots and roots of the three studied species when subjected to different exposures of gamma rays. A similar induction of APOX was reported in two sugar cane varieties grown under γ-irradiation and in tobacco exposed to UV-B. Inhibition of CAT activity was also reported under irradiation stress. The present increase in APOX activity was reported to compensate for the progressive drop in catalase activity. Peroxidase was considered to be the key enzyme for the decomposition of H$_2$O$_2$, especially under CAT inactivation. Pasternak attributed peroxidase activation to membrane injury and the resulting shift in cellular Ca$^{2+}$ levels. According to Karpinski, APOX activation in Arabidopsis subjected to oxidative stress occurred through induction of APOX 1 and APOX 2 gene transcription. Zaka further reported enhanced expression of APOX gene in cells undergoing low chronic γ-irradiation stress. Several studies attributed enzyme induction either to up-regulation of encoding genes or to activation of existing enzyme pools by a modulatory effect of enzyme structure.

The present results further revealed induction of SOD in shoots and roots of the three investigated species at different exposures of gamma (Tables 1, 2 and 3). Similar reports were given by Zaka who reported that when Stepa capillata plants were exposed to high gamma irradiances, SOD activity increased (67%). High SOD activity had been associated with stress tolerance in plants where overproduction of
(O$_2^-$) was involved \cite{4}. Inze and Van Montagu \cite{35} attributed SOD stimulation to positive regulation of SOD genes in response to low external chronic irradiation. Other reports related the enhanced SOD and POD activities to induction of specific isozymes \cite{10}.

The results presented in Tables 1, 2 and 3 revealed that GR activity in shoots and roots of the three studied species significantly increased above the control values when exposed to different doses of gamma rays. Increased activity of this enzyme has been reported earlier in cotton when subjected to elevated atmospheric O$_2$ \cite{36}, in Mg$^{++}$ deficient bean leaves \cite{26} and in peas fumigated with ozone \cite{37}. Higher GR activity of salt stressed cotton was reported to be due to an increase in glutathione turnover rate \cite{38}. Foyer\cite{25} reported an increase in GR activity in higher plants as a result of enhancement of the transcription rate of encoding genes.

The results of the present investigation further revealed that the three studied species responded differently to gamma irradiation stress. The data presented in Tables 1, 2 and 3 revealed that T. stellata was more radio-sensitive than T. hamosa and T. anguina as it showed higher stimulation in APOX, SOD and GR activities under irradiation stress. The results were consistent with the findings of Xu-Meifen and Xu-Weijie \cite{39} who reported radio-sensitivity differences between wheat cultivars. Several studies related radio-resistance to the ability of living systems to eliminate the reactive substances or to suppress their formation \cite{10}. Other reports related the differences in stress tolerance among plant species to the varied development of antioxidant defense systems under stress conditions \cite{40}.

**CONCLUSION**

Gamma irradiation affected antioxidant enzyme activities in the three investigated species of wheat. It activated the antioxidant defense system against oxidative stress in order to increase their capacity in scavenging AOS. Significant cultivar differences were observed. Accordingly, antioxidant enzyme activities could be used as an index of radio-sensitivity.

**REFERENCES**

1. Foyer, C.H., P. Decourvieres and K.J. Kunerk, 1994. Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. Plant Cell Environ., 17: 507-523.

2. Xienia, U., G.C. Foote, S. Van, P.N. Devreotes, S. Alexander and H. Alexander, 2000. Differential developmental expression and cell type specificity of dictystelium catalases and their response to oxidative stress and UV light. Biochem. Biophys. Acta, 149: 295-310.

3. Salter, L. and C.N. Hewitt, 1992. Ozone-hydrocarbon interactions in plants. Phytochem., 31: 4045-4050.

4. Bowler, C. M. Van Montagu and D. Inzi, 1992 Superoxide dismutase and stress tolerance. Ann. Rev. Plant Physiol. Plant Mol. Biol., 43: 83-116.

5. Larson, R.A., 1988. The antioxidants of higher plants. Phyto Chem., 27: 969-978.

6. Lin, C.C. and C.H. Kao, 2000. Effect of NaCl stress on H$_2$O$_2$ metabolism in rice leaves. Plant Growth Regul., 30: 151-155.

7. Singh, R.K., P. Chandra, J. Singh and D.N. Singh, 1993 Effect of gamma-ray on Physio-biochemical parameters of sugar cane. J. Nucl. Agric. Biol., 22(2): 65-69.

8. Lee, H.Y., J.S. Kim, M.H. Baek, J.C. Yoo and S.T. Kwon, 2003. Effects of low dose γ-irradiation on physiological activities of radish (Raphanus sativus) during early growth and reduction of UV-B stress. J. Korean Society Horticul. Sci., 44(3): 314-320.

9. Kim, J.H., M.H. Baek, B.Y. Chung, S.G. Wi and J.S. Kim, 2004. Alteration in the photosynthetic pigments and antioxidant machineries of red pepper (Capsicum annuum L.) seedlings from gamma-irradiated seeds. J. Plant Biol., 47(4): 314-321.
15. Stoeva, N., Z. Zlatev and T. Bineva, 2001. Physiological response of bean (*Phaseolus vulgaris* L.) to gamma irradiation treatment. II. Water exchange, respiration and peroxidase activity. J. Environ. Protect. Ecol., 2(2): 304-308.

16. Sheila Collette, 1985. An Illustrated Guide to the Flowers of Saudi Arabia, Flora Publication No. 1. London, Scorpion Publishing Ltd.

17. Reasat, M., J. Karapetyan and A. Nazirzadeh, 2003. Karyotypic analysis of Trigonella genus of Fars Province. Iran. J. Rangeland and Forests Plant Breed. Genet. Res., 11(1): 127-145.

18. Costa, H., S.M. Gallego and M. L. Tomaro, 2002. Effect of UV-B radiation on antioxidant defense system in sunflower cotyledons. Plant Sci. 169: 939-945.

19. Nakama, Y. and K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. Plant Cell Physiol., 22: 867-880.

20. Becana, M., P. Aparicio-Tejo, J.J. Irigoyeu and M. Sanchez-Diaz, 1986. Some enzymes of hydrogen peroxide metabolism in leaves and root nodules of *Medicago sativa*. Plant Physiol., 82: 1169-1171.

21. Fryer, M.J., J.R. Andrews, K. Oxborough, D.A. Clower and N.R. Baker, 1998. Relationship between CO$_2$ assimilation, photosynthetic electron transport and active O$_2$ metabolism in leaves of maize in the field during periods of low temperature. Plant Physiol., 116: 571-580.

22. Chance B., H. Sies and A. Boveris, 1979. Hydroperoxide metabolism in mammalian organs. Physiol. Acv., 59: 527-605.

23. Steel, R.G.D. and J.H. Torrie, 1980 Principles and Procedures of Statistics, McGraw-Hill Book Co., New York.

24. Zaka, R., C. Chanal and M.T. Misset, 2002. Study of external low irradiation dose effects on induction of chromosome aberrations in *Pisum sativum* root tip meristem. Mutat. Res., 517:87-99.

25. Pasternak, D., 1987. Salt tolerance and crop induction, a comparative approach. Ann. Rev. Phyto. Path., 25: 271-291.

26. Cara, M. Lelandais, C. Galap and K.J. Kunert, 1991. Effects of elevated cytosolic glutathione reductase activity on the cellular glutathione pool and photosynthesis in leaves under normal and stress conditions. Plant Physiol., 97: 863-872.

27. Sharabash, M.T.M., S.S.M.Gaweesh, I.O.A. Orabi and A.H.A. Hammad, 1988. Physiological response of wheat, maize and cotton to gamma irradiation. Proc. 4$^{th}$ conf. Nucl. Sci. and Appl. II (P-3.2.213), 478-482.

28. Sah, N.K., S. Pramanik and S.S. Raychandhuri, 1996. Peroxidase changes in barley induced by ionizing and thermal radiation. Int. J. Radiant. Biol., 69(1): 107-111.

29. Blokhina, O., E. Virolaineu and K. Fagerstedt, 2003. Antioxidant oxidative damage and oxygen deprivation stress. Ann. Bot., 91: 179-194.

30. Allen R.G., 1998. Oxidative stress and superoxide dismutase in development, aging and gene regulation. Age, 21: 47-76.

31. Willekens, J.H., W. Van Camp, M. Van Montagu, D. Inzi, J.H. Sandermann and C. Langebartils, 1994. Ozone, sulfur dioxide, and ultra violet B have the same effects on mRNA accumulation of antioxidant genes in *Nicotiana plumbaginifolia*. Plant Physiol., 106: 1007-1014.

32. Liang, Y.E., Y.G. Hui and Z. Qi, 2000. Response of the antioxidant systems and xanthophyll cycle in *Phaseolus vulgaris* to the combined stress of high irradiation and high temperature. Photosynthetica, 38(2): 205-210.

33. Foster, J.G. and J.L. Hess, 1980. Response of superoxide dismutase and glutathione reductase activities in cotton leaf tissue exposed to an atmosphere enriched in oxygen. Plant Physiol., 66: 482-487.

34. Hurkman, W.J. and C.K. Tanaka, 1987. The effect of salt on the pattern of protein synthesis in barley roots. Plant Physiol., 33: 517-524.

35. Xu-Meifen and Xu-Weijie, 1986. Effect of gamma radiation on sub1 wheat plants and cytological analysis. Nucl. Tech., 10: 49-51.

36. Wu, F., G. Zhang and P. Dominy, 2003. Dour barley genotypes respond differently to cadmium: Lipid peroxidation and activities of antioxidant capacity. Environ. Exper. Bot., 50: 67-78.