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

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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_2O_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_2O_2 and oxygen [6]. Catalases were synthesized in a tissue specific and age dependent manner, scavenge H_2O_2 generated during photo-respiration and ß-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_2O_2 in the oxidation of various inorganic and organic substrates [8]. The role of GR in H_2O_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, (C*apsicum annum*) yeomyang variety and a decrease in joheung variety [14]. Irradiation was reported to enhance POX activity of two *Phaseolus*

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vulgaris cultivars (Plovdiv 10 and Plovdiv 11). Cultivar Plovdiv 10 was more tolerant to irradiation than cultivar Plovdiv 11 which was more radio-sensitive [15].

The species selected for the present study *Trigonella stellata, Trigonella hamosa* and *Trigonella anguina* were annual herbs which belong to the family Leguminosae. Several reports revealed their wide distribution in the Kingdom of Saudi Arabia[16], and others considered them as medicinal plants and forage crops [17].

At present our knowledge concerning the role of the antioxidant systems in protecting plants under gamma irradiation stress were limited and studies on this matter have been made on very few plant species and none were reported so far for Trigonella. In the present study, an attempt had been made to elucidate the irradiation induced changes in the activities of ascorbate peroxidase, superoxidase dismutase, glutathione reductase and catalase, the enzymes involved in oxidative stress defense in three species of Trigonella.

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 µ mol 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_2O_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_2O_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 Krads (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	Units mg^{-1} Protein 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
Roots	0	32.1 ± 1.03	84.33 ± 0.63	5.83 ± 0.44	14.48 ± 0.75
	40	34.29 ± 0.91*	95.87 ± 0.90**	6.73 ± 0.52*	13.38 ± 0.65
	60	39.73 ± 0.94**	101.20 ± 0.60**	7.05 ± 0.55**	10.62 ± 0.75**
	80	43.44 ± 0.97**	121.96 ± 0.79**	8.18 ± 0.47**	8.62 ± 0.73**
	100	52.04 ± 0.75**	146.68 ± 0.71**	9.28 ± 0.42**	6.59 ± 0.76**
LSD at 5%		1.680	1.33	0.88	1.32
LSD at 10%		2.39	1.90	1.25	1.85

** 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	Units mg^{-1} Protein 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**	23.25 ± 1.50**
	80	85.62 ± 1.33**	240.01 ± 11.38**	13.59 ± 0.76**	17.69 ± 1.58**
	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
Roots	0	42.81 ± 5.72	115.23 ± 8.34	7.94 ± 0.76	23.68 ± 1.13
	40	49.08 ± 5.43	132.61 ± 10.87	8.39 ± 0.56	22.87 ± 1.04
	60	52.96 ± 5.91*	139.46 ± 8.64*	9.36 ± 0.59*	20.99 ± 1.09*
	80	58.42 ± 4.59**	167.70 ± 9.55**	10.31 ± 0.65**	17.89 ± 1.07**
	100	63.63 ± 4.67**	173.29 ± 10.42**	11.53 ± 0.67**	14.23 ± 0.91**
LSD at 5%		9.50	17.48	1.18	1.91
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 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	Units mg^{-1} Protein 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%		23.30	23.35	3.79	9.11
Roots	0	30.23 ± 5.10	129.76 ± 10.30	10.31 ± 0.90	22.65 ± 1.58
	40	32.61 ± 4.64	134.50 ± 9.34	10.57 ± 0.69	21.55 ± 1.52
	60	36.71 ± 5.21	140.49 ± 11.78	10.89 ± 0.80	19.72 ± 1.72
	80	39.86 ± 4.92	156.01 ± 9.31*	12.30 ± 0.79*	18.42 ± 1.71*
	100	41.98 ± 5.40*	169.05 ± 11.72**	13.40 ± 0.77**	16.04 ± 1.74**
LSD at 5%		9.20	19.16	1.44	3.01
LSD at 10%		13.09	28.80	2.05	4.28

** 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. aguina*) irradiated with gamma rays

Source of Variance	APOX	SOD	GR	CAT
Dose (D)	**	**	**	**
Species (S)	**	**	**	**
Organ (O)	**	**	**	**
D X S	**	**	NS	NS
D X O	**	**	**	**
S X O	**	NS	NS	**
D X S X O	**	**	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 [12], SOD [24] and GR [25] by gamma irradiation treatment. Gamma irradiation was shown to induce oxidative stress with overproduction of reactive oxygen species [2]. Generation of ROS, particularly H_2O_2 had been proposed to be part of the signaling cascades that lead to protection from stresses[5]. Environmental stresses were shown to upset the balance between the production of ROS and quenching activity of antioxidants [26]. Induction of antioxidant enzyme activities was reported to be a general strategy adopted by plants to overcome oxidative stresses [1].

Involvement of APOX [27], CAT [28], SOD [24] and GR [14] enzymes in maintaining the overall defense mechanisms against the effect of irradiation was reported. Blokhina [29] 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_2O_2 produced by SOD and other reactions [1]. Allen [30] associated the oxidative bursts as well as the 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 [9] 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 [24].

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 [12] and in tobacco exposed to UV-B [31]. Inhibition of CAT activity was also reported under irradiation stress [32]. 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_2O_2, especially under CAT inactivation. Pasternak [33] attributed peroxidase activation to membrane injury and the resulting shift in cellular Ca^{2+} levels. According to Karpinski [34] APOX activation in Arabidopsis subjected to oxidative stress occurred through induction of APOX 1 and APOX 2 gene transcription. Zaka [24] 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 [25] 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 [24] 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 [4]. Inze and Van Montagu [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 [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 [36], in Mg^{++} deficient bean leaves [26] and in peas fumigated with ozone [37]. Higher GR activity of salt stressed cotton was reported to be due to an increase in glutathione turnover rate [38]. Foyer[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 [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 [10]. Other reports related the differences in stress tolerance among plant species to the varied development of antioxidant defense systems under stress conditions [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.

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