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Effects of γ-irradiation on antioxidant activity in soybean seeds

Research Article

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Abstract: There are some reports that low doses of γ-irradiation could induce antioxidant activities in plant material, including soybean. Irradiation, required for the inactivation of some pathogens and induction of mutations, may have adverse effects on sensorial, nutritional and antioxidant qualities. The effects of different γ-irradiation doses (100-200 Gy) on antioxidant properties of soybean seeds was investigated. In this study, we report the results obtained by analysis of antioxidant enzyme activities, reduced glutathione, malonyldialdehyde (MDA) and hydroxyl ($HO^{\cdot}$) radical quantities, soluble protein content, and total antioxidant activity in irradiated soybean seeds. Antioxidant enzyme activities were affected due to high irradiation intensity. Significant changes of total antioxidant activity and MDA and $HO^{\cdot}$ quantities were observed only under the highest irradiation dose, with a 15.7% reduction in total antioxidant activity, MDA quantity increase of 21.6%, and $HO^{\cdot}$ radical quantity increase of 79.3% compared to the non-irradiated control. The total soluble protein content increased slightly.

Keywords: *γ-irradiation • Soybean • Antioxidant • Lipid peroxidation • Proteins*

1. Introduction

Food irradiation uses radiant energy to rid food of harmful microorganisms, insects, fungi and other pests and could therefore reduce the need for some hazardous pesticides, fumigants and preservatives. Irradiation processing by γ-irradiation up to a dose of 1 kGy has been recommended for quarantine treatment of legumes including soybeans [1]. Gamma irradiation can be also used to develop economically improved sorts of different plants (varietes with high productivity potential, resistant to dieases, salt and environmental factors) [2-5]. Research on the effects of γ-irradiation on food has been done with a little information avaliable on antioxidant system in plants.

γ-irradiation affects biomolecules by causing conformational changes, oxidation, rupture of covalent bonds, and formation of free radicals [6]. The hydroxyl (HO•) and superoxide anion ($O_2^{\cdot-}$) radicals that are generated by irradiation could modify the molecular properties of the proteins and lipids, causing oxidative modifications of the proteins and lipid peroxidation (LP) [7,8]. Chemical changes of the proteins that are caused by γ-irradiation include fragmentation, cross-linking, aggregation and oxidation resulting from oxygen radicals that are generated in the radiolysis of water [9].

The formation of reactive oxygen species (ROS) is prevented by an antioxidant system involving the activity of low molecular mass antioxidants (ascorbic acid, glutathione, tocopherols), enzymes regenerating the reduced forms of antioxidants, and antioxidant enzymes such as superoxide dismutases (SOD),

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peroxidases (Px) and catalase (CAT). In plant tissues many phenolic compounds, in addition to tocopherols, are potential antioxidants, including flavonoids, tannins and isoflavons which may act as ROS-scavenging compounds. Antioxidants act as a cooperative network, employing a series of redox reactions [3].

Soybean seeds are widely used in the food industry for producing healthy food items. In addition, due to the high phenol content of soybean seeds, they are used in pharmaceutical industries. In the present study, an attempt had been made to elucidate the irradiation-induced changes in antioxidant system of soybean and to select the parameters which could be useful as indicators of radiosensitivity. The effects of gamma irradiation (up to 200 Gy) on the antioxidant and free radical scavenging system and protein content of imbibed soybean seeds were investigated.

2. Experimental Procedures

2.1 Biological material and extraction procedure

In this study, seeds of soybean (*Glycine max* L. Merrill) genotype Novosadjanka were obtained from the Institute of Field and Vegetable Crops in Novi Sad. Dry seeds were treated with γ-irradiation (100, 120, 140 and 200 Gy) using ^{60}Co (TCT Gammatron S 65-K). The dose rate was 0.919 Gy/min. Seed irradiation was performed at the Irradiation Laboratory of the Oncology Institute in Sremska Kamenica near Novi Sad. Irradiated and non-irradiated soybean seeds were imbibed in Petri dishes on filter paper soaked with distilled water for 48 h at 24ºC in the dark. The extraction procedure of antioxidant enzymes and nonenzymatic antioxidants, hydroxyl radicals and soluble proteins was performed as previously described [10]. One g of imbibed seeds was ground with quartz sand in a cold mortar. The ground material was suspended in 5 ml 0.1 M K_2HPO_4 at pH 7.0. Samples were centrifuged at 15 000 g for 10 minutes at 4ºC, and aliquots of the supernatant were used for determination of antioxidant enzymes and total antioxidant activity. Non-irradiated seeds were used as a control.

2.2 Methods

2.2.1 Determination of antioxidant enzyme activity

All the antioxidant enzyme activities were determined by spectrophotometric analysis of plant extracts in phosphate buffer (pH 7) at 25ºC. Enzymatic specific activity is expressed as μmol of the substrate converted in minute/mg protein, with the exception of superoxide dismutase activity.

Superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovics [10] based on the inhibition of conversion of adrenaline to adrenochrome at pH 10.2. Unit SOD can be regarded as the amount of enzyme which causes a 50% inhibition in the extinction change in 1 min compared to the control [11]. Measurements were made at 480 nm.

Guaiacol peroxidase (GPx) activity was determined by the method of Matkovics *et al.* [12], using guaiacol as the substrate at 436 nm. Catalase (CAT) activity was determined by the method of Simon *et al.* [13], at 240 nm. The decomposition of H_2O_2 was followed by a decrease in absorbance. One unit of catalase specific activity is defined in terms of μmol of H_2O_2 consumed in minute mg^{-1} protein and GPx specific activity as μmol of guaiacol in minute mg^{-1} protein.

Glutathione peroxidase (GSH-Px) activity was determined by the method of Chiu *et al.* [14], using cumene hydroperoxide and reduced glutathione (GSH) as substrates at 412 nm. One unit of enzyme activity was defined as the amount of enzyme that resulted in oxidation of 1 μmol of GSH per minute.

2.2.2 Determination of GSH, MDA and HO˙ quantities, soluble protein content and total antioxidant activity

The quantity of reduced glutathione (GSH) was determined with Ellman reagent at 412 nm, by the method of Sedlak and Lindsay [15]. Soluble protein content was determined using the Bradford assay [16].

Malonyldialdehyde quantity (MDA) was determined by the TBA reaction with minor modification of the method of Dhindsa *et al.* [17]. The absorbance at 532 nm was monitored spectrophotometrically and MDA quantity is expressed as nmol of MDA mg^{-1} protein. Hydroxyl radical quantity was determinated by the inhibition of deoxyribose degradation and expressed as nmol HO˙/mg protein [18].

Total antioxidant activity was estimated according to the FRAP (Ferric Reducing Antioxidant Power) assay [19]. Total reducing power is expressed as FRAP units. FRAP unit is equal to 100 μmol/dm^3 Fe^{2+}.

2.2.3 Data Analysis

Each experiment was performed twice and all determinations were obtained from triplicate measurements (N=6). Results are expressed as mean ± standard error. Experimental data were statistically analyzed using software Origin, by one-way ANOVA. Differences between means and control were evaluated for significance by using Tukey-test ($P<0.05$).

3. Results and Discussion

The results obtained from the analysis of effects of γ-irradiation on some antioxidant properties of soybean seed are presented in Figures 1-9.

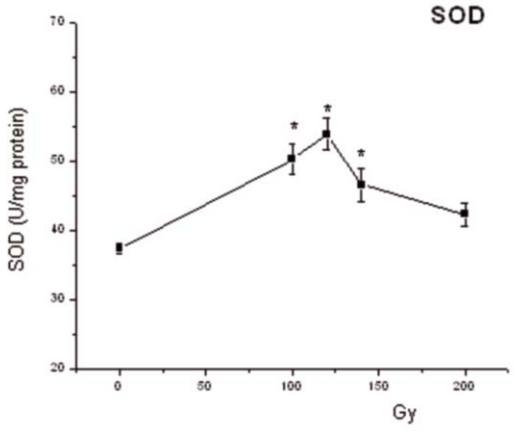

Figure 1. Effect of γ-irradiation on SOD activity in imbibed soybean seeds. Each data point represents the mean±SE. *ANOVA, Tukey-test, P<0.05.

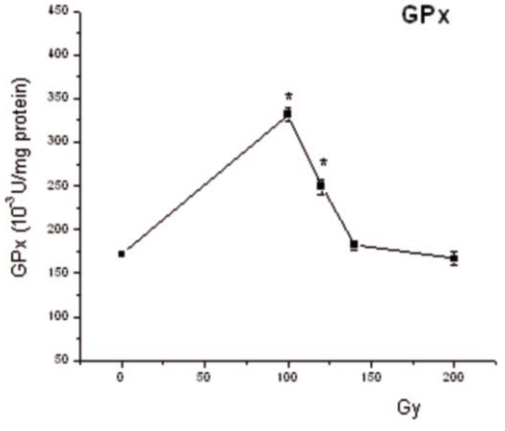

Figure 2. Effect of γ-irradiation on GPx activity in imbibed soybean seeds. Each data point represents the mean±SE. *ANOVA, Tukey-test, P<0.05.

All doses of γ-irradiation resulted in the increase of SOD activity in soybean seeds. The maximum increase of SOD activity was observed at a dose of 120 Gy (44.3% compared to the control value), (Figure 1). The maximum increase of GPx activity was observed at 100 Gy (93.4%). Higher doses (120-200 Gy) gradually decreased GPx activity to the control level (Figure 2).

Similar effect of hormesis (adaptive reaction) is observed during many stresses (UV light, heavy metals, herbicides, *etc.*) and was published by other authors [20,21]. They observed that plants can cope with different stressful conditions to a certain extent (depending of plant species and genotype), after which the ability of defense declines (β-shaped hermetic response). A similar phenomenon was observed during our experiment in regards to SOD and GPx activities. Other authors also showed that irradiation of plants and photosynthetic microorganisms with relatively low-doses of irradiation resulted in accelerated cell proliferation, germination rate, cell growth, stress resistance, enzyme activity and increased crop yields [4,5,22-25].

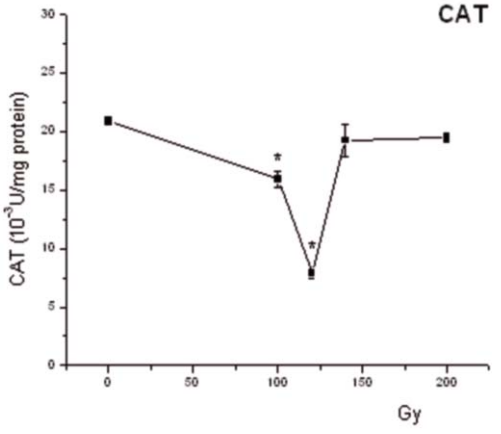

Figure 3. Effect of γ-irradiation on CAT activity in imbibed soybean seeds. Each data point represents the mean±SE. *ANOVA, Tukey-test, P<0.05.

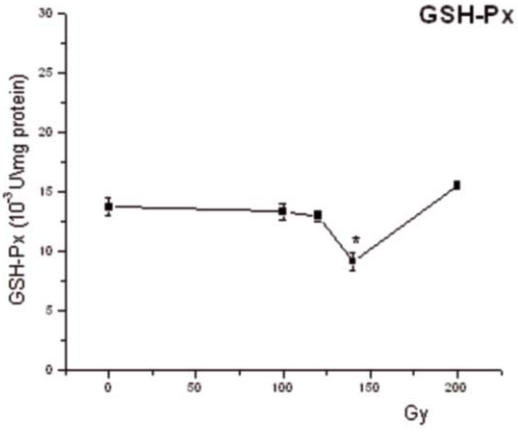

Figure 4. Effect of γ-irradiation on GSH-Px activity in imbibed soybean seeds. Each data point represents the mean±SE. *ANOVA, Tukey-test, P<0.05.

All doses provoked the decrease of CAT activity (significant only at 100 and 120 Gy) and the maximum decrease was detected at 120 Gy (62.4%), (Figure 3). GSH-Px activity was not significantly changed as a result of irradiation treatment, except by a dose of 140 Gy which caused a decrease in GSH-Px activity by 33.7%, (Figure 4). It has been reported that low doses of γ-irradiation can modulate antioxidant enzyme activities [23,26]. For CAT and GSH-Px *vice versa* hormetic response (U-shape) occurred in contrast to SOD and GPx. Decline of their activities was observed under lower doses, but further increase of irradiation dose caused the increase of their activities. Catalase, which is involved in the degradation of H_2O_2, is the major antioxidant enzyme in all aerobic organisms [27]. Under higher irradiation it appears that CAT takes over central role in controlling H_2O_2 level and oxidative stress when SOD and GPx activities start to decline [7]. Our results indicate that irradiation of genotype Novosadjanka seeds provoked changes in all antioxidant enzymes, depending on applied dose of irradiation.

The GSH quantity increased as irradiation dose increased to 140 Gy (27.0% greater than the control) and then decreased at the highest irradiation dose (36.0% less than the control), which is also a hermetic type of response under applied doses of irradiation (Figure 5). Total antioxidant activity determined by FRAP method decreased, especially at a dose of 200 Gy (35.9%) (Figure 6).

LP intensity and HO$^{\bullet}$ quantities increased in response to increased radiation intensity (Figures 7,8). The maximum changes of LP intensity and HO$^{\bullet}$ quantity were observed by the highest irradiation dose where LP

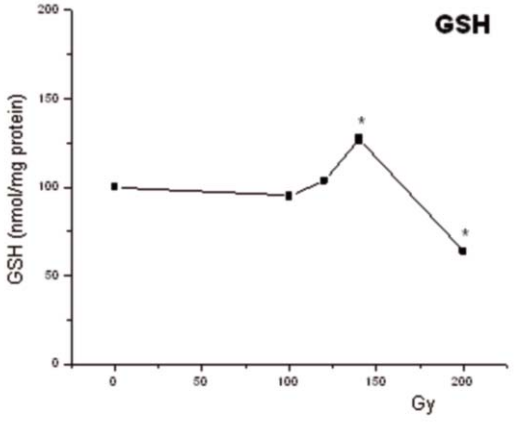

Figure 5. Effect of γ-irradiation on GSH quantity in imbibed soybean seeds. Each data point represents the mean±SE. *ANOVA, Tukey-test, P<0.05.

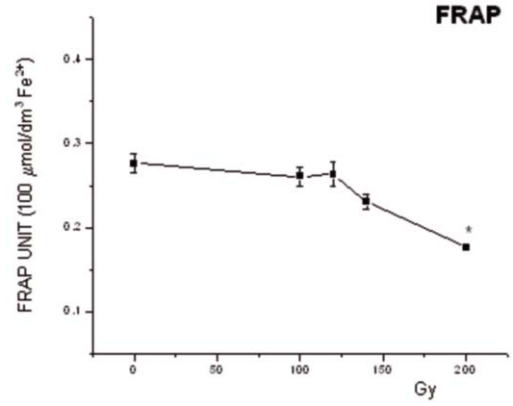

Figure 6. Effect of γ-irradiation on total antioxidant activity in imbibed soybean seeds. Each data point represents the mean±SE. *ANOVA, Tukey-test, P<0.05.

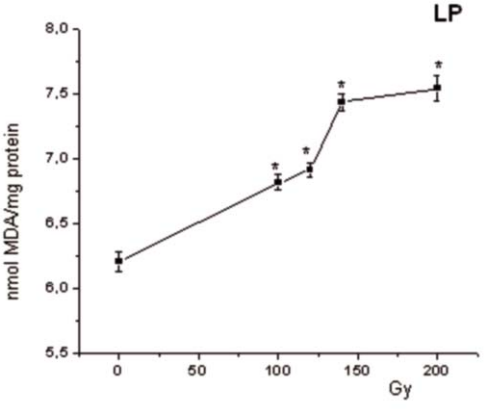

Figure 7. Effect of γ-irradiation on LP intensity in imbibed soybean seeds. Each data point represents the mean±SE. *ANOVA, Tukey-test, P<0.05.

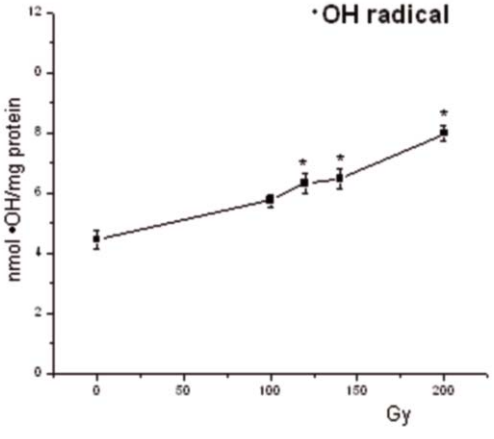

Figure 8. Effect of γ-irradiation on •OH radical quantity in imbibed soybean seeds. Each data point represents the mean±SE. *ANOVA, Tukey-test, P<0.05.

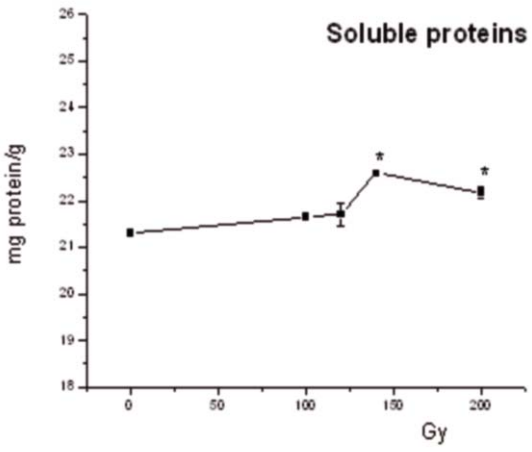

Figure 9. Effect of γ-irradiation on soluble protein content in imbibed soybean seeds. Each data point represents the mean±SE. *ANOVA, Tukey-test, P<0.05.

increased by 21.6% and HO˙ quantity by 79.3% . The soluble protein content slightly increased at all applied doses, and the maximum increase was observed at a dose of 140 Gy (6.1%), (Figure 9). Our results showed that the genotype Novosadjanka was most sensitive at the highest applied dose of 200 Gy where the maximum increase of lipid peroxidation intensity and HO˙ quantity and decrease of total antioxidant activity were detected. Gamma irradiation causes oxidative stress and affects biomolecules by causing conformational changes, oxidation, rupture of covalent bonds and formation of free radicals [6]. The hydroxyl (HO•) and superoxide anion (O2•-) radicals that are generated by radiation could modify the molecular properties of the proteins and lipid peroxidation (LP) [7].

The amount of reduced glutathione, which is one of the most important non-enzymatic antioxidants, was positively affected by γ-irradiation dose of 140 Gy. It was reported that low level doses of γ-irradiation enhance glutathione reductase activity [22] which could explain the increase of GSH quantity in soybean irradiated with a dose of 140 Gy. On the other hand, a decrease in the FRAP value indicated a decrease of non-enzymatic antioxidants (vitamins C and E and polyphenol constituents) [28]. The increase of LP intensity was well correlated with the increase of HO˙ quantity and decrease of total antioxidant capacity in irradiated soybean, which appear to be a consequence of irradiation treatment [29].

4. Conclusion

Our results indicate that all antioxidant parameters were affected differently by γ-irradiation. Under low doses of irradiation, a decrease in the activity of antioxidant enzymes CAT and GSH-Px was observed. However, an increase in SOD and GPx occurred follow irradiation treatment. The increase of LP intensity, a major indicator of oxidative stress, resulted from HO˙ radical accumulation under irradiation. A decrease in FRAP value was observed but the decrease in non enzymatic antioxidants in soybean seeds did not appear to affect the soluble protein biosynthesis. Our study indicated that irradiation of soybean seeds by dose of 200 Gy induced oxidative stress manifested by high HO˙ and MDA quantities. Irradiation also activated different antioxidant enzymes depending of irradiation dose, so they act cooperatively in antioxidant defense.

Further studies are needed to explain the mechanism of antioxidants radio-induction in plants and developing radio-resistance. In future prospects, investigations of the effects of γ-irradiation on soybean could enable the production of varietes with superior qualities to produce important antioxidants.

Acknowledgements

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