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Physiological and Biochemical Response of Gamma Irradiated *Sesamum indicum L.* Seed Grown in Heavy Metal Contaminated Soil

Eman E. Selem[1]; Reda E. Abdel Hamed[1]; Hedaya A. Kamel*[2] and Hegazy S. Hegazy[1]

[1]Botany Department, Faculty of Science, Zagazig University, Zagazig, **Egypt**
[2]Radioisotope Department, Atomic Energy Authority, Dokki 12311, Giza, **Egypt**

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***Correspondence:** hedayakamel@yahoo.com **Accepted: 18** May 2018 **Published online:** 24 June. 2018

This study was performed to assess the physiological and biochemical changes in Dry seeds of sesame exposed to different doses of gamma radiation (0.0, 10, 25, 50, 100 and 200 Gy) emitted from ^{60}Co and cultivated in heavy metal contaminated soil. Seed irradiation with gamma rays significantly affects photosynthetic pigment fractions, total free amino acids and soluble protein content. Also, accumulation of the reactive oxygen species such as hydrogen peroxide and malondialdehyde was observed as oxidative stress indicator. Moreover, increasing gamma doses improved the activity of enzymatic (superoxide dismutase, peroxidase and polyphenol oxidase) or non-enzymatic (total free proline, phenolic and flavonoid compounds) antioxidant. Ascorbic acid content in sesame plant was decreased in the control and irradiated plants with the elapse of time; also significantly and gradually decreased by increasing gamma dose from 0.0-200 Gy.

Keywords: *Sesamum indicum* L., gamma irradiation, antioxidant enzymes, heavy metals, contaminated soil.

INTRODUCTION

Gamma radiation is one of common physical mutagens imposing considerable effects on physiological and biochemical processes in plants (Heidarieh et al., 2012). The biological effect of gamma-rays is mediated through their interaction with atoms or molecules in the cell, particularly water, to produce free radicals (Kovacs and Keresztes, 2002). These radicals in turn can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the intensity of radiation (Ashraf et al., 2003). The synthesis of protein, chlorophyll, lipid peroxidation, enzyme activity and accumulation of phenolic compounds are affected by irradiation of seeds with gamma rays (Hameed et al., 2008). Gamma rays cause changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds (Ashraf, 2009). The treatment of plants with low doses of γ-irradiation stimulated the cell proliferation, germination rate, cell growth, enzyme activity, stress resistance and crop yields (Moussa, 2010 and El-Beltagi et al., 2011). On the other hand, the irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein, hormone balance, leaf gas-exchange, water exchange and enzyme activity (Aly and El-Beltagi, 2010). Seed irradiation is one of the most effective methods to improve plant production, yield components and chemical composition (Selenia and Stepanenko, 1979). Many studies report that low doses of gamma rays stimulated seed germination, plant growth and oil production (Moussa, 2006).

The aim of the present work is to study the physiological and biochemical response of gamma irradiated sesame seeds which grown in heavy metals contaminated soil.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The sesame seeds *(Sesamum indicum* L.) cv. Shandawel 3 was purchased from the Legume Research Department, Field Crops Research Institute, Agricultural Research Centre (ARC), Ministry of Agriculture, Giza, Egypt. Seeds irradiation was performed by using ^{60}Co source (gamma radiation emitter) at Cyclotrone, Nuclear Research Centre, Egyptian Atomic Energy Authority, Cairo, Egypt. The dose rate of the source was 1.29744 kGy/h. A uniform dry seeds (size and color) of sesame were exposed to gamma irradiation doses 0.0, 10, 25, 50, 100 and 200 Gy. Then sown immediately in rows, 15 cm a part and in six plots. The dimension of each part was 8x4 meter long and width. This study was conducted at sewage water irrigated site. The source for treated waste water irrigation was from Bahr El Bqar, Belbis, Sharkia. Bahr El Bqar was used in large-scale by farmers. The experiment was carried out within the period from May to September 2013 growing season. Plant samples were collected randomly from each plot after 30, 54 and 90 days from sowing (vegetative, flowering and fruiting stage, respectively. The plants were carefully uprooted with minimum disturbance of roots. The roots were washed with tap water. At each stage, plant samples were divided into root, stem and leaves. Roots and shoots were separated. Fresh samples were frozen at -4ºC for the required analysis. Other samples were cut into pieces, and then oven-dried for 48 hours at 65ºC till constant dry weight. The oven-dried samples were taken out and ground to fine powder.

Chemical analysis:

Photosynthetic pigment content (Chlorophyll *a*&*b*) was estimated by the method of Metzner et al. (1965). Total soluble protein was determined according to Lowry et al. (1951). Proline was determined following the method of Bates et al. (1973). Total phenolic was estimated by the method of Jindal and Singh (1975). Free amino acid was determined according to the method of Lee and Takahashi (1966). H_2O_2 content was determined according to the method of Alexievia et al. (2001). lipid peroxidation (malondialdehyde, MDA) was estimated by the method of Heath and Packer (1968). Total flavonoid content was measured by the method of (Zou et al., 2004) and vitamin C was estimated according to the method of Bajaj and Kaur (1981). Superoxide dismutase (SOD) activity was measured by the method of Dhindsa et al. (1981). Polyphenol oxidase activity (PPO) was measured according to Beyer and Fridovich (1987). Peroxidase activity (POX) was measured according to Racusen and Foote (1965).

Statistical analysis:

The data were compared for significance by one way ANOVA using Duncan's test by SPSS software version 10 (SPSS, Richmond VA, USA) as described by Dytham (1999).

RESULTS

Photosynthetic pigments.

Significant and non significant changes were observed in the pigment contents at different growth stages as shown in Figure 1. Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity (Parekh et al., 1990). Regarding the results of the present study, Borzouei et al. (2010) found that the irradiation of wheat seeds resulted in a significant enhancement in the contents of Chl. *a*, Chl. *b*, and total chlorophyll in leaves with plant age up to flowering stage and thereafter declined steadily which is in accordance of our results. The maximum increase in Chl. *a*, Chl. *b,* and total chlorophyll due to 100 Gy dose at flowering stage followed by gradual decrease at the higher doses (200, 300 and 400 Gy). A dose of 400 Gy reduced the total chlorophyll content. A significant increase in the chlorophyll content has been observed in *Terminalia arjuna* Roxb seedlings treated with γ-irradiation (100 Gy) as compared to the control and decreased slightly thereafter (Akshatha et al., 2013).

Free proline content

The data of the effect of γ-irradiation on free proline of sesame is shown in Figure 2A. Gamma irradiation exhibited a significant increase in free proline content as compared to the control plants. A dose of 100 Gy exhibited the maximum free proline content.

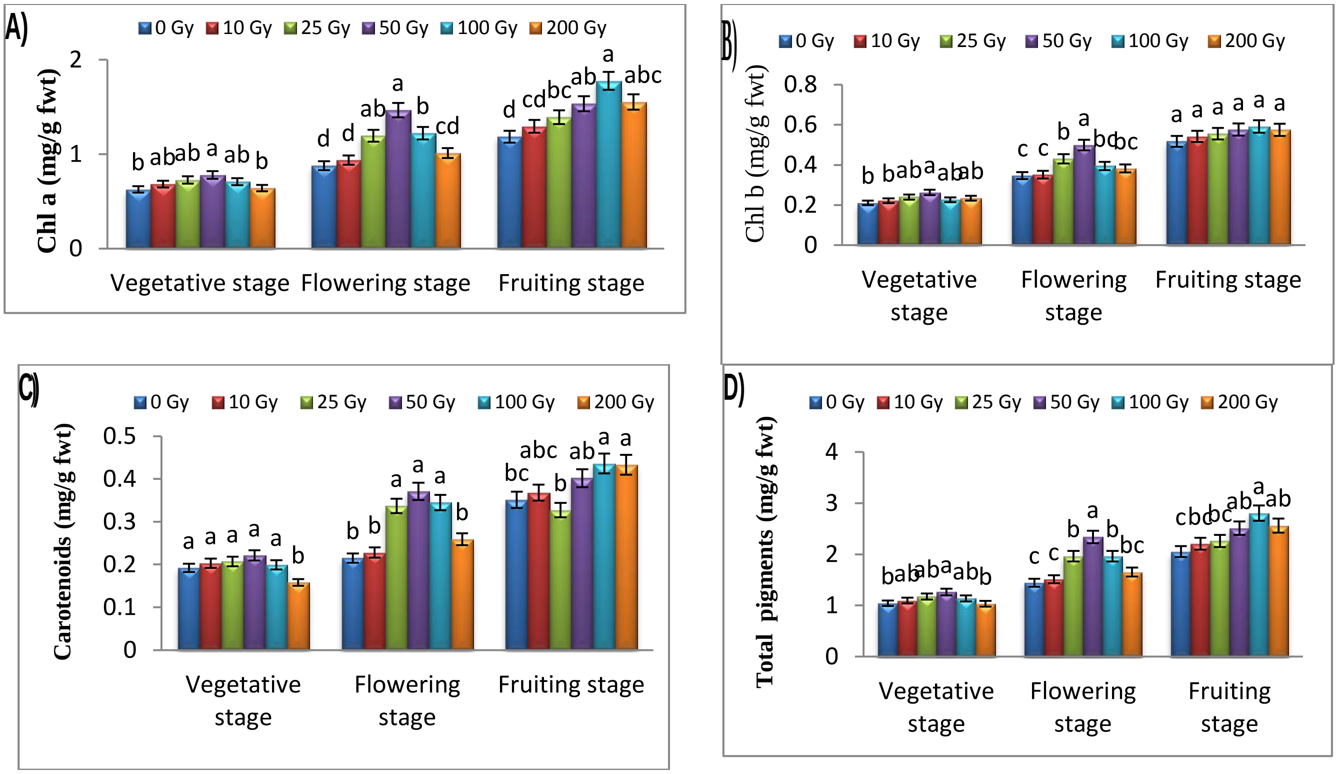
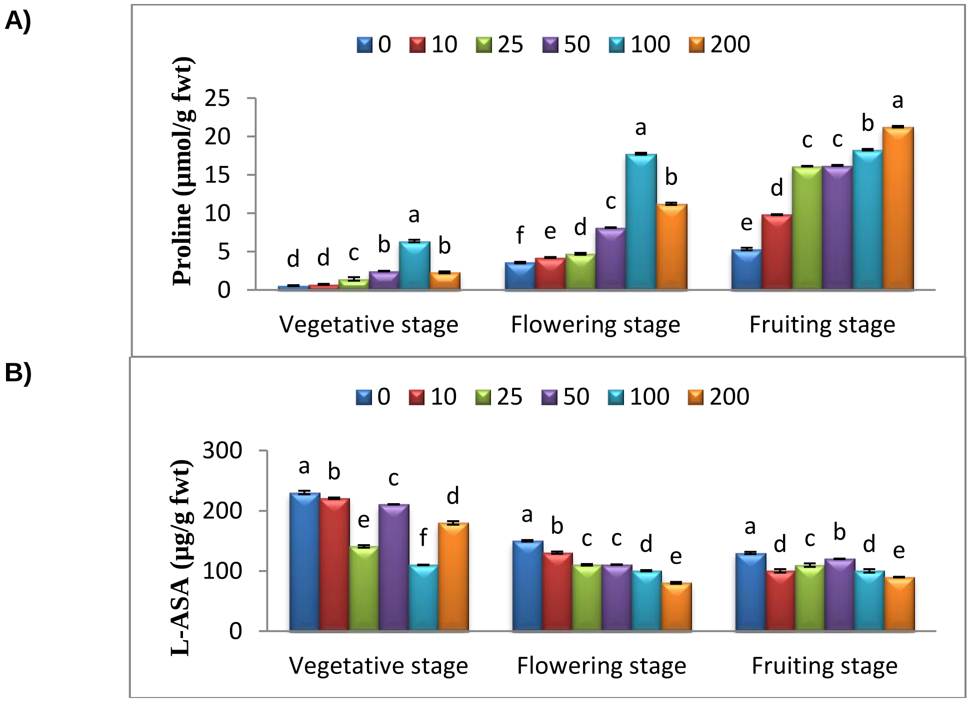


Figure 1: Effect of seed irradiation on (A) Chlorophyll "*a*", (B) Chlorophyll "*b*", (C) Carotenoids, and (D) Total pigments (mg/g fwt) content in leaves of sesame grown on soil irrigated with wastewater.



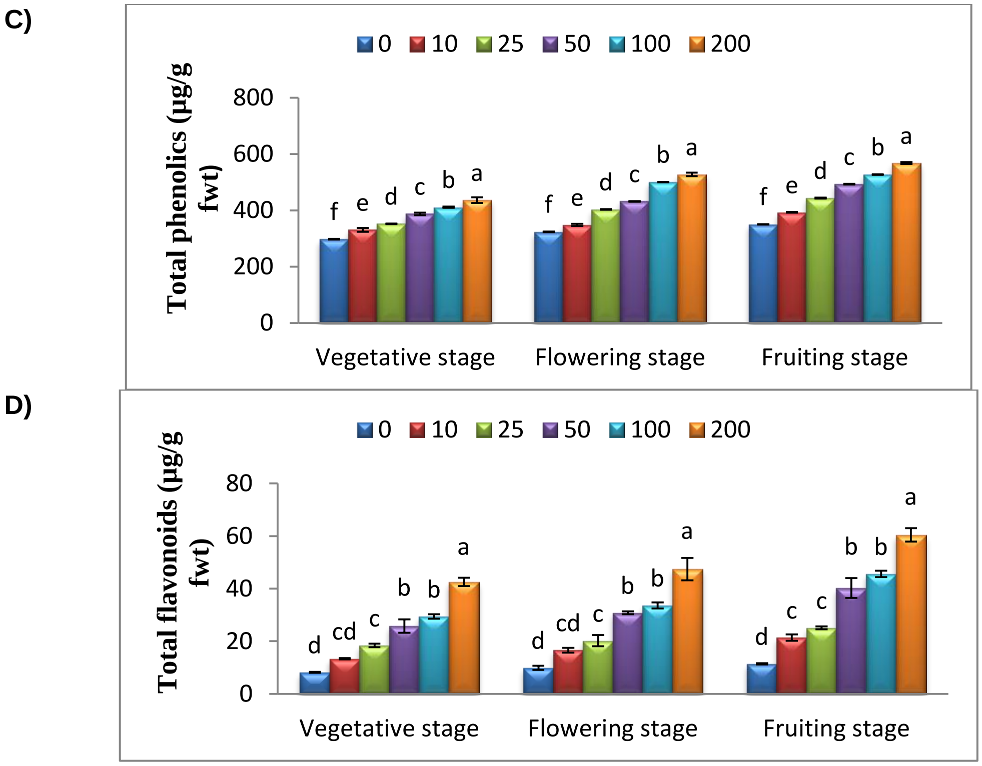


Figure 2: Effect of seed irradiation on (A) proline, (B) L-ascorbic acid, (C) total phenolics content, and (D) Total flavonoids content in leaves of sesame grown on soil irrigated with wastewater.

The significant increase in free proline was supported by the results of Hernandez et al. (2000) under several stresses. Qi et al. (2015) investigated the physiological and molecular mechanisms of heavy metal stress mitigated by low-dose of γ-irradiation (25 to 150 Gy) in *Arabidopsis thaliana.*

L-Ascorbic acid (vitamin C)

The data in Figure 2B showed significant changes in L-ascorbic acid (ASA) content as a result of the application of different γ-irradiation doses to the sesame seeds as compared with the control. ASA content was decreased in control and γ-irradiated plants with the elapse of time; also decreased by increasing γ-irradiation doses from 0.0-200 Gy. Moreover, Rai et al. (2004) measured a decline in the content of ascorbic acid of *Ocimum tenuiflorum* after radiation. Also, Huang et al. (2010) reported that Initial increase and subsequent decrease in response to heavy metal stress was observed in *Kandelia candel* and *Bruguiera gymnorrhiza.*

Total phenolics content

The data of the effect of γ-irradiation on the total phenolics in leaves of sesame is shown in Figure 2C, which revealed that throughout the different growth stages, the maximum total phenolics content was recorded due to the effect of 200 Gy dose and the minimum content was recorded at the control. Moreover, the obtained data revealed significant changes between each dose as compared with the control and among the different doses. The antioxidant activity of phenolic compounds is mainly attributed to their redox actions, neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Increase of phenolic acid due to the free radicals formation during irradiation in *Pterocarpus santalinus* (Akshatha and Chandrashekar, 2013). Results obtained in this research showed an increase in phenolics content was observed at 25 and 200 Gy (Mohajer et al., 2014).

Total flavonoids content

The data of the effect of γ-irradiation on total flavonoids in leaves of sesame is shown in Figure 2D. The total flavonoid content was increased significantly due to irradiation as compared with control and also increased gradually with increasing γ-doses till maximum contents were observed at a dose of 200 Gy. Flavonoid content in sesame leaves was increased in control and irradiated plants with the elapse of time so the fruiting stage has the maximum content. Under γ-irradiation stress flavonoids have a broad spectrum of chemical and biological activities including radical scavenging properties and improve the plant's ability of self-protection by inhibiting formation of MDA and by retaining membrane permeability (Porter and Grodzinski, 1985).

Total free amino acids content

The data of total free amino acids in control and γ-irradiated sesame are shown in Figure 3A. Total free amino acids content in sesame plants was gradually and significantly increased within each stage with increasing γ-dose from 0.0-200 Gy. El-Beltagi et al. (2013) illustrated a significant increase in the content of free amino acids in leaves of γ-irradiated cowpea plants under salinity stress as compared to the control. There was a significant increase in the level of amino acids in *Vigna unguiculata* L. plants under water stress (Lobato et al., 2008a). Hussein et al. (2012) observed that treating seeds of salt stressed Ambrosia maritima by γ-irradiation doses (20, 40 and 80 Gy dose) increased amino acids content above the control (Hussein, 2010).

Total soluble proteins content

The data of the influence of different doses of γ-irradiation (0.0-200 Gy) on total soluble proteins content in leaves of sesameare shown in Figure 3B. Generally, results implicated significant increases in total soluble proteins content in sesame plants grown from γ-irradiated seeds as compared with the control plants (Singh and Datta, 2010). The increase in protein content is possible due to de novo synthesis of stress proteins provoked by metal exposure (Verma and Dubey, 2003). These stress proteins may constitute enzymes involved in glutathione and phytochelatins biosynthesis and those required for Krebs cycle, as well as antioxidants and some heat shock proteins (Mishra et al., 2006b).

Hydrogen peroxide (H_2O_2) content

The data in Figure 3C revealed the accumulation of H_2O_2 in leaves of sesame by application of different γ-irradiation doses. Significant and gradual increases in the H_2O_2 content were observed in sesame plants with increasing γ-irradiation doses from 0.0-200 Gy. H_2O_2 content was increased in control and irradiated plants with the elapse of time so the fruiting stage attained the highest H_2O_2 content. H_2O_2, a key player in oxidative stress, is required for a variety of physiological processes associated with cell wall biosynthesis (Wi et al., 2005). H_2O_2 is a normal metabolite and not particularly cytotoxic at optimal concentrations, when these concentrations are increased by environmental stresses and ionizing radiation, they lead to cell lethality (Halliwell, 1974). Qi et al. (2015) showed that the H_2O_2 content in Arabidopsis thaliana under heavy metal stress decreased as compared with the control at a dose of 50-Gy of γ-irradiation.

Malondialdehyde (MDA) content

The extent of damage to lipid membrane with exposing to γ-irradiation as measured by MDA content is presented in Figure 3D. MDA content in leaves of sesame exhibited variation among the different doses of γ-irradiation. Hameed et al. (2008) investigated that γ-irradiation100 to 1000 Gy decreased significantly in desi and kabuli chickpea.

Antioxidant Enzymes

The data in Figure 4A showed the effect of radiation with different doses of γ-rays on superoxide dismutase (SOD) activity in sesame leaves at different growth stages. The obtained results exhibited significant increases in the activity of SOD. The obtained results revealed that peroxidase activity (POX) activity was generally increased after γ-irradiation as compared with the control (Figure 4B). Control recorded the lowest POX activity. The effect of irradiation with different doses of γ-rays on the activity of polyphenol oxidase (PPO) in leaves of sesame is shown in Figure 4C. Generally, the obtained data revealed significant changes in leaf PPO activity at all the growth stages. To avoid oxidative damage, plants have evolved various protective mechanisms to counteract the effects of reactive oxygen species (ROS) in cellular compartments (Kiong et al., 2008). One of the protective mechanisms was the antioxidative system (Kovacs and Keresztes, 2002).

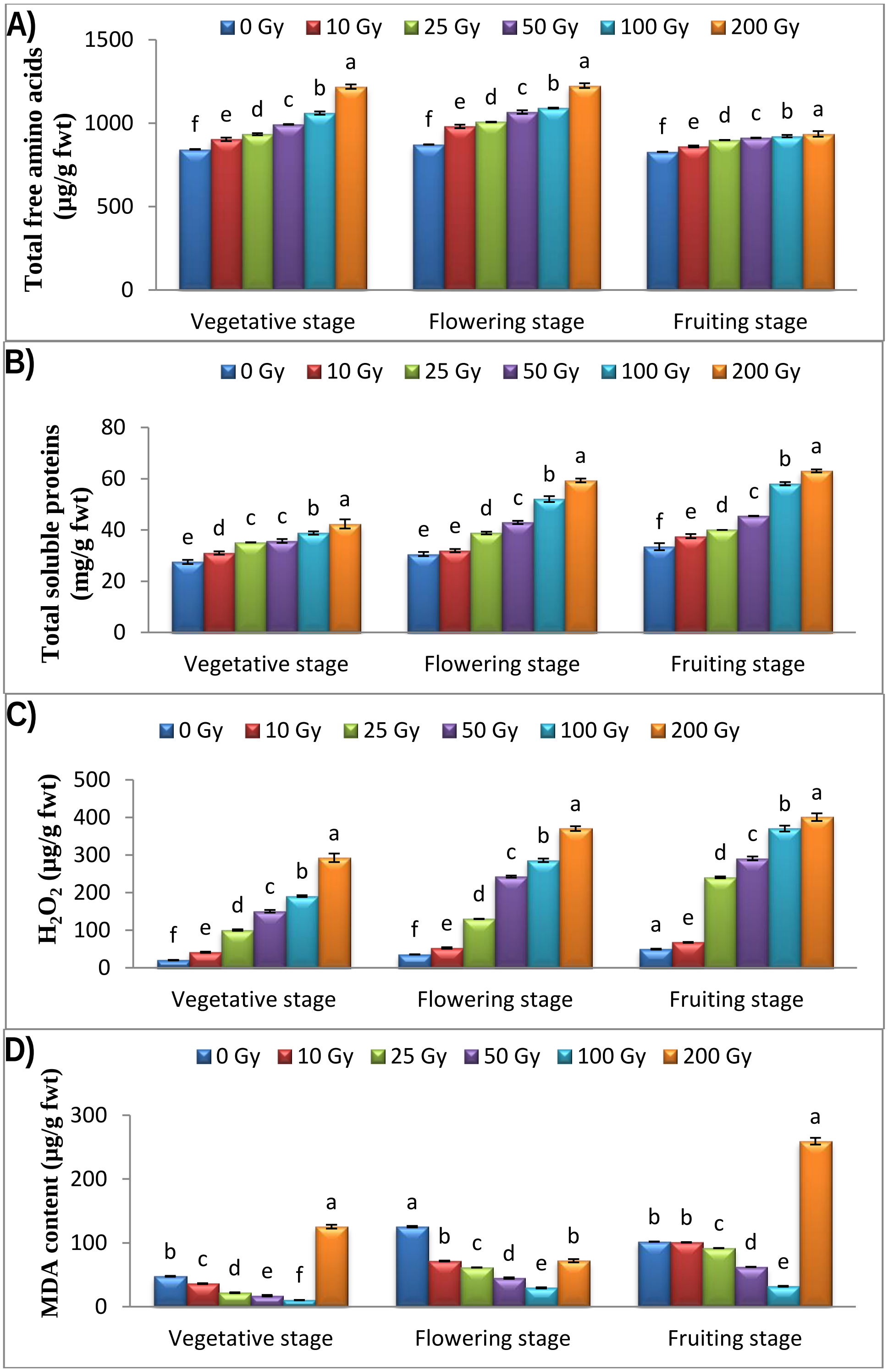


Figure 3: Effect of seed irradiation on (A) total free amino acids, (B) total soluble proteins, (C) H_2O_2 content, and (D) MDA content in leaves of sesame grown on soil irrigated with wastewater.

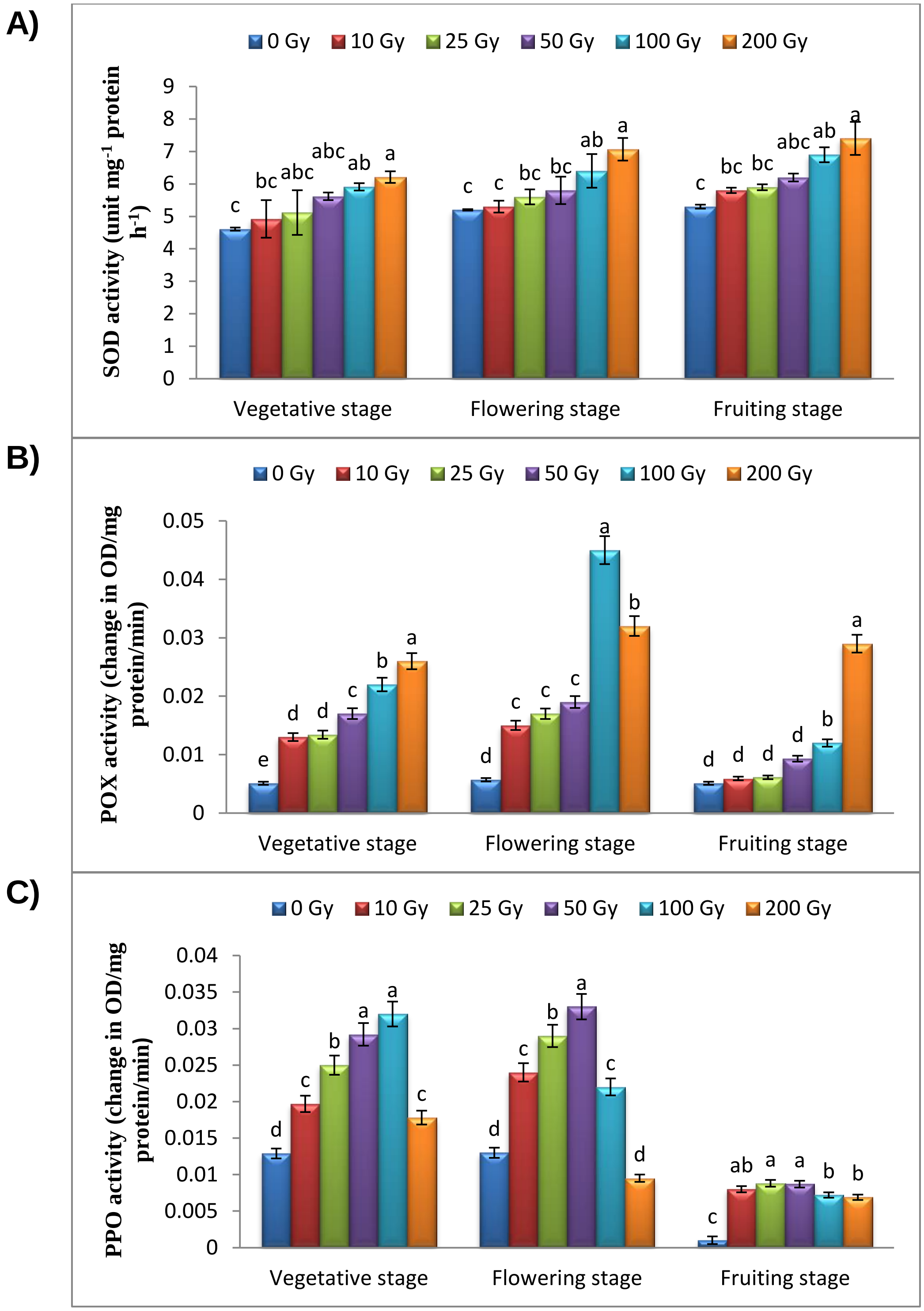


Figure 4: Effect of seed irradiation on (A) SOD activity, (B) POX activity, and (C) PPO activity of sesame leaves grown on soil irrigated with wastewater at different growth stages.

The decreases in the activity of antioxidants may result in the accumulation of ROS which can cause severe damage

SOD activity results in H_2O_2 production that should be detoxified by some other oxidative enzymes such as APX, GPX and CAT to H_2O and O_2 (Anuradha and Rao, 2007). Although any change in GPX activity can be considered as a typical response to oxidative stress, but diversity in POX activity under heavy metals stress depends on plant species (physiological status and genetic potential of plant), time of treatment and metal concentration (Tamàs et al., 2008). Sreedhar et al. (2013) established changes in the activity of the scavenging enzymes of groundnut seedlings subjected to γ-irradiation (0.0, 10, 20, 40, 50 and 100 Gy). The observed increase of SOD and POX of the antioxidant system indicated that increase in oxidative stress caused by radiation may be over whelmed by this enzymatic system (Afify et al., 2012).

CONCLUSION

Treatment of sesame seeds with low dose of γ-irradiation has a beneficial effect in ameliorating the harmful effect of heavy metals stress in the contaminated soil.

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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