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RESEARCH ARTICLE

Effects of gamma irradiation on biochemical and antioxidant defense system in wheat (Triticum aestivum L.) seedlings

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Abstract

This investigation was carried out to determine the effect of gamma radiation on germination and physiological characteristics of wheat seedlings. For this purpose soaked grains were irradiated with ^{60}Co at dose of 0.5, 1, 2, and 5 KGy. The germination percentages as well as the growth parameters of wheat seedling (shoot and root fresh and dry weights, and shoot length) were significantly decreased by increasing the irradiation dose. Non-enzymatic Amadori products content was increased in germinating grains in response to the increase of γ-irradiation dose. Increasing of gamma irradiation dose lead to the decrease of the relative water content (RWC) and the membrane integrity (MI) of wheat leaves. There was a gradual decline in leaf chlorophyll content with increasing irradiation dose. In contrast, there was a significant increase of carotenoids content and Carot./Chl.a + Chl.b ratio. Gamma rays induced a distortion of the pattern of grana and thylakoids, dilation of nuclear membrane and chromatin materials as well as a gradually degeneration of mitochondria. Antioxidant enzymes (superoxide dismutase, and peroxidase) activities were significantly increased in wheat leaves with increasing gamma doses, whereas catalase activity was decreased. Total phenolics and flavonoids content were significantly increased in wheat leaves in response to γ-irradiation which was associated with a significant increase of PAL activity. There was a significant increase in superoxide radicals, malondialdehyde content, and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging ability in accordance with high doses of γ-radiation.

Introduction

Application of radiation has revolutionized current day research in the field of agricultural science and food technology (**Sommers and Fan, 2002; Bari et al., 2003**). Radiation treatment is gaining attention as a substitute for conventional decontamination methods (heating/chemical sterilization) and high doses of ionizing radiation have been shown to inhibit growth of the infesting microbes on seeds (**El-Bazza et al., 2001; Rajkowski et al., 2003**). Plants often face the challenge of several environmental conditions which include such stresses as drought, salinity, pesticides, low temperature and irradiation, all of which exert adverse effects on plant growth and development (**Foyer et al., 1994**).Gamma rays belong to the ionizing electromagnetic group of radiations and are highly penetrating because of low linear energy transfer, it improves the hygienic quality of various foods and herbal materials and reduces the losses due to microbial contamination and insect damage (**Farkas, 1998**). Gamma irradiation is one of common physical mutagens imposing considerable effects on physiological and biochemical processes in plants (**Begum and Dasgupta, 2011; Heidarieh et al., 2012**). The deleterious effects of ionizing radiation in biological systems arise directly via the interaction between radiation and target macromolecules or indirectly via the generation of free radicals (**Chandrasekharan et al., 2009**). Gamma radiation can interacts with

atoms and molecules to create oxidative stress with overproduction of reactive oxygen species (**Xienia et al., 2000**), that are able to modify important components of plant cells (**Hameed et al., 2008**).

In dry seeds, enzymatic reactions may play little role in seed ageing, because dry seeds lack active enzymatic metabolism. However, certain non-enzymatic reactions, such as Amadori and Maillard reactions, could occur even at very low moisture content (**Wettlaufer and Leopold, 1991; Sun and Leopold, 1995**). Amadori and Maillard reactions refer to a series of complex reactions that occur following an initial carbonyl-amine reaction. The Maillard reaction occurring between amino group and carbonyl group produces neoformed compounds having certain levels of antioxidant activity depending on the reaction conditions and the type of reactants **(Castilho et al., 1994)**. The non-enzymatic modifications of proteins through Amadori and Maillard reactions play an important role in the loss of seed viability during storage (**Murthy and Sun, 2000**). **Strelec et al. (2008)** observed molecular alterations due to accumulation of Amadori and Maillard products in wheat seeds during storage, especially at elevated storage temperatures.

To avoid oxidative damage, plants have evolved various protective mechanisms to counteract the effects of reactive oxygen species in cellular compartments (**Bowler et al., 1992**). Antioxidant defense enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) are the systems designed to minimize the concentrations of superoxide and hydrogen peroxide. Furthermore, several non-enzymic antioxidants such as phenolics and flavonoids are synthesized during several environmental stresses **(El-Beltagi et al., 2010; El-Beltagi et al., 2011). Dixon and Paiva (1995**) reported that phenylalanine ammonia lyase (PAL) is a key enzyme in the first step of the phenylpropanoid pathway responsible for the synthesis of plant phenylpropanoids or phenolics, many of which play important roles in plant defense against pathogens and herbivores.

Considering the effects of radiation on plants, the present study was conducted to determine the effect of γ-radiation (0.5, 1, 2, and 5kGy) on wheat (Triticum aestivum L.) germination and some key physiological and biochemical characteristics in wheat seedlings.

MATERIALS AND METHODS

Plant materials and gamma irradiation

Wheat grains (Triticum aestivum L. cv. Sakha 94) obtained from Ministry of Agriculture, Egypt, were subjected to surface sterilization with 0.1% sodium hypochlorite solution for 5 min and then rinsed several times with distilled water, soaked for 2h in distilled water, then air dried and stored in polythene bags for irradiation following the method described by **Cuero et al. (1986**). The presoaked grains were irradiated at the Department of Physics, University of Taibah (KSA).The grains were exposed to a ^{60}**Co** gamma source with a dose rate of 120 Gy/h for irradiation at the room temperature. All experiments were repeated three times and 3 replicates of the grains received each dose (0.5, 1, 2, and 5 kGy). The non-irradiated grains were served as control. After irradiation, 10 grains from each treatment were sown in Petri dishes containing 20 ml of distilled water. Petri dishes were placed in an incubator for 3days at 25^{o}C. Number of germinated seeds was recorded during the 3 days. The germinating seeds were then transferred to plastic pots (20x15x10 cm) filled with acid- washed quartz sand, and irrigated with half strength Hoagland´s nutrient solution (**Hoagland and Arnon, 1950**) each even day during the experimental period. The pots (in triplicates) were placed in growth chamber under 12h light/12h dark cycle at 23-18±2°C during the light/dark period for 18 days.

Growth parameters

After harvesting (21-days), fresh and dry masses (FM, DM), and shoot height were measured for control and irradiated plants. Fresh samples of leaves were subjected for estimation of relative water content and membrane integrity, other leaves samples were used for biochemical analysis and other samples were dried in oven at 70 °C till constant weight.

Measurement of Amadori products

Amadori products were measured according to **Wettlaufer and Leopold (1991).**Twenty mg of germinated grains were homogenized in a centrifuge tube with 1.2 ml of phosphate buffer (50 mM, pH 7.2). An aliquot (200 µl) of 10% streptomycin sulphate dissolved in 50 mM HEPES (pH 7.2) was added to the homogenate to precipitate nucleic acids. After vortexing and centrifuging at 15 000 g for 15 min, another 200 µl streptomycin was added and the suspension was centrifuged again. To minimize the interference of non-protein components, seed proteins in the supernatant were precipitated with ammonium sulphate (0.55 g ml^{-1}). The precipitated proteins after centrifugation were redissolved in 3.3 ml phosphate buffer (50 mM, pH 7.2). Extracted proteins were used for measurement of Amadori products using the nitroblue tetrazolium (NBT) method**.** One ml of NBT solution (0.5 mM NBT in 100 mM sodium carbonate, pH 10.3) was added to 0.2 mg of extracted protein and incubated at 40 °C in a water bath. The absorbance was read at 550 nm after 10 and 20 min of incubation. The increase in absorbance (ΔOD) was taken as the measure of Amadori products.

Determination of relative water content (RWC)

The relative water content was estimated according to **Turner** (**1981) ,**and was evaluated from the equation:

RWC (%) = (FW – DW) / (TW – DW) x100, where FW is the fresh weight of the leaves, TW is the weight at full turgor, and DW is the weight estimated after drying the leaves.

Determination of membrane integrity (MI)

Membrane integrity was determined by recording the electrical conductivity of leaf leachates in double distilled water at 40 and 100^{o}C (**Deshmukh et al., 1991**). Leaf samples (0.1 g) were cut into discs of uniform size and taken in test tubes containing 10 ml of double distilled water in two sets. One set was kept at 40^{o}C for 30 minutes and another set at 100^{o}C in boiling water bath for 15 minutes and their respective electric conductivities C_1 and C_2 were measured by Conductivity meter. **Membrane Integrity (%) = [1- (C_1/C_2)] x 100**

Determination of chlorophylls, and carotenoids content

Chlorophyll (Chl) and carotenoids (Carot.) contents were determined by the method described by **Lichtenthaler (1987)**. Leaf tissues (50 mg) were homogenized in 10 ml chilled acetone (80 %). The homogenate was centrifuged at 4,000 g for 12 min. Absorbance of the supernatant was recorded at 663, 647 and 470 nm (using a T80 UV–Vis spectrophotometer - double beam) for Chl.a, Chl.b and carotenoids. The contents were expressed as mg Chl. or carotenoids g^{-1} fresh weight (FM).

Determination of superoxide radical ($O_2^{\bullet}$) and malondialdehyde (MDA)

The production rate of $O_2^{\bullet}$ was measured by the method described **by Elstner and Heupel (1976).** The level of lipid peroxidation in leaves was measured by determining the malondialdehyde (MDA) contents and that assayed by thiobarbaturic acid reactive substance contents (**Buege and Aust, 1978**).

Determination of radical-scavenging activity (DPPH)

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay was carried out similar to earlier report by **Rajurkar and Gaikwad (2010**). Briefly 100 µL extract of the sample (non-irradiated and irradiated leaves) was mixed with 2 ml of 0.1 mM DPPH solutions in methanol. The reaction mixture was kept in dark at room temperature. The change in colour (from deep violet to light yellow) was measured at 517 nm on a UV-visible light spectrophotometer (T80+ UV-Vis spectrometer, double beam). The radical scavenging activity was measured as a decrease in the absorbance of DPPH and % scavenging was calculated by using the following equation:

% Scavenging = [(A_0 – A_1)/ A_0] × 100

Where A_0 is the absorbance of the control reaction (containing all reagents except the test compound) and A_1 is the absorbance of the test compound.

Determination of total phenolics content

The total phenolics content were determined with the Folin-Ciocalteu method (**Wolfe et al., 2003**). The reaction mixture containing 200 µl of diluted extract, 800 µl of freshly prepared diluted Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The final mixture was diluted to 7 ml with deionized water. Mixtures were kept in dark at ambient conditions for 2 h to complete the reaction. The total phenolic content was expressed as mg gallic acid equivalents g^{-1} DM.

Determination of total flavonoids content

Total flavonoids content of the methanolic extract were estimated according to **Ismail et al. (2010**). Total flavonoids content was determined using aluminum chloride ($AlCl_3$) and rutin (standard). The plant extract of 0.1 ml was added to 0.3 ml distilled water followed by 0.03 ml of 5% $NaNO_2$, then, 0.03 ml, of 10% $AlCl_3$ was added, after 5 min at 25°C. After further 5 min, the reaction mixture was treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The results were expressed as mg rutin g^{-1} DM.

Assay of enzymes activities

Phenyl alanine ammonia lyase (PAL) activity

PAL activity was determined following the method used by **Jiang and Joyce (2003**). Five grams of fresh plant material was homogenized in 20 ml of borate buffer (0.1M, pH8.0) containing 5mM b-mercaptoethanol and 2mM EDTA. Homogenate was centrifuged for 20 min and supernatant was collected for enzyme assay. For determining PAL activity,0.5ml of supernatant was incubated for 1h at 30°C in 2ml of 0.1M borate buffer (pH8.0) containing 1mlof L-phenylalanine (0.1M). Increase in OD 290 nm due to the formation of trans- cinnamate was measured spectrophotometrically.PAL activity was expressed as change in OD_{290} g^{-1} FM min^{-1}.

Superoxide dismutase (SOD) activity

SOD activity was determined following the method used by **Giannopolitis and Ries (1977**) where its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT) was measured. One unit of SOD activity is defined as the amount of enzyme causing 50% inhibition of the initial rate of reaction in the absence of enzyme. The enzymatic activity was expressed as U g^{-1} FM.

Catalase (CAT) activity

CAT activity was assayed according to the method of **Zhang et al. (2005)**. One unit of CAT activity was expressed as µmol H_2O_2 g^{-1} FM min^{-1}.

Peroxidase (POD) activity

POD activity was determined using the method described by **Noreen and Ashraf (2009)**. Changes in absorbance of the reaction solution at 470nm were determined after every 20 s. The enzymatic activity was expressed as ΔOD_{470} g^{-1} FM min^{-1}.

Ultra-structural analysis

Leaves of non-irradiated and irradiated seedlings (control and 5KGy) were cut into 1-2 mm^2, and prepared according to method of **Loreto et al. (2001)** for electron microscopy processing. The ultra-structure visualization and photographing were carried out using the transmission electron microscope (JEOL-TEM 100 CX) at the Electron Microscopic Unit, Faculty of Science, Alexandria University.

Statistical analysis

Based on the data obtained from the experiment, the results presented are the mean ± standard deviation (SD) gained from at least three replicate samples using Microsoft Office Excel 2007. Statistical analysis by the least significant difference (LSD) for multiple comparisons, taking $P \leq 0.05$ as significant, was calculated by SPSS 13.0.

RESULTS

The results of the Petri dishes experiment (Table 1) showed that irradiation of the soaked wheat grains with 1KGy caused an insignificant effect on germination capacity, whereas high doses resulted in a significantly decreased of the germination percentage compared to non-irradiated grains. This observation was accompanied with a significant increase of Amadori products in the germinated wheat grain. The Amadori products were 1.4- and 2.6-fold of control in 1KGy and 5 KGy-treated wheat grains respectively.

The results in Table 2 showed that irradiation with 1 KGy caused a 42% and 33% increase in fresh mass of the shoot and roots respectively compared to control plants. Conversely, irradiation with 5KGy provoked a significant decrease of shoots and roots fresh mass; the decrease reached to 50% and 46% respectively, compared to non-treated ones. Additionally, the leaves of wheat plants issued from seeds irradiated with 1KGy dose maintained a higher RWC and more stable membrane structure (MI) than the non-treated plants. Whereas, increasing the irradiation up to 5KGy resulted in a significant decrease in the shoot height, leaf RWC and MI.

In the present study, treatments of wheat grains with γ rays caused a significant decrease of Chl.a and Chl.b content (Figure 1A). In contrast, there was a significant increase of carotenoids content and Carot./Chl.a+Chl.b ratio (Figure 1B). At 5 KGy the decrease of Chl.a and Chl.b content was 56% and 81% respectively compared to the control, whereas the increase of carotenoids content was 1.5-fold of control.

Exposure of soaked wheat grains to γ-irradiation resulted in a significant increase the production of superoxide radicals ($O_2^{\bullet}$) and lipid peroxidation as indicated by estimation of MDA content in the 21-day old leaves (Figure 2 A,B). The increase of $O_2^{\bullet}$ content in the leaves of plant tissues from seeds irradiated with 1 KGy and 5KGy was 1.3- and 4.2-fold of untreated plants, respectively. The corresponding values for MDA were 1.5- and 2.8-fold respectively. The enhancement of the generation of superoxide radicals and MDA in response to γ- irradiation was accompanied with a significant increase of scavenging ability of DPPH (Figure 2 C) revealing the positive regression between $O_2^{\bullet}$ and MDA with DPPH scavenging (Figure 3B).

There was a significant increase of total phenolic and flavonoid contents in the wheat leaves in response to γ-irradiation. At 5KGy the total phenolic and flavonoid contents were 4.5- and 4.9-fold respectively compared to control. These observations were associated with a significant increase of PAL activity (Figure 3A, 4)

In the present study exposure of wheat grains to γ-irradiations resulted in a significant increase of SOD and POD activities in 21-d old leaves (Figure 4).The increase in SOD in the leaves of 1KGy and 5 KGy-irradiated wheat leaves was 2.1- and 2.7-fold respectively, compared to control, whereas the increase in POD activity was 1.6- and 1.8-fold respectively. Conversely, there was a significant decrease of CAT activity in the leaves with increasing γ doses. The decrease of CAT activity in the leaves of 1 and 5 KGy was 19% and 56% respectively compared to control.

The leaf material from non-irradiated plant showed a typical ellipsoid chloroplasts with well defined chloroplastic membrane and definite grana, developed mitochondria (M), clear nucleus (N) surrounded with nuclear membrane (NM) and containing a dense chromatin material (CM). In contrast, the chloroplast structure in leaves of plants issued from γ-irradiated wheat grains showed a disturbance in thylakoids and dilation of grana as well as chloroplastic membranes, disintegration of mitochondria and chromatin materials (Figure 5).

Table 1: Effects of γ-irradiation treatments on the germination percentage and Amadori products of wheat grains.

Treatment (KGy)	Germination %	Amadori Products (ΔOD)
0	100	0.017[a]
0.5	98	0.019[a]
1	89	0.024[b]
2	76	0.031[c]
5	63	0.044[d]

Each reading represents the mean of the three replicates.
Different letters indicate significant differences at $p \leq 0.05$.

Table 2: Effects of γ-irradiation treatments on the fresh and dry biomasses of the shoot and root, RWC and MI of leaves in 21-day-old wheat plants.

Treatment (KGy)	FM (g plant $^{-1}$)		DM (g plant $^{-1}$)		Shoot height (Cm)	RWC %	MI %
	Shoot	Root	Shoot	Root			
0	6.27±0.332[b]	2.33±0.043[b]	1.01±0.033[a]	0.48±0.061[b]	15.6±1.231[b]	53	11.31
0.5	7.61±0.445[ab]	2.97±0.121[a]	1.44±0.045[a]	0.54±0.025[ab]	16.4±0.881[ab]	64	16.37
1	8.92±0.465[a]	3.11±0.322[a]	1.88±0.051[a]	0.67±0.034[a]	17.7±1.293[a]	72	17.58
2	4.38±0.680[c]	1.84±0.134[c]	0.66±0.073[b]	0.24±0.013[c]	13.2±0.988[c]	51	10.9
5	3.14±0.883[c]	1.26±0.032[c]	0.53±0.021[c]	0.18±0.033[d]	10.8±0.789[d]	48	8.54

Each reading represents the mean of the three replicates ±SD.
Different letters indicate significant differences at $p \leq 0.05$.

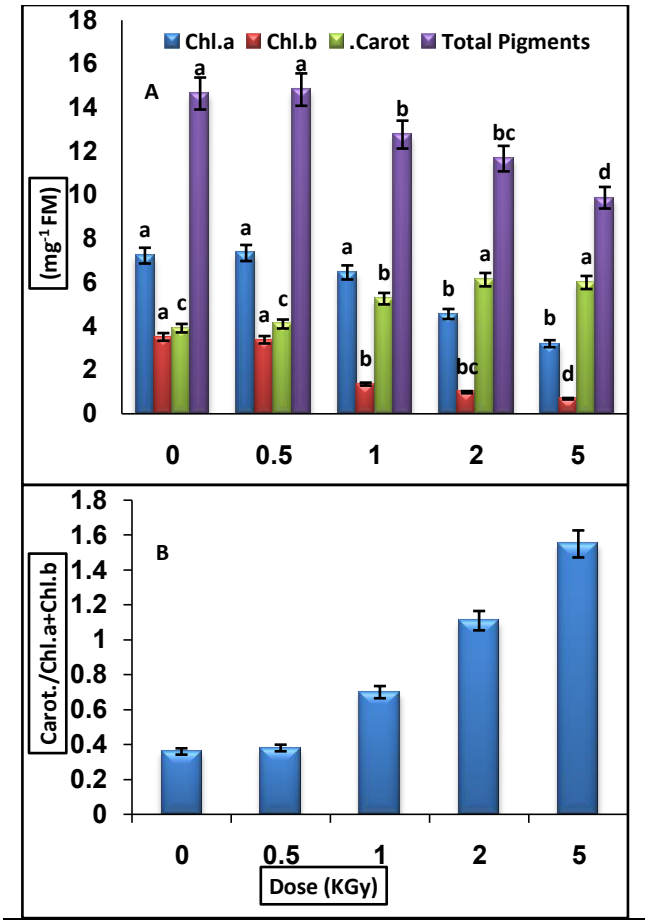

Figure 1: Effects of γ-irradiation treatments on photosynthetic pigments contents of wheat leaves. Each point represents the mean of three replicates±SD. Different letters indicate significant differences at $p \leq 0.05$.

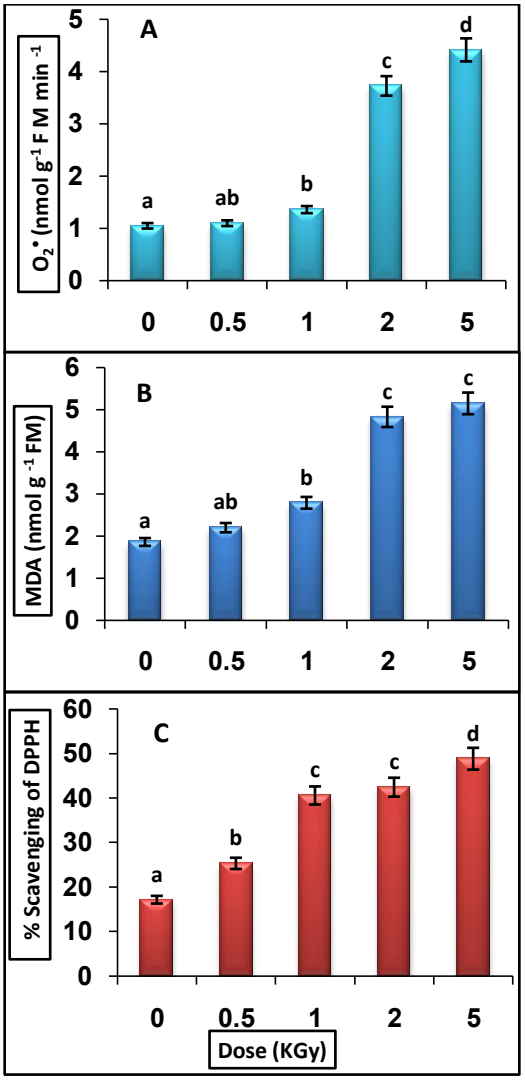

Figure 2: Effects of γ-irradiation treatments on **(A)** $O_2^{\bullet}$ generation rate**, (B)** MDA concentration, and **(C)** % scavenging of DPPH in wheat leaves. Each point represents the mean of three replicates±SD. Different letters indicate significant differences at $p \leq 0.05$.

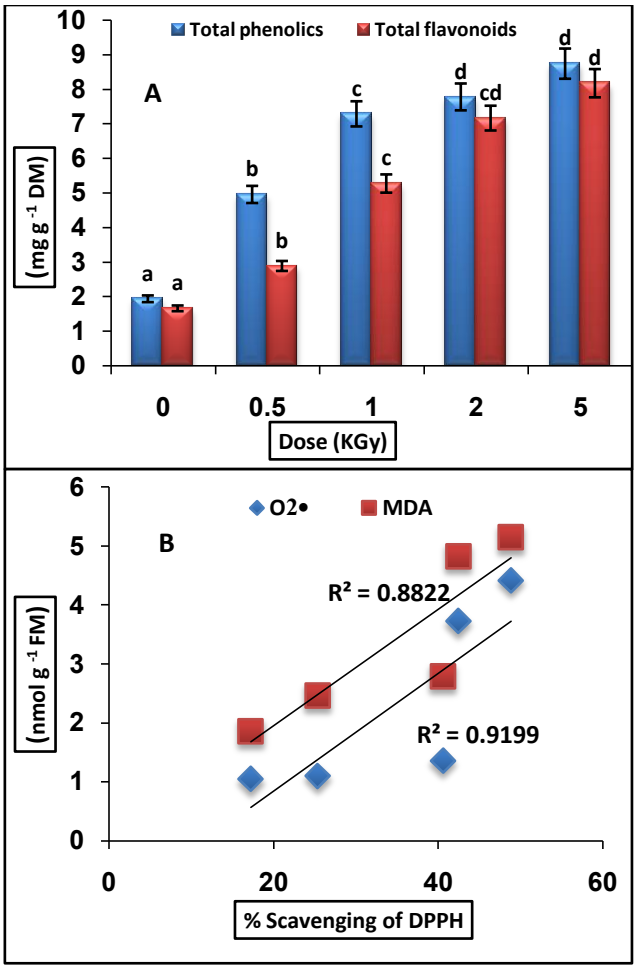

Figure 3: Effects of γ-irradiation treatments on (**A)** total phenolics and total flavonoids content, and (**B**) linear regression analysis between $O_2^{\bullet}$ and MDA and % scavenging of DPPH of wheat leaves. Each point represents the mean of three replicates±SD. Different letters indicate significant differences at $p \leq 0.05$.

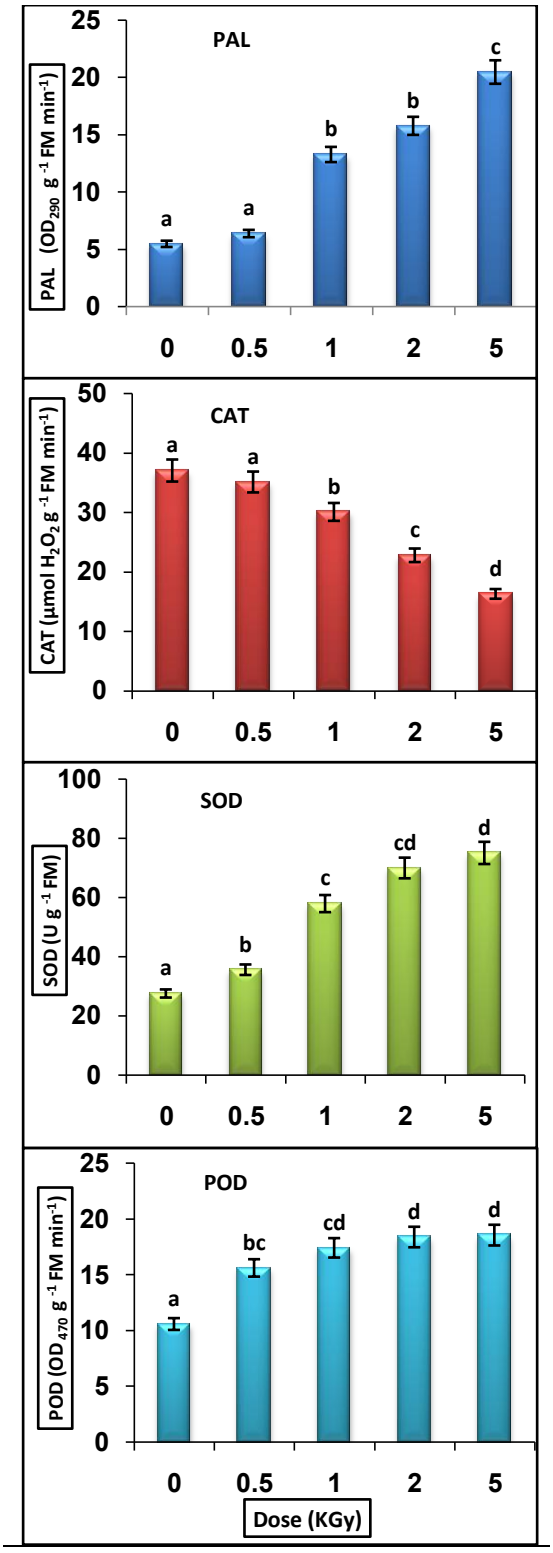

Figure 4: Effects of γ-irradiation treatments on the activity of Phenyl alanine ammonia lyase (**PAL**), Catalase (**CAT**), Superoxide dismutase (**SOD**), and Peroxidase (**POD**) in wheat leaves. Each point represents the mean of three replicates ±SD. Different letters indicate significant differences at $p \leq 0.05$.

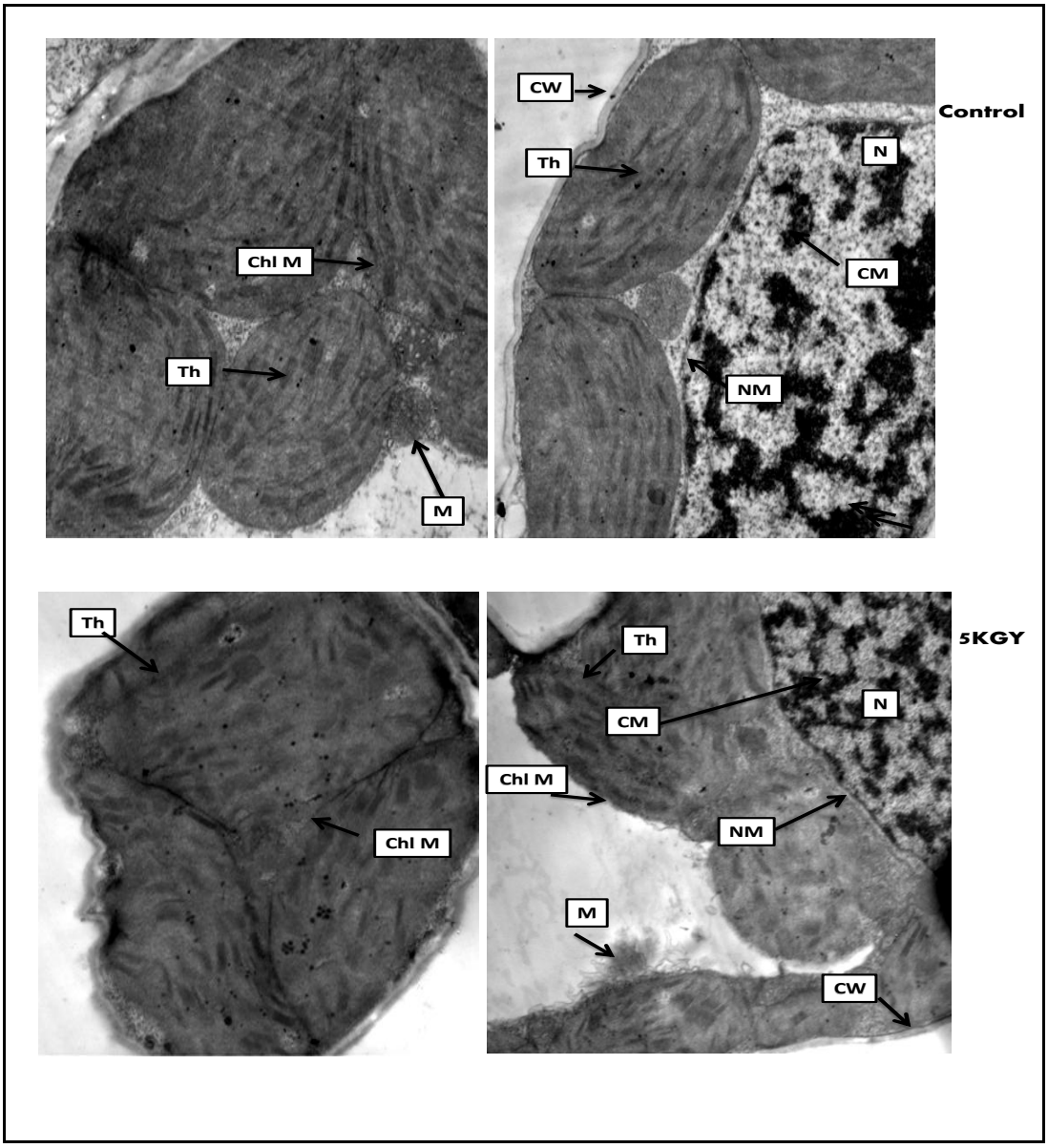

Figure 5: Transmission electron micrographs of leaf mesophyll cells irradiated with 5 KGy gamma rays and non-irradiated (control) from wheat plant.
Note: (**CW**) cell wall, (**N**) nucleus, (**NM**) nuclear membrane, (**CM**) chromatin material, (**Th**) thylakoid, (**Chl M**) chloroplast membrane, **(M)** mitochondria.

DISCUSSION

There was a significant decrease of germination percentage (G %) with increasing γ- radiation doses (Table 1). This observation was coincide with those reported by other investigators (**Chaudhuri, 2002**; **Irfaq and Nawab, 2003; Elangovan and Pavadai, 2014**) who reported that γ- irradiations caused adverse effects on the germination of various seeds. In this study, the significant decrease of G% might be related to the synthesis of some components implicated in the inhibition of germination and decrease of the multiplication of genetic materials and cell division. **Thapa (2004)** reported that γ- rays are well known with their effects on plant growth and development by inducing cytological, physiological and morphological changes in cells and tissues, while **Jagetia (2007**) and **Askari et al. (2014)** concluded that the most important target in the cell damaged by gamma radiation is genomic DNA. In the present study, there was a significant accumulation of Amadori products in the germinating wheat grains in response to γ- irradiation. This observation might be related to non enzymatic reaction of reducing sugar formed from

hydrolysis of storage carbohydrates, and the secondary products of lipid peroxidation with terminal amino acids of proteins (**Ory and Angelo**, **1982**; **Sun and Leopold, 1995**). Furthermore, **Murthy and Sun (2000**) reported that increasing of reducing sugars during storage might initiate Amadori reactions which accumulated and in turn causing protein and DNA damage. Earlier, **Castilho et al. (1994)** stated that the formation of Schiff bases was observed between peroxidized phospholipids and membrane proteins which suspected that the second products of peroxidation might participate in non-enzymatic protein and DNA degradation through Amadori and Maillard reactions. Therefore, the inhibition of germination percentage of γ-irradiated wheat grains could relate to degradation of protein and DNA through Amadori products.

In the present study**,** 1KGy improved the measured growth parameters (FM, DM, and shoot height) of wheat plants issued from irradiated grains. This observation was accompanied with a significant increase of RWC, MI, and DPPH as well as a significant decline in $O_2^{\bullet}$ and MDA contents. Thus, this improvement of plant growth could be attributed to increase of water content and plasma membrane stability. **Melki and Marouani (2009)** reported that high water content in the tissues of wheat plants issued from 20Gy γ rays- irradiated grains and grown under drought stress has protective effects on plasma membranes stability. On the other hand, the significant reduction of measured growth parameters of wheat plants under higher γ doses (Table 1) might be related to deleterious effect of highly γ rays doses on the cell ultra- structure (Figure 5) and various biochemical reactions. It is clearly demonstrated that at high irradiation, there was a significant accumulation of $O_2^{\bullet}$ and MDA content revealing the production of toxic ROS which exert an inhibitory effect of most biochemical reactions and destruction of plasma membrane integrity **(Khattak and Simpson, 2010).** Gamma irradiation was reported to induce oxidative stress with overproduction of reactive oxygen species which react rapidly with almost all structural and functional organic molecules (**Al-Rumaih and Al-Rumaih, 2008**).

It is clearly demonstrated that Chl.a and Chl.b as well as total photosynthetic pigments in the leaves of wheat plants issued from various γ-irradiated grains were significantly decreased compared to untreated plants. These observations in agreement with other reports of many investigators (**Strid et al.,1990; Kiong et al., 2008; Saha et al.,2010**).The reduction of Chl.a and Chl.b ,in the present study, could be attributed to inhibition of chlorophyll biosynthesis or enhancement of their degradation (**Sreedhar et al., 2013)**.In addition the early increase of Amadori products during germination of wheat grains might be introduced in the inhibition of Chl.a and Chl.b biosynthesis via a decrease the chloroplastic-associated proteins and degeneration of DNA. Also, the accumulation of $O_2^{\bullet}$ and MDA in wheat leaves might indicate the destruction of chloroplast and thylakoid membranes. On the other hand, total carotenoids and Carot./Chl.a+Chl.b of wheat leaves, in the present study, were markedly increased under γ-irradiation compared to untreated plants. **De Pascale et al. (2001)** reported that carotenoids react with lipid peroxidation products to terminate chain reactions, while **Loggini et al. (1999)** concluded that carotenoids directly reacted with singlet oxygen. These observations might reveal the protective role of carotenoids against the oxidative stress caused by γ-radiation on photosynthetic apparatus of wheat plants.

Sandeep and Nair (2010) reported that methanolic extract of γ- irradiated Acorus calmus increased the scavenging activity of DPPH radical, in a concentration dependant manner, depending upon the inhibition of degradation of DNA. Furthermore, **Rajurkar et al., (2012)** showed a significant change in DPPH activity after γ-irradiation of Justicia adhatoda and maximum increase was found at 5 KGy. They concluded that this increase in DPPH activity might be attributed to Amadori and Millard reaction products formed during irradiation of samples, in which these products are able to scavenge OH^- and $O_2^{\bullet}$ radicals. In agreement with these observations, there was a marked increase of scavenging of the stable free radical DPPH in wheat leaves up to 1KGy which indicating scavenging of OH^- and superoxide radicals preventing them to induce a marked damage of chloroplastic apparatus, hence improved the growth.

In the present study, there was a significant accumulation of total phenolic and flavonoid contents in the leaves of γ-irradiated wheat plants. This accumulation was accompanied with an increase of POD activity (Figure 4) revealing the enhancement of antioxidant system for the scavenging the generated ROS, due to gamma radiation, using phenolic compounds as reductants. Many authors (**Horvathova et al., 2007; Costa de Camargo et al., 2012; Akshatha et al., 2013)** have been concluded that phenolic compounds and flavonoids act as antioxidants via their ability to donate hydrogen atoms or electrons, and also to their stable radical intermediates which prevent the oxidation of fatty acids. Thus, the increase of phenolic compounds content in wheat leaves, in the present study, could be attributed to the enhancement the synthesis of phenolics and degradation of the large phenolics into stable intermediates by γ-irradiation. The data in Figure 4 demonstrated that a significant increase of PAL activity with increasing the doses of γ-irradiation indicating the induction of phenolics biosynthesis and therefore increasing their accumulation.

In the present study, both SOD and POD were significantly increased with increasing γ doses. Similar observations were mentioned by many authors (**Hameed et al., 2008; Kiong et al., 2008; Borzouei et al., 2013**).

Ling et al. (2008) cited that the induction of SOD and POD by irradiation would be one of the defense systems activated ROS-mediated cellular signaling. As observed in the present study, the significant increase of phenolic compounds and radical scavenging (DPPH) was accompanied with an increase of SOD and POD activities indicating the inductions of enzymic and non-enzymic defense systems in the wheat leaves upon γ-irradiations. In contrast, the decrease of CAT activity might be related to the inhibition of posttranscriptional of CAT protein by Amadori products at early germination state. There are some reports showing that the activities of enzymes involved in reactive oxygen species scavenging were altered by several environmental stresses, including gamma irradiation (**El-Beltagi et al., 2011; Afify et al., 2012**).

As shown in Figure 5, non-irradiated leaf was characterized by well defined cellular components including chloroplasts, grana structure, mitochondria, nucleus and nuclear membrane. In contrast, 5KGy irradiation resulted in a severe damage in chloroplast structure leading to lose its integrity. The pattern of grana and thylakoids as well as chloroplast membrane has been dilated. Furthermore, a serious ultra-structure was induced by gamma rays included destruction of nuclear membrane and dilation of chromatin materials as well as gradually degeneration of mitochondria. In the present study, those destruction effects might be related to generation of ROS which resulted in impairment of photosynthesis and ATP production, due to destruction of chloroplast, and mitochondria, therefore, the suppression of wheat growth. **Khattak and Simpson (2010**) reported that gamma irradiation induced oxidative stress to various cellular components with over production of ROS causing disturbance of cellular structure and metabolism. In addition **Yoshikawa et al. (2000)** stated that radiation stress can comprise energy transduction by faultily synthesized proteins or lead to DNA fragmentation and decrease membrane potential. In the present study, non-enzymatic synthesis of Amadori products and generation of ROS due to exposure of wheat grains to gamma irradiation might result from devastation of cellular components including chloroplasts, nucleus and mitochondria leading to decrease the growth of wheat plants.

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REFERENCES

Afify, A.M.R., El-Beltagi, H.S., Aly, A.A. and El-Ansary, A. E. (2012): Antioxidant enzyme activities and lipid peroxidation as biomarker compounds for potato tuber stored by gamma radiation. Asian Pacific Journal of Tropical Biomedicine, S1548-S1555.

Al-Rumaih, M.M. and Al-Rumaih, M.M. (2008): Influence of ionizing radiation on antioxidant enzymes in three species of Trigonella. Am. J. Environ. Sci., 4: 151–156.

Akshatha, Chandrashekar, K.R., Somashekarappa, H.M. and Souframanien, J. (2013): Effect of gamma irradiation on germination, growth, and biochemical parameters of Terminalia arjuna Roxb. Radiat Prot Environ., 36:38-44.

Askari, M.B., Askari, N., Vahid, M. M.A., Mohsen, M. and Nasrin, A. **(2014):** Gamma rays destroy plant stress resistance genes. Greener Journal of Biochemistry and Biotechnology, 1 (1):018-022.

Bari, M.L., Nazuka, E., Sabina, Y., Todoriki, S. and Isshiki, K. (2003): Chemical and irradiation treatments for killing Escherichia coli O157: H7 on alfalfa, radis, and mung bean seeds. J. Food Prot., 66 (5): 767–774.

Begum, T. and Dasgupta, T. (2011): Effect of mutagens on character association in sesame (Sesamum indicum L.). Pak. J. Bot., 43: 243-251.

Buege, J.A and Aust S.D. (1978): Microsomal lipid peroxidation. Methods in Enzymology, 52: 302–310.

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

Borzouei, A., Kafi, M., Sayahi, R., Rabiei, E. and Sayad Amin, P. (2013): Biochemical response of two wheat cultivars (Triticum aestivum L.) to gamma radiation. Pak. J. Bot., 45(2): 473- 477.

Castilho, R.F., Meinicke, A.R., Almeida, A.M., Hermes-Lima, M. and Vercesi, A.E.(1994): Oxidative damage of mitochondria induced by Fe(II)citrate is potentiated by Ca^{2+} and includes lipid peroxidation and alteration in membrane proteins. Archives of Biochemistry and Biophysics, 308**:** 158–163.

Chandrasekharan, D.K., Kagiya, V.T. and Nair, C.K.K. (2009): Radia - on protect- on by 6- palmitoyl ascorbic acid- 2-glucoside: studies on DNA damage in vitro, ex vivo, in vivo and oxidative stress in vivo. J Radiat Res., 50: 203- 212.

Chaudhuri, K.S. (2002): A simple and reliable method to detect gamma irradiated lentil (Lensculinaris Medik.) seeds by germination efficiency and seedling growth test. Radiat. Phys.Chem., 64: 131-136.

Costa de Camargo, A., Ferreira de Souza Vieira, T.M., Regitano-D Arce, M.A.B., Calori-Domingues, M.A. and Canniatti-Brazaca, S.G. (2012): Gamma radiation effects on peanut skin antioxidants. Int. J. Mol. Sci., 13: 3073–3084.

Cuero, R.G., Smith, J.E. and Lacey, J. (1986): The influence of gamma irradiation and sodium hypochloride sterilisation on maize seed micro flora and germination. Food Microbiol, 3: 107–113.

Deshmuukh, P.S., Sairam, R.K. and Shukla, D.S. (1991): Measurement of ion leakage as a screening technique for drought resistance in wheat genotypes. Indian journal of plant physiology, 34: 89-91.

De Pascale, S., Maggio, A., Fogliano, V., Ambrosino, P. and Ritieni, A. (2001): Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. Journal of Horticultural Science and Biotechnology, 76: 447–453.

Dixon, R.A. and Paiva, N.L. (1995): Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085-1097.

EL-Bazza, Z.E.M., Farrag, H.A., El-Fouly, M.E.D.Z. and El-Tablawy, S.S.Y.M. (2001): Inhibitory effect of gamma radiation and Nigella sativa seeds oil n growth, spore germination and toxin production of fungi. Radiation physics and chemistry, 60 (3): 181-189.

El-Beltagi, H.S., Mohamed, A.A., and Rashed, M.M. (2010): Response of antioxidative enzymes to cadmium stress in leaves and roots of Radish. Not. Sci. Biol., 2: 76-82.

El-Beltagi, H.S., Ahmed, O.K. and El-Desouky, W. (2011): Effect of low doses γ -irradiation on oxidative stress and secondary metabolites production of rosemary (Rosmarinus officinalis L.) callus culture. Radiat Phys Chem., 80: 968-976.

Elangovan, R. and Pavadai, P. (2014): Effect of gamma rays on growth and yield parameters in Behendi (Abelmoschus Escuelntus (l.) Moench) in M1 generation. Elagovan . Int.J.Res.Ins., 1(1): 27-32.

Elstner, E.F. and Heupel, A. (1976): Inhibition of nitrite formation from hydroxylammonium-chloride: a simple assay for superoxide dismutase. Anal Biochem., 70:616-620.

Farkas, J. (1998): Irradiation as a method for decontaminating food: A review. International Journal of Food Microbiology, 44: 189–204.

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

Giannopolitis, C. N. and Ries, S. K. (1977): Superoxide dismutase 11. Purification and quantitative relationship with water-soluble protein in seedlings. Plant Physiology, 59:315-318.

Hameed, A., Shah, T.M., Atta, B.M., Ahsanul Haq, M. and Sayed, H. (2008): Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in Desi and Kabuli Chickpea. Pak J Bot., 40(3): 1033-1041.

Heidarieh, M., Borzouei, A., Rajabifar, S., Ziaie, F. and Shafiei, Sh. (2012): Effects of gamma irradiation on antioxidant activity of Ergosan. Iran. J. Radiat. Res., 9: 245-249.

Hoagland, D.R. and Arnon, D.I. (1950): The water culture method for growing plants without soil. California Agricultural Experiment Station,Circular, 347.

Horváthová, J., Suhaj, M. and Polovka, M. (2007): Effect of gamma irradiation on trichromatic values of spices . Chemical Papers, 61: 282–285.

Irfaq, M. and Nawab, K. (2003): A Study to Determine the Proper Dose of Gamma Radiation for Inducing Beneficial Genetic Variability in Bread Wheat (Triticum aestivum L.)**.** Asian Journal of Plant Science, 2(13): 999-1003.

Ismail, H.I., Chan, K.W., Mariod, A.A. and Ismail, M. (2010): Phenolic content and antioxidant activity of cantaloupe (Cucumis melo) methanolic extracts. Food Chem., 119: 643-647.

Jagetia, C.G. (2007): Radioprotective potential of plants and herbs against the effect of ionizing radiation. J. Clin. Biochem. Nutr. 40: 74–81.

Jiang, Y. and Joyce, D.C. (2003): ABA effects on ethylene production. PAL activity, anthocyanin and phenolic contents of strawberry fruit. Pl. Grow. Regula., 39: 171-174.

Khattak, K. F. and Simpson, T. J. (2010): Effect of gamma irradiation on the antimicrobial and free radical scavenging activities of Glycyrrhiza glabra root. Radiation Physics and Chemistry, 79: 507-512.

Kiong, A.L.P., Lai, A.G., Hussein, S. and Harun, A.R. (2008): Physiological responses of Orthosiphon stamineus plantlets to gamma irradiation. Am-Eurasian. J. Sustain. Agric. 2:135–149.

Lichtenthaler, H.K. (1987): Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. 148:350–382.

Ling, A.P.K., Chia, J.Y., Hussein, S. and Harun, A.R. (2008): Physiological responses Ling APK, Chia JY, Hussein S, Harun AR. Physiological responses of Citrus sinensis to gamma irradiation. World Appl Sci J., 5(1): 12-19.

Loggini, B., Scartazza, A., Brugnoli, E. and Navari-Izzo, F. (1999): Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiology, 119:1091–1099.

Loreto, F., Mannozzi, M., Maris, C., Nascetti, P., Ferranti, F. and Pasqualini, S. (2001): Ozone quenching properties of isoprene and its antioxidant role in leaves. Plant Physiol., 126: 993–1000.

Melki, M. and Marouani, A. (2009): Effects of gamma rays irradiation on seed germination and growth of hard wheat. Environ Chem Lett., Doi: 10.1007/s10311-009-0222-1.

Murthy, U.M.N. and Sun, W.Q. (2000): Protein modi®cation by the Amadori and Maillard reactions during seed storage: roles of sugar hydrolysis and lipid peroxidation. Journal of Experimental Botany, 51: 1221-1228.

Noreen, Z. and Ashraf, M. (2009): Changes in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (Raphanus sativus L.), Environ. Exp. Bot., 67: 395–402.

Ory, R.L. and Angelo, A.J. St. (1982): Effects of lipid oxidation on proteins of oilseeds. In: Cherry JP, ed. Food protein deterioration: mechanisms and functionality. Washington.

Rajkowski, K. T., Boyed, G. and Thaylar, D.W. (2003): Irradiation D- value E. coli O157:H7 and Salmonella sp on inoculated broccoli seeds and effects of irradiation on broccoli sporut keeping quality and seed viability, 66(5):760–766.

Rajurkar, N.S. and Gaikwad, K. (2010): Effect of Gama radiation on Free Radical Scavenging Activity of Sphaeranthus Hirtus Willed. J. Indian Counc. Chem., 27: 1–3.

Rajurkar, N. S., Gaikwad, K. N., and Razavi, M. S. (2012): Evaluation of free radical scavenging scavenging activity of Justicia adhatoda: A gamma radiation study**.** International Journal of Pharmacy and Pharmaceutical Sciences**,** 4 (4): 93-96.

Saha, P., Raychaudhuri, S.S., Chakraborty, A. and Sudarshan, M. (2010): PIXE analysis of trace elements in relation to chlorophyll concentration in Plantago ovata Forsk. Appl. Radiat. Isot., 68: 444–449

Sandeep, **D. and Nair, C.K.K. (2010**)**:** Protection of DNA and membrane from γ-radiation induced damage by the extract of Acorus calamus Linn.: An in vitro study. Environmental Toxicology and Pharmacology, 29:302–307.

Sommers, C.I.I. and Fan, X. (2002): Antioxidant power, lipid oxidation, color and viability of Listeria monocytogenes in beef bologna with gamma radiation and containing various levels of glucose. J. Food Prot., 65: 1750–1755.

Sreedhar, M., Anurag, C., Aparna, M., Pavan, K.D., Singhal, R.K. and Venu-Babu,P. (2013): Influence of γ-radiation stress on scavenging enzyme activity and cell ultrastructure in groundnut (Arachis hypogaea L.) Advances in Applied Science Research, 4(2):35-44.

Strelec I., Ugarčić-Hardi, Ž. and Hlevnjak, M. (2008): Accumulation of Amadori and Maillard products in wheat seeds aged under different storage conditions. Croat Chem Acta, 81: 131-137.

Strid, A., Chow, W.S. and Anderson, J.M. (1990): Effects of supplementary gamma irradiation on photosynthesis in Pisum sativum. Biochem., 1020: 260–268.

Sun, W.Q. and Leopold, A.C. (1995): The Maillard reaction and oxidative stress during ageing of soybean seeds. Physiologia Plantarum, 94**:**94–105.

Thapa, C.B. (2004): Effect of acute exposure of gamma rays on seed germination and seedling growth of Pinus kesiya Gord and P.wallichiana A.B. Jacks. Our Nature 2:13-17.

Turner, N.C. (1981): Techniques and experimental approaches for the measurement of plant water status, Plant and Soil, 58, 339–366.

Wettlaufer, S.H. and Leopold, A.C. (1991): Relevance of Amadori and Maillard products to seed deterioration. Plant Physiology, 97**:**165–169.

Wolfe, K., Wu, X. and Liu, R. H. (2003): Antioxidant activity of apple peels. Journal of Agriculture and Food Chemistry, 51: 609-614.

Xienia, U., Foote, G.C., Van, S., Devreotes, P.N., Alexander, S. and Alexander, H. (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.

Yoshikawa, T., Toyokuni, S. Yamamoto, Y. and Naito, Y. (eds) Free Radicals in Chemistry Biology and Medicine, OICA International, London, 2000.

Zhang, H., Jiang, Y., He, Z., Ma, M. (2005): Cadmium accumulation and oxidative burst in garlic (Allium sativum), J. Plant Physiol., 162: 977–984.