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Antioxidant enzyme activities and lipid peroxidation as biomarker compounds for potato tuber stored by gamma radiation

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ABSTRACT

Objective: To study the capability of gamma irradiation for inhibiting sprouting of potato tubers. **Methods:** The enzymes activities *i.e.* peroxidase (POD), polyphenol oxidase (PPO), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT), in addition to lipid peroxidation level were tested in potato tubers stored for 3, 6 and 9 weeks. Gamma irradiation with five treatments (0, 30, 50, 100 and 200 Gy) was used to control germination process of potato tubers. **Results:** Gamma radiation was able to maintain potato tuber for 6 weeks. The main biomarkers for validity of potato tuber during storage were studying antioxidant enzyme activities *i.e.* POD, PPO, GST, SOD, CAT enzyme activities as well as lipid peroxidation during storage time. **Conclusions:** The optimum dose was 50 Gy which prevented the sprouting initiation all over the storage period without casting undesirable rotting for potato tubers. At this dose all antioxidant enzyme activities *i.e.* POD, PPO, GST, SOD, CAT enzyme activities as well as lipid peroxidation level during storage time recorded the best rates.

1. Introduction

Sprouting of potato tuber represents a loss of material to the tubers and accelerated loss of water through the permeable surface of the sprout. Due to increasing concern for consumer health and safety, there is considerable interest in finding effective potato sprouting suppressants that have a negligible environmental impact. Appropriate and efficient post harvest technology and marketing are critical to the entire production-consumption system of potato because of its bulkiness and perish ability. It's very important to use natural products compounds such as essential oils as well as the pure compound derived from essential oils or alcoholic extracts[1-5]. On the same time it's not advisable to use pesticides because of its bad impact on the health of consumer[6,7]. According to FAO Egypt is the first producer and exporter of potatoes in Africa[8]. In 2009, Egypt produced 4 million metric tons of potatoes and exported 52 908 metric tons valued at nearly 106 587 $ US million to Europe and the Arab countries.

Potatoes being a living organism require an effective management for storage. Quality of the potatoes cannot improve during storage. Bruise prevention is an important part of keeping quality of potatoes with minimum weight loss and storage diseases. Irradiation is a physical process that could be applied to harvested fruits and seeds to eliminate microorganisms, insects and plagues as well as delay ripening or spoilage, in turn lengthening its shelf life[11,12]. Without sprout suppressants, potatoes for the fresh market would have to be stored at temperatures cold enough to prevent sprouting or snipped long distances from winter production areas. Potatoes for processing cannot be stored at cold temperatures because of the resulting accumulation of reducing sugars that negatively affect quality of the processed product. Processing would then be limited to the period from harvest to dormancy break and the output of all processing facilities would be drastically reduced. Previous research has shown that gamma radiation can be an effective sprout suppressant[13]. Thomas has summarized much of the early research on potato irradiation and indicated a dose of 10 krad (100 Gy) sufficient to inhibit

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sprouting regardless of variety and storage temperature[14]. This dose is effective in preventing sprout development regardless of the radiation source. Gamma radiation from radionuclide sources and radiation from a linear accelerator have both been used with great success[15]. Todoriki and Hayashi indicate potatoes treated with low energy electrons have lower concentrations of reducing sugars after three months in storage than potatoes stored at cold temperatures (5 ℃)[16]. For potatoes destined for the fresh market, reducing sugar concentrations are not an important quality aspect. However, potatoes destined for processing may require a lower concentration of reducing sugars than the concentration that may occur as a result of irradiation for sprout inhibition.

The radiation dose of 50 Gy was the most effective dose that gave the lower percentage of rotting and completely inhibited the sprouting during the storage period[17]. Lipid peroxidation is a major cause for the deterioration of fat-containing food. It initiates other undesirable changes in food, affecting its nutritional quality, color, flavor and texture. Auto-oxidation of polyunsaturated lipids involves a free radical chain reaction, generally initiated by exposure of the lipids to salt, Fe deficiency, drought, light, roasting, metal ions or metaloprotein catalysts, lead toxicity, nematode infection, organisms and micro-organisms[18-28]. Therefore, the inhibition of free radical oxidation by antioxidants is of great practical importance in preserving polyunsaturated lipids from deterioration[29,30]. In the case of moderate stress, such as low dose of gamma radiation the adaptability capacity of the plants is preserved and changing in protein solubility and trypsin inhibitor activity were carried out[31].

The aim of the present investigation is to study the capability of gamma irradiation for inhibiting sprouting of potato tubers (*Solanum tuberosum* L.) cv. Diamont during storage and extending its shelf life instead of using pesticides chemicals which have bad impact on human consumer[32]. Doses levels of γ-irradiation 0, 30, 50, 100 and 200 Gy were applied to maintain potato tuber for longer times which reached 6 months. One of the main biomarker for validity of potato tuber during storage is studying antioxidant enzyme activities: peroxidase, polyphenol oxidases, glutathione-S-transferase, superoxide dismutase, catalase enzymes activities as well as lipid peroxidation.

2. Materials and methods

Potato tubers (*Solanum tuberosum* L. ssp. tuberosum) cv. Diamont of a uniform size (60-65 mm) was obtained at harvest time from the farm of Faculty of Agriculture Cairo University. They were washed and allowed to dry at room temperature and then divided to groups according to the different treatments.

2.1. Experimental design

Five stored boxes with small iterance door (32.5 cm × 32.5 cm × 39.5 cm) were manufactured, 5 for samples were treated by gamma radiation, in addition to control.

2.2. Sprout inhibitor treatments

Potato tubers cv. Diamond were packed in polyethylene high density bags and radiated at different dose levels of γ-irradiation (0, 30, 50, 100 and 200 Gy) at room temperature (25±1 ℃). Gamma irradiation was performed using a gamma cell 200 apparatus equipped with a Co6o γ source (dose rate, 6.5 kGy/h) at the National Center for Irradiation Research and Technology, Cairo, Egypt. Packed tubers samples without irradiation served as the control.

2.3. Biochemical analysis

2.3.1. Preparation of enzyme extracts

Ground samples, (10.0 g each) were crushed into fine powder using liquid nitrogen. Soluble protein was extracted by homogenizing the powder in 10 mL of 50 mM phosphate buffer (pH 7.8) containing 1 mM EDTA and 1% PVP, with the addition of 1 mM ascorbate in the case of APX assay at 4 ℃. The homogenate was centrifuged at 15 000 × g for 20 min, and the supernatant was used for the following enzyme activity assay.

2.3.2. Assay of total soluble protein

Soluble proteins were measured by the Bio-Rad micro assay modification of the Bradford, procedure using crystalline bovine serum albumin as a reference[33].

2.3.3. Assay of peroxidase activity

Peroxidase activity (POD; EC 1.11.1.7) was assayed by monitoring the increase in absorbance at 430 nm due to the oxidation of pyrogallol (→= 2.6 mM-1 cm-1)[34]. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 20 mM pyrogallol, 5 mM H2O2 and 20 μL of enzyme extract. POD activity was expressed as U/100g tuber f.w. One unit of enzyme was the amount necessary to decompose 1 μmol of substrate per minute at 25 ℃.

2.3.4. Assay of polyphenol oxidase activity

Polyphenol oxidase activity (PPO; EC 1.14.18.1) was determined by spectrophotometer at 20 ℃ in triplicate. The reaction mixture consisted of 0.1 M sodium phosphate buffer pH 6.8, 20 mM 4-dihydroxy L-phenylalanine (L-DOPA, Merck) and 50 μL of the sample. The increase in absorbance was measured in a 1 cm light path cuvette at 475 nm, in a final volume of 1 mL. PPO activity was calculated considering molar extinction coefficient for dopaquinone of 3 600 M^{-1} cm^{-1}[35]. Polyphenol oxidases activity was expressed as (U/g tuber f.w.).

2.3.5. Assay of glutathione-S-transferase activity

Glutathione-S-transferase activity (GST; EC 2.5.1.18) was measured by following the changes in the absorbance at 340 nm in a mixture containing 0.17 mM sodium phosphate buffer, pH 6.5, 1 mM GSH, 1 mM 1-chloro-2,4 dinitrobenzene (CDNB) in ethanol and enzyme extract[36]. EU refers to the amount of enzyme that catalyses the formation of 1 μmol of S-2,4- dinitrophenylglutathione min-1. Glutathione-S-transferase was expressed as (U/100 g tuber f.w.).

2.3.6. Assay of superoxide dismutase activity

Superoxide dismutase activity (SOD; EC 1.15.1.1) was measured by the photochemical method[37]. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of nitro blue tetrazolium (NBT) reduction at 560 nm in the presence of riboflavin and light. The reaction mixture contained 45 mM potassium phosphate buffer, pH 7.0, containing 0.1 mM EDTA and 13 mM methionine, 0.17 mM NBT in ethanol, 0.007 mM riboflavin and enzyme aliquot. Blanks were kept in the dark and the others were illuminated for 15 min. One unit of SOD is the amount of extract that gives 50% inhibition to the rate of NBT reduction. Superoxide dismutase was expressed as (U/100 g tuber f.w.).

2.3.7. Assay of catalase activity

Catalase activity (CAT; EC 1.11.1.6) was determined as H_2O_2 consumption measured as the decrease in absorbance at 240 nm[38]. The assay contained 50 mM KH_2PO_4/K_2HPO_4 (pH 7.0), 10 mM H_2O_2 in phosphate buffer. Extinction coefficient of 39.4 mM-1 cm-1 was used to calculate activity. Catalase activity was expressed as (U/100g tuber f.w.)

2.3.8. Lipid peroxidation (MDA contents)

The lipid peroxidation was measured in terms of malondialdehyde (MDA) content by thiobarbituric acid (TBA) reaction. The level of lipid peroxidation was expressed as mmol of MDA formed using an extinction coefficient of 155 mM^{-1} cm^{-1}[39]. MDA was expressed as (nmol g^{-1} tuber f.w.).

2.4. Statistical analysis

All analysis was performed in triplicate (n=3). Statistical analysis was done using SPSS (version 10) program. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples. *P*-values <0.05 were considered significant.

3. Results

Chemical control of potato tuber is a common method for tuber disinfestations but its extensive use has led to many problems such as the presence of undesirable residues which are conceded as potential health hazard. Development of insect resistance or tolerance to chemical and chemical fumigations fails to kill the larva that live inside the stored tuber. Therefore, three types of method including essential oils, iodine vapor and gamma irradiation were applied to store potato tuber for longer time. Since the reactive oxygen species (ROS) removal rate is controlled by antioxidant enzymes and by a variety of low molecular weight antioxidants, it is of interest to determine the global change of antioxidant activity present in potato tubers during different time of storing[40,41].

3.1. Peroxidase enzyme activity (POD)

Data presented in Figure 1 revealed that non-treated potato tubers were decayed progressively during the different storage periods and the decay percent reached its maximum value at the end of experiment. While, exposing potato tubers to gamma irradiation exerted promising effect for retarding the decay during storage and the most effective applied dose was 50 Gy. The activity of POD enzyme increased or decreased depending on gamma irradiation dose. The different applied doses of (50,100 and 200 Gy) had the ability to store potato tuber for nine weeks, therefore, the low dose of 50 Gy represents the preferable and advisable one. The activities of POD enzymes after nine weeks were 14.98, 17.41, 30.77 (U/100g tuber f.w.) corresponding to gamma rays dose levels of 50, 100, 200 Gy, respectively.

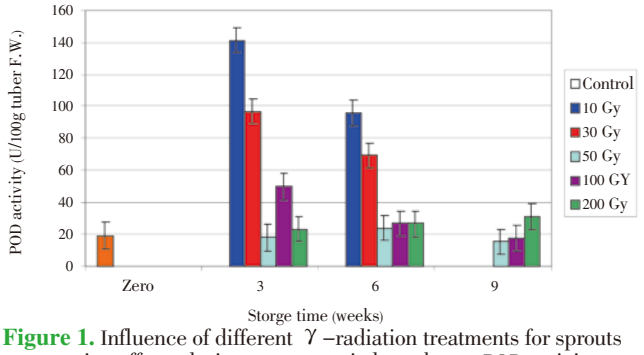

Figure 1. Influence of different γ-radiation treatments for sprouts suppression effects during storage period (weeks) on POD activity (U/100g tuber F.W.).

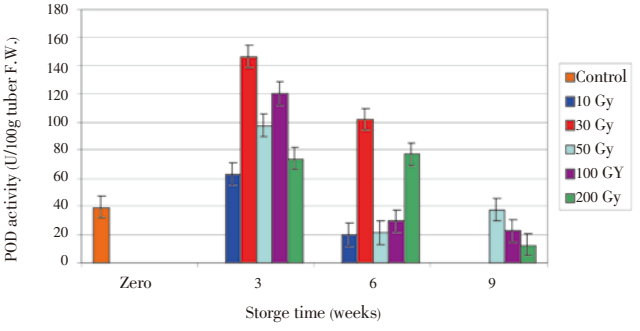

Figure 2. Influence of different γ-radiation treatments for sprouts suppression effects during storage period (weeks) on PPO activity (U/100g tuber F.W.).

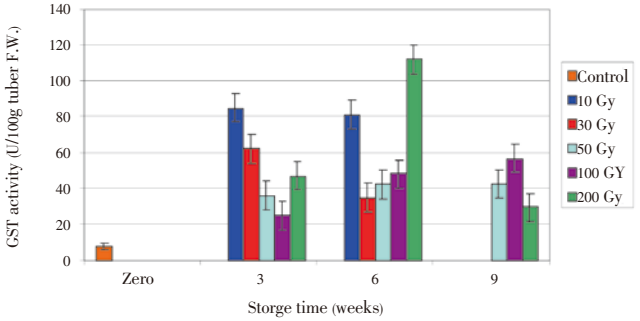

Figure 3. Influence of different γ-radiation treatments for sprouts suppression effects during storage period (weeks) on GST activity (U/100g tuber F.W.).

3.2. Polyphenol oxidase (PPO) activity

Radiation with 50 Gy represents the best treatment to store potato tuber for 9 weeks within the radiation doses ranged between 10 to 200 Gy. The activity of polyphenol oxidase initiated in the beginning of radiation especially with 30 Gy (146.4) (Figure 2). On the other hand the activity of PPO gradually increased with radiation treatments 50 to 100 Gy on week 3 with 97.3 and 120.0, while, it decreased and reached to 73.9 at 200 Gy. In contrast, it declined to reach 37.8, 22.6 and 12.6 at 50, 100 and 200 Gy, respectively, on the week 9. The general conclusions after gamma radiation with 50, 100 and 200 Gy were recorded as increment of PPO activity after 6 weeks.

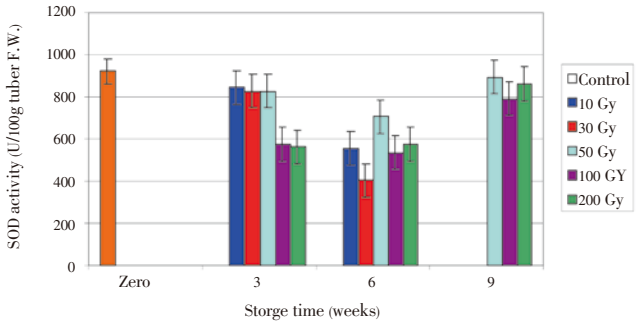

Figure 4. Influence of different γ-radiation treatments for sprouts suppression effects during storage period (weeks) on SOD activity (U/100g tuber F.W.).

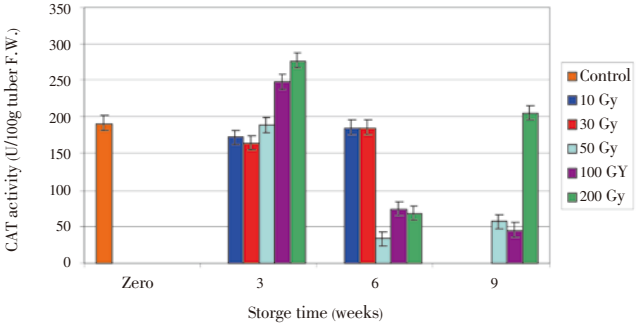

Figure 5. Influence of different γ-radiation treatments for sprouts suppression effects during storage period (weeks) on CAT activity (U/100g tuber F.W.).

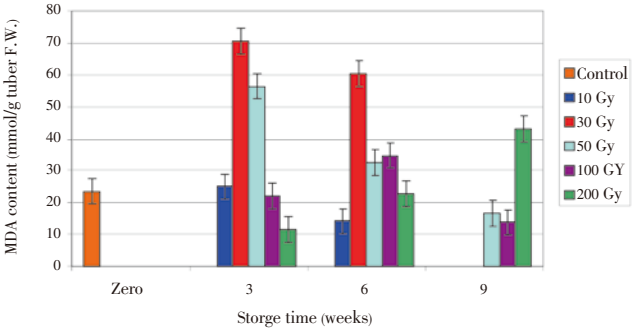

Figure 6. Influence of different γ-radiation treatments for sprouts suppression effects during storage period (weeks) on MDA content (nmol/g tuber F.W.).

3.3. Glutathione-S-transferase enzyme activity (GST)

Data in Figure 3 showed that exposing potato tubers to gamma radiation within 50 Gy represented the most effective treatment, exerted promising effect for retarding the decay during storage and inhibiting sprouting. The activity of GST enzyme activities increased or decreased depending on gamma radiation dose. We can notice that in week 6, the activities of GST enzymes with the doses of 10 and 30 Gy were higher than the control with values 81.0 and 35.0, respectively and after that tubers sprouted. On the other hand the activities of GST enzyme after nine weeks were 42.4, 56.6 and 29.5 with radiation of gamma dose 50, 100 and 200 Gy, respectively.

3.4. Superoxide dismutase enzyme activity (SOD)

It is very important to note that the activity of SOD in potato tubers after treatment with gamma radiation was small decreased in all treatment even potato maintained for 6 or nine weeks (Figure 4). With the doses of 10 and 30 Gy values enzyme activity was a little lower than control 920 with 554.2 and 404.2, respectively and it couldn't inhibit potato sprouting. However, the doses of 50, 100 and 200 Gy gave 895.8, 791.7 and 860.7, respectively and could inhibit sprouting for 9 weeks.

3.5. Catalase enzyme activity (CAT)

The data in Figure 5 showed that the activity of CAT enzyme increased after treatment and gave values of 185.0 and 184.95 with the doses of 10 and 30 Gy, respectively on the sixth week then decreased until reached the minimum 44.4 with 100 Gy. Radiation dose of 100 Gy gave the lowest activity of CAT with 44.4 but the best dose was 50 Gy which gave 56.8 CAT activity because it is the lowest dose inhibited sprouting for nine weeks.

3.6. Lipid peroxidation (MDA)

Data in Figure 6 showed that MDA which represents the oxidation process was decreased as appeared in the sixth

week 14.0 with the doses 10 and also and for doses 50, 100 and 200 which gave 16.8, 13.9 and 43.0, respectively. MDA was increased after 6 weeks then decreased in the ninth weeks with dose even 50 or 100 Gy.

4. Discussion

4.1. Peroxidase enzyme activity (POD)

Our results are in agreement with previous study, who found those irradiation doses of 300 and 600 Gy exerted lower effects for maintaining potato tubers against decay[42]. This could be attributed to their effect on the tissues of the treated tubers as it resulted in a softening for the tubers tissues making it easy to be infected by storage pathogens. Exposing potato tubers to different doses of gamma radiation significantly lowered the sprouting percent during storage and irradiation doses of 150, 300 and 600 Gy completely inhibited sprouting of potato during storage periods.

In our study the optimum dose was 50 Gy which prevented the sprouting initiation all over the storage period without causing undesirable rotting for potato tubers. The ability of gamma irradiation for inhibition sprouting could be attributed to its effect on mitotic activity and on the indole acetic acid synthesis. This was clear in our results for the different 10, 30, 50, 100 and 200 Gy. Radiation dose of 50 Gy was the best one to inhibit sprouting and at the same time kept potato from decay. On the other hand, 10, 30 Gy didn't inhibit sprouting over six weeks but the dose levels of 100, 200 Gy didn't keep tubers against decay. Various γ-irradiation doses (0, 5, 10, 15 and 20 Gy) showed a highly metabolic modification of chemical constituents and various antioxidant defense enzymes (ascorbate peroxidase, catalase and superoxide dismutase), which gradually increased in response to radiation doses[43]. The same results were obtained by other study, who found that the dose of 50 Gy irradiation treatment on the 10th day after harvest resulted in complete sprout inhibition of tubers at 8 ℃ storage and 150 Gy dose while inhibiting sprouting at 16 ℃, caused greater loss of ascorbic acid[44]. While radiation extend its shelf life by inhibition the sprouting from 20 to 200 Gy[45]. On the other hand, Hameed et al found that POD activity was enhanced by 400 and 600 Gy gamma irradiation dose in chickpea, and concluded that POD was involved in the compensatory mechanisms of inhibition of free radicals formed upon gamma irradiation of seeds[30]. In addition, other study cited that the induction of POD by the irradiation would be one of the defense systems activated ROS-mediated cellular signaling[46]. As observed in the present study, it was suggested that the increase in gamma doses corresponded to an increase in specific activity of peroxidase. Extreme increased in POD activity and the highest amount of specific activity of peroxidase was obtained in plantlets irradiated at 50 Gy. POD activity increased in a linear fashion at both doses 100 and 800 Gy[47]. Similarly, the enhancement in POD activity by radiation (5, 10 and 20 KR) has also been reported in Citrus limon[48]. It has been suggested by Rumaih that the activity and isozyme patterns of POD in Nicotiana debneyi and Nicotiana tabacum were increased in response to gamma irradiation treatment[49]. In addition, an extreme increase in POD activity was observed after gamma irradiation especially at high dosages like 50, 60 and 70 Gy[50]. Other type of ionizing radiation is UV-B which found that it increased ascorbate peroxidase, also found that under the same conditions, solar UV-B increased the total activity of ascorbate peroxidase enzyme that destroy active oxygen species and are usually increased under stress conditions[51,52]. The activity of free radical scavenging enzymes viz., peroxidase, catalase and superoxide dismutase showed inverse relationships with ageing period and direct proportion to reductions in the seed germination[53]. Moreover, antioxidant enzyme activities superoxide dismutase, catalase, ascorbate peroxidase were enhanced during the advanced phase of aging[54]. Irradiation doses of 1 or 2 kGy produced bio-chemical changes in cellular contents as well as in the cell wall constitutive networks at the same time, the mentioned changes involved an increase in the antioxidant capacity of red beet root tissue[55]. A higher activity of POD in the germinating aged alfalfa seeds was observed[56]. Other studies have shown that, apart from the accumulation of various patatin isoforms, the tuberization process is also associated with the over expression of ascorbate peroxidase[57]. In their study, Wang et al found that the activity of POD in maize pollen was decreased by the elevated UV-B radiation[58]. Superoxide dismutase, catalase and peroxidase polyphenol oxidase and glutathione-S-transferase are key enzymes of the antioxidant defense system in potato tuber well be changed in their activities after treatment with essential oils[5]. Superoxide dismutase accelerates the conversion of superoxide to H_2O_2, while catalase and POD catalyze H_2O_2 breakdown. The decreased activities of superoxide dismutase, catalase, peroxidase and DPPH-radical scavenging indicate that UV-B radiation impaired the antioxidant ability of the pollen.

4.2. Polyphenol oxidase (PPO) activity

Jimenez et al found that green onion exposure to γ-radiation produces slight increases in the polyphenol concentrations[59]. On the other hand, other study found that, there were no significant differences in the residual PPO activity after the radiation dose[60]. While Latorre et al observed that there was no change in PPO activity with 1 kGy irradiation while it increased significantly for 2 kGy[55]. Gamma rays belong to ionizing radiation and interact to atoms or molecules to produce free radicals in cells. These radicals 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 irradiation level. These effects include 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[61].

4.3. Glutathione-S-transferase enzyme activity (GST)

This result shows that higher γ-radiation kept GST values low and therefore inhibited sprouting for nine weeks. Our results are in agreement with El-Beltagi et al who found that, reduced GST is positively correlated with the increased doses of γ-radiation[43].

4.4. Superoxide dismutase enzyme activity (SOD)

Kim et al found that interesting results were noted in the investigation of ascorbate peroxidase and superoxide dismutase activities[47]. Significant differences in the changes in the two antioxidant enzymes activities were noted according to the developmental stage.

Superoxide dismutase activity was conversely increased from 2.9 U/mg protein (non-irradiation) to 11.3 U/mg protein (800 Gy treatment) at the reproductive stage. However, SOD levels of irradiated plants were lower than non-irradiated plants at vegetative stage. Our results are approved by Cakmak et al who cited that, decreased germination ability of the aged legume seeds was well correlated with the increase in lipid peroxidation levels and the decreased in the antioxidants[56]. At the same time during germination, SOD activity did not show any significant differences between the aged and non-aged seeds .The activity of SOD decrease during artificial aging of some aged seeds such as beech[62,63]. SOD activity gradually increased throughout the experiment of potato storage while treated with thiourea a dormancy breaker occurred[64]. The activity of free radical scavenging enzymes viz., peroxidase, catalase and superoxide dismutase showed inverse relationships with ageing period and direct proportion to reductions in the seed germination[53]. The results approved by Kibinza et al reported a sharp increase in the activities of SOD which was observed with increasing water content[65]. Agrawal et al have shown that, apart from the accumulation of various patatin isoforms, the tuberization process is also associated with the over expression of superoxide dismutase activity which was elevated by radiation (5, 10 and 20 KR)[57].

4.5. Catalase enzyme activity (CAT)

The activity of free radical scavenging enzymes viz., peroxidase, catalase and superoxide dismutase showed inverse relationships with ageing period and direct proportion to reductions in the seed germination[53]. Also, stated that the main reasons for loss of storability, which occurs due to decreased levels of antioxidants, reduced activity of free radical and peroxide scavenging enzymes. Antioxidant enzyme activities SOD, CAT, APX were enhanced during the advanced phase of aging[54]. CAT activity was low significantly ($P<0.01$) in the aged dry seeds of alfalfa as compared to non-aged ones[56]. A sharp increase in the activities of superoxide dismutase was observed with increasing water content[65]. Apart from the accumulation of various patatin isoforms, the tuberization process is also associated with the over expression of CAT[57]. The activity levels of CAT, the principal H_2O_2 scavenger in the peroxisomes, were reduced by 100 Gy treatments; however, the CAT activity levels were increased by 800 Gy treatments at both stages: vegetative and reproductive stage[47]. Helaly and El-Hosieny has also reported, enhancement in CAT activity by radiation (5, 10 and 20 KR) in Citrus limon[48].

4.6. Lipid peroxidation (MDA)

We could conclude from our results that accelerated ageing due to increased lipid peroxidation, decreased activities of several free radicals and peroxide scavenging enzymes and consequently MDA value as approved by Rao et al who stated that MDA content increased with degree of seed[53]. Cakmak et al reported that the level of MDA, a product of the lipid peroxidation, was significantly ($P<0.01$) high in the aged dry seeds, when compared to controls[56]. Kibinza et al found that MDA content did not markedly enlarge until water content reached 0.248 g H2Og-1 DM, and then it increased reaching 282% after 7 days of ageing at a seed[65].

Conflict of interest statement

We declare that we have no conflict of interest.

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