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# Effect of $UV_{A+B}$ on Germination Consequences, Oxidative Stress and Antioxidant Defence Mechanisms of Wheat *Triticum aestivum* L.

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**Abstract:** Stratospheric ozone depletion will increase the solar ultraviolet radiations especially in the range UV-A and UV-B. Increased levels of UV radiations affect and cause damage on cellular level on various organisms. Current research study was targeted to evaluate the effect of seed pretreatment with ultraviolet radiation on germination, oxidative damage and some antioxidant enzymes of three common wheat (*Triticum aestivum* L.) cultivars namely; Sakha-94, Gemmiza-9 and Giza-168. Seeds were irradiated to ultraviolet radiation with wavelength from 300-410 nm and maximum wavelengths of 350 nm for 6 different exposure doses (1, 5, 10, 30, 60 and 120 s). Various germination indices, oxidative stress and protective enzymes were estimated and recorded every two weeks till harvest of experiment. Ultraviolet radiation induced increases in germination consequences of wheat cultivars especially seed germination percentage and germination rate. Seed pretreatment with ultraviolet radiations induced a significant decrease on various oxidative stress damage consequences both hydrogen peroxide level and lipid peroxidation, which were monitored biweekly till harvest. Antioxidant enzymes estimated; superoxide dismutase, catalase and ascorbic acid peroxidase showed a huge and significant activation after seed pretreatment with different doses of UV-light. Data provide a new trend in priming using ultraviolet radiations of wavelengths range UV-A and UV-B

**Keywords:** priming, ultraviolet radiation; UV-A, UV-B, $UV_{A+B}$, oxidative stress, antioxidant; superoxide dismutase, catalase, ascorbic acid peroxidase APX, $H_2O_2$

## 1 Introduction

Depletion of stratospheric ozone will increase the solar ultraviolet in the range of 290-320 nm (UV-B) that reaches the surface of the earth. The increased UV-B radiation affected directly organisms living, led to variation of morphs and structure, physiological metabolic activities, genetic properties and growth cycle of many animals and plants, and might be further threaten human beings. Therefore, it is extremely important to uncover the mechanisms of effects of UV-B on organisms, especially on crops **[**1**]**.

UV-B radiation is harmful to most cultivated plants, depending on the plant species because it reduces plant height and leaf area and increases leaf thickness **[**2**]**. The impacts of UV-B radiations on plants are commonly observed by decline in chlorophyll, flavonoids, proline content, which heavily effects plant productivity **[**3,4,5**]**. Higher doses of UV-B radiation in plant cells increase reactive oxygen species, which cause ambivalent plant reactions: a part of reactive oxygen species causes oxidative stress and leads to irreversible oxidative damage of leaf tissues; another part activates the plant protection systems of different character **[**6,7**]**.

UV effect on plants occurs within the regulatory systems controlling plant response to stress causing factor **[**8,9**]**. The active oxygen species (AOS) potentially induced by UV-B radiation include not only free radicals such as superoxide ($O_2^-$) and hydroxyl radicals (•OH), but also hydrogen peroxide ($H_2O_2$) and singlet oxygen ($1O_2$). These AOS can cause oxidative damage to membrane lipids, nucleic acids, and proteins **[**10**]**. Generation of ROS is one major process for UV-B radiation to cause damages to plants. ROS is harmful to plant cells affecting plant growth and development and physico-chemical reactions **[**11-13**]**. UV-B radiation induced oxidative injury and the impact on the antioxidant system have been studied on modern hybrid rice cultivars including IR74 **[**14**]**, *Sasanishiki*, *Norin*, *Surjamkhi* **[**15**]** and *Lemont* and *Dular* **[**9**]**. Unfavourable environmental conditions lead to generation of ROS, which cause damage to cell membrane, protein and DNA **[**16**]**. MDA has been used as a reliable biomarker for measuring oxidative injury level as the content is correlated to the level of superoxidation of membrane lipid **[**17-19**]**.

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One important consequence of abiotic stresses is an increase in the cellular levels of reactive oxygen species (ROS), which show toxicity to the metabolic functions after conversion to $H_2O_2$ [20]. There is compelling evidence about the biological activity of ROS with emphasis on the function of hydrogen peroxide ($H_2O_2$) as a signal molecule in plants [21].

An important source of $H_2O_2$ during light stress is the photochemical quenching of excess light by the Mehler's reaction and by photorespiration. In $C_3$ plants, photorespiratory $H_2O_2$ production would account for the majority of total $H_2O_2$ formed [22]. The abundant $H_2O_2$ production is mainly counteracted by peroxisomal catalase, although other antioxidative enzyme is active in the leaf peroxisome [23]. Catalase is a tetrameric iron porphyrin that catalyses the dismutation of $H_2O_2$ to water and oxygen. Peroxisomal catalase, perturbed by mutation or gene silencing, results in decreased $H_2O_2$ scavenging during [24]. Constantly exposed to changing climatic conditions and abiotic factors, plants have evolved protective defence mechanisms including enzymatic and non-enzymatic antioxidants and production of secondary metabolites to counteract the destructive effect of ROS [25].

The main enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPX), ascorbate peroxidase (APX) and glutathione reductase (GR), whereas non-enzymatic portion comprised of low molecular weight antioxidants i.e. proline, thiol, ascorbic acid and glutathione [26]. Plants might use and scavenge ROS and various metabolites (glutathione, ascorbate etc.) to regulate gene expression and plant function [27]. According to Pappa *et al.* [28], superoxide dismutase (producing hydrogen peroxide from superoxide) and glutathione peroxidase (removing the hydrogen peroxide) has several postulated defences functions protecting the corneal epithelium against UV-induced oxidative damage, including the detoxification of peroxidic aldehydes, the scavenging of free radicals, and the direct absorption of ultraviolet (UV) radiation.

To minimize oxidative damage, plants have evolved various enzymatic and non-enzymatic defence mechanisms to detoxify free radical and reduce oxidative stress induced by a biotic stress (e.g. extreme temperatures, drought, salinity, UV-B, ozone and heavy metals); the antioxidant defence system includes enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidases (POD), while non-enzymatic antioxidants include ascorbic acid, proline, etc. Among the defences superoxide dismutase (SOD) are a group of enzymes that accelerate the conversion of superoxide radical to $H_2O_2$. Catalase is known to play a key role in protecting cells against oxidative stress. CAT is one of the main $H_2O_2$-scavenging enzymes that dismutate $H_2O_2$ into water and $O_2$-. Peroxidases (POD) are enzymes that catalyse the $H_2O_2$-dependent oxidation of a wide variety of substrates, mainly phenolics [29]. Understanding the mechanisms for removal of AOS is important for UV studies because increasing evidence suggests that AOS are involved in the damage caused by UV-B radiation.

For example, UV-B radiation has been shown to increase AOS levels [30-31] and lipid peroxidation in plants [32-33]. Although; it is not known how plants irradiated with UV-B generate AOS, it is thought that NADPH oxidase may be involved in the generation of AOS [34]. Other studies have shown that UV-B radiation may have an impact on the non-enzymatic antioxidants such as AsA [35-37] and GSH [35]. Differences in UV-B sensitivity between cultivars of the same species have been investigated in rice (*Oryza sativa* L.) cultivars [9,38], wheat (*Triticum aestivum* L.) cultivars [39] and cucumber (*Cucumis sativus* L.) cultivars [40].

One way of increasing germination in stress condition is priming method [41]. Seed priming is the process of regulating germination by managing the temperature and seed moisture content; in order to maximize the seed's potential. Seed priming can improve germination rate, reduce time of germination and seedling emergence and improve plant establishment. There are evidence regarding the use of chemical stimuli in accelerating growth and germination [42-43]. Growth hormone is normally used for seed priming, including auxin, abscisic acid, polyamines, ethylene, salicylic acid and ascorbic acid [44]. Primed seeds with gibberellic acid usually increases the emergence, growth and extensive of root systems. In addition, seed priming with gibberellic acid accelerates flowering, maturity and yield of plants [45]. Seed priming can increases the glucose and proline content and can improve quality of the germination and germination index in dry conditions [46,43].

Therefore, the aim of this research was to assess the priming effects of seed pretreatment with $UV\text{-}_{A+B}$ on three different wheat cultivars and determine the activity of antioxidative enzymes in response mechanisms to the UV-B radiation.

## 2 Materials and Methods

### *2.1. Plant Materials and Experimental condition*

Three common cultivars of wheat (*Triticum aestivum* L.) were selected namely; Sakha-94, Gemmiza-9 and Giza-168. Seeds were collected from the National Agricultural Research Centre, Egypt (NARC, Egypt). Seeds of each cultivar were randomly divided into two main groups; (1) control group and (2) Ultraviolet radiation group in which seeds were pretreated with different irradiation time of $UV_{A+B}$ with wavelength of 300-410 nm and maximum wavelength of 350 nm (Table 1). Plants were collected every two weeks in order to analyse various biochemical parameters. Experiments were cultivated *in vivo* on experimental site, and the germination and morphological parameters were recorded.

**Table 1:** Experimental conditions treatment of three *Triticum aestivum* cultivars

| Experimental conditions | Details |
|---|---|
| Radiation type | Ultraviolet radiations ($UV_{A+B}$) |
| UV Lamp Model | Turbo Black Light Blue FL20T8/BLB |
| UV lamp dimensions/Specs | Diameter: 38.10 mm<br>Length: 609.60 mm<br>Watts: 20 watt |
| Wavelengths | 300 - 410 nm |
| $\lambda_{max}$ | 350 nm |
| UV-Irradiation dose | 0, 1, 5, 10, 30, 60 and 120 s |
| Genotypes treated | *Triticum aestivum* L. c.v. Sakha-94<br>*Triticum aestivum* L. c.v. Gemmiza-9<br>*Triticum aestivum* L. c.v. Giza-168 |

### 2.2 Germination parameters

Standard germination data were recorded two times after 5 and 7 days following seed sowing and various indices were calculated following Ciupak *et al.* [47]. Based on the results obtained, the percentage of germinated seeds ($N_k$) was calculated by the following formula:

$$N_k = \frac{n_k}{n_c}.100\%$$

Where: $n_k$ = number of germinated seeds, $n_c$= total number of seeds sown.

Number of germinated seeds ($n_k$) was expressed as the absolute number of germinated seeds and is presented as such in all figures and tables.

The germination rate $S_k$ (seed/h) of wheat was calculated by the following equation:-

$$S_k = \frac{n_{max}}{\Delta_t}$$

Where: $n_{max}$= maximum number of germinated seeds recorded during one count, Δt= time interval between two successive counts.

The relative germination rate coefficient $W_k$ of various treated and untreated wheat cultivars was determined by the following equation:

$$W_k = \frac{n(t)}{n_{control}}$$

Where: $n_{(t)}$= number of treated seeds germinated in time t, $n_{control}$ = number of untreated seeds germinated in given time t.

### 2.3 Oxidative damage

### 2.3.1 Determination of Lipid Peroxidation

Lipid peroxidation estimated by spectrophotometric method using Thiobarbituric acid (TBA)-Malondialdehyde (TBA-MDA assay). Extraction of lipid peroxides were carried out using 500 mg fresh shoot tissues with 0.3 ml of 1% metaphosphoric acid of pH=2.0 plus 1 ml of 0.6% TBA (Thiobarbituric acid). The TBA-chromogen colour measured spectrophotometically at 532 nm [48].

### 2.3.2 Determination of hydrogen peroxide level

Leaf $H_2O_2$ concentration following irradiation with UV was measured by the FOX method [49]. 500µg of fresh leaves were extracted in trichloro-acetic acid (TCA). 500 µL of the extraction solution was added to 500 µL of assay reagent (500 µM ammonium ferrous sulphate, 50 mM $H_2SO_4$, 200 µM xylenol orange, and 200 mM sorbitol). Absorbance of the $Fe^{3+}$-xylenol orange complex was detected after 45 min at 560nm. The standard curves of $H_2O_2$ were performed using different dilution of $H_2O_2$. Data were expressed as µM $H_2O_2$ per gram of fresh weight of explants. Each data point was the average of three independent samples.

### 2.4 Antioxidant enzymes activity

The enzymes extracts were prepared by homogenizing broad bean plant in a previously chilled mortar in 20 ml chilled phosphate buffer (pH= 7.5). Centrifugation of the obtained enzyme extract carried out at 6000 rpm for 20 minutes at 5°C. Enzyme assays conducted immediately following extraction.

### 2.4.1 Superoxide dismutase activity

Superoxide dismutase (SOD) measured by the photochemical method as described by Winter *et al.* **[50]**. Assays carried out under illumination. One unit SOD activity defined as the amount of enzyme required to cause 50% inhibition of the rate of p-nitro blue tetrazolium chloride reaction at 560 nm. Cu/Zn-SOD measured by the photochemical method as described by Giannopolitis and Ries [51]. Assays carried out under illumination. One unit of SOD activity defined as the amount of enzyme required to cause 50% inhibition of the rate of p-nitro blue tetrazolium chloride reduction at 560 nm.

### 2.4.2 Catalase activity

Activity of catalase enzyme assessed by method following Aebi [52]. Catalase activity was assayed spectrophoto-metrically by following the hydrogen peroxide decomposition at 240 nm. The absorbance was recorded versus a control cuvette including enzyme solution plus $H_2O_2$-free-$PO_4$ buffer (M/15). 3 ml of $H_2O_2$-$PO_4$ transferred into the experimental cuvette, and mixed with the sample. Δt for absorbance decrease from 0.45 to 0.40 recorded, Δt used in calculations.

### 2.4.3 Ascorbic acid peroxidase (APX) activity

Ascorbic acid peroxidase (APX) activities assessed by using Nakano and Asada method [53]. The APX activity in broad bean following the pre-treatment with proline and irradiation with $UV_{A+B}$ radiations was assayed by following the hydrogen peroxide-dependent dissociation of ascorbate at 290 nm, one millilitre of the reaction mixture contained 50 mM potassium phosphate (pH=7), 0.5 mM ascorbate, 0.1 mM EDTA and 0.1 mM hydrogen peroxide. The reaction was initiated by addition of hydrogen peroxide, and oxidation of ascorbate followed by the decrease in absorbance at 290 nm at 30 seconds interval for 5 min. One unit of ascorbic acid peroxidase enzyme activity is expressed as the amount of APX enzyme that oxidizes 1 µmol of ascorbate per min at room temperature.

### *2.5 Statistical analyses*

$UV_{A+B}$ irradiation experiments were performed using completely randomized design based on 3 repetitions. Statistical analyses were performed using SPSS version 22 software in probability level of 0.05 and 0.01 and with the help of Microsoft excel 2016.

## 3 Results

### *3.1 Germination parameters*

Effects of ultraviolet radiation on the percentage of germinated seeds were presented in table (2), showing the germination percentage calculated after 120 and 168 hours following the seed sowing, calculated germination percentage after five and seven days were presented in figures (1 and 2). Seed pretreatment with $UV_{A+B}$ induced significant changes in various germination indices of the three wheat cultivars (Sakha-94, Gemmiza-9 and Giza-168). The differences between cultivars response to $UV_{A+B}$ of various parameters were assessed by two-way analysis of variance (ANOVA). A maximum of 100% germination percentages were recorded in Giza-168 at 60, 120 seconds after seven days (Table, 2). Generally, $UV_{A+B}$ significantly enhanced the germination percentage of wheat cultivars.

Germination rate were calculated from germination data according to the equation provided and were expressed as seed.h$^{-1}$ for both control and irradiated seeds. The germination rate express the rate of seed germination in time, and were presented in figure (3). The three studied wheat cultivars showed a different pattern in germination rate in response to irradiation with ultraviolet radiation. The cultivar Sakha-94 showed a clear increase in germination rate after irradiation with all irradiation doses of ultraviolet radiation, while the other two cultivars showed negative response (Figure 3). The cultivar Giza-168 showed no change in germination rate between the control and UV-treated plants and the cultivar, while, cultivar gemmiza-9 showed a decrease after 1s and then back to normal germination rate after all other irradiation doses.

**Table 2:** Germination percentage (%) of three wheat cultivars (sakha-94, gemmiza-9, giza-168) pretreated with different irradiation doses of $UV_{A+B}$ (1, 5, 10, 30, 60 and 120 s), data were recorded after 5 and 7 days (120, 168 h) and calculated as a mean of three replicas.

| The percentage of germinated seed (%) | | | | | | |
|---|---|---|---|---|---|---|
| UV-Radiation dose (s) | After 120 hours | | | After 168 hours | | |
| | S-94 | G9 | G168 | S-94 | G-9 | G-168 |
| 0 | 67.0 | 82.0 | 92.0 | 78.0 | 87.0 | 95.0 |
| 1 | 80.0 | 74.0 | 88.0 | 86.0 | 85.0 | 93.0 |
| 5 | 86.0 | 75.0 | 87.0 | 90.0 | 90.0 | 97.0 |
| 10 | 84.0 | 76.0 | 87.0 | 90.0 | 89.0 | 98.0 |
| 30 | 91.0 | 83.0 | 87.0 | 94.0 | 92.0 | 97.0 |
| 60 | 92.0 | 82.0 | 91.0 | 94.0 | 93.0 | 100.0 |
| 120 | 97.0 | 83.0 | 90.0 | 97.0 | 93.0 | 100.0 |
| Two Way ANOVA | | | | | | |
| Among cultivars | F-ratio<br>*P-value* | 57.9<br>0.000* | | F-ratio<br>*P-value* | 133.0<br>0.000* | |
| Among treatments | F-ratio<br>*P-value* | 184.0<br>0.000* | | F-ratio<br>*P-value* | 386.5<br>0.000* | |

* Significant at $p$-value<0.05

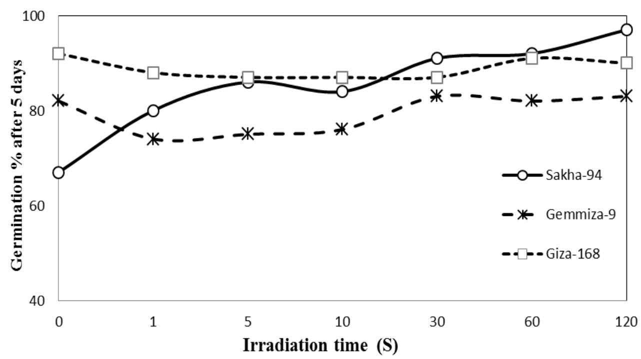


**Figure 1:** The percentage of germinated seed (%) after 5 days of three wheat cultivars (sakha-94, gemmiza-9, giza-168) pretreated with different irradiation doses of $UV_{A+B}$ (1, 5, 10, 30, 60 and 120 seconds), data were recorded after 5 days (120 h) and calculated as a mean of three replicas.

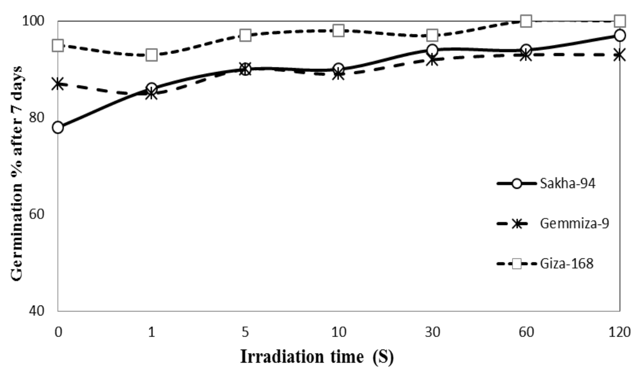


**Figure 2:** The percentage of germinated seed (%) after 7 days of three wheat cultivars (Sakha-94, Gemmiza-9, Giza-168) pretreated with different irradiation doses of $UV_{A+B}$ (1, 5, 10, 30, 60 and 120 seconds), data were recorded after 7 days (168 h) and calculated as a mean of three replicas.

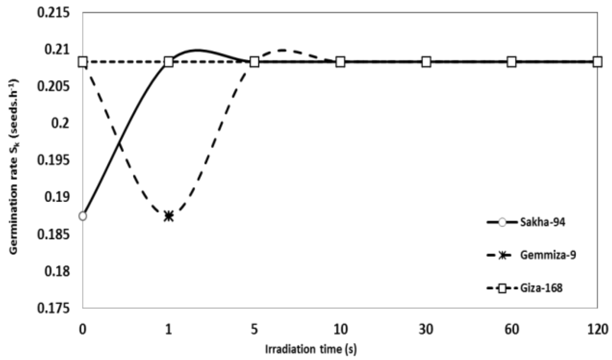


**Figure 3:** Germination rate ($S_k$, seed.$h^{-1}$) of three wheat cultivars (Sakha-94, Gemmiza-9, Giza-168) after seed pretreatment with different irradiation doses of ultraviolet radiations ($UV_{A+B}$; 1, 5, 10, 30, 60 and 120 seconds), data were recorded after 5, 7 days (120, 168 h) and calculated as a mean of three replicas.

**Table 3:** Relative germination rate coefficient ($W_k$) of three wheat cultivars (Sakha-94, Gemmiza-9, Giza-168) pretreated with different irradiation doses of $UV_{A+B}$ (1, 5, 10, 30, 60 and 120 seconds), data were recorded after 5, 7 days (120, 168 hours; respectively) and calculated as a mean of three replicas.

| The relative germination coefficient ($W_k$) | | | | | | |
|---|---|---|---|---|---|---|
| UV radiation dose (s) | After 120 hours | | | After 168 hours | | |
| | S-94 | G-9 | G-168 | S-94 | G-9 | G-168 |
| 0 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 1 | 1.19 | 0.90 | 0.96 | 1.10 | 0.98 | 0.98 |
| 5 | 1.28 | 0.91 | 0.95 | 1.15 | 1.03 | 1.02 |
| 10 | 1.25 | 0.93 | 0.95 | 1.15 | 1.02 | 1.03 |
| 30 | 1.36 | 1.01 | 0.95 | 1.21 | 1.06 | 1.02 |
| 60 | 1.37 | 1.00 | 0.99 | 1.21 | 1.07 | 1.05 |
| 120 | 1.45 | 1.01 | 0.98 | 1.24 | 1.07 | 1.05 |
| Two Way ANOVA | | | | | | |
| Among cultivars | F-ratio | 69.07 | | F-ratio | 11.42 | |
| | *P- value* | 0.000* | | *P-value* | 0.000* | |
| Among treatments | F-ratio | 2.91 | | F-ratio | 1.93 | |
| | *P-value* | 0.018* | | *P-value* | 0.099 | |

* Significant at $p$-value<0.05

## *3.2 Oxidative damage*

### *3.2.1 Lipid peroxidation level*

The data in figures (4a,b,c) showed that generally, Lipid peroxidation level significantly increased with the increasing irradiation time of $UV_{A+B}$. Using spearman rank correlation for assessment of correlation between increasing the irradiation time of $UV_{A+B}$ and Lipid peroxidation level, the test statistics analysis revealed there were a strong significant correlation for Giza-168 at the harvest phase; where correlation coefficient R at the end of experiment were (0.812) and $p$-value (0.000*); (where * is significant at $p \leq 0.05$). Using two-way analysis of variance, the differences between studied wheat cultivars were assessed. There were significant differences in Lipid peroxidation level between the three studied wheat cultivars (F=288.9, $P$-value=0.000*) at the harvest phase.

### *3.2.2. Hydrogen peroxide level ($H_2O_2$)*

The level of hydrogen peroxide accumulation in wheat leaves was estimated at different time point (2,4,6,8,10,12 weeks) for three wheat cultivars (Sakha94, Gemmiza-9 and Giza168) after seed pretreatment with ultraviolet radiations. Data of hydrogen peroxide levels were presented in figures (5a,b,c). Hydrogen peroxide level significantly increased with increasing irradiation time of $UV_{A+B.}$ Using spearman rank correlation for assessment of correlation between increasing the irradiation time of $UV_{A+B}$ and hydrogen peroxide level, the test statistics analysis revealed there were a strong significant correlation for all studied cultivars (Sakha-94, Gemmiza-9 and Giza-168) at the harvest phase; where correlation coefficient R at the end of experiment were (0.903, 0.987, 0.716) and $p$-value (0.000*, 0.000*, 0.000*); respectively (where * is significant at $p \leq 0.05$). Using two-way analysis of variance, the differences between studied wheat cultivars were assessed. There was a significant difference in hydrogen peroxide level between the three studied wheat cultivars (F=238148.6, $P$-value=0.000*) at the harvest phase.

## *3.3. Antioxidant enzymes activity*

### *3.3.1. Superoxide dismutase (SOD) activity*

Superoxide dismutases are enzymes that catalyse the dismutation of superoxide into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defence in nearly all plant cells. SOD were estimated in leaves of wheat cultivars exposed to ultraviolet radiation doses, data were presented in figures (6a,b,c). Data of superoxide dismutase revealed that wheat grain pre-treatment with $UV_{A+B}$ induced a significant increase in superoxide dismutase activity (unit/g-FW) in all wheat cultivars with increasing irradiation time at most time intervals compared with control. Sakha-94 was the most affected $UV_{A+B}$ treatments where it was recorded maximum level of SOD activity (40.69 unit/g-FW) at 120 s after 12 weeks and minimum level of SOD activity (2.5 unit/g-FW) at 5 s after 4 weeks (see figure 10). Using spearman rank correlation for assessment of correlation between increasing the irradiation time of $UV_{A+B}$ and SOD activity, the test statistics analysis revealed there were a strong significant correlation for all cultivars (Sakha-94, Gemmiza-9 and Giza-168) at the harvest phase; where correlation coefficient R at the end of experiment were (0.880, 0.865, 0.718) and $p$-value (0.000*, 0.000*, 0.000*) respectively.

The results of spearman rank correlation between increasing irradiation time of $UV_{A+B}$ and SOD activity also strong significant correlation at different time intervals were observed in all three cultivars and all times intervals except Sakha-94 after 4 weeks; where correlation coefficient R for wheat cultivars (Sakha-94, Gemmiza-9 and Giza-168) after 2, 4, 6, 8, 10 and 12 weeks were (0.718*, 0.977*, 0.752*), (0.248, 0.991*, 0.800*), (0.988*, 0.989*, 0.976*), (0.991*, 0.977*, 0.946*), (0.956*, 0.971*, 0.989*) and (0.880*, 0.865*, 0.718*); respectively. Using two-way analysis of variance, the differences between studied wheat cultivars were assessed. There was a significant difference in SOD activity between the three studied wheat cultivars (F=1799.4, *p*-value=0.000*) at the harvest stage.

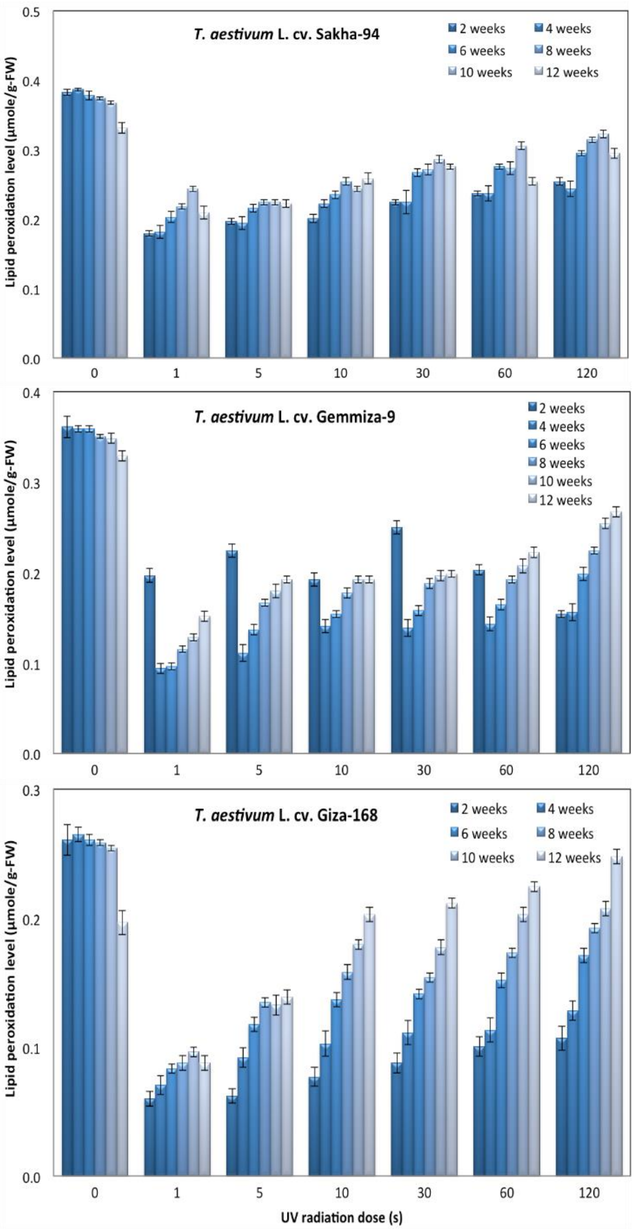


**Figure 4(a-c):** Lipid peroxidation level (µmole/g-FW) of *T. aestivum* cvs. Sakha-94, Gemmiza-9 and Giza-168; respectively, after seed pre-treatment with $UV_{A+B}$ (exposure doses of 1, 5, 10, 30, 60, and 120 s). Error bars represent calculated standard error at different time points (2, 4, 6, 8, 10 and 12) weeks of seed germination.

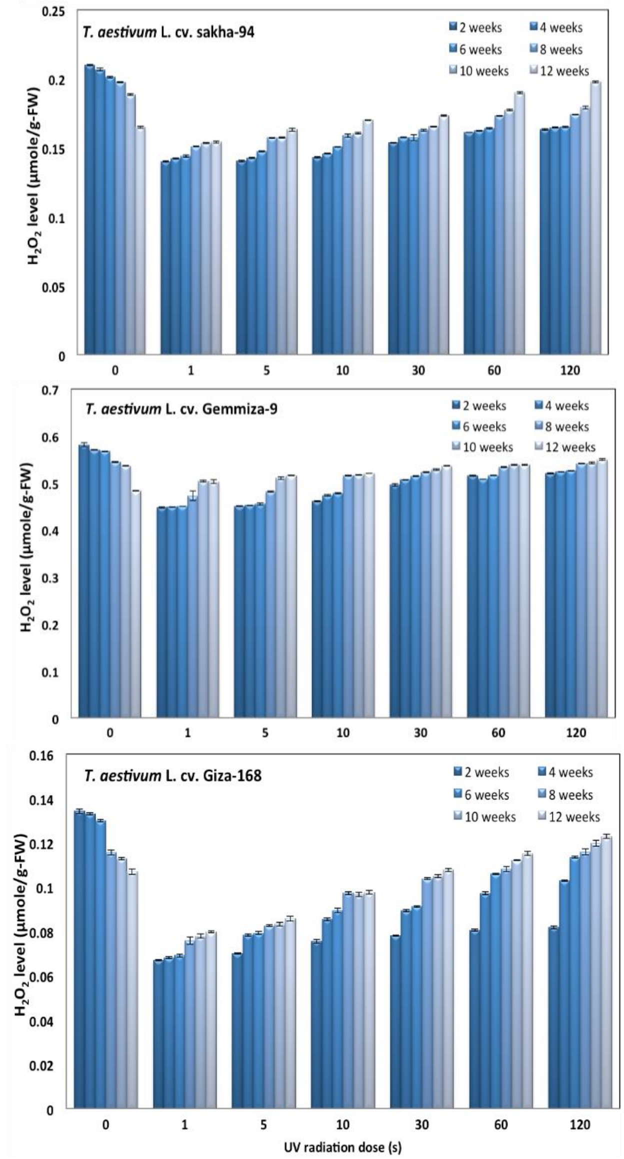


**Figure 5(a,b,c):** Hydrogen peroxide level (µmole/g-FW) of *T. aestivum* c.v. Sakha-94, Gemmiza-9 and Giza-168; respectively, after seed pretreatment with $UV_{A+B}$ (exposure doses of 1, 5, 10, 30, 60, and 120 s). Error bars represent calculated standard error at different time points (2, 4, 6, 8, 10 and 12) weeks of seed germination.

### *3.3.2. Catalase (CAT) activity*

The $UV_{A+B}$ radiation stimulated a significant decrease in catalase activity (unit/g-FW) in almost all wheat grain pre-treatment cultivars at most time intervals compared with control (figures from 13 to 15). Where, the decreasing in CAT activity was observed in Giza-168 at all times intervals with increasing the $UV_{A+B}$ irradiation time. While; in Gemmiza-9 $UV_{A+B}$ irradiation stimulated a significant increase in CAT activity after 2 weeks at all doses then; the decreasing in CAT activity was recorded after 4, 6, 8, 10 and 12 weeks at all doses except at 10 s after 4 and 6 weeks. Also; in Sakha-94 the increasing in CAT activity was detected at all doses after 2 weeks and at 10, 60, and

120 s after 4 weeks compared with control. Using spearman's rank correlation for assessment of correlation between increasing $UV_{A+B}$ irradiation time and CAT activity, the test statistics analysis revealed there were a significant correlation for all cultivars (Sakha-94, Gemmiza-9 and Giza-168) at the harvest phase where correlation coefficient R at the harvest were (-0.482, -0.683, -0.785) and *p*-value (0.027*, 0.000*, 0.000*); respectively (where * is significant at $p<0.05$). The differences between studied wheat cultivars were assessed by two-way analysis of variance. There was a significant difference in CAT activity between the three studied wheat cultivars ($F=29.87$, *p*-value=0.000*) at the harvest phase.

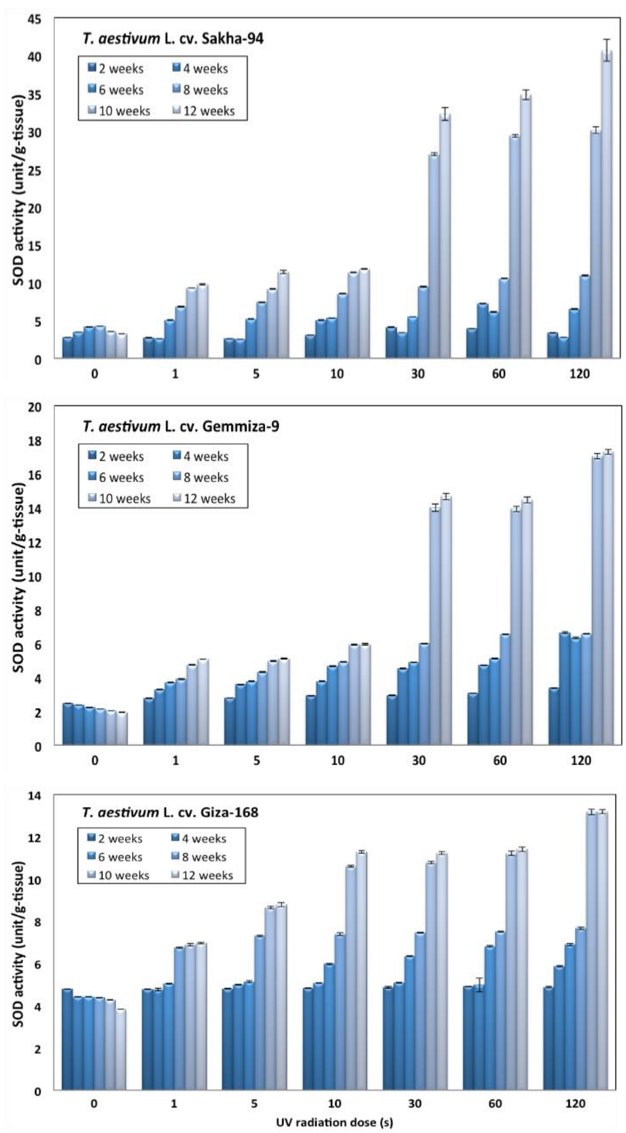


**Figure 6(a,b,c):** Superoxide dismutase activity (unit/g-FW) of *T. aestivum* c.v. Sakha-94, Gemmiza-9 and Giza-168; respectively, after seed pretreatment with $UV_{A+B}$ radiation (exposure doses of 1, 5, 10, 30, 60, and 120 s). Error bars represent calculated standard error at different time points (2, 4, 6, 8, 10 and 12) weeks of seed germination.

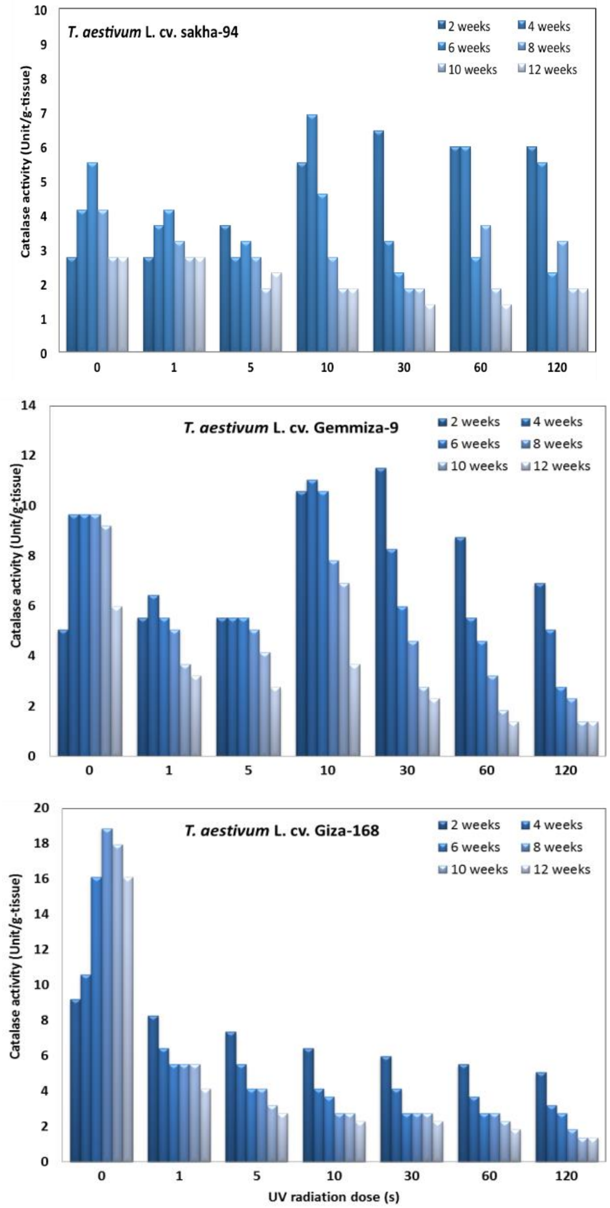


**Figure 7(a,b,c):** Catalase activity (unit/g-FW) of *T. aestivum* c.v. Sakha-94, Gemmiza-9 and Giza-168; respectively, after seed pretreatment with $UV_{A+B}$ radiation (exposure doses of 1, 5, 10, 30, 60, and 120 s). Error bars represent calculated standard error at different time points (2, 4, 6, 8, 10 and 12) weeks of seed germination.

### *3.3.3. Ascorbic acid peroxidase (APX) activity*

A major hydrogen peroxide detoxifying system in plant cells is the ascorbate-glutathione cycle, in which, ascorbate peroxidase (APX) enzymes play an important and key role in catalysing the conversion of $H_2O_2$ into $H_2O$, using ascorbate as a specific electron donor.

The enzyme ascorbic acid peroxidase activity (APX) was monitored after pretreatment with ultraviolet radiations. The data in figures (8a-c) showed that the activities of ascorbic peroxidase enzyme (APX) were slightly changing around APX activities in the control untreated plants in almost all wheat grains at most time intervals. In Sakha-94; $UV_{A+B}$ radiation induced a significant increase in APX activity after 2, 4, 6 and 8 weeks at all doses except 60 s after 2 weeks, 10 s, 30 s after 4 weeks, 5 s after 6 weeks and 10 s after 8 weeks; then a significant decreased in APX activity was detected at all doses after 10 and 12 weeks compared with the control. However, the high doses of $UV_{A+B}$ radiations stimulated a significant increase in APX activity after 2, 4 and 6 weeks in Gemmiza-9 (doses of 30, 120 s), while a significant increase in APX activity was observed in Gemmiza-9 for all doses after 8 weeks (Figure 8).

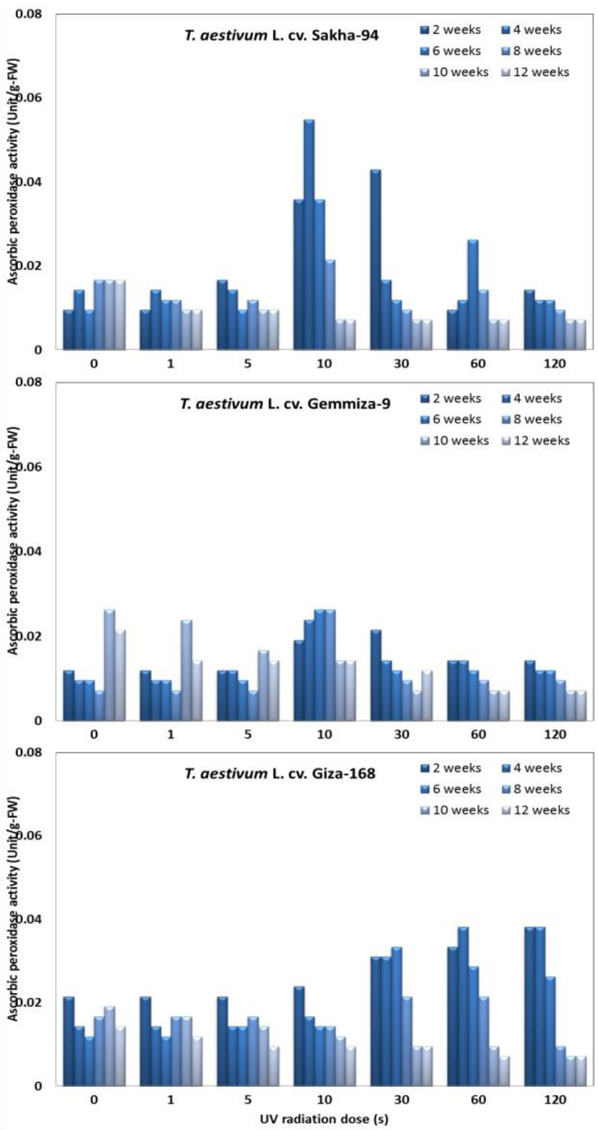


**Figure 8(a-c).** Ascorbate peroxidase activity (unit/g-FW) of *T. aestivum* c.v. Giza-168, Gemmiza-9 and Giza-168; respectively, after seed pretreatment with $UV_{A+B}$ radiation (exposure doses of 1, 5, 10, 30, 60, and 120 s). Error bars represent calculated standard error at different time points (2, 4, 6, 8, 10 and 12) weeks of seed germination.

The same trend was detected in Giza-168 where the high doses of $UV_{A+B}$ radiation stimulated a significant increase in APX activity after 2, 4 weeks (10–120 s) and 8 weeks (30, 60 s), a significant increase in APX activity was recorded after 6 weeks at all doses except 1 s while; $UV_{A+B}$ radiation stimulated a significant decrease in APX activity at all doses in Gemmiza-9 and Giza-168 after 10 and 12 weeks. Using spearman rank correlation for assessment of correlation between increasing doses of $UV_{A+B}$ radiation and APX activity, the test statistics analysis revealed there were a strong significant correlation for all cultivars (Sakha-94, Gemmiza-9 and Giza-168) at the harvest stage where correlation coefficient R at the harvest were (-0.552, -0.662, -0.526) and *p*-value (0.009*, 0.001*, 0.014*); respectively.

The differences between studied wheat cultivars were assessed by two-way analysis of variance. A significant difference in APX activity between the three studied wheat cultivars were noticed (F=3.55, *p*-value=0.038*) at the harvest phase. Using post-hoc least significant difference test statistic to comparing APX activity of treated plant groups of wheat cultivars with the untreated wheat. The data presented non-significant differences between low doses of $UV_{A+B}$ radiation treated and untreated plant group while; a significant differences were recorded between high doses of $UV_{A+B}$ radiation treated and untreated plant group.

## 4 Discussion

Ozone layer is continuously being damaged resulting in increasing the levels of UV radiation, which can be harmful for all life forms especially higher plants. UV radiation often causes different changes in physiological parameters especially in antioxidant system among plant species and genotypes. The objective of current research was to use the ultraviolet radiations ($UV_{A+B}$) as a priming tool and the possibility of seed pretreatment with UV to enhance germination, growth, anti-oxidative stress system which were monitored through the whole study at different time points (2,4,6,8,10,12 weeks).

Results of germination showed improved germination percentages and germination rates of some wheat cultivars especially Sakha-94, these were in accordance with results of Nangle *et al.* **[**54**]** in which UV-B radiations had positive impacts on germination parameters. Restricting light treatments to UV-A and UV-B wavelengths could have enhanced the biologically activity spectrum **[**55**]**. Wavelengths and energy associated with UV light may have caused a response from the light receptor phytochrome A, especially at low fluency rates, which may have irreversibly triggered germination **[**56**]**. As already noted, UV-C (100–290 nm) has been reported to inhibit germination of sunflower seeds **[57]** to inhibit germination of sunflower seeds **[**57**]**.

Hydrogen peroxide and lipid peroxidation results showed increasing levels with increasing the ultraviolet radiations doses, however, pretreatment with ultraviolet radiations may improve the response and the levels of lipid peroxidation and hydrogen peroxide. The overall increase in oxidative stress was at all doses less than the level of control group, giving positive impact of ultraviolet radiation priming.

In some cases, plants exposed to various stresses can increase $H_2O_2$ content as a strategy to trigger the activity of multiple functional enzymes as well as many metabolic pathways **[**58**]**. Generation of ROS (such as $H_2O_2$, $OH^{\bullet}$ and $O^{\bullet}$) causes rapid cell damage by triggering a chain of reactions to protect themselves from the harmful effects of oxidative stress, plants develop ROS- scavenging mechanism that involve detoxification process carried out by an integrated system of the non-enzymatic molecules and the enzymatic antioxidant **[**58-60**]**.

$H_2O_2$ received much attention as a signal molecule in response to different stresses **[**61-65**]**. $H_2O_2$ mediated the regulation of transcription in response to UV-B exposure as an important early upstream signal **[**66**]**. Activation of endogenous protective mechanisms can in turn tolerate or delete excess ROS burst. They found that the enhanced $H_2O_2$ level under the stresses was followed by the up-regulation of the enzyme activities. This suggests that $H_2O_2$ may act more as a signal molecule than directly inducing oxidative damage.

Antioxidant enzyme activities monitored after seed pretreatment with ultraviolet radiations revealed that there were an increase in level of anti-oxidative activities at different ultraviolet radiation doses. The potential long-term effects are considered to be physiological and plants do possess response mechanisms for dealing with UV-B light. These are mainly antioxidants such as superoxide dismutase **[**67,68**]**. These systems repair damage caused by excited radicals due to excess energy from UV-B radiation. There are compounds however, that may absorb the light directly and aid the reduction of UV-B light attenuation and energy dissipation in the plant such as flavonoids and carotenoids **[**69,70**]**.

The activity of antioxidant system revealed that there were a huge activation of catalase and ascorbic acid peroxidase in genotype sakha-94, gemmiza-9 and giza-168. Increasing levels of ROS due to enhancing of UV trigger the activity of several antioxidant enzymes such as superoxide dismutase, catalase and peroxidase **[**71**]**. Hollòsy **[**72**]** stated that the overall UV-B sensitive of the cells is determined by the balance of damage that occurs and the efficiency of the repair processes that can restore the impaired functions protection against oxidative stress caused by UV exposure is complex process and includes both enzymatic and non-enzymatic antioxidant **[**72**]**.

Therefore the criteria of peroxidase went opposite to the degree of tolerance among the $UV_{A+B}$ stressed genotypes. Consequently the differences in the activity of peroxidase can be used as suitable marker in the genotypic variation under stress, indicating sensitivity rather than tolerance of genotypes or varieties under different stresses.

Mishra *et al.* **[**71**]** suggest that excess UV-B radiation could promote and stimulate the generation of ROS leading to increase in the activities of antioxidant enzymes as a defence system induced antioxidant defences protecting plant against major fatal effects of ROS.

The impact of seed pretreatment with ultraviolet radiation was found to be positive effect in case of germination consequences and anti-oxidative enzyme activity and decreasing the oxidative damage of wheat plants. The priming role of ultraviolet radiation of wavelength range of UV-A and UV-B is very interesting and need more investigations especially against various biotic and abiotic stresses

## 5 Conclusion

The impact of ultraviolet radiations on germination, oxidative stress and antioxidants activities were intensively studied and monitored on three common wheat (*Triticum aestivum* L.) cultivars namely; Sakha-94, Gemmiza-9 and Giza-168. Ultraviolet radiations pretreatment induced increases in germination consequences of wheat cultivars especially grain germination percentage and germination rate. Grain pretreatment with ultraviolet radiations induced a significant decrease on various oxidative stress damage consequences both hydrogen peroxide level and lipid peroxidation, which were monitored biweekly till harvest. Antioxidant enzymes estimated; superoxide dismutase, catalase and ascorbic acid peroxidase showed a huge and significant activation after grain pretreatment with different doses of UV-light. Results provide a perspective trend in the light of effect of enhanced levels of ultraviolet radiations and in seed priming using ultraviolet radiations of wavelengths range UV-A and UV-B.

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