The Impact of Short-Wave UV Radiation on Peroxidase Activity in Soft Wheat Seeds

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Abstract. The effect of UV radiation on plants provokes oxidative stress, accompanied by the production of reactive oxygen species that can have a direct destructive effect on cellular structures and initiate free radical oxidation of proteins, lipids, and nucleic acids. There is an antioxidant defense system in plant cells that includes a complex of low and high molecular weight compounds. Antioxidants with high molecular weight are enzymes, the key of which is peroxidase [PO]. The paper studies changes in PO activity in soft spring wheat seedlings due to grain treatment with small and medium doses of short-wave UV radiation. A UV-dosed irradiation system (Bio-Link Vilber) with the exposure time 0–60 minutes in increments of 10 minutes was used for seed treatment. Pre-soaked wheat seeds were germinated in Petri dishes. PO activity was determined colorimetrically in three-day-old wheat seedlings, according to A. L. Boyarkin [3]. When the source was irradiated for up to 30 minutes, a clear dependence of the PO activity on the exposure time was not established. The maximum increase in peroxidase activity (by 25.9% relative to the control) was detected with 30-minute UV irradiation. A further increase in exposure time led to a significant decrease in enzyme activity. Moreover, vital feedback was revealed between the activity and exposure time in the range of 30–60 minutes.

Keywords: Ultraviolet radiation · Oxidative stress · Antioxidant system · Peroxidase · Soft wheat · Agricultural production

1. Introduction
During metabolism in cells and tissues of plants, active forms of oxygen [AFO] are formed. They directly affect cells and initiate free radical oxidation of proteins, lipids, and nucleic acids. AFO production in aerobic organisms occurs continuously. Therefore, in cells, there is a protective system against their harmful effects. The cells are protected from excess oxygen radicals and oxidative damage caused by an active antioxidant system, including high molecular weight compounds and low molecular weight antioxidants, including vitamins, proline, melatonin, glutathione, etc. High molecular weight components of antioxidant protection are enzymes that defend the cell from the oxidation of vital substances and components of intracellular structures. The antioxidant enzymes that neutralize AFO are superoxide dismutase [SOD], catalase, peroxidase, etc. [2, 9]. Oxidative stress occurs and develops when the balance between the formation of AFO and the functioning of the plant’s antioxidant defense system is broken due to various influences [15, 21].

The interaction of active radicals with proteins, lipids, and nucleic acids leads to mutagenesis, disruption of the structure and function of membranes (primarily in chloroplasts), a change in the
activity of enzymes, the intensity of photosynthesis, water metabolism, and, ultimately, cell cycle arrest and death. For this reason, the ability of plants to control the level of ROS in the cell can mostly correlate with their resistance to various damaging effects.

The functioning of the antioxidant defense system depends not only on the type of plant but also on the type of stressor, the degree, and duration of its action. In laboratory conditions, along with herbicides’ action, UV radiation is a convenient modulator of oxidative stress. Thus, it can be used to test the adaptive potential of plants [5, 9]. Many studies are devoted to treating seeds with long- and medium-wave UV radiation [4, 10, 11, 14, 19, 22]. A relatively smaller number of them cover the use of short-wave UV radiation for pre-sowing seed treatment. According to some authors, seed treatment with UV-C radiation leads to increased laboratory germination, activation of growth processes, and stimulation of grain productivity [7], which is associated with activation of the antioxidant system in response to ROS production [18, 20]. Pre-sowing treatment of wheat with a mercury-quartz source resulted in an increase in the mass of raw sprouts by 12%–13% and an increase in catalase activity by 6%–18% compared with the control. G. I. Ali-Zade [1] showed that hard UV-C rays, even at low doses, reduce catalase activity and prevent antioxidants from participating in the compensatory mechanisms of plant adaptation to stress. Additionally, short-wave UV rays inhibit the formation of chlorophylls a and b but stimulate the production of carotenoids [6].

One of the critical enzymes of plant antioxidant protection is peroxidase [PO]. It is involved in metabolic processes associated with breathing. An essential feature of the PO’s catalytic activity is producing free radicals to enter into spontaneous reactions. Moreover, peroxidases are sensitive indicators of environmental stresses on plants; they have high heat resistance [8]. Peroxidase increases dramatically with damage to plant cells, activating the respiration process. This enzyme is the main one in maintaining the redox balance in stressed plants [9].

UV radiation is one of the environmental factors that can induce oxidative stress. Because the software can act as a regulator of oxidative processes, it seems relevant to study changes in peroxidase activity in soft spring wheat seedlings after pre-sowing treatment of grains with small and medium doses of short-wave UV radiation.

2. Materials and Methods

The study object was soft spring wheat of the Altai Reaper variety. Grain was treated with shortwave ultraviolet radiation (λ = 254 nm) using a UV dosed irradiation system (Bio-Link Vilber) with exposure times of 10, 20, 30, 40, 50, and 60 minutes. Pre-soaked wheat seeds were germinated in Petri dishes according to GOST 12038-84 “Seeds of crops. Germination determination methods.” The total peroxidase activity was determined in 3-day-old seedlings of soft spring wheat colorimetrically at a wavelength of 590 nm [3] based on determining the reaction rate of benzidines oxidation to forming a blue oxidation product of a specific concentration. The enzyme activity (A) was calculated from the found reaction rate [16]. The experiment was performed in triplicate biological and analytical replicates. Statistical processing of experimental data was carried out using the methods of variation statistics in Microsoft Office Excel.

3. Results

UV-C radiation is an influential factor that provokes oxidative stress in plant cells. In response to the stressor’s action, it is necessary to include an antioxidant plant protection system (including antioxidant enzymes) [15, 20, 21].

According to the results, different doses of UV radiation have a multidirectional effect on peroxidase activity in common wheat seedlings (Table 1, Figure 1).
Table 1. The change in peroxidase activity (relative to the control) in wheat seedlings as a result of pre-sowing UV treatment ($\lambda = 254$ nm).

| UV exposure time, min | $\Delta A \cdot 10^3$, $E\cdot cm^{-1}dcm^{-1}$ | $\Delta A$, % |
|----------------------|---------------------------------------------|--------------|
| 10                   | 4.9                                         | + 9.3        |
| 20                   | -1.2                                        | -2.2         |
| 30                   | 13.8                                        | +25.9        |
| 40                   | 5.1                                         | + 9.7        |
| UV exposure time, min | $\Delta A \cdot 10^3$, $E\cdot cm^{-1}dcm^{-1}$ | $\Delta A$, % |
| 50                   | -9.2                                        | -17.3        |
| 60                   | -20.9                                       | -39.2        |

Source: Compiled by the authors.

Pre-sowing grain treatment for 10 minutes led to a slight increase (by 9.3%) in peroxidase activity in seedlings. With a 20-minute exposure, a decrease in enzyme activity was recorded. Moreover, in this case, the antioxidant activity was 2.2% lower than in the control samples. Then, with an increase in UV exposure to 30 minutes, a sharp increase in peroxidase activity was observed – by 25.9% relative to the control. A further increase in the time of pre-sowing treatment (40, 50, and 60 minutes) provoked a gradual but significant decrease in peroxidase activity. Irradiation of seeds for 50 and 60 minutes led to the fact that the seedlings’ enzyme activity was significantly lower than the enzyme activity in control (by 17.3% and 39.2%) (Table 1).

Thus, according to the obtained data, 30 minutes is the time of UV irradiation at which the activity of peroxidase in wheat seedlings was maximal.

When irradiating the grain before germination in the range from 30 to 60 minutes, there is a clear inverse relationship between the exposure time and the activity of PO, which is also confirmed by the correlation analysis of the data ($r = 0.9$) (figure 1).

![Figure 1. The dynamics of changes in peroxidase activity, depending on the time of UV exposure, %.
Source: Compiled by the authors.](image-url)

When exposed to small doses of UV radiation (up to 30 minutes), a clear relationship between the
exposure time and peroxidase activity was not detected.

4. Discussion

UV irradiation of wheat seeds provokes an increase in lipid peroxidation [LPO]. A decrease in the intensity of free radical processes is possible due to the rise in the synthesis of many antioxidants, i.e., the activation of the antioxidant system in response to UV exposure [17].

According to the obtained data, small doses of UV radiation (irradiation during 10–20 minutes) may provoke free radical processes. Nevertheless, they still slightly affect the biosynthesis of antioxidant enzymes since the peroxidase activity of seedlings does not significantly differ from the values of the control samples. An increase in peroxidase activity is observed with an increase in the irradiation time, which may be due to the activation of oxidative processes, during which hydrogen peroxide is formed, which initiates the synthesis of enzymes of the antioxidant system [12].

The maximum level of peroxidase activity in three-day-old wheat seedlings was detected during 30-minute UV irradiation, which indicates an increase in the intensity of peroxidation reactions due to a change in the physiological and biochemical processes in response to the stressor.

A further increase in UV exposure time leads to a significant decrease in peroxidase activity. This can be caused by a reduction in the intensity of hydrogen peroxide formation reactions and an increase in the metabolic processes in which $\text{H}_2\text{O}_2$ is used. These processes lead to a decrease in concentration and weaken the synthesis of enzymes of the antioxidant system. With prolonged UV irradiation, compensatory mechanisms are activated. They prevent the formation of free radicals in germinating wheat seeds, which, in addition to antioxidants, also use other tools to suppress LPO. This is confirmed by the study, according to the results of which, after 30 minutes of UV irradiation in germinating seeds, the content of malondialdehyde decreased, although the antioxidant activity in them was even lower than the control level [17].

Based on the obtained results, it can be assumed that pre-sowing UV treatment of soft spring wheat grains for 30 minutes will contribute to a more viable seed material, since it is with this treatment option that high peroxidase activity is observed in seedlings, indicating a more active synthesis and transformation of substances with the consumption of hydrogen peroxide. However, the question of selecting the optimal parameters for pre-sowing treatment requires further research.

5. Conclusion

Thus, it was found that after pre-sowing treatment of soft spring wheat grains with small and medium doses of short-wave UV radiation, the maximum increase in peroxidase activity was observed at an exposure time of 30 minutes. With an increase in exposure from 30 to 60 minutes, a significant decrease in the enzyme activity was noted. A linear relationship was observed between the activity of PO and the exposure time in this range.

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