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Research Article

Investigation of UV Protection of Pigments Obtained from DifferentBacteria on Wheat Plant

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Abstract

In this study, UV protection of the same type of pigments obtained from different types of bacteria on wheat (*Triticum aestivum* cv Kirik) plant was investigated. The seedlings were grown in pots under optimal conditions for a total of 15 days. Pigments obtained from bacteria were applied on the 12th day, and after 24 hours, all UV was applied except for the control group. The plants harvested after the fifteenth day were used as experimental material. As a result of the application, the amounts of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) increased in the seedlings that were only UV applied. In the pigment applications we use as a preservative, it has been determined by the measurements that it reduces the UV damage. In addition, antioxidant enzyme activities; Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) were examined and it was observed that these enzymes increased in pigment applications and showed protection against UV.

Keywords: Bacterial pigments; UV; Plant resistance; *Triticum aestivum*.

1. Introduction

Pigments have been used as coloring agents for aesthetic purposes since ancient times [1]. natural pigments; it is obtained from animals, plants, algae and microorganisms. Microbial pigments are used in fields such as medicine, food and cosmetics. The reason for this is that they are easily obtained, they are more effective and their antioxidant levels are not high. It is very popular especially in the cosmetic industry due to its UV protection. Microbial pigments; They are secondary metabolites produced by microorganisms and the pigment microorganisms produced act as a shield against the harmful effects of UV [2,3,4,5,6]. UV harms not only microorganisms, but also humans, animals and plants. Especially in higher plants; by disrupting the DNA structure, it causes biochemical damage by disrupting the molecular, chlorophyll and cell membrane structure [7,8].

UV stress causes an increase in reactive oxygen species (ROS) [1]. ROS causes many harmful effects such as an increase in the amount of malondialdehyde (MDA) and DNA damage due to cell membrane destruction in plants. In order for the plant to cope with it; They also produce many antioxidant-rich compounds such as flavonoids, ascorbic acid, phenolic substances and carotenoids, and high molecular weight enzymes such as superoxide dismutase (SOD) catalase (CAT) peroxidase (POD). [9].In recent years, new methods have been sought and used against UV damage. Among these methods, microbial pigments come first. It is seen that especially bacterial pigments are used in these studies. Based on this, the UV protection effect of yellow pigment obtained from different bacteria on wheat plants was investigated in our study.

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2. Materials and Methods

Bacteria (*Pantoea agglomerans* OG3 Y1 * and *Enterecoccus mundtii* OG4* Y2) used in the study were obtained from the microbiology culture collections of Atatürk University, Faculty of Science, Department of Biology, Microbiology Laboratory.

*The microorganisms used in the study are our own isolates and an application has been made for the accession number and the result is awaited.

2.1. Extraction of pigments

Bacterial pigments; It was obtained from bacteria grown in a medium prepared by adding 3 g peptone to Nutrient Broth. Bacteria were inoculated to OD_{600} = 1 and incubated at 150 rpm at 30°C for 3 days. After

incubation, the culture was centrifuged at 6000 rpm for 15 minutes. This process was repeated twice by washing with distilled water to remove the nutrient residue. Then, sterile distilled water was placed on the precipitated bacteria and sonicator was added for 1 minute, then ethanol was added and the pigment was allowed to pass into the solvent at 28 ˚C for 1 hour. Then, bacteria were removed by centrifugation at 6000 rpm for 15 minutes [10]. Obtained pigments were stored at +4 degrees to prevent deterioration.

2.2. Plant growth and pigment applications

In this study, Kirik wheat variety (*Triticum aestivum*) was used. Seed sterilization, washing the dry seeds several times with tap water, then soaking in 10% commercial bleach for 5 minutes, and finally sterilizing by washing thoroughly with distilled water, the seeds were grown in sand culture. Plants were grown at 22/20°C and a 12/12 hour light-dark period for 15 days in an environment containing 70% humidity.

Microbial yellow pigments were dissolved in ethanol and covered with distilled water, and then sprayed on 14-day-old wheat leaves. (2ml ethanol and 1g/l pigment). After 24 hours, they were exposed to a UV-B lamp (Philips TL100W/12) with a brightness of 3.3 Wm^{-2} for 3 minutes. One day later, the plants were harvested for various analyses.

2.3. Determination of lipid peroxidation and hydrogen peroxide

Lipid peroxidation was determined by Heath and Packer 1968 [11] method, and the amount of H_2O_2 was determined according to Patterson et al. 1984 [12].

2.4. Determination of antioxidant enzyme activity

To determine the activities of antioxidant enzymes, fresh leaves (0.5 g) were ground with a mortar and pestle under chilled conditions in the presence of phosphate buffer (0.1 M, pH 7.5) containing 0.5 mM EDTA. The homogenate was centrifuged at 12,000 g for 10 min at 4 ˚C, and the resulting supernatant was used for the enzyme assay. SOD activity was assayed using the method of Agarwal and Pandey 2004 [13]. POX activity was measured according to the method of Zhang and Kirkham 1994 [14]. CAT activity was performed according to Qiu et al. 2011 [15].

2.5. Statistical analysis

All experiments were performed 6 times and the average of values was presented. The data were analyzed by analysis of variance, and means were compared by using Duncan's Multiple Range Test at $p < 0.05$ significance level.

3. Results and Discussion

It was determined by the measured H_2O_2 and LPO amount that UV, which is an abiotic stress factor, causes stress in the wheat plant. Bacterial yellow pigments we use to reduce the harmful effect of UV; It was observed that the amount of H_2O_2 decreased by approximately 25% and 15%, respectively.

When we look at the amount of MDA; It was observed that the bacterial yellow pigments applied significantly decreased the amount of MDA compared to UV, while UV alone increased compared to the control.

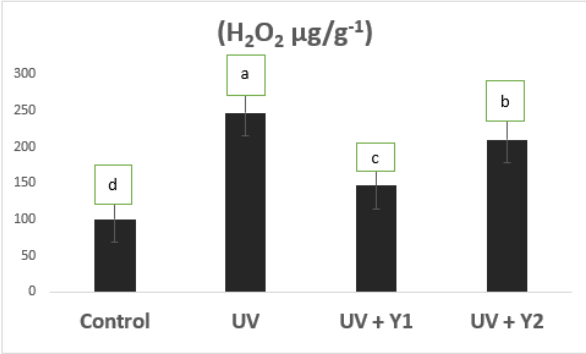

Figure 1. H_2O_2 amount of pigments applied in wheat plant exposed to UV stress. Indicates a significant difference from the control and UV application value at the $P < 0.05$ level according to the Duncan Test.

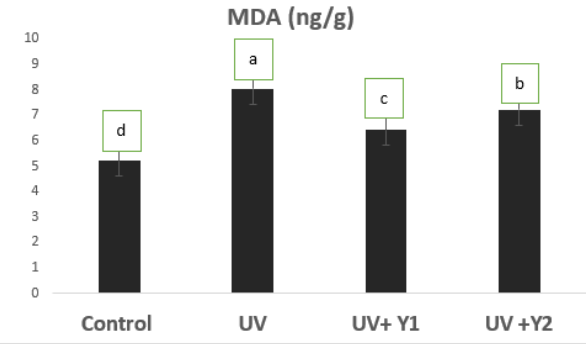

Figure 2. MDA amount of pigments applied in wheat plant exposed to UV stress. Indicates a significant difference from the control and UV application value at the $P < 0.05$ level according to the Duncan Test.

SOD enzyme activity increased in both UV and pigment applications.(Figure3). In the CAT activity, the enzyme activity was measured, which was 26.44 in the control, and it was determined as 24.05 in the UV application. In pigment applications, these rates were determined as 37 and 39 enzyme amounts, respectively (Figure 4). When we look at the POX activity, another antioxidant enzyme, no significant difference was observed in the control and UV applications, but the bacterial pigments we applied, especially the Y1 pigment, increased this rate significantly (Figure 5).

Since plants are immobile organisms, they are exposed to UV stress more than other living things, and therefore, an increase in the amount of ROS is observed, which causes cellular activities to stop and even plant death. Plants have developed many adaptation mechanisms to cope with UV stress (antioxidant substances and antioxidant enzymes) [16]. Recent studies are looking for new methods to

eliminate or minimize the harmful effects of UV. In particular, plants synthesize pigments such as carotenoids to protect them from UV damage. The reason they produce pigment is to absorb different wavelengths and to minimize physiological damage**.** [17]. It has been observed that the amounts of H_2O_2 and MDA increase in plants exposed to various abiotic stress factors [18,19]. As a result of our study, there was a significant increase in the amounts of H_2O_2 and MDA in wheat seedlings exposed to UV stress compared to the control. It was determined that there was a significant decrease in these rates in yellow pigment applications, whose UV protection effect was investigated: (Figure 1 and Figure 2).

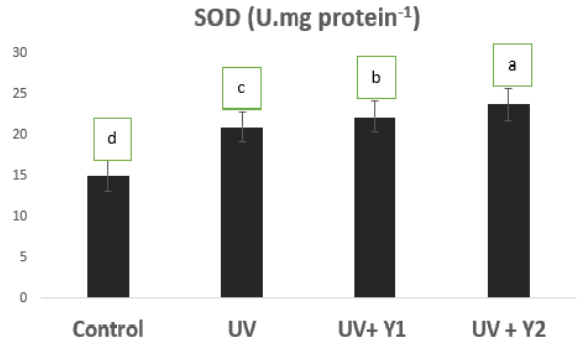

Figure 3. SOD enzyme activity. Indicates a significant difference from the control and UV application value at the $P < 0.05$ level according to the Duncan Test.

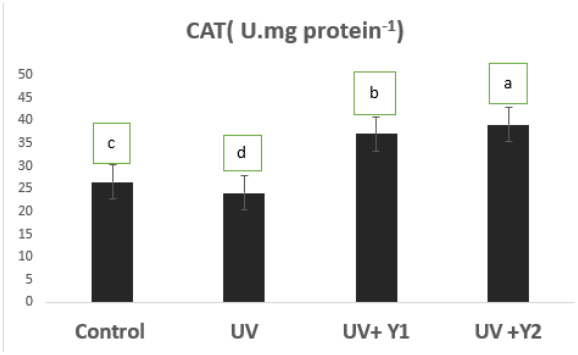

Figure 4. CAT enzyme activity. Indicates a significant difference from the control and UV application value at the $P < 0.05$ level according to the Duncan Test.

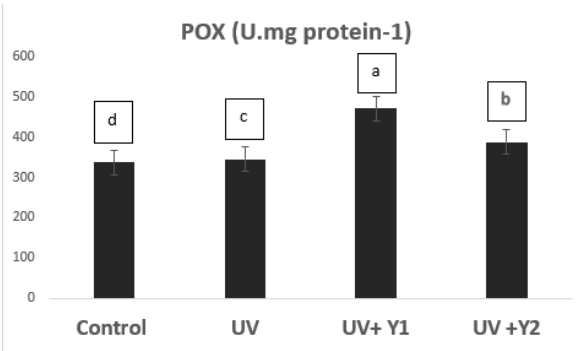

Figure 5. POX enzyme activity.Indicates a significant difference from the control and UV application value at the $P < 0.05$ level according to the Duncan Test.

The existence of microorganisms that provide resistance to plants against various biotic and abiotic stress factors has been reported in literature studies. Especially bacteria and fungi that promote plant growth; It has been seen in studies that it both promotes plant growth and provides resistance by secreting substances such as auxins, cytokinins, gibberellic acid and pigments [20]. When we look at the literature studies, it has been seen that the amount of ROS increases in plants exposed to UV stress and antioxidant enzyme (SOD; CAT; POX) activities are activated to combat this. It has been shown in many studies that the substances applied to protect the plant from the harmful effects of ROS increase, decrease or remain unchanged in these enzyme activities [18]. In our study, it was shown that the yellow pigments we applied increased the enzyme activities, and that bacterial pigments gave the plant a protective feature against UV (Figure 3, 4 and 5).

4. Conclusions

Bacterial pigments have shown that they give plants resistance to UV and increase the plant's chances of surviving under stress conditions.

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Conflict of Interest

The authors declare no conflict of interest.

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