
**THE INFLUENCE OF UV-B TYPE RADIATION OF DIFFERENT WAVELENGTHS ON
THE ACTIVITY OF THE ANTIOXIDANT ENZYMES THROUGHOUT THE
DEVELOPMENT OF THE ZEA MAYS L. HYBRID PLANTS
UNDER LOW TEMPERATURE CONDITIONS**

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Abstract

The elevated levels of the reactive oxygen species (ROS), determine the significant increase of the antioxidant enzymes' activity, a process by which the plants are protected against the damaging effects of the oxidative stress. The activity increase of the antioxidant defense system, due to negative effects of environmental stressors, develops over a long period of time, a process called adaptation. Many agricultural plants of subtropical origin (eg, corn, rice, tomatoes, peppers, squash, cucumbers etc.) are also grown in the temperate zone, but in less optimal conditions. In this case, the reproduction of these plants must also take into consideration selecting genotypes tolerant to the new conditions, and through them creating tolerant varieties. Through our experiments we sought to answer the question whether the treatment with UV-B type radiations, of different wavelengths of 280-310 nm, under low temperature conditions (6-8°C), has a stressful effect on plants and induces the activation of the antioxidant enzymes, and if there are differences in this regard between the control and treated plants. We should note that in the specialty literature there is no data regarding the complex characterization of the 287 nm UVB effect, and our data bring an important contribution to the characterization of the UVB spectrum from a biochemical point of view.

Keywords: oxidative and thermal stress, antioxidant enzymes, spectrophotometric method, wavelengths, temperature conditions, UV radiations.

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1. INTRODUCTION

In recent years, considerable interest has been shown regarding the UV radiations in the aquatic systems from regions with low temperatures, where there can be noticed a significant increase in the UVB level (280-310 nm) resulting from the depletion of the protective ozone layer. (Mandronich, 1995). In literature, there are some data obtained from superior plants, according to which low temperatures determine a severe photoinhibition, under the influence of the available photosynthetic radiation (Krause 1988, 1994). Based on the data available from the literature, we questioned whether the low temperatures and UVB influence induce changes dependent on the spectrum used on the

antioxidant system on plants. The harmful effects on plants caused by the abiotic stress factors in conjunction with the UV stress, is reflected in alterations of the plant's physiology, causing a reduction in their growth and a decrease in their bioproductivity (Khan, 2003). Chloroplast damage by overexposure to UV-B radiation can lead to the decrease in the chlorophyll content; this involves ultrastructural changes, a decrease of the photosynthetic protection pigments, thus affecting the photosynthesis process (Sullivan and Rozema, 1999). Different culture species have the capacity of tolerating UV-B radiation and retaining chlorophyll in leaves, the results varying for monocotyledonous, in comparison to dicotyledonous ones (0-33% in monocotyledonous species, compared to 10-

78% in dicotyledonous species).(Tevini et al., 1981)

2. MATERIALS AND METHODS

The experimental part

The effect of the thermal stress but also the activity of the antioxidant system has been investigated for two hybrids *Zea Mays L.*, Helga and ZP471.

In order to extract and evaluate the activity of the enzymes, 0.5 g of plant material (leaves without the main nervure) was triturated with quartz sand, adding 2.5 ml of MgCl₂ solution, with a concentration of 3 mM, cold, EDTA 1 mM, containing 0.5 mM of TRIS-HCl (pH 7.4) buffer solution, in a chilled mortar. The homogenized mixture was centrifuged (4°C, 20 minutes, 1500 rpm), then the supernatant was divided into Eppendorf tubes. Until

measurements were due, the samples were stored on ice, measurements being conducted at room temperature. The enzyme activity is given by the change in absorbance, caused by 1 g of enzyme protein, throughout 1 minute ($\Delta A \text{ min}^{-1} \text{ g}^{-1} \text{ protein}$).

In order to determine the enzyme activity, the absorbance changes were monitored at wavelengths between 280-310 nm, with precise specifications at 287 nm. The enzymatic activity was calculated using the formula: $A[U/mg] = (dA/dt \times V \text{ total} \times 1000) / \epsilon \times V \text{ enzimei}$; ϵ = extinction coefficient.

The enzymatic activity was measured on the 4th day, from the samples taken from the 3rd leaf, and analyzed comparatively for the two hybrids, under different UV-B type wavelengths, within a temperature interval of Δt (6-8°C).

Table 1. The effect of the UV-B wavelnghts on the enzyme activity of the HELGA and ZP471 hybrid.

Temperature	Reaction environment	UV-B Wavelength	Absorbance alterations		Enzyme
			Helga hybrid	ZP471 hybrid	
6°C-8°C	Phosphate buffer solution 0,1 M ,pH 7,5), (EDTA) 1 mM, dinitrobenzoic acid (DTNB) 0,75 mM, adenine dinucleotide phosphate NADPH 0,1 mM, The total volume of the mixture was 1 ml, containing 50 µl of plant sample.	287nm	0,75 **	0,35 *	Glutathione reductase (GR)
6°C-8°C	Sodium phosphate buffer solution of 72,7 mM concentration (pH 6,5), reduced glutathione of 3,6 mM concentration (GSH), 1-chloro-2, 4 - dinitrobenzene (2,4-D) of 1mM concentration and enzymatic extract (2,75 ml reactive mixture containing 100 µl plant sample)	287nm	0,17 **	0,45 *	Glutathione -S- transferase (GST)
6°C-8°C	The total volume of the reactive mixture was 3ml, containing 0,5 mM TRIS (pH 7,4) buffer solution, H ₂ O ₂ 10 mM , to which we added 50 µl plant sample. The reaction was triggered by adding H ₂ O ₂ .	287nm	0,048 **	0,45 *	Catalase (CAT)
6°C-8°C	Buffer solution TRIS (0,2 mM pH 7,8) containing 25 mM of ascorbic acid and H ₂ O ₂ 0,5 mM. The reactive mixture volume was 2,25ml, to which we added 50 µl plant sample.	287nm	0,39 **	0,45 **	Ascorbate Peroxidase (APX)
6°C-8°C	The reactive mixture measures a total volume of 3 ml to which was added 50 µl of plant sample, 0,1 mM acetate buffer solution (pH 5,5), H ₂ O ₂ 10 mM and guaiacol of 1 mM concentration.	287nm	0,29 **	0,17 **	Guaiacol peroxidase (POD)

3. RESULTS AND DISCUSSION

The GR enzyme activity in the control group was induced at the 280 nm wavelength for the two hybrids, and it peaked at 287 nm for the ZP471 hybrid. Under the effect of the UV-B treatment, the enzymatic activation had been noticed only for the 280 nm wavelength, slightly increasing and exceeding the control group values. For the ZP471 hybrid, only the 287 nm wavelength induces the GST activity increase with significant differences (table 1 and figure 1), suggesting an increased tolerance of this hybrid which has also been noticed in other experimental circumstances.

The CAT activity following the exposure of both the control group as well as the treated group to UV-B had initially increased, with significant differences at 287 nm, but constantly remained at low values, without significant differences for other wavelengths and the same temperature gradient. There is a significant increase in the case of the Helga hybrid, which leads to the conclusion that this hybrid is more resilient to the oxidative stress, after which it gradually returns to lower values, insignificantly different compared to the witness group. (table 1 and figure 1).

The APX activity did not change significantly for the control plants, regardless of the low temperature variation imposed by the experimental conditions. However, under the effect of the treatment for all wavelengths, the

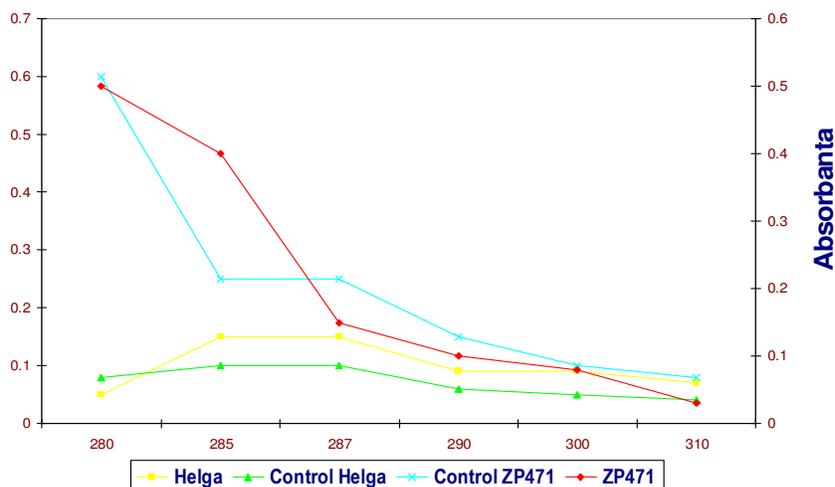
APX enzymatic activity registered higher and more significant values compared to the control group. The highest activity was registered for the 280-290 nm wavelengths (table 1, figure 1).

The POD activity also showed no major changes for the different wavelengths imposed for the witness group. However, under the effect of the treatment, at the 287 nm wavelength, it reached its maximum enzymatic activity, exceeding the values recorded in the control group (table 1 and figure 1).

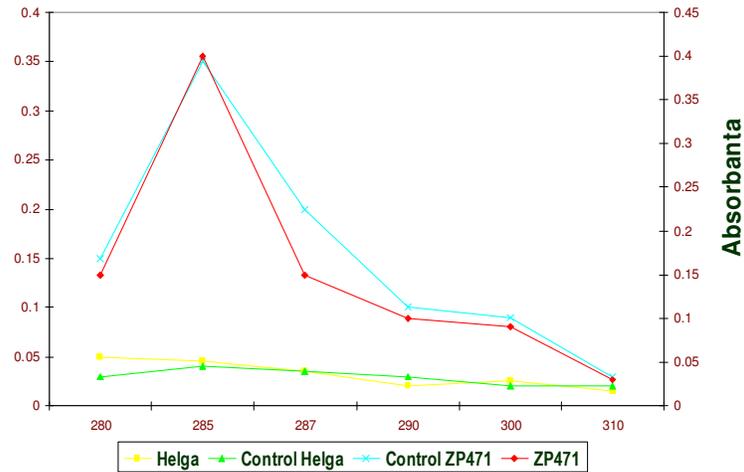
When plants are subjected to a biotic or abiotic stress, the reactive oxygen species will accumulate excessively leading to the oxidative alteration of the cells. In this respect, the antioxidants and the antioxidant enzymes function to interrupt the uncontrolled oxidation in each organ.

4. CONCLUSIONS

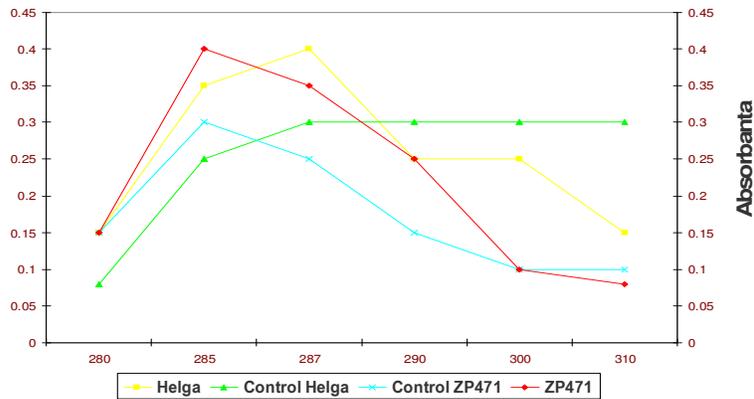
The data obtained from the experiments suggest that at low temperatures, in the imposed experimental conditions, there is major stress manifested upon the plants. In contrast, the exposure to different wavelengths determines the accumulation of peroxide in the plant cells, which becomes toxic and leads to the activation of the antioxidant enzymes.



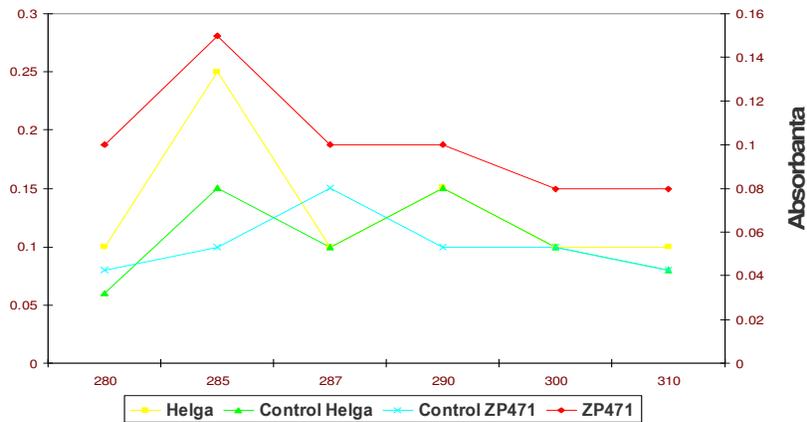
a) The GST enzyme activity



b) The CAT enzyme activity



c) The APX enzyme activity



d) The POD enzyme activity

Figure 1. The degree of change of the antioxidant system enzymes' activity in the plant extract of *Zea mays L.*, hybrids Helga and ZP471, under the influence of the UV-B type radiation and of low temperatures

These effects are obvious for the 287 nm wavelength (Figure 1.) The studied UVB spectrum determines the increase of the APX concentration from the plant extract at low temperatures, indicating the high level of peroxide at the cellular level, compared to the plants irradiated under normal temperature circumstances.

We should note that in the specialty literature there is no data regarding the complex characterization of the 287 nm UVB effect, and our data bring an important contribution to the characterization of the UVB spectrum from a biochemical point of view.

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