

BRIEF COMMUNICATION

Influence of ultraviolet-B radiation on peroxidase activity of *Allium schoenoprasum* leaves

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Abstract

An approximately 7 % difference in biologically effective ultraviolet-B (UV-B) radiation did not significantly influence leaf length or leaf peroxidase activity of chives (*Allium schoenoprasum* L.). However, correlation and regression analyses with different climatic parameters revealed that increased UV-B radiation enhanced ascorbate peroxidase activity in chive leaves whereas guaiacol peroxidase was inhibited.

Additional key words: ascorbate peroxidase, growth, guaiacol peroxidase, oxidative stress.

Ascorbate peroxidases (APOXs, EC 1.11.1.11) are antioxidative enzymes that detoxify H₂O₂ using ascorbate as a reducing agent and thus protect plants from oxidative stress (Asada 1992, Smirnoff 1995). Guaiacol peroxidases (GPOXs, EC 1.11.1.7) are enzymes performing several functions, including the control of leaf elongation by promoting cross-linking of matrix polymers in growing cell walls (Fry 1986). Because they utilize H₂O₂ GPOXs are also sometimes considered antioxidative (e.g. Kronfuss *et al.* 1996). We investigated the influence of ultraviolet-B (UV-B) radiation on the specific APOX and GPOX activities of chive leaves to elucidate if GPOX could have an antioxidative function in the case of UV-B stress. As GPOXs are supposed to be involved in the UV-B-induced reduction of leaf elongation (Ros 1990, Tevini and Teramura 1989), we also investigated the UV-B effect on leaf length and compared it with GPOX activity.

From June 1997 until September 1998, chive plants (*Allium schoenoprasum* cv. Grobröhrig Hilds Polycross) were grown in a substrate used for green rooftops ("Rotgrand", Bott, Dobel, Germany; for details see Egert

and Tevini 2002), in two greenhouses covered with either 3-mm thick or 5-mm thick plexiglass sheets (GS 2458, Röhm, Darmstadt, Germany), in the botanical garden of Karlsruhe. Biologically effective UV-B radiation (UV-B_{BE}, Caldwell 1971) was approximately 7 % lower in the greenhouse covered with thicker plexiglass. UV-B levels in the greenhouses were ca. 88 and 82 % of ambient UV-B, respectively. There were no differences in photosynthetically active radiation (PAR; ca. 90 % of ambient PAR) or air temperature between the two greenhouses.

Daily PAR and UV-B doses were measured with a broad band radiometer (ELDONET-dosimeter, Häder *et al.* 1999) on the top of a building located ca. 500 m distant from the greenhouses. The daily maximum air temperature, T_{max}, was determined in the greenhouses with a thermohygrograph.

Enzyme activities were measured from crude extracts obtained by homogenizing 3 g of leaves in 15 cm³ of 50 mM K-phosphate buffer (pH 5.5 for GPOX; pH 8.0 for APOX, with 1 mM ascorbate), followed by gauze-filtration and centrifugation (4 °C, 20 000 g) for 20 min.

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Abbreviations: APOX - ascorbate peroxidase; GPOX - guaiacol peroxidase; PAR - photosynthetically active radiation; ROS - reactive oxygen species; T_{max} - daily maximum air temperature; UV-B - ultraviolet-B radiation; UV-B_{BE} - biologically effective UV-B radiation.

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Volumes of 0.05 cm³ (GPOX) or 0.10 cm³ (APOX) of the supernatant in a final volume of 3 cm³ were used for determination of absorbance with a spectrophotometer (*Uvikon 931, Kontron, Neufahrn, Germany*) at 25 °C. GPOX activity was measured with 13.4 mM guaiacol and 3.3 mM H₂O₂ in 50 mM K-phosphate buffer (pH 5.5), by following the increase of absorption at 470 nm. APOX activity was measured with 0.5 mM ascorbate and 0.1 mM H₂O₂ in 50 mM K-phosphate buffer (pH 6.0), by following the decrease of absorption at 290 nm. Specific activities were calculated by referring changes in absorbance to the protein content of the extract.

The 7 % difference in UV-B_{BE} did not influence leaf length. The average length of 200 leaves grown for 1 month under relative higher UV-B radiation in the summer of 1998 was not significantly different than an equal amount of leaves grown with less UV-B radiation (8.1 ± 2.1 vs. 8.8 ± 1.1 cm). Specific peroxidase activities were also not affected significantly by the different UV-B levels (Fig. 1). There were also no effects on the leaf concentrations of chlorophyll, carotenoids, UV-radiation-absorbing substances, thiobarbituric-acid-reactive substances and leaf lipoxygenase activity (data not shown; for these parameters see Egert and Tevini 2002). Chives are apparently not susceptible to small changes in near ambient UV-B radiation, as has also been shown for other plants such as *Dactylis glomerata* and *Trifolium repens* (Cayenberghs *et al.* 2001).

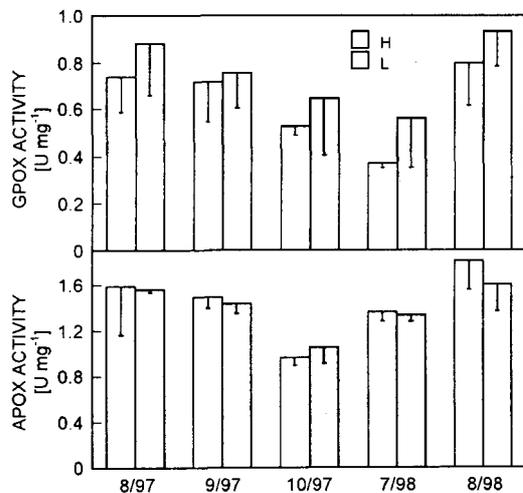


Fig. 1. Influence of an approximately 7 % higher biologically effective UV-B radiation (H) on specific GPOX and APOX activity of chive leaves at different dates (month/year). Means ± SD, n = 3 - 4, L = relatively lower UV-B radiation.

Nevertheless, the average GPOX activity under higher UV-B radiation was always lower than under lower UV-B radiation (Fig. 1), and this trend was significant ($P < 0.05$, Wilcoxon test). Moreover, it can be deduced from Fig. 1 that at least APOX activity in chive leaves might be correlated with climatic parameters that changed

over the course of a year, as the activities decreased from summer to autumn of 1997 and increased again in the summer of 1998. To investigate the influence of different climatic parameters in more detail, we harvested leaves from the more UV-B penetrable greenhouse in the morning at different dates in 1997 and 1998 and correlated GPOX and APOX activity with PAR dose, UV-B dose and the maximum air temperature of the previous day (PAR₁, UV-B₁, T_{max1}) or of the 3 previous days averaged (PAR₃, UV-B₃, T_{max3}). While APOX activity was significantly positively correlated with all investigated climatic parameters, GPOX activity was significantly negatively correlated with UV-B₁, UV-B₃, and T_{max3} (Table 1). Based on combined consideration of rank correlation and regression coefficients, GPOX activity was best correlated with UV-B₃ and APOX

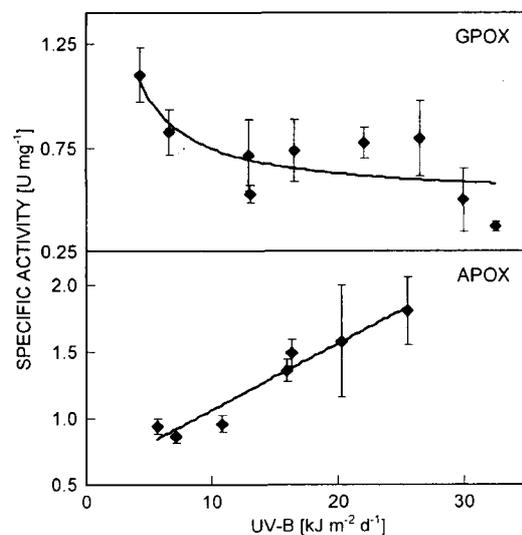


Fig. 2. Correlation of specific GPOX and APOX activity of chive leaves with UV-B dose of the previous day (APOX) or of the 3 previous days averaged (GPOX). Means ± SD, n = 3 - 4.

Table 1. Results of correlation analyses of GPOX (n = 9) and APOX (n = 7) activities [U mg⁻¹(protein)] in chive leaves against different climatic parameters (see text); ρ_{xy} - rank correlation coefficient (Spearman), r² - regression coefficient for hyperbolic (GPOX) and linear (APOX) regression curves, n.s. - not significantly correlated, n.d. - not determined, * - P < 0.05, ** - P < 0.01, *** - P < 0.001.

Parameter	GPOX		APOX	
	ρ _{xy}	r ²	ρ _{xy}	r ²
UV-B ₁	-0.70*	0.49	0.96***	0.94
UV-B ₃	-0.68*	0.59	0.96***	0.73
PAR ₁	n.s.	n.d.	0.93**	0.86
PAR ₃	n.s.	n.d.	0.75*	0.55
T _{max1}	n.s.	n.d.	0.93**	0.72
T _{max3}	-0.58*	0.56	0.82*	0.58

activity with UV-B₁ (Fig. 2). GPOX activity in chive leaves is obviously inhibited by UV-B radiation, and this inhibition might be a cumulative effect (in the range of days). In contrast, APOX activity in chive leaves is enhanced by UV-B radiation, and this seems to be a short-term effect. The positive correlations of APOX activity with PAR dose, air temperature and UV-B dose are consistent with the antioxidative function of APOXs, as these parameters potentially increase the formation of reactive oxygen species (ROS) in plants. That the best correlations were found with the UV-B doses underlines the potential of this environmental stressor to cause ROS formation. Increased APOX activities due to elevated

UV-B radiation have also been reported for other plants, e.g., flavonoid-deficient *Arabidopsis* mutants (Rao *et al.* 1996). An antioxidative function of GPOXs in chives is questionable, at least with UV-B, PAR or air temperature as stressors, because in this case positive correlations would have been expected. Also in the case of drought-stressed chives an antioxidative GPOX function could not be shown (Egert and Tevini 2002). As there was no UV-B effect on leaf length, the role of GPOXs in this connection remains unclear, although the significant negative correlation with UV-B dose argues against a major role of this enzyme in UV-B-induced reduction of leaf elongation.

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