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DISSERTATION SUMMARY

In vivo ROS detection in UV-stressed leaves

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During their life cycle plants are daily exposed to adverse environmental impacts, since light, temperature or water conditions are often far from optimal. Various stress conditions affect a number of metabolic functions in plants through the generation of reactive oxygen species (ROS). Under field conditions, plants are often exposed to high PAR irradiances as well as to ultraviolet (UV) radiation. The solar radiation reaching the Earth's surface is divided into UV-B (280-320 nm), UV-A (320-400 nm) and visible (PAR: 400-700 nm) radiation, the latter one being used for photosynthesis by the vegetation. ROS induction is known to be the early plant response to UV exposure. Previous studies showed, that high levels of UV-B radiation induce lipid peroxidation and oxidative membrane damage (Cen et al. 1994; Takeuchi et al. 1995). In isolated thylakoid membranes UV-B exposure triggered hydroxyl radical generation, but the presence of carbon centred (methyl-like), and peroxyl radicals were also reported (Hideg et al. 1996; Hideg et al. 1999). Singlet oxygen was not found in the same preparation. The difference in the action site of UV-A and UV-B in photosynthesis has been intensely discussed, attributing smaller damaging effect to the UV-A irradiation, than to the UV-B (Turcsányi et al. 2000; Flint et al. 2003).

Since the ROS generating abilities of various UV energies have not been compared yet, our goal was to study the nature of ROS involved in the stress by both UV-A and UV-B radiation *in vivo*, in leaves.

In order to understand the physiological functions of ROS generated under oxidative stress by UV radiation, their direct measurement in leaves is of special importance. Therefore, the short-lived ROS were detected by fluorescent sensors: the fluorescence of the sensors decreased upon their reaction with ROS. Spinach leaves were infiltrated with either the singlet oxygen sensitive DanePy or with the singlet oxygen and superoxide reactive HO-1889NH, then exposed to $2x10^{22}$ photons of quasi-monochromatic (±8 nm around central wavelength) UV radiation in the 280-390 nm range, corresponding to 18-36 µmol m^{-2} s^{-1}. The effect of UV radiation on the photosynthesis of various leaves was estimated from the relative decrease in their variable chlorophyll fluorescence.

We have found, that reactive oxygen production in leaves exposed to UV radiation was heterogeneous. Superoxide production was characteristic to the UV-B wavelength region but not to UV-A. The correlation between the UV-induced superoxide production and photosynthetic activity decrease of the leaves indicated, that the superoxide yielding primary reaction is localized in the chloroplasts, possibly in the thylakoid membranes. Singlet oxygen evolution was a characteristic UV-A induced physiological response, which we identified in plants for the first time. It was typical for irradiation by 340-390 nm, however its source is probably not localized inside the chloroplasts, since no correlation was found between the damage of the photosynthetic apparatus and the singlet oxygen production (Barta et al. 2004).

In summary, we have shown, that UV stress is a complex oxidative stress, inducing the production of various reactive oxygen species in plants.

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