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Effect of preillumination with red light on photosynthetic parameters and oxidant-/antioxidant balance in *Arabidopsis thaliana* in response to UV-A

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ABSTRACT

The effect of preillumination with low intensity (10 μmol quanta m^{-2} s^{-1}, 10 min) light of different wavelengths in the spectral range of 550–730 nm on photosynthesis and activity of PSII, the content of photosynthetic pigments and H_2O_2, as well as the peroxidase activity in the leaves of 26-d-old *Arabidopsis thaliana* wild-type (WT) plants in response to UV-A radiation was studied. UV-A decreased the activity of the PSII, the content of Chl *a*, Chl *b* and carotenoids, as well as increased the peroxidase activity and H_2O_2 level in the WT leaves. Preillumination of the leaves with red light (RL, λ_{max} = 664 nm) reduced the inhibitory effect of UV radiation on photosynthesis and activity of the PSII, indicated by delayed light emission as well as the H_2O_2 level, but increased the peroxidase activity in the leaves compared to illumination by UV radiation only. Illumination with RL alone and the subsequent exposure of plants to darkness increased the peroxidase activity and the transcription activity of genes of the transcription factors APX1 and HYH. Preillumination of leaves with RL, then far red light (FRL, λ_{max} = 727 nm) partially compensated the effect of the RL for all studied parameters, suggesting that the active form of phytochrome (P_{FR}) is involved in these processes. Preillumination with the wavelengths of 550, 594 and 727 nm only did not have a marked effect on photosynthesis. The *hy2* mutant of *Arabidopsis* with reduced synthesis of the phytochrome B chromophore showed decreased resistance of PSII to UV-A compared with the WT of *Arabidopsis*. UV radiation reduced Chl *a* fluorescence much faster in the *hy2* mutant compared to the WT. Preillumination of the *hy2* mutant with RL did not affect the PSII activity and H_2O_2 level in UV-irradiated leaves. It is assumed that the formation of the increased resistance of the photosynthetic apparatus of *Arabidopsis* to UV-A radiation involves P_{FR} and the antioxidant system of plants, partly by inducing transcriptional activity of some antioxidant and transcription factors genes.

1. Introduction

UV radiation present in sunlight is absorbed by proteins, lipids, nucleic acids, and various physiologically active substances such as vitamins and quinones, which leads to their oxidation, structural modifications of chlorophyll-protein complexes of the photosystems and, in many cases, to the damage of the photosynthetic apparatus. First of all, the components of the PSII such as Q_A, Q_B, PQ, D1 and D2 proteins as well as the Mn_4CaO_5 cluster are damaged [1–6].

At the same time, UV radiation induces the synthesis of various compounds that protect a plant from UV radiation, such as antioxidant enzymes, chaperone proteins and low molecular weight antioxidants, including carotenoids and flavonoids [7,8]. The activation of the protective systems can be induced not only by UV radiation, but also with visible light of low intensity acting together with UV [9], particularly in the blue [8], and red [10] spectral range. It is assumed that in the latter case, the protective effect is caused by the generation of the red light-(RL)-induced formation of the far-red (FR)-absorbing active form of phytochrome – P_{FR} [11]. It was found that this process results in less Chl degradation and loss of PSII activity, and to a reduced violation of the integrity of thylakoid membranes [10,12–14].

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Phytochromes (Phy) are well studied key photoreceptors, which are involved in many processes of photomorphogenesis, which occur in variety of organisms ranging from unicellular green algae to higher plants. Five types of Phy are identified, between them PhyA and PhyB are basic types. PhyA is the primary photoreceptor responsible for perceiving and mediating various responses to far-red light (FRL), whereas PhyB is the predominant phytochrome regulating responses to RL [15–17].

There are several classes of phytochrome responses, known as low-fluence responses (LFRs), very low-fluence responses (VLFRs) without any RL/FRL reversibility and high-irradiance responses (HIRs). VLFRs and HIRs are unique for PhyA [18]. PhyB is involved in the low-energy reactions of the classical type (LFR), which are manifested during growth, photomorphogenesis and some other processes [19]. However, there is a lack of knowledge regarding the role of PhyB for the development of stress-protective mechanisms in plants [20], and, in particular, in their photosynthetic apparatus. Meanwhile, the photosynthetic apparatus, first of all PSII, is a key element in the formation of plant resistance to oxidative stress [21,22].

There is evidence that the physiologically active form of PhyB that is formed upon illumination with low-energy RL can enhance the activity of antioxidant enzymes [11] and increases the content of low molecular weight antioxidants such as carotenoids and UV-absorbing pigments. This is one of the most common hypotheses about the mechanism of the action of RL, which has been confirmed in several studies. Thus, Sharma et al. [23] showed that phytochrome regulates the peroxidase activity of maize. Short-term RL (λ_m – 650 nm; 500 mW cm^{-2} s^{-1}) increased the enzyme activity by 50–70%, measured one day after exposure. The mechanism of the stimulating action of the RL at peroxidase might be explained by the fact that the RL activates the *de novo* peroxidase synthesis. Activation of peroxidase and other antioxidant enzymes during the action of RL could be a result of the higher transcriptional levels of the genes encoding for peroxidase and other enzymes. One can also assume that there is a link between the state of the phytochrome system, in particular, the content of PhyB and its active form, and the resistance of the photosynthetic apparatus to a variety of natural stressors. However, this problem needs further investigation and is not the focus of the work presented here.

According to an earlier study, the phytochrome-deficient *hy1* and *hy2* (long hypocotyl) mutant plants of *Arabidopsis* are defective in phytochrome chromophore biosynthesis [24]. Here, we suggested that the *hy2* mutation also affects photosynthetic apparatus resistance to UV-induced oxidative stress.

The aim was to study a link between changes in the content of P_{FR} induced by RL exposure and the photosynthetic apparatus resistance to UV-A radiation in the wild type and the *hy2* mutant plants of *A. thaliana*. For this purpose, the effects of UV-A, RL, and the illumination schemes RL → UV-A and RL → FRL → UV were measured by registration of the photosynthetic parameters, the content of H_2O_2 and the peroxidase activity in the leaves, as well as the transcriptional activity of some genes involved in UV-signaling and Phy-signaling systems.

2. Materials and methods

2.1. Plant material

Experiments were performed using 26-days-old wild-type and *hy2* mutant (from the European Arabidopsis Stock Centre (Nottingham, UK)) plants of *Arabidopsis thaliana* (ecotype Columbia-0). Plants were grown under controlled conditions with 12-h photoperiod (12 h light, 25 °C; 12 h dark, 21 °C).

2.2. Characteristics of the applied light sources, exposure and exposure conditions

Plants were grown under white fluorescent lamps (120 μmol quanta m^{-2} s^{-1}). UV-A was obtained by an ultraviolet lamp T8 18W BLB (Selecta) with λ_{max} = 365 nm (I = 8–12 W m^{-2} on the leaf surface, half-width 24 nm). The plants were illuminated with RL (λ_{max} – 664 nm, half-width 32 nm, 10 min) and green light (λ_{max} = 550 nm, half-width 15 nm, 10 min), yellow–orange light (λ_{max} = 594 nm, half-width 16 nm) and FRL (λ_{max} – 727 nm, half-width 40 nm, 10 min), obtained with the help of LEDs. The light intensity was 10 μmol quanta m^{-2} s^{-1} on the surface of leaves.

Immediately after the last dark period, plants were exposed to UV-A (UV) for 2 h. To transform PhyB in a physiologically active form [19] one group of plants was preilluminated with RL, and in some experiments additionaly with far-red light or green light, and then subjected to UV irradiation. Another group of plants was not subjected to the light treatments as control. After irradiation, plants were kept in the dark for at least 20 min up to 24 h. Immediately before and after exposure, as well as during the subsequent dark incubation of seedlings, the photosynthetic activity of PSII, the content of photosynthetic pigments and UV-absorbing pigments (UAPs), as well as the H_2O_2 level and the activity of the peroxidase were measured.

In addition, bleaching curves of the Chl *a* fluorescence were registered with a home-built setup based on a Nikon TI Eclipse wide-field fluorescence microscope equipped with 10× objective. Excitation was performed via a dichroic cube (425 DCXRU, AHF Analysentechnik Tübingen) with an additional bandpass filter for the selection of the excitation range (ET Bandpass 360/40×, AHF Analysentechnik, Tübingen) and an additional emission filter (BrightLine HC 680/22, AHF Analysentechnik, Tübingen). The filter set was used to select the desired UV-A range from the emission of a mercury lamp and to remove the stray laser signals from fluorescence detection. The emission filter (BrightLine HC 688/31, AHF Analysentechnik, Tübingen, λ_{max} = 688 nm, half-width 31 nm) ensures that mainly Chl *a* luminescence is monitored. The images were projected on a CCD camera (Andor-Luca). The intensity of the UV-A light (λ_{max} = 360 nm, half-width 40 nm)) was 5000 W m^{-2}.

The methods of delayed light emission (DLE) of Chl *a* and CO_2 gas exchange were used for assessment of the state of the photosynthetic apparatus under normal physiological and stress conditions.

2.3. Delayed fluorescence

Induction curves of millisecond DLE were obtained using a disc phosphoroscope and an analog-to-digital converter to store the data with a personal computer. Excitation times for recording fluorescence and dark period between them were 2 ms, 2 ms and 4.5 ms, respectively. The intensity of the acting light (during the excitation time) was 2000 μmol photons m^{-2} s^{-1}. The phosphoroscope device is described in detail in [25].

The apparent photosynthetic rate was determined using an infrared gas analyzer Infralit-4 in a closed system with saturating light intensity

2.4. Determination of enzyme activity

Total peroxidase activity in the *Arabidopsis* leaves was studied spectrophotometrically by calculating the changes in optical density at λ_m = 450 nm for 1 min, assuming an extinction coefficient E = 4.4 $mM^{-1}cm^{-1}$. All values are expressed relative to the control. The substrate used was 3,3′-diaminobenzidine. Ascorbate