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Effects of salicylic acid on UV-B-induced oxidative stress and ultrastructure of leaves in cucumber seedlings

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1. Introduction

Numerous studies have demonstrated several detrimental effects of UV-B on plants, including the inhibition of photosynthetic activity and growth. Therefore, such an increase in UV-B is expected to have a negative impact on plants.

Salicylic acid (SA) is a natural plant phenolic. Although SA has received particular attention because of its ability to induce protection against plant pathogens, it might also play an important role in controlling plant sensitivity to different types of abiotic stress. The oxidative stress defence system of higher plants is composed of both enzymatic and nonezymatic mechanisms. The objective in this study, we examined the effect of UV-B irradiation on plant to oxidative stress, using the free radical generator, salicylic acid. We also investigated the effects of salicylic acid on UV-B-induced antioxidative enzymes and ultrastructure of leaves, and discuss their possible role in cucumber seedlings.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of cucumber (*Cucumis sativus* L. cv.) were sown on a mixture of vermiculite, peat moss and perlite in plastic pots. The seedlings were grown in a growth chamber (Eyelatron, FLI-301N, Japan) with a temperature of 25℃, 70±7% RH, 160μmol $m^{-2}s^{-2}$ PAR, and with a 12h light period. When about 15-days old, seedlings were transferred to anther chamber to be growth chamber a 20/15℃ (light/dark) temperature regime. Seedlings were containing 10ml distilled water or equal amounts of water solution from the required SA concentrations (100μM, 300μM, 500μM, 700μM, 1mM), stock of SA (Sigma Chem. Co.). The solution were changed every 24h.

2.2. UV-B irradiation

Approximately 15-day-old seedlings were used for the UV-B treatments. The UV-B fluence rate, at the height of seedlings, was measured to be 6 W m^{-2} s^{-1} using a UV spectroradiometer (Li-1800, Lycosa).

2.3. Chlorophyll fluorescence

The quantum yield of PSII electron transport was assessed by measuring the chlorophyll fluorescence (Fv/Fm) with a pulse amplitude modulation fluorometer (PAM 2000; Heinz Walz GmbH, Germany). Leaves were dark adapted for 15 min prior to measurement.

2.4. Transmission electron microscopy (TEM)

The ultrastructure was assayed for 50 random chloroplast section photographed at the same magnification. The chloroplast ultrastucture was analysed from the electron micrographs as described by Silaeva and Silaev (1979) and Evans (1986).

3. Results and Discussion

3.1. Effect of UV-B on cucumber seedlings

However, visible injury, such as chlorosis, which is likely to have been mediated by the generation of free radicals, was not observed throughout the 15 d of UV-B treatment. Under carefully controlled growth chamber conditions elongation of UV-B-induced leaves lagged significantly behind that of conrols between the 1 d and 7d, but final leaf length on day 7 d did not differ between treatments and fresh weight was significantly greater for UV-B-induced leaves than for controls at most developmental stage.

3.2. Photosynthetic damage of cucumber first leaves

To investigate the effect of UV-B on the electron transport of photosystem, we measured the Fv/Fm ratio of cucumber first leaves during the 7 d period of Fv/Fm ratio for the first 5 d. However, the Fv/Fm ratio decreased by 13% compared to the control after 2 d, and was kept to be lower than the control level until 5 d.

In first leaves of cucumber seedlings grown with supplementary UV-B irradiation, the contents of total SA concentraion began to rise from about 1 h after the start of UV-B irradiation, and reached a maximum level (1.4-fold) after 1 d of UV-B irradiation.

3.3. Effect of SA treatment on UV-B-induced leaf damage syptoms

We examined whether SA treatment could protect cucumber seedling against UV-B-induced oxidative stress. After reaching the full vegetative state in a growth chamber, the plants were treated with or without of SA (100μM, 300μM, 500μM, 700μM, 1.0mM), 24h before being subjected to 1 d under UV-B-induced stress.

3.4. Ultrastructure of chloroplasts

The inner structure of chloroplast was characterized by thylakoid destruction. Gradual

changs in chloroplast ultrastructure may result in an inhibition of photosynthetic electron transport around photosystem II (PSII), as suggested by Maslenkova and Toncheva (1998).

4. Abstract

Cucumber (*Cucumis sativus* L.) seedlings were irradiated or not irradiated with UV-B for several days in environment controled growth chambers. The first leaves irradiated with UV-B were retarded in growth but simultaneously acquired a remarkably high tolerence to oxidative stress, as induced by SA treatment, compared with the non irradiated leaves. Observed within 1 d after the start of UV-B and was maintained during the 5 d period of UV-B treatment.

There were low rates of growth and photosynthesis in cucumber plants when treated with SA, suggesting alterations in anatomy and ultrastructure organisation of chloroplasts. Exogenous application of SA diminished chloroplast photosynthetic activity.

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