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The mechanisms by which phenanthrene affects the photosynthetic apparatus of cucumber leaves

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HIGHLIGHTS

- Short-term phenanthrene treatment inhibited the photosynthesis of cucumber leaves.
- Phenanthrene suppressed PSII activity.
- Phenanthrene caused ROS accumulation and D1 protein degradation under high light.

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ABSTRACT

Phenanthrene is a polycyclic aromatic hydrocarbon (PAH) that is widely distributed in the environment and seriously affects the growth and development of plants. To clarify the mechanisms of the direct effects of phenanthrene on the plant photosynthetic apparatus, we measured short-term phenanthrene-treated cucumber leaves. Phenanthrene inhibited Rubisco carboxylation activity, decreasing photosynthesis rates (Pn). And phenanthrene inhibited photosystem II (PSII) activity, thereby blocking photosynthetic electron transport. The inhibition of the light and dark reactions decreased the photosynthetic electron transport rate (ETR) and increased the excitation pressure (1−qP). Under high light, the maximum photochemical efficiency of photosystem II (F_v/F_m) in phenanthrene-treated cucumber leaves decreased significantly, but photosystem I (PSI) activity ($\Delta I/I_o$) did not. Phenanthrene also caused a J-point rise in the OJIP curve under high light, which indicated that the acceptor side of PSII Q_A to Q_B electron transfer was restricted. This was primarily due to the net degradation of D1 protein, which is caused by the accumulation of reactive oxygen species (ROS) in phenanthrene-treated cucumber leaves under high light.

This study demonstrated that phenanthrene could directly inhibit photosynthetic electron transport and Rubisco carboxylation activity to decrease net Pn. Under high light, phenanthrene caused the accumulation of ROS, resulting in net increases in D1 protein degradation and consequently causing PSII photoinhibition.

1. Introduction

Phenanthrene is an important polycyclic aromatic hydrocarbon (PAH), a group of organic pollutants that are highly persistent in the environment and has spread with human urbanization and industrialization. PAHs have accumulated gradually in the ecosystem and are widely distributed in the atmosphere, soil and water (Srogi, 2007). They have mutagenic and teratogenic properties and thus pose a substantial threat. PAHs can be absorbed by plants and enter the food chain, thereby threatening human health (Wang and Freemark, 1995; Dalla valle et al., 2004).

Plants can suffer long-term exposure to PAHs (Simonich and Hites, 1994). Many studies have shown that this type of contamination can affect plant germination and biological production (Henner et al., 1999; Kummerová and Kmentová, 2004; Smith et al., 2006; Kummerová et al., 2012), the production of reactive oxygen species (Alkio et al., 2005; Pašková et al., 2006; Liu et al., 2009) and root growth (Kummerová and Kmentová, 2004; Merkl et al., 2005; Kummerová et al., 2013). Photosynthesis is the energy source for plant metabolism. Damage to the photosynthetic apparatus will affect the physiological activity of plant cells, inhibiting growth and

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reducing yield. Previous researches have found that PAHs can cause the degradation of photosynthetic pigments (Kummerová et al., 2006; Oguntimehin et al., 2010; Jajoo et al., 2014), inhibition of photosynthetic carbon assimilation (Oguntimehin et al., 2010) and decreases in photosynthetic products (Li et al., 2008; Muratova et al., 2009). However, little research has been done on the effect of PAH treatment on energy absorption and conversion or on the mechanism by which PAHs inhibit photosynthesis.

PAHs can accumulate in the plant body in two ways. First, PAHs can be taken up by roots; however, they are lipophilic molecules that are not easily transported to the shoot by transpiration, so they aggregate near the surfaces of roots. Second, PAHs can settle from the atmosphere onto the surface of the leaf and then enter through the waxy cuticle (Wild and Jones, 1992; Simonich and Hites, 1995; Kipopoulou et al., 1999; Kirkwood, 1999; Barber et al., 2004). To study the direct impact of phenanthrene on cucumber photosynthesis, leaves were measured after a short-term phenanthrene treatment. This treatment eliminated the possible negative effects of phenanthrene on the roots, which may have indirect effects on photosynthesis.

Chlorophyll fluorescence analyses, gas exchange measurements, oxygen electrode measurements and protein analyses were used to investigate the impact of phenanthrene on photosynthetic light energy absorption and conversion, as well as the mechanism by which phenanthrene inhibits photosynthesis.

2. Materials and methods

2.1. Plant materials and growth conditions

Cucumber (*Cucumis sativus* L.) seedlings were planted in pots (7 cm in diameter, 10 cm in height) with vermiculite and watered with Hoagland solution every three days. The plants were grown in a greenhouse at Shandong Agriculture University. The environmental conditions within the greenhouse were as follows: day, 18–30 °C; night, 12–18 °C; and maximum photosynthetic photon flux density (PPFD) at plant height during the day, 1000 μmol m^{-2} s^{-1}. Plants were used for experiments when their first true leaves were fully expanded.

2.2. Phenanthrene preparation and treatment

Phenanthrene (Aladdin, China) was dissolved in 80% aqueous acetone to a concentration of 1000 mg/l and diluted in distilled water to final concentrations of 0.2, 1, 5 and 10 mg/L. Every phenanthrene solution and the control solution contained a final concentration of 0.8% acetone. A preliminary study showed that 0.8% acetone, as used in these mixtures, had no adverse effect on cucumber seedlings (data not shown). Phenanthrene has low water solubility of 1.2 mg/L at 25 °C (Perlman et al., 1984). The oversaturated solutions (5 and 10 mg/L) of phenanthrene were used where some amount of phenanthrene was present in the samples as stable suspensions of fine compound. All the phenanthrene solutions and the control solutions were new prepared. In the period of our studies, there was no sediment in our solutions.

The whole plants were horizontally placed and the first fully expanded true leaves were submerged in phenanthrene or control solutions for 4 h and then were removed and allowed to recover for 12 h in the dark at room temperature (25 °C) in the air. All measurements were carried out after this treatment. The high-light treatment took place under 1000 μmol m^{-2} s^{-1} PPFD for 2 h at room temperature. A red and blue (8:1) light emitting diode (LED) (SP501-N, SanPeng, China) was used to provide illumination.

2.3. Photosynthetic gas exchange measurements

The net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (E) and substomatal CO_2 concentrations (Ci) were measured using a CIRAS-3 portable photosynthesis system (PP Systems, USA) at 25 °C, 1000 μmol m^{-2} s^{-1} PPFD, 65%–70% relative humidity and ambient CO_2 (380 μmol mol^{-1}) between 8.00 a.m. and 11.00 a.m. in the morning.

To obtain the apparent quantum yield of photosynthesis (AQY), the net assimilation rate was measured under ambient CO_2 (380 μmol mol^{-1}) and under PPFDs of 400, 300, 200 and 100 μmol m^{-2} s^{-1}. The leaves were kept under each PPFD for 3 min to let photosynthesis reach steady state, after which the net assimilation rate was recorded. To obtain apparent carboxylation efficiency (CE), the net assimilation rate was measured under a PPDF of 1000 μmol m^{-2} s^{-1} and under CO_2 concentrations of 300, 200, 150 and 100 μmol mol^{-1}. The leaves were kept under each CO_2 concentration for 5 min to let photosynthesis reach steady state, after which the net assimilation rate was recorded. The net assimilation rates exhibited significant linear correlations with the Pn-PPDF and Pn-Ci responses, and the initial slop of these responses were used to calculate AQY and CE, respectively. All gas exchange measurements were performed at 25 °C.

2.4. Measurements of chlorophyll fluorescence

Modulated chlorophyll fluorescence was measured using a FMS-2 pulse-modulated fluorometer (Hansatech, Norfolk, UK). The light-fluorescence measurement protocol was as follows: light-adapted leaves (1000 μmol m^{-2} s^{-1}, 20 min) were continuously illuminated by actinic light at 1000 μmol m^{-2} s^{-1} from the FMS-2 light source, steady-state fluorescence (Fs) was recorded after 40 s of illumination, and a 0.8-s saturating light pulse of 8000 μmol m^{-2} s^{-1} was applied to obtain the maximum fluorescence in the light-adapted state (F_m'). The actinic light was then turned off for 3 s, and the minimum fluorescence in the light-adapted state (F_o') was determined by a 3 s illumination with far-red light. The intensity of metrical light used in all trials was the same and did not exceed 1 μmol m^{-2} s^{-1}.

The following parameters were then calculated (Maxwell and Johnson, 2000): (i) Quantum yield of electron transfer though PSII, $\varphi PSII = (F_m' - F_s)/Fm'$; (ii) Electron transport rate, $ETR = \varphi PSII \times PPFD \times 0.5 \times 0.84$; (iii) Excitation pressure, $1 - qP = 1 - (F_m' - F_s)/(F_m' - F_o')$.

2.5. Measurements of the chlorophyll a fluorescence transient and 820-nm transmission

The chlorophyll *a* fluorescence transient (OJIP) was measured using a Multifunctional Plant Efficiency Analyzer, M-PEA (Hansatech Instrument Ltd., UK), with dark-adapted leaves (in dark for 1 h) under ambient CO_2 levels at room temperature (25 °C). Saturating red light at 5000 μmol m^{-2} s^{-1} was produced by an array of four LEDs with a peak of 650 nm. During 2 s of illumination with saturating red light, the chlorophyll *a* fluorescence intensity in dark-adapted leaves rose rapidly from an initial minimal level, F_o (O step), to the maximal level, Fm (P step). Two intermediate steps, designated J and I, appeared at 2 and 30 ms, respectively; a rapid rise in chlorophyll *a* fluorescence, termed O-J-I-P, was obtained. Chlorophyll *a* fluorescence transients were analysed by using the original data from polyphasic fluorescence transients according to the JIP test (Haldimann and Strasser, 1999). The following fluorescence parameters were calculated: (i) Maximum quantum yield of PSII, $Fv/Fm = 1 - (F_o/F_m)$; (ii) Relative variable fluorescence at any time, $V_t = (F_t - F_o)/(F_m - F_o)$.