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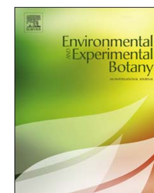

Aggravation of photoinhibition during variegated leaf development in *Actinidia kolomikta* (Rupr. & Maxim.) Maxim.

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A B S T R A C T

Variegated leaves of *Actinidia kolomikta* (Rupr. & Maxim.) Maxim. are white, pink and pale green according to the order of leaf development, respectively. However, the pale-green leaves are the most sensitive to high-light stress. To clarify the photoinhibitory mechanism in pale-green leaves, we investigated leaf pigment, spectral properties, gas exchange and chlorophyll fluorescence. During variegated leaf development, the chlorophyll content and light absorption increased rapidly, but only pink leaves contained higher anthocyanin contents. With increasing light absorption, the photosynthetic capacity and the actual quantum yield of photosystem II (PSII) in variegated leaves increased slightly, while the non-photochemical quenching (NPQ) significantly decreased. Under high-light conditions, the maximum quantum yield of PSII photochemistry in white and pink leaves decreased less, while in pale-green leaves it decreased significantly together with a substantial increase in malonaldehyde (MDA) and hydrogen peroxide (H_2O_2) levels. Therefore, during variegated leaf development, although the light absorption increased significantly in pale-green leaves, the photosynthetic capacity and thermal dissipation were not significantly enhanced, which may result in serious photoinhibition under high-light conditions.

1. Introduction

Photoinhibition is an inevitable phenomenon in all oxygen-evolving photosynthetic organisms exposed to high light. The primary damage of photoinhibition occurs within the reaction centers of photosystem II (PSII). Newly initiating leaves are often exposed to full sunlight in the topmost canopy or at the top of branches during leaf development. However, juvenile leaves have lower photosynthetic capacity compared with mature leaves (Juvany et al., 2013), which results in more excessive excited energy (Krause et al., 1995; Choinski et al., 2003; Jiang et al., 2005; Zhang et al., 2012; Allorent and Petroutsos, 2017). Excessive excited energy can lead to the production of reactive oxygen species (ROS), which can damage the photosynthetic apparatus (Zhu et al., 2016; Zhang et al., 2017). Thus, photoinhibition may readily take place in juvenile leaves (Jiang et al., 2006; Juvany et al., 2013). During leaf expansion, photosynthetic capacity and PSII photochemical activity increase gradually, and excessive excited energy decreases rapidly. Therefore, the extent of photoinhibition in developing leaves decreases gradually during leaf development. In addition to studies focused on photoinhibition and photoprotective mechanisms during normal green leaf development, there have also been studies in variegated leaf development (Esteban et al., 2008; Chen et al., 2013).

Variegation is characterized by the presence of white, red, purple, yellow or pale-green areas in a normally green leaf or tissue. Many variegated plants often have green and white sectors in normally green leaves. A large number of variegated leaf mutants have been screened in the model plant *Arabidopsis*. In these mutants (such as *immutans*, *spotty*, *var1* and *var2*), high-light favors white sector formation, while weak-light favors green sector formation (Rosso et al., 2009). Thermal dissipation in the *immutans* mutant decreases owing to the lack of carotenoids (Yu et al., 2007; Rosso et al., 2009; Foudree et al., 2012; Pogorelko et al., 2016). Therefore, high-light conditions give rise to severe photoinhibition in the *immutans* mutant. Accordingly, the formation of white sectors is the result of photooxidation. Additionally, leaves of some plant species in tropical or sub-tropical forests usually display red at the beginning of leaf development, and then turn green as development continues (Karageorgou and Manetas, 2006; Zhang et al., 2016; Csepregi et al., 2017). The red of leaves is mainly caused by

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anthocyanin accumulation (Steyn et al., 2002; Kyparissis et al., 2007). Anthocyanins may confer significant protection against photooxidative damage during variegated leaf development (Hughes et al., 2005). On one hand, anthocyanins act as light attenuators, absorbing blue–green light and reflecting red light (Albert et al., 2009; Zhang et al., 2014). On the other hand, anthocyanins have strong antioxidant potentials and can scavenge the reactive oxygen molecules effectively (Neill et al., 2002; Bi et al., 2014). Because of the anthocyanin accumulation in red leaves, a very slight photoinhibition occurs in the field (Li et al., 2014).

Besides the variegated leaves mentioned above, in some plants, leaf color undergoes very complex changes in pigment levels and leaf structure during leaf development (Sheue et al., 2012). Thus, to cope with high-light conditions, photoinhibition and photoprotective mechanisms in these plants may be more complex than in others.

Actinidia kolomikta (Rupr. & Maxim.) Maxim. is an important fruit germplasm resource in China. It is mainly distributed in mixed coniferous and broad-leaved forest environments in both north-eastern and south-western China. One of the most important characteristics of *A. kolomikta* is the complex change in leaf color that occurs during leaf development, which is a rare phenomenon among other plant species. Generally, *A. kolomikta* has fully white or green leaves with obvious white patches on the adaxial surfaces from late spring to early summer, yet the abaxial surfaces of variegated leaves are green. By mid-summer, the white leaves turn pink, and then the pink leaves become pale green. Interestingly, the white and pink leaves of *A. kolomikta* have a strong tolerance to full sunlight. However, pale-green leaves are very sensitive to high-light conditions and often suffer serious photoinhibition and even photodamage. To clarify the special photoinhibitory mechanism in pale-green leaves of *A. kolomikta*, we carefully investigated leaf pigment, spectral properties, gas exchange and chlorophyll fluorescence. This study will have great significance in understanding photoinhibition and photoprotective mechanisms in variegated leaves of *A. kolomikta* during leaf development.

2. Materials and methods

2.1. Plant materials

Actinidia kolomikta (Rupr. & Maxim.) Maxim. seedlings came from the Institute of Special Wild Economic Animal and Plant Science. Vegetatively propagated *A. kolomikta* (13118) vines were used for studies at Zuojia, China. The location is between 44°04′ N and 126°05′ E, has an annual precipitation of 679 mm, and annual high and low temperatures of 35 °C and −30 °C, respectively. In this area, plants were exposed to full sunlight; the maximum photosynthetic photon flux density (PPFD) on a clear day is about 1800 ± 152 μmol m^{-2} s^{-1}. The maximum light intensity underwent no significant changes during leaf development. All field trials were conducted from May to July 2016. The humidity during the experimental period was 85% in the evening and 60% in the daytime. Each field plot was divided into three subplots, and the seedlings were planted in each plot (3 m × 4 m). Plot soil, and concentrations of nitrogen, phosphorous and potassium were similar. Plant materials were of a uniform size. White, pink, green and pale-green leaves were collected during late May (late spring), early June (early summer) and early July (mid-summer).

2.2. Methods

2.2.1. Chlorophyll distribution measurement

Leaf sections were fixed in 5 mL formalin, 6 mL acetic acid, 89 mL 50% alcohol and 5 mL glycerol (Deng et al., 2010), and stored in 70% ethyl alcohol at 4 °C. Leaves were prepared for serial paraffin sectioning. Sections were dehydrated through a graded ethanol series, infiltrated and embedded in Leica Historesin. Next, serial sections were cut at 10–12 μm with a rotary microtome. Sections were observed under an epifluorescence microscope (Nikon ECLIPSE 80i, Nikon, Tokyo, Japan) equipped with an FITC filter (excitation 560 nm) (Peer et al., 2001). Photomicrographs were taken with a Zeiss Axiolab and a digital camera (Nikon DXM1200, Nikon Corporation,Tokyo, Japan).

2.2.2. Pigment analysis

Ten leaf discs were excised using a standard hole punch, immediately sealed in pre-labeled aluminum envelopes and placed in liquid nitrogen. Tissues were stored at −80 °C until analysis, and then extracted in a solvent mixture of acetone, methanol and water (80:15:5, v/v/v.). The chlorophyll content was measured at 663 nm and 645 nm using an UV–vis spectrophotometer UV-1601 (Shimadzu, Kyoto, Japan) according to Porra (2002). Total chlorophyll content was calculated by the formula [Total chlorophyll] = $(20.29 \times A_{645}) - (8.05 \times A_{663})$, in which A denotes the absorbance at the chlorophyll peaks (663 nm and 645 nm).

For anthocyanin extraction, nine leaf discs were immersed in boiling methanol/H_2O/HCl (90:10:1, v/v/v) for 10 min. The relative amount of anthocyanins was assessed from their peak absorbance levels (530 nm and 657 nm), after correcting for the contribution of pheophytins. The anthocyanin concentration was determined by the formula [Anthocyanin] = $A_{530} - 1/3A_{657}$ (Mancinelli et al., 1975; Kytridis and Manetas, 2006; Wang et al., 2016), in which A denotes the absorbance at the anthocyanin peaks (530 nm and 657 nm).

2.2.3. Leaf absorbance measurement

Reflectance was measured on three leaves at different developmental stages using a bifurcated fiber optic cable and a leaf-clip of a Unispec spectrometer (PP Systems, Amesbury, MA, USA) according to Xue et al. (2014). The leaves were irradiated from one side with a tungsten halogen lamp in the spectrometer using the bifurcated fiber cable. The experimental set-up provided a spectral range of 400–700 nm. Transmittance was measured according to the method of Konoplyova et al. (2008). Absorbance (A) was calculated as A = 1–(Reflectance + Transmittance).

2.2.4. Gas exchange measurement

Net photosynthetic rate (P_N) was analyzed with a portable *CIRAS-2* photosynthesis system (PP Systems, Amesbury, MA, USA) between 8:00 am and 11:00 am on a sunny day. The CO_2 concentration, air humidity and leaf temperature were maintained at 360 μmol mol^{-1}, 800 Pa and 25 °C, respectively. To obtain photosynthetic light-response curves, the PPFDs were set in the order of 1200, 1000, 800, 600, 400, 200, 100, 50 and 0 μ mol m^{-2} s^{-1}. The P_N at each PPFD was recorded when it was stable (usually 3–5 min).

2.2.5. Chlorophyll fluorescence measurement

2.2.5.1. Light responses of quantum yield of PSII (ΦPSII) and non-photochemical quenching (NPQ). Modulated chlorophyll fluorescence was measured with an FMS-2 pulse-modulated fluorometer (Hansatech Instruments, King's Lynn, Norfolk, UK). Initial fluorescence (F_0) was recorded in leaves adapted to dark for 60 min. A 0.7-s pulse of saturating white light (> 3000 μmol m^{-2} s^{-1}) was applied to obtain maximum fluorescence (F_m). The fluorescence measurement protocol was as follows: the light-adapted leaves were continuously illuminated by actinic light from the FMS-2 light source. The steady-state fluorescence level (F_s) and the maximum fluorescence in the light-adapted state (F_m') during exposure to different light intensities were also measured (Jiang et al., 2005). The parameters listed below were then calculated at different light intensities. The PFDs were set in the order of 2100, 1600, 1200, 900, 600, 400, 300, 200, 150, 100, 70, 50, 25, 15 and 0 μmol m^{-2} s^{-1} to obtain ΦPSII–PFD and NPQ–PFD response curves.

$$\Phi PSII = (F_m' - F_s)/F_m'; \quad (1)$$

$$NPQ = (F_m - F_m')/F_m'. \quad (2)$$

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