Seasonal variations in photosystem I compared with photosystem II of three alpine evergreen broad-leaf tree species

Wei Huang *, Ying-Jie Yang, Hong Hu, Shi-Bao Zhang

Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China

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ABSTRACT

Low temperature associated with high light can induce photoinhibition of photosystem I (PSI) and photosystem II (PSII). However, the photosynthetic electron flow and specific photoprotective responses in alpine evergreen broad-leaf plants in winter is unclear. We analyzed seasonal changes in PSI and PSII activities, and energy quenching in PSI and PSII in three alpine broad-leaf tree species, *Quercus guyavifolia* (Fagaceae), *Rhododendron decorum* (Ericaceae), *Euonymus tingens* (Celastraceae). In winter, PSII activity remained stable in *Q. guyavifolia* but decreased significantly in *R. decorum* and *E. tingens*. *Q. guyavifolia* showed much higher capacities of cyclic electron flow (CEF), water-water cycle (WWC), non-photochemical quenching (NPQ) than *R. decorum* and *E. tingens* in winter. These results indicated that in alpine evergreen broad-leaf tree species the PSII activity in winter was closely related to these photoprotective mechanisms. Interestingly, unlike PSII, PSI activity was maintained stable in winter in the three species. Meanwhile, photosynthetic electron flow from PSII to PSI (ETRII) was much higher in *Q. guyavifolia*, suggesting that the mechanisms protecting PSI activity against photoinhibition in winter differed among the three species. A high level of CEF contributed the stability of PSI activity in *Q. guyavifolia*. By comparison, *R. decorum* and *E. tingens* prevented PSI photoinhibition through depression of electron transport to PSI. Taking together, CEF, WWC and NPQ played important roles in coping with excess light energy in winter for alpine evergreen broad-leaf tree species.

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1. Introduction

Under conditions in which absorbed light is in excess of the requirements for photosynthesis, photoinhibition occurs in leaves [50]. Low temperature is a typical climatic condition in winter in alpine regions. Combination of high light and low temperature can lead to photoinhibition of photosystem I (PSI) and photosystem II (PSII) not only in chilling-sensitive species such as *Arabidopsis thaliana* [76], tobacco [3,4] and cucumber [30,54–57], but also in chilling-tolerant species such spinach [51,73]. Alpine evergreen broad-leaf tree species have to regularly encounter high irradiance and low temperature in winter. However, the seasonal changes in PSI and PSII activities in alpine evergreen broad-leaf plants are unclear.

At present, there are three schemes involved in the mechanism of PSII photoinhibition. In the "excess-energy hypothesis", strong light induces the production of reactive oxygen species (ROS), which directly inactivate the photochemical reaction center of PSII. In the "two-step hypothesis", photodamage is initiated by the direct effect of light on the oxygen-evolving complex and ROS inhibit the repair of

photodamaged PSII (for review [38]). The third scheme propose that both "excess-energy hypothesis" and "two-step hypothesis" are involved in photoinactivation of PSII, and the relative contribution from each mechanism depends on growth conditions or plant species [43, 44]. Generally, PSII photoinhibition primarily occurs at oxygen-evolving complex (OEC) [11,45,73]. Inactivation of OEC suppresses the electron transport to P680 and consequently leads to high levels of P680$^+$. Since P680$^+$ is highly oxidative, high levels of P680$^+$ can cause damage to the PSII reaction center [65,67]. Under environmental stresses, inhibition of the Calvin cycle aggravates the production of reactive oxygen species (ROS). In cyanobacteria, flavodiiron proteins (FLVs) functions alternative electron flow with a high electron flux, diminishing the production of ROS when photosynthesis is inhibited [14,74,75]. By comparison, in higher plants, the restriction of the Calvin cycle at low temperature leads to the depletion of NADP$^+$ and then increases the generation of ROS at PSI acceptor side [38]. Subsequently, PSII photoinhibition was aggravated at chilling-light stress [1]. In addition to PSII, PSI is sensitive to chilling-light stress in some chilling-sensitive plants such as cucumber [55,57], tobacco [3,4], and *Arabidopsis thaliana* [76]. At chilling temperature, alternative electron flow via Mehler reaction results in the formation of ROS, which increase the risk of PSI photoinhibition [2,22]. Recently, Takagi et al. [63] reported that the ROS production site, but not the ROS production rate, is critical for PSI photoinhibition in isolated chloroplasts of spinach leaves. However,

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* Corresponding author at: Chinese Academy of Sciences, Kunming Institute of Botany, Key Laboratory of Economic Plants and Biotechnology, 132# Lanhei Road, Heilongtan, Kunming, Yunnan 650201, China.

E-mail address: huangwei@mail.kib.ac.cn (W. Huang).

we found that PSI photoinhibition is more related to electron transfer from PSII to PSI rather than PSI redox state in intact leaves of *Psychotria rubra* [23]. In order to survive under conditions of high light and low temperature, alpine evergreen broad-leaf plants should have several mechanisms to control the accumulation of ROS in thylakoid membrane and then protect PSI and PSII against photodamage in winter.

In higher plants, several mechanisms are documented to diminish PSI and PSII photoinhibition, such as cyclic electron flow (CEF) [6,18, 19,36,37,61,66,69], water-water cycle (WWC) [2,35], photorespiration [60,64], non-photochemical quenching [39–41], and antioxidant system [32,59,60]. During CEF, electrons from either NADPH or ferredoxin are cycled around PSI into the plastoquinone pool, which is coupled to the generation of ΔpH [25,26]. Under ambient conditions, the WWC plays an important role in balancing ATP/NADPH energy budget and NPQ activation via ΔpH formation [33]. For a widely studied alpine herbaceous species *Ranunculus glacialis*, alternative electron flow and antioxidant system play important roles in photoprotection for PSII [32]. However, it is unclear whether the WWC activity plays an important role in photoprotection for evergreen broad-leaf tree species. Furthermore, the role of CEF in photosynthetic regulation in winter for alpine evergreen broad-leaf plants is little known.

In the model plant *Arabidopsis thaliana*, activation of CEF helps generation of proton gradient across thylakoid membranes (ΔpH) that is essential for activation of NPQ and oxidation of P700 reaction centers [36,37,66]. In the *pgr5* mutants, impairment of PGR5-dependent CEF pathway leads to depression of NPQ and over-reduction of PSI acceptor side, which then causes severe photoinhibition of PSI and PSII when illuminated at high light or fluctuating light conditions [36,61,66,69]. At chilling-light stress, *Arabidopsis thaliana* displayed slightly activation of CEF, leading to a significant PSI photoinhibition [76]. These previous studies suggest that activation of CEF is an important mechanism for preventing PSI from photoinhibition at chilling temperature. Because PSI photoinhibition is dependent on active electron flow from PSII to PSI, depression of linear electron flow is another strategy to avoid PSI photoinhibition at chilling-light stress, which was used by tropical tree species [17]. Since PSI activity is critical for photoprotection, photosynthesis and growth of the whole plants [77], it is necessary to maintain stable PSI activity in winter for alpine evergreen broad-leaf plants. However, the specific mechanisms involved in PSI photoprotection in alpine evergreen broad-leaf species are unclear.

When CO_2 assimilation is limited or inhibited, the water-water cycle (WWC) can maintain electron flow through photosynthetic apparatus [16,33,72]. During WWC, the splitting of water molecules at PSII produces electrons, which are transported to oxygen through PSI, resulting in the formation of superoxide radicals. The superoxide radicals are converted to O_2 and H_2O_2 by a membrane-attached copper/zinc superoxide dismutase (Cu/Zn SOD). Subsequently, a membrane-bound ascorbate peroxidase converts the hydrogen peroxide back into water. Although the WWC induces production of ROS, the WWC-dependent generation of ΔpH helps ATP synthesis and NPQ activation, furthermore, the WWC activity optimizes the redox state of plastoquinone pool. As a result, the WWC is essential for chloroplast photoprotection in the absence and present of abiotic stresses ([2,47,52]). The WWC-dependent generation of ΔpH helps to activate NPQ and regulates the redox state of the PQ pool, and thus alleviates PSII photoinhibition [15,53]. In alpine regions, combination of high light and low temperature in winter limits photosynthesis, which increases the risk of PSII photoinhibition in chloroplasts of alpine evergreen broad-leaf plants. Therefore, we speculate that alpine evergreen broad-leaf plants should have high WWC activity to control the ROS content in chloroplast and then protect PSII activity in winter.

Although the roles of CEF and WWC were inferred from a number of studies, the functions of these pathway in protecting PSI and PSII against low temperature and high light are little known in alpine evergreen broad-leaf species, excluding the evergreen broad leaf alpine species *Geum montanum* [34]. In the present study, we examined seasonal variations in PSI and PSII activities in three alpine evergreen broad-leaf tree species, *Quercus guyavifolia* (Fagaceae), *Rhododendron decorum* (Ericaceae), and *Euonymus tingens* (Celastraceae). Furthermore, the CEF and WWC activities in winter were examined in the three species. Three main questions were investigated: (1) did PSI and PSII activities were maintained stable in winter in alpine evergreen broad-leaf tree species? (2) how did alpine evergreen broad-leaf tree species protect PSI against photoinhibition in winter? (3) did CEF and WWC play important roles in photoprotection for PSII in alpine evergreen broad-leaf tree species? The differences in photoprotective responses in winter among alpine evergreen broad-leaf tree species are discussed.

2. Materials and Methods

2.1. Study Site and Plant Materials

This study was conducted at Lijiang Forest Ecosystem Research Station which located at elevations from 3200 to 3500 m. In the present study, we chose three evergreen tree species grown at an altitude of 3290 m (E 27°00′11.37″, N 100°10′49.59″) for photosynthetic measurements. *Quercus guyavifolia* H. Léveillé (Fagaceae) is a dominant (in number) tree species that grows in mountainous oak forests or pine-oak mixed forests of southwestern China at elevations of 2500 to 4300 m. As an important component of evergreen broad-leaved forests, plants of this species can reach 15 m height. For our investigation, we sampled 3–4 m tall trees. *Rhododendron decorum* Franchet (Ericaceae) is an important tree species that grows in mountainous at elevations of 1000 to 3500 m. The maximum height of *R. decorum* can reach 6 m. Plants of this species with height of 1–3 m were chosen for study. *Euonymus tingens* Wallich (Celastraceae) is an endemic tree species that grows at elevations of 1300–3700 m. Plants of *E. tingens* can reach 8 m height, but we chose 1–2 m tall plants for study. Plants of the three species grow well in the study site. To investigate the seasonal changes in PSI and PSII activities, we chose mature sun leaves that had flushed in summer in 2014 for photosynthetic measurement. After cutting from plants, the twigs were immersed in water and leaves were measured.

2.2. Chlorophyll Fluorescence and P700 Measurements

We measured photosynthetic parameters in January in 2014 (winter) and August in 2015 (summer). To estimate the activity of WWC in winter, we measured the photosynthetic parameters at approximately 0 °C in winter in a snowy day (19 January in 2014). In summer, we measured the photosynthetic parameters at approximately 15 °C in two clear days (25 and 26 August 2015). The maximum quantum yield of PSII (F_v/F_m), the maximum photo-oxidizable P700 (P_m), and light response curves were measured by a Dual-PAM-100 (Walz, Germany) connected to a computer with control software. To facilitate the measurements of light response curves, twigs from four to six individuals per species were cut from the trees and immediately inserted in a plastic bottle filled with water. To generate a light response curve, we initially exposed the mature leaves to 759 μmol photons m^{-2} s^{-1} for 15 min to obtain steady-state levels of Y(II). Afterward, photosynthetic parameters were evaluated at 2-min intervals at photosynthetic photon flux densities (PPFDs) of 1809, 1455, 1178, 923, 759, 501, 330, 172, 94, or 36 μmol photons m^{-2} s^{-1}. Values for predawn F_v/F_m and P_m were measured after one night dark adaptation. At 7:30 a.m. before sunrise, detached leaf samples were obtained and we measured F_v/F_m and P_m immediately.

The PSII activity was calculated as $F_v/F_m = (F_m - F_o)/F_m$, where F_o and F_m represent the minimum and the maximum fluorescence after one night dark adaptation, respectively. The effective quantum yield of PSII was calculated as $Y(II) = (F_m' - F_s)/F_m'$ [10], where F_s is the light-adapted steady-state fluorescence and F_m' is the maximum light-adapted fluorescence. F_m and F_m' are measured upon illumination of a pulse (300 ms) of saturating light (10,000 μmol photons m^{-2} s^{-1}).