Photoinhibition and Post-Recovery Effects of Photosystem II in Three Evergreen Broad-Leaved Tree Species Under Low Temperature Stress

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Abstract

One of the primary effects of winter low temperature stress on the photosynthetic activity of subtropical evergreen broadleaf trees is manifested as low temperature photoinhibition of the photosynthetic apparatus. To elucidate the degree of photoinhibition and photoprotective mechanisms of Photosystem II (PSII) in evergreen broadleaf trees under winter low temperature stress, this experiment investigated the effects of natural winter low temperature stress (subzero freezing injury and above-zero chilling injury) on PSII photoinhibition in three subtropical evergreen broadleaf species—Photinia × fraseri, Eriobotrya japonica, and Cinnamomum bodinieri—and their recovery following spring warming. The results demonstrated that freezing and chilling stresses significantly reduced PSII activity in Cinnamomum bodinieri, with PSII experiencing relatively severe photoinhibition that failed to fully recover after the low temperature stress was alleviated. Photinia × fraseri exhibited the smallest decline in PSII activity and the mildest degree of photoinhibition, with PSII activity increasing significantly and photoinhibition decreasing markedly in spring. The degree of PSII activity and photoinhibition in Eriobotrya japonica was intermediate between that of Cinnamomum bodinieri and Photinia × fraseri. Under low temperature stress, the non-photochemical quenching (NPQ) of Photinia × fraseri remained near normal temperature levels; the NPQ of Eriobotrya japonica decreased slightly but returned to normal in spring; while Cinnamomum bodinieri had the lowest NPQ, which still could not fully recover after low temperature relief in spring. Moreover, a significant negative correlation existed between NPQ and the degree of PSII photoinhibition in the three evergreen broadleaf species during both winter low temperature stress and spring recovery periods. Comprehensive analysis of these results indicates that winter

low temperature had minimal impact on PSII of Photinia × fraseri, moderate impact on Eriobotrya japonica that could be promptly recovered with spring warming, and significant photoinhibitory effects on Cinnamomum bodinieri with a slower recovery process. Furthermore, NPQ makes an important contribution to protecting PSII of evergreen broadleaf trees from winter low temperature photoinhibition.

Full Text

Preamble

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Photoinhibition and Recovery of Photosystem II in Three Broad-leaved Evergreen Species under Low Temperature Stress

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Abstract

Winter low temperature stress on subtropical evergreen trees primarily limits photosynthesis through photoinhibition of the photosynthetic apparatus. To elucidate the extent of photosystem II (PSII) photoinhibition and the underlying photoprotective mechanisms in evergreen trees under winter low temperature stress, we investigated PSII photoinhibition and recovery in three broad-leaved evergreen species: *Photinia × fraseri*, *Eriobotrya japonica* (Thunb.) Lindl, and *Cinnamomum bodinieri* Levl. Our results demonstrated that *C. bodinieri* experienced severe PSII depression and photoinhibition under both freezing and chilling temperatures, with PSII function failing to fully recover even when spring temperatures returned to normal. *P. × fraseri* exhibited the most stable PSII function and the least photoinhibition, while *E. japonica* showed an intermediate response. Correspondingly, *P. × fraseri* maintained non-photochemical quenching (NPQ) levels nearly equivalent to those under normal temperature conditions. In *E. japonica*, NPQ decreased slightly but recovered upon return to normal temperatures. *C. bodinieri* displayed the lowest NPQ under low temperature stress and was unable to achieve complete recovery. Furthermore, all three species exhibited a strong negative correlation between NPQ and PSII photoinhibition, as indicated by the maximum potential photochemical efficiency of PSII (Fv/Fm) and the quantum yield of non-regulated energy dissipation (Y(NO)). Collectively, these results demonstrate that *P. × fraseri* possesses greater resistance to low temperature stress than *E. japonica* and *C. bodinieri* with respect to PSII function. NPQ plays a crucial role in protecting PSII of these evergreen species from photoinhibition under low temperature conditions.

Key words: physiology of photosynthesis, PSII, photoinhibition, low temperature stress, NPQ, photoprotection

Introduction

Low temperature is a critical environmental factor that limits plant geographic distribution and physiological activities (Sharma et al, 2005; Ensminger et al, 2012). Freezing and chilling injuries not only induce water crystallization in plant cells, alter osmotic pressure, and damage membrane systems, but also reduce enzyme activities, slow metabolic processes, and decrease photosynthetic apparatus activity through oxidative stress (Graham and Patterson, 1982; Öquist and Hüner, 2003; Zhang et al, 2013). Photosystem II (PSII) of the photosynthetic apparatus on chloroplast thylakoid membranes is particularly sensitive to environmental changes (Aro et al, 1993; Tyystjärvi, 2013). Under environmental stresses such as low temperature, plants absorb and transfer excess light energy, generating large quantities of reactive oxygen species that cause oxidative damage to the D1 protein in PSII reaction centers at a rate exceeding its repair, thereby reducing photochemical efficiency and inducing photoinhibition (Vass and Cser, 2009; Sonoike, 2011). Non-photochemical quenching (NPQ) represents an important defense mechanism against oxidative stress, wherein light-harvesting antenna complexes dissipate absorbed excess light energy as heat to prevent reactive oxygen species accumulation (Takahashi and Murata, 2008; Murchie and Niyogi, 2011; Pinnola and Bassi, 2018).

Unlike deciduous trees, evergreen species retain their leaves for photosynthesis during winter. Low temperatures reduce the activity of CO_2 fixation-related enzymes, leading to accumulation of NADPH reducing power and generating substantial reactive oxygen species in chloroplasts (Sharma et al, 2005; Verhoeven, 2013). Consequently, most coniferous evergreen trees exhibit significantly reduced PSII activity and markedly upregulated NPQ during winter (Bigras et al, 2001; Derks et al, 2015). This phenomenon of sustained PSII photoinhibition in winter serves as an important protective mechanism for plants against low temperature stress (Öquist and Hüner, 2003; Míguez et al, 2017). Recent studies have reported that above-zero low temperatures cause significant PSII photoinhibition in tropical and subtropical evergreen broad-leaved trees (Hu et al, 2005; Huang et al, 2010; Li et al, 2018). However, reports on the extent of PSII photoinhibition following sub-zero freezing injury and the role of NPQ in photoprotection for subtropical evergreen broad-leaved species such as *Photinia × fraseri*, *Eriobotrya japonica*, and *Cinnamomum bodinieri* remain scarce. This study investigated PSII photoinhibition and activity recovery in these three evergreen broad-leaved species under freezing and chilling stress, and examined the role of NPQ in protecting PSII from photoinhibition.

1. Materials and Methods

1.1 Plant Materials

Plant materials consisted of eight-year-old mature individuals of *Photinia* × *fraseri*, *Eriobotrya japonica* (Thunb.) Lindl, and *Cinnamomum bodinieri* Levl. cultivated at the Poyang Lake Botanical Garden (116°5.2 E, 29°40.5 N, altitude 25 m), a branch of the Lushan Botanical Garden, Jiangxi Province and Chinese Academy of Sciences. During the winter and spring seasons of 2018, mature leaves and branches from the same position on the sun-exposed side were collected between 09:00 and 11:00 AM during both low temperature stress and recovery periods. After 30 minutes of dark adaptation, chlorophyll fluorescence measurements were immediately performed on the leaves using a LI-COR 6800 portable photosynthesis system. A minimum of three leaf replicates were measured for each species.

From January 27 to 30, 2018, the Poyang Lake Botanical Garden experienced continuous snowfall and freezing rain resulting in sub-zero freezing injury. On February 8, temperatures rose to above-zero chilling conditions, and by April 9, temperatures had essentially returned to normal, serving as a control for low temperature stress relief and photosynthetic activity recovery. Experiments were conducted on January 30, February 8, and April 9, with daily minimum temperatures of -5.3°C, 4.5°C, and 9.2°C, and daily mean temperatures of -1.1°C, 7.2°C, and 18.5°C, respectively. Temperature data were recorded by the botanical garden' s meteorological station.

1.3 Chlorophyll Fluorescence Detection

For dark-adapted leaves, minimum fluorescence (Fo) and maximum fluorescence (Fm) under a saturating pulse of 10,000 mol photons $\cdot$ $m^{-2} \cdot s^{-1}$ were measured, and the maximum potential photochemical quantum yield of PSII was calculated as Fv/Fm = (Fm - Fo)/Fm. Subsequently, actinic light was activated to measure minimum fluorescence (Fo), maximum fluorescence (Fm), and steady-state fluorescence (Fs) under light-adapted conditions. The following parameters were then calculated: actual photochemical quantum yield of PSII, Φ(II) = (Fm - Fs)/Fm ; relative electron transport rate of PSII, rETR(II) = photosynthetically active radiation (PAR) × Φ(II) × 0.84 × 0.5; non-photochemical quenching, NPQ = (Fm - Fm)/Fm ; qN = 1 - (Fm - Fo)/(Fm - Fo); and quantum yield of non-regulated energy dissipation of PSII, Y(NO) = Fs/Fm. Rapid light-response curves of chlorophyll fluorescence parameters were obtained by exposing leaves to photosynthetically active radiation levels of 0, 25, 50, 100, 150, 300, 500, 1000, 1500, and 2000 mol photons $\cdot$ $m^{-2} \cdot s^{-1}$ for 90–120 s at each intensity.

1.4 Curve Fitting

The rETR(II) rapid light-response curves were fitted using the equation of Jassby and Platt (1976): $P = Pm \times \tanh(\alpha \times PAR/Pm)$, where α represents the initial slope of the curve, reflecting light use efficiency; Pm is the fitted potential maximum relative electron transport rate; and $Ik = Pm/\alpha$ is the half-saturation light intensity, reflecting the sample' s tolerance to high light. Curves were fitted using the least squares method with Origin 8.5 software.

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2. Results

2.1 PSII Activity Changes

Figure 1 [Figure 1: see original paper] presents the light-response curves of PSII actual photochemical quantum yield [Φ(II)] and PSII relative electron transport rate [rETR(II)] for *Photinia* × *fraseri*, *Eriobotrya japonica*, and *Cinnamomum bodinieri* during sub-zero freezing injury (January 30), above-zero chilling injury (February 8), and recovery (April 9) periods, illustrating the effects of low temperature on PSII activity. The results show that freezing injury caused varying degrees of PSII activity decline in all three species. In *P.* × *fraseri*, Φ(II) remained similar to recovery-period levels following freezing injury, while rETR(II) maintained a high level, even exceeding that during the recovery period. In *E. japonica*, Φ(II) and rETR(II) were slightly lower than in *P.* × *fraseri* after freezing injury. *C. bodinieri* exhibited the most severe PSII impairment, with the lowest Φ(II) and rETR(II) values. Under above-zero chilling injury, PSII activity in all three species remained at or below the levels observed during the freezing injury period. When spring temperatures rose to 18.5°C (April 9), Φ(II) in *P.* × *fraseri* and *E. japonica* recovered to normal levels, whereas Φ(II) and rETR(II) in *C. bodinieri* failed to achieve complete recovery. Fitting of the rETR(II) rapid light-response curves revealed that the initial slope (α) and maximum photosynthetic rate (Pm) of *C. bodinieri* were significantly lower than those of *P.* × *fraseri* and *E. japonica* during low temperature stress. Even after temperature recovery, the maximum photosynthetic rate [P(m)] and high-light tolerance capability (Ik) of *C. bodinieri* remained lower than those of the other two species (Table 1).

2.2 PSII Photoinhibition and Recovery

Figure 2 [Figure 2: see original paper] illustrates changes in PSII photoinhibition in the three species during low temperature stress and recovery. Under freezing and chilling conditions, *P.* × *fraseri* maintained high Fv/Fm and low Y(NO) values, indicating only slight PSII photoinhibition that fully recovered upon return to normal temperatures. Freezing injury resulted in Fv/Fm and Y(NO) values in *E. japonica* that were respectively lower and higher than those in *P.* × *fraseri*. PSII photoinhibition in *E. japonica* was alleviated under chilling conditions and fully recovered when temperatures normalized. *C. bodinieri* experienced severe PSII photoinhibition from freezing injury, with Fv/Fm and Y(NO) reaching the lowest and highest values, respectively. Moreover, the degree of photoinhibition continued to intensify as freezing stress was relieved but chilling persisted, with only partial recovery observed after temperatures returned to normal.

2.3 Relationship between NPQ and PSII Photoinhibition

Non-photochemical quenching (NPQ) is an important photoprotective mechanism in plants that can be assessed through chlorophyll fluorescence parameters NPQ and qN. Figure 3 [Figure 3: see original paper] shows the light-response curves of NPQ and qN for *P.* × *fraseri*, *E. japonica*, and *C. bodinieri* during sub-zero freezing injury, above-zero chilling injury, and temperature recovery periods. The results indicate that NPQ and qN decreased to varying degrees in all three species under freezing and chilling stress, with partial recovery observed after temperature normalization. Relatively speaking, freezing and chilling stress had minimal impact on NPQ and qN in *P.* × *fraseri*. Values for *E. japonica* were lower than those for *P.* × *fraseri*, while *C. bodinieri* exhibited the lowest NPQ and qN. Following temperature recovery, NPQ and qN in *P.* × *fraseri* and *E. japonica* returned to relatively high levels, whereas those in *C. bodinieri* remained unable to fully recover. Analysis of Fv/Fm in relation to NPQ and Y(NO) under 500 mol photons $\cdot$ $m^{-2} \cdot s^{-1}$ photosynthetically active radiation across freezing, chilling, and normal temperature conditions revealed a strongly significant negative correlation between Y(NO), Fv/Fm, and NPQ in all three species under all three conditions, indicating a significant negative relationship between PSII photoinhibition and non-photochemical quenching (Figure 4 [Figure 4: see original paper]).

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3. Conclusions and Discussion

Freezing and chilling stress induce PSII photoinhibition in overwintering evergreen broad-leaved trees, representing a major cause of reduced photosynthetic activity during winter (Verhoeven, 2013). Reactive oxygen species generated under low temperature and light conditions cause oxidative damage to the photosynthetic apparatus and other biomolecules, not only reducing photosynthetic activity but also leading to leaf yellowing, browning, and necrosis (Sharma et al, 2005). This study investigated PSII photoinhibition and recovery in *P.* × *fraseri*, *E. japonica*, and *C. bodinieri* under winter freezing and chilling stress, demonstrating differential PSII photoinhibition among these three subtropical evergreen broad-leaved species. Overall, low temperature had minimal impact on PSII in *P.* × *fraseri*, intermediate effects in *E. japonica*, and caused significant photoinhibition with slow recovery in *C. bodinieri*. A negative correlation existed between PSII photoinhibition and NPQ activity across all three species.

Previous research has demonstrated that Fv/Fm and Y(NO) effectively reflect the degree of PSII photoinhibition (Baker, 2008; Huang et al, 2010; Míguez et al, 2015). Our results show that higher PSII activity during low temperature stress and recovery periods corresponded to higher Fv/Fm and lower Y(NO) across the three evergreen broad-leaved species, confirming that these two chlorophyll fluorescence parameters can reliably reflect the physiological status of PSII photoinhibition. Although PSII activity in *P.* × *fraseri* was not significantly reduced under low temperature stress, rETR(II) exhibited a decline after spring temperature recovery (Figure 1). This may be attributed to the rapid decrease in the Φ(II) light-response curve with increasing photosynthetically active radiation during the recovery period, as rETR(II) is calculated from both photosynthetically active radiation and Φ(II).

Despite varying degrees of PSII activity reduction under low temperature stress, all three species showed declining Fv/Fm and increasing Y(NO), indicating that PSII experienced different levels of photoinhibition. This suggests that the three evergreen broad-leaved species employed sustained PSII photoinhibition as a protective mechanism during winter. These results support Míguez' s (2015) conclusion that overwintering evergreen plants generally exhibit sustained PSII photoinhibition at temperature thresholds around 0°C. Future research on the effects of low temperature stress on PSI photoinhibition will further elucidate the characteristics and interrelationships of photoinhibition between PSII and PSI under low temperature conditions.

In summary, winter freezing and chilling stress caused differential PSII photoinhibition in the three subtropical evergreen broad-leaved species *P.* × *fraseri*, *E. japonica*, and *C. bodinieri*. The photoprotective mechanism of non-photochemical quenching plays an important physiological role in protecting PSII of overwintering tree species from photoinhibition.

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Note: Figure translations are in progress. See original paper for figures.