Moderate photoinhibition of PSII and oxidation of P700 contribute to chilling tolerance of tropical tree species in subtropics of China


State Key Laboratory for Conservation and Utilization of Subtropical Agri-Bioresources and Guangxi Key Laboratory of Forest Ecology and Conservation, College of Forestry, Guangxi University, 530004 Nanning, Guangxi, China*

School of Life Science, Devi Ahilya University, 452017 Indore, India**

Department of Agricultural Botany, Tanta University, 72513 Tanta, Egypt#

Institute of Ecology and Evolution, University of Oregon, Eugene, OR 97403, USA***

Abstract

In the subtropics, a few tropical tree species are distributed and planted for ornamental and horticultural purposes; however, the photosynthesis of these species can be impaired by chilling. This study aimed to understand how these species respond to chilling. Light-dependent and CO$_2$ assimilation reactions of six tropical tree species from geographically diverse areas, but grown at a lower subtropical site in China, were monitored during a chilling (≤ 10°C). Chilling induced stomatal and nonstomatal effects and moderate photoinhibition of PSII, with severe effect in Ixora chinensis. Woodfordia fruticosa was little affected by chilling, with negligible reduction of photosynthesis and PSII activity, higher cyclic electron flow (CEF), and oxidation state of P700 (P700$^+$). Photoinhibition of PSII thus reduced electron flow to P700, while active CEF reduced oxidative damage of PSI and maintained photosynthesis during chilling. Studied parameters revealed that coupling between light-dependent and CO$_2$ assimilation reactions was enhanced under chilling.

Keywords: chilling; cyclic electron flow; oxidation of P700; photoinhibition; photosynthesis.

Highlights

● Chilling induced stomatal and nonstomatal effects and moderate photoinhibition of PSII
● Photoinhibition of PSII, photosynthesis control, and CEF sustained oxidation of P700
● Oxidation of P700 reduced oxidative damage and maintained photosynthesis during chilling

Abbreviations: CE – carboxylation efficiency; CEF – cyclic electron flow; C – internal CO$_2$ concentration; ETR$_{0}$ – photosynthetic electron flow through PSI; ETR$_{II}$ – photosynthetic electron flow through PSII; Fd – ferredoxin; F$_{v}$/F$_{m}$ – maximum quantum yield of PSII in the dark-adapted state; g – stomatal conductance; LEF – linear electron flow; NPQ – nonphotochemical quenching; P680 – chlorophyll a of PSII reaction centers; P680$^+$ – oxidized P680; P680$^*$ – excited P680; P700 – chlorophyll a of PSI reaction centers; P700$^+$ – oxidized P700; P700$^*$ – excited P700; PC – plastocyanin; P$_{m}$ – maximum photooxidizable P700; P$_{c}$ – net photosynthetic rate; PQ – plastoquinone; RISE – reduction-induced suppression of electron flow; Y$_{(I)}$ – effective photochemical quantum yield of PSI; Y$_{(II)}$ – effective photochemical quantum yield of PSII; Y$_{(CEF)}$ – effective quantum yield of CEF; Y$_{(NA)}$ – acceptor-side limitation of PSI; Y$_{(ND)}$ – donor-side limitation of PSI; Y$_{(NA)}$ – yield of regulated heat dissipation of PSII; Y$_{(NPQ)}$ – effective quantum yield of NPQ or regulated nonphotochemical quenching; ΔpH – proton gradient.

Acknowledgments: We thank Hans Lambers of the University of Western Australia, Australia for critical comments, corrections, and editing and Junjie Zhu of Guangxi University, China for the technical advice on Dual PAM-100 operations. This work was supported by the National Natural Science Foundation of China (31861133008, 31470469), and by the postdoctoral fellowship from Guangxi University, China, granted to John Sunoj Valiaparambil Sebastian, and the Bagui Scholarship (C336000992001) granted to Kun-fang Cao.

Conflict of interest: The authors declare that they have no conflict of interest.

Received 19 May 2022
Accepted 9 August 2022
Published online 15 September 2022

Corresponding author
e-mail: johnsunoj.valiaparam@ag.tamu.edu
kunfangcao@gxu.edu.cn

DOI 10.32615/ps.2022.039
PHOTOSYNTHETICA 60 (4): 476-488, 2022
PHOTOSYNTHESIS IN TROPICAL TREE SPECIES UNDER CHILLING STRESS

Introduction

Tropical plants grow in hot and humid climatic conditions with minor seasonal temperature variations. However, the chilling tolerance of some tropical plant species enables them to survive in marginal tropical and lower subtropical areas (subtropics with relatively low latitudes), where short-term chilling events (≤ 10°C for a few days) frequently occur during winter. A few selected tropical tree species have been planted in marginal tropical and lower subtropical areas for ornamental and horticultural purposes (Jalili et al. 2010, Li et al. 2016, Mau et al. 2018). Chilling during winter in the lower subtropics is a major factor limiting the latitudinal distribution and poleward migration of tropical plant species, despite global warming (Li et al. 2016, Wen et al. 2018). Chilling stress is complex and adversely affects the morphological, physio-biochemical, and molecular processes in plants, thus it reduces their growth and development (Allen and Ort 2001, Liu et al. 2018, Elsheery et al. 2020, Li et al. 2021, Mathur et al. 2021). Earlier studies have revealed the influence of chilling in combination with varying incident light intensities on photosynthesis and photoprotection mechanisms of plants, including diverse tropical tree species (Someralo and Krause 1990, Barth and Krause 1999, Elsheery et al. 2008, Huang et al. 2010a,b; 2011, 2017; Zheng et al. 2016, Yang et al. 2017).

During photosynthesis, the antenna complexes of PSI and PSII absorb light energy and drive the electron flow to generate energy-rich compounds (ATP and NADPH), which are utilized to fix CO₂, converting it into carbohydrates (Miyake 2020). Under chilling, due to stomatal and nonstomatal control of CO₂ fixation, Chl a of reaction centers of the photosystems (P700 in PSI [excited; P700*] and P680 in PSII [excited; P680*]) cannot be de-excited to their ground state (Allen and Ort 2001). The failure of de-excitation of reaction centers of photosystems alters the photosynthetic electron transport. Such circumstances result in a reduction of O₂ (Mehler reaction) and produce reactive oxygen species (ROS) such as superoxide (O₂⁻) and hydrogen peroxide (H₂O₂). The production of ROS contributes to the generation of hydroxyl radicals (OH⁻), which can damage DNA, proteins, and lipids. In contrast, the oxidized reaction centers of chlorophyll P680 in PSII (P680⁺) are reduced by accepting electrons, resulting from the oxidation of water and forming singlet P680 (³P680⁺). Failure to de-excite ³P680⁺ leads to the production of triplet chlorophyll (³P680⁺), which can transfer the energy to ground state oxygen (O₂), resulting in the production of singlet oxygen (O₂), a harmful ROS similar to O₂⁻. High concentrations of ROS trigger a higher level of photooxidation or inactivation of photosystems which is detrimental to plant survival under chilling (Allen and Ort 2001, Müller et al. 2001, Rutherford and Krieger-Liszkay 2001, Miyake 2010, 2020). However, among photosystems, PSI is more sensitive to chilling than PSII in a range of tropical tree species (Someralo and Krause 1990, Barth and Krause 1999, Huang et al. 2010a,b).

Photoinhibition of PSI occurs when PSII transfers electrons beyond the electron-accepting capacity of PSI. Compared with PSII, the PSI reaction centers require more time to recover from ROS-mediated oxidative damage (Zhang et al. 2004, Zivcak et al. 2015), which can be lethal due to the inefficiency of plants to cope with extensive loss/inhibition of PSI (Sonoike 1996, 2011; Guidi et al. 2019). Therefore, protection of PSI from oxidative damage by keeping P700 in an oxidized state (P700⁺) is crucial to the survival of plants under chilling (Kubo et al. 2011, Sonoike 2011). Photoinhibition of PSII and subsequent reduction of electron flow to PSI in tropical trees under chilling protects PSI from chilling injury by supporting a sustainable P700⁺ state (Huang et al. 2010a, Sonoike 2011, Miyake 2020). Hence, moderate photooxidation of PSIII throughout unfavorable conditions is considered a first-level photoprotection mechanism (Murchie and Niyogi 2011, Tikkanen et al. 2014). However, higher, and prolonged photoinhibition of PSI and PSII leads to the accumulation of ROS, which damages DNA, proteins, and lipids. Damage to the lipids of the thylakoid membrane increase membrane fluidity, resulting in the destruction of the photosynthetic apparatus and thus the impairment of photosynthesis and growth (Derks et al. 2015, Elsheery et al. 2020).

Irrespective of the photosystems, the change in the magnitude of photoinhibition from moderate to severe is a consequence of the intensity and duration of chilling, the high intensity of incident light during the stress period, lower CO₂ fixation, and linear electron flow (LEF) between PSI and PSII and the failed de-excitation of photosystem reaction centers (P700⁺ and P680⁺) to an oxidized state (P700⁺ and P680⁺). In other words, the extent of photoinhibition is intensified by a combination of the longer duration of excitation status of P700 and P680, high content of ROS, inability to avoid oxidative damage by activation of various photoprotection mechanisms, nonenzymatic scavenging of ROS, and delayed recovery from chilling injuries (Asada 2006, Khatoon et al. 2009, Murata et al. 2007, 2012; Miyake 2010, 2020; Huang et al. 2011).

ROS are strong signaling molecules involved in plant growth and development as well as primary signals for stress responses; their excess production and high content can have a negative impact on plant development (Foyer and Shigeoka 2011, Zheng et al. 2019). Effective regulation of the excess energy in photosystems before the surplus production of ROS, and timely removal of ROS, relies on photoprotection mechanisms such as (1) cyclic electron flow (CEF) around PSI, (2) nonphotochemical quenching (NPQ) to dissipate excess absorbed light energy, (3) water–water cycle (WWC) or the Mehler–ascorbate peroxidase pathway (MAP) (Miyake 2010, Neto et al. 2017), and (4) photorespiration (Asada 2006). Hence, CEF, NPQ, and WWC under stress conditions are vital for stress tolerance and the subsequent recovery from stress. The activation of the above-mentioned photoprotective mechanisms under chilling is entirely dependent on the magnitude of the chilling tolerance of a plant, and the efficiency of such
mechanisms determines the chilling tolerance of a plant. Other important mechanisms, such as regulation of the stomatal opening and closing (stomatal behavior) (Raven 2014, Jarczyk et al. 2019) and reducing side heterogeneity of PSII, antenna size heterogeneity of PSII (Bukhov and Carpenter 2000, Belgio et al. 2014, Mathur et al. 2021), and anatomical and morphological alterations (Gratani et al. 2013, Wu et al. 2022), are equally crucial for chilling tolerance. The chilling tolerance of plant species can be genetic, which is permanent within the lifespan of a plant species, and phenotypic, which is reversible according to the existing microclimatic conditions, called evolutionary adaptation and acclimation or morphophysiological adjustments, respectively (Körner 2016).

A few studies have been conducted to understand the photoprotection mechanisms and sensitivity of photosystems in tropical tree species found in the marginal tropics of China (Huang et al. 2010a,b; 2011, 2017; Zheng et al. 2016, Yang et al. 2017). However, such studies were conducted at the seedling stage with artificial chilling treatments for a short duration (hours), and their results demonstrate the activation of photoprotection mechanisms, i.e., CEF and NPQ. Furthermore, these studies have also demonstrated reduced maximum photochemical efficiency of PSII (Fv/Fm), the effective photochemical quantum yield of PSII [Yll] and PSI [Ylo], nonphotochemical quenching (NPQ), PSI acceptor-side limitation [YNA], and foliar gas exchange along with higher PSI donor-side limitation [YNl] and quantum yield of nonregulated energy dissipation [YNl] in response to chilling. At the same time, maximum photooxidizable P700 (Pm) was higher or stable in tropical trees under chilling. In this study, we sought to provide photosynthetic responses of geographically diverse tropical tree species to a realistic seasonal chilling event under the prevailing ambient microclimatic conditions in open fields of a lower subtropical site in China during winter, rather than using seedlings and artificial induction of chilling.

Our primary target was to grade the tested tropical plant species in a lower subtropical site in China according to the magnitude of their chilling tolerance and compare and validate the physiological response of the current study with the earlier studies. The specific objectives of this study were to address the following questions: (1) How is the magnitude of chilling tolerance of tropical tree species in the lower subtropics related to the physiological mechanisms involved in photosynthesis? And (2) how are light-dependent and CO2 assimilation reactions coupled to each other in tested tropical trees under a chilling event during winter as compared with summer? We tested the hypothesis that, under chilling, maintaining the P700+ state in PSI and related suppression of oxidative damage retains limited photosynthesis, and the tight coupling of light-dependent and CO2 assimilation reactions support the chilling survival of tropical trees in the lower subtropics.

Materials and methods

Plant materials and growth conditions: The present study was conducted in January 2018 (winter; average day/night temperature: 13/11°C) and July 2018 (summer; average day/night temperature: 28/25°C) on the campus of Guangxi University, Nanning, China (22.83°N, 108.28°E). For this experiment, adult plants of six tropical tree species grown in an open field were selected based on geographical diversity (see text table below).

Physiological traits were recorded for fully mature leaves, which were exposed to sunlight during winter and summer. Before the physiological traits were recorded the experimental site received 11.7 mm of rainfall from 4 to 7 January 2018 and 78.9 mm from 1 to 15 July 2018 (Fig. 1S, supplement). Therefore, the soil was wet during the measurements in both seasons. A temperature drop to ≤ 10°C in day/night for four days during winter was considered a chilling event.

Photosynthetic rate, stomatal conductance, and carboxylation efficiency: In both seasons (winter and summer), photosynthetic rate (Pn), stomatal conductance (gs), and internal CO2 concentration (Ci) of six tree species were recorded using a portable photosynthesis system (LI-6800, LICOR, Lincoln, NE, USA). From each species, a minimum of nine measurements were recorded between 9:00 to 11:00 h from three individual plants. The leaf was illuminated by PAR of 1,000 µmol(photons) m−2 s−1 provided by a red-blue light-emitting diode (LED), whereas ambient CO2, temperature, and relative humidity (RH) were used during the measurement of gas exchange. Carboxylation efficiency (CE) was calculated from the ratio of Pn to Ci (Rymbai et al. 2014).

Chlorophyll (Chl a) fluorescence and P700 measurements: Chl fluorescence and P700 measurements were

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Family</th>
<th>Geographic distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ixora chinensis Lam.</td>
<td>Rubiaceae</td>
<td>Southern China and southeastern Asia</td>
</tr>
<tr>
<td>Aglaia odorata Lour.</td>
<td>Meliaceae</td>
<td>Native to Taiwan and subtropical mountain regions of southern China</td>
</tr>
<tr>
<td>Lagerstroemia speciosa L.</td>
<td>Lythraceae</td>
<td>Tropical southern Asia</td>
</tr>
<tr>
<td>Dypsis lutescens (H. Wendl.)</td>
<td>Areceae</td>
<td>Madagascar, Andaman Islands, Reunion, El Salvador, Cuba, Puerto Rico, the Canary Islands, southern Florida, Haiti, the Dominican Republic, Jamaica, the Leeward Islands, and the Venezuelan Antilles</td>
</tr>
<tr>
<td>Markhamia stipulata (Wall.)</td>
<td>Bignoniaceae</td>
<td>South China to Southeast Asia</td>
</tr>
<tr>
<td>Woodfordia fruticosa L.</td>
<td>Lythraceae</td>
<td>Tanzania, Madagascar, Comores, Saudi Arabia, Oman, Myanmar (Burma), Bhutan, Indonesia, China, India, Sri Lanka, Nepal, Pakistan, and Vietnam</td>
</tr>
</tbody>
</table>
performed in both seasons using a Dual PAM-100 (Heinz Walz, Effeltrich, Germany). The transients were recorded at 25°C from the leaves of six detached branches of three individual trees. Immediately after detaching branches from the trees, basal parts of branches were immersed in distilled water and transported to the laboratory. First, dark-adapted $F_0$ and $F_m$ were determined in fully exposed mature leaves after 30 min of dark adaptation and by applying a saturation pulse of 10,000 µmol(photon) m$^{-2}$ s$^{-1}$ for 300 ms. Photochemical efficiency of PSII ($F_{v}/F_{m}$) was calculated as $F_{v}/F_{m} = (F_{m} - F_{0})/F_{m}$, where $F_{0}$ is the minimum fluorescence and $F_{m}$ the maximum in the dark-adapted state.

After determining $F_0$ and $F_m$, light-adapted Chl fluorescence transients and P700 measurements were measured. The mode of collection of branches, number of replications, and recording temperature was the same as for dark-adapted measurements. Before the light-adapted measurements, leaves were light-adapted for 30 min under PAR of 500 µmol(photon) m$^{-2}$ s$^{-1}$ and then Chl fluorescence and P700 transients were recorded after 3 min exposure to light intensity of 10,000 µmol(photon) m$^{-2}$ s$^{-1}$ by placing the leaf between the measuring head of the Dual PAM-100. The light-adapted Chl fluorescence transients were calculated as $Y_{(II)} = (F_{m} - F_{0})/F_{m}$, $Y_{(ND)} = F_{m}/F_{m}$, and $Y_{(NPQ)} = 1 - Y_{(II)} - Y_{(ND)}$ (Genty et al. 1989, Oxborough and Baker 1997, Kramer et al. 2004), where $Y_{(II)}$ is the effective photochemical quantum yield of PSII, $Y_{(ND)}$ is the quantum yield of nonregulated energy dissipation, $Y_{(NPQ)}$ is the fraction of energy dissipated as heat through regulated nonphotochemical quenching (NPQ), $F_{m}$ is light-adapted state maximum fluorescence, and $F_{m}$ is light-adapted state steady-state fluorescence (Kramer et al. 2004).

The maximum photooxidizable P700 in PSI ($P_{a}$) was determined by applying far-red light for 10 s, followed by a saturation pulse of 10,000 µmol(photon) m$^{-2}$ s$^{-1}$ for 300 ms after 30 min of dark adaptation. The $P_{a}$ represents the maximum change of the P700 signal from fully reduced state P700 (minimum signal; P700) to the fully oxidized state P700 (maximum; P700) upon application of a saturation pulse. $P_{a}$ allows the scaling of the P700 signal and is an essential prerequisite for the determination of P700 transients, similar to $F_{m}$ of chlorophyll fluorescence transient. Other PSI transients were calculated from the light-adapted transients and predetermined P700 as follows: $Y_{(I)} = 1 - Y_{(ND)} - Y_{(NPQ)}$, where $Y_{(ND)} = 1 - Y_{(II)} - Y_{(ND)} = 1 - Y_{(II)} = (P_{m} - P_{n})/P_{m}$, $Y_{(II)}$ is the effective photochemical quantum yield of PSI, which is defined by the fraction of overall P700 reduced in a given state and not limited by the acceptor side $Y_{(NPQ)}$. Donor-side limitation $Y_{(ND)}$ is the fraction of overall P700 oxidized in a given state and $Y_{(NPQ)}$ (acceptor-side limitation) represents the fraction of P700 that cannot be oxidized by a saturation pulse in a given state due to a lack of acceptors; $Y_{(NPQ)}$ reflects the inability of far-red light to oxidize all P700. At the same time, maximum photooxidizable P700 in a given light state ($P_{a}$) upon application of the saturation pulse is similar to $F_{m}$ of Chl fluorescence at light-adapted state. P700(red), which was determined in a given state with the help of the saturation pulse, represents the fraction of overall P700 reduced for a given state (Klugehammer and Schreiber 1994, 2008).

**Estimation of LEF and CEF:** Linear electron flow from PSII to PSI ([µmol(e−) m$^{-2}$ s$^{-1}$]: electron transport rate of PSII $ETR_{(II)}$ [µmol(e−) m$^{-2}$ s$^{-1}$] and electron transport rate of PSI $ETR_{(I)}$ [µmol(e−) m$^{-2}$ s$^{-1}$], respectively) in the six selected species were calculated using the following formula: $ETR_{(II)} = Y_{(II)} \times ab \times PAR \times 0.5$ and $ETR_{(I)} = Y_{(I)} \times ab \times PAR \times 0.5$; where, ab 1 (0.84) is the light absorptance ratio of a leaf and 0.5 is a theoretical factor based on the assumption that reaction centers of the chlorophylls in both photosystems absorb 50% of incident light (Maxwell and Johnson 2000). The value of CEF was estimated as: $CEF = ETR_{(I)} - ETR_{(II)}$ [data not shown in the manuscript as $Y_{(CEF)}$ is representative of CEF], and $Y_{(CEF)}$ was estimated as: $Y_{(CEF)} = Y_{(I)} - Y_{(II)}$ (Miyake et al. 2005a,b).

The outputs from the Dual PAM-100 may somewhat underestimate LEF; consequently, they overestimate CEF as the Dual PAM-100 measures Chl a fluorescence from leaf mesophyll cells near the leaf surface while P700 comprises the signal from the whole leaf (Huang et al. 2011). Despite this inaccuracy, earlier researchers and we believe that relative changes in LEF and CEF in response to chilling are reliable to understand the relative seasonal changes (Miyake et al. 2005a,b; Huang et al. 2010a,b; 2011, 2015; Gao and Wang 2012).

**Statistical analysis:** Correlation analysis between ambient light-independent reaction values and potential light-dependent reaction values of both seasons was executed using Sigma Plot (ver. 10, Systat Inc., USA). For statistical analysis of the data, three biological replications per species were used for each collected parameter and season. We used analysis of variance (ANOVA) to test for significant differences in all measured physiological parameters among species and between seasons. Generalized linear models (GLM) in SPSS (ver. 10, SPSS Inc., USA) were used to assess the effects of species and season on the measured physiological parameters. For the statistical comparison between the two seasons for each species, a Student’s $t$-test was conducted.

**Results**

We observed significant differences in various physiological parameters and responses of the studied tree species between seasons (Table 1). $F_{a}/F_{m}$ was significantly reduced under chilling in all species, except for *Woodfordia fruticosa*, which displayed no change in $F_{a}/F_{m}$ and the smallest reduction in photosynthesis compared to the other species (Fig. 1). Among the six species, three species had $F_{a}/F_{m}$ values of 0.6–0.7 during the chilling period; *Ixora chinensis* had an $F_{a}/F_{m}$ value of 0.43, and *W. fruticosa* maintained an $F_{a}/F_{m}$ value above 0.78 during both seasons. $Y_{(NPQ)}$ was reduced in all species studied and the smallest reduction was observed in *Aglaia odorata* and *W. fruticosa*.
Table 1. Effect of chilling event on light-energy utilization in PSII, redox state of PSI, and cyclic electron flow (CEF) in comparison with those in summer. The values in parentheses indicate the percentage change in winter compared with summer. Statistical comparison between both seasons for individual species indicated by * and ** corresponding to significance at P<0.05 and P<0.01, respectively. NS indicates nonsignificant. ETR$_{(1)}$ – photosynthetic electron flow through PSI; ETR$_{(2)}$ – photosynthetic electron flow through PSII; LEF – linear electron flow; P$_m$ – maximum photooxidizable P700; Y$_{(I)}$ – effective photochemical quantum yield of PSI; Y$_{(II)}$ – effective photochemical quantum yield of PSII; Y$_{(CEF)}$ – effective quantum yield of CEF; Y$_{(NPQ)}$ – effective quantum yield of NPQ; Y$_{(NA)}$ – effective quantum yield of NPQ or regulated nonphotochemical quenching.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ixora chinensis</th>
<th>Aglaia odorata</th>
<th>Lagerstroemia speciosa</th>
<th>Dypsis lutescens</th>
<th>Markhamia stipulata</th>
<th>Woodfordia fruticosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Winter</td>
<td>Summer</td>
<td>Winter</td>
<td>Summer</td>
<td>Winter</td>
<td>Summer</td>
</tr>
<tr>
<td>Y$_{(I)}$</td>
<td>0.05</td>
<td>0.07</td>
<td>0.04</td>
<td>0.06</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>(-57%)*</td>
<td></td>
<td>(-33%)*</td>
<td></td>
<td>(-29%)*</td>
<td>(-14%)*</td>
</tr>
<tr>
<td>ETR$_{(I)}$</td>
<td>26.0</td>
<td>52.5</td>
<td>31.8</td>
<td>49.9</td>
<td>31.8</td>
<td>49.9</td>
</tr>
<tr>
<td></td>
<td>(-50%)*</td>
<td></td>
<td>(-36%)*</td>
<td></td>
<td>(-36%)*</td>
<td>(-36%)*</td>
</tr>
<tr>
<td>Y$_{(II)}$</td>
<td>0.07</td>
<td>0.14</td>
<td>0.08</td>
<td>0.13</td>
<td>0.08</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>(-50%)*</td>
<td></td>
<td>(-38%)*</td>
<td></td>
<td>(-38%)*</td>
<td>(-36%)*</td>
</tr>
<tr>
<td>ETR$_{(II)}$</td>
<td>11.9</td>
<td>27.9</td>
<td>15.7</td>
<td>24.1</td>
<td>15.7</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td>(-57%)*</td>
<td></td>
<td>(-35%)*</td>
<td></td>
<td>(-40%)*</td>
<td>(-17%)*</td>
</tr>
<tr>
<td>Y$_{(NPQ)}$</td>
<td>0.23</td>
<td>0.71</td>
<td>0.32</td>
<td>0.71</td>
<td>0.12</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>(-68%)*</td>
<td></td>
<td>(-55%)*</td>
<td></td>
<td>(-83%)*</td>
<td>(-75%)*</td>
</tr>
<tr>
<td>Y$_{(ND)}$</td>
<td>0.74</td>
<td>0.22</td>
<td>0.64</td>
<td>0.23</td>
<td>0.81</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>(+336%)*</td>
<td></td>
<td>(+178%)*</td>
<td></td>
<td>(+268%)*</td>
<td>(+122%)*</td>
</tr>
<tr>
<td>P$_m$</td>
<td>2.4</td>
<td>0.6</td>
<td>1.8</td>
<td>0.5</td>
<td>2.7</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>(+322%)*</td>
<td></td>
<td>(+239%)*</td>
<td></td>
<td>(+435%)*</td>
<td>(+306%)*</td>
</tr>
<tr>
<td>Y$_{(CEF)}$</td>
<td>0.04</td>
<td>0.06</td>
<td>0.04</td>
<td>0.07</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>(-33%)*</td>
<td></td>
<td>(-43%)*</td>
<td></td>
<td>(-43%)*</td>
<td>(-43%)*</td>
</tr>
<tr>
<td>Y$_{(NA)}$</td>
<td>0.85</td>
<td>0.70</td>
<td>0.85</td>
<td>0.63</td>
<td>0.71</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>(+21%)*</td>
<td></td>
<td>(+35%)*</td>
<td></td>
<td>(+35%)*</td>
<td>(+35%)*</td>
</tr>
<tr>
<td>LEF [µmol(e) m$^{-2}$ s$^{-1}$]</td>
<td>37.9</td>
<td>80.4</td>
<td>47.5</td>
<td>74.1</td>
<td>106.0</td>
<td>104.0</td>
</tr>
<tr>
<td></td>
<td>(-53%)*</td>
<td></td>
<td>(-36%)*</td>
<td></td>
<td>(+2%)*</td>
<td>(+2%)*</td>
</tr>
<tr>
<td>Y$_{(NPQ)}$</td>
<td>0.08</td>
<td>0.17</td>
<td>0.07</td>
<td>0.24</td>
<td>0.14</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>(-53%)*</td>
<td></td>
<td>(-71%)*</td>
<td></td>
<td>(-26%)*</td>
<td>(-31%)*</td>
</tr>
<tr>
<td>ETR$_{(NPQ)}$ [µmol(e) m$^{-2}$ s$^{-1}$]</td>
<td>35.5</td>
<td>54.7</td>
<td>35.5</td>
<td>54.7</td>
<td>55.5</td>
<td>54.7</td>
</tr>
<tr>
<td></td>
<td>(-35%)*</td>
<td></td>
<td>(-35%)*</td>
<td></td>
<td>(+15%)*</td>
<td>(+15%)*</td>
</tr>
</tbody>
</table>
PHOTOSYNTHESIS IN TROPICAL TREE SPECIES UNDER CHILLING STRESS

(Table 1). In contrast, $P_m$ was 15 to 462% higher across species under chilling compared to measurements made in summer (Table 1). CEF under chilling was the highest in *W. fruticosa* followed by *Lagerstroemia speciosa*, and lower in *Markhamia stipulata*, *A. odorata*, and *I. chinensis*.

**Effect of chilling event on gas exchange and their relationship with $F_v/F_m$:** Besides the significant reduction of $F_v/F_m$ in five of the species (Fig. 1), $P_N$, $g_s$, and CE were significantly reduced in all species under chilling, and positively correlated with $F_v/F_m$ across species during each season, respectively (Fig. 2). *Lagerstroemia speciosa* showed the greatest reduction in $P_N$ and CE, while *W. fruticosa* showed higher $P_N$ and CE than any other species in both seasons, with the lowest reduction under chilling; and the lowest $P_N$ and CE was in *A. odorata* (Fig. 1).
Effect of chilling event on light-energy utilization by PSII, redox state of PSI, and CEF: We observed lower values of $Y_{i(i)}$ and $Y_{i(i)}$ under chilling across species, except for $W. fruticosa$, which had higher values than any of the other species for the above parameters (Table 1; Fig. 2S, supplement). Concomitantly, $Y_{(NPQ)}$ was reduced across all species, whereas $Y_{(NA)}$ increased. The difference in $Y_{(NA)}$ and CEF among species was minimal during summer compared with the difference under chilling. A faster rate of photosynthetic electron flow through PSI and PSII [LEF; ETR$_{(i)}$ and ETR$_{(ii)}$] was recorded in $W. fruticosa$ during both seasons among all species studied. In contrast, LEF was low in *I. chinensis* under chilling. Furthermore, we measured a higher CEF during summer compared to chilling in all species except for *W. fruticosa* and *L. speciosa*. Finally, $Y_{(ND)}$ and $P_m$ increased, whereas $Y_{(NA)}$ decreased in all species under chilling. In contrast, $Y_{(ND)}$ and $P_m$ were lower in *W. fruticosa* (Table 1).

Relationship of light energy utilization in PSII with the redox state of PSI and CEF: We detected negative correlations between $Y_{(ND)}$ and $Y_{(II)}$, and $Y_{(ND)}$ and LEF across species in each season; however, the correlation was stronger during winter. Furthermore, the negative correlation between $Y_{(ND)}$ and CEF across species was only observed during winter (Fig. 3). In contrast, there were no correlations between $Y_{(NA)}$ and CEF in either season, whereas CEF showed significant positive correlations with LEF and $Y_{(II)}$ only during winter (Fig. 4). We observed negative correlations between $Y_{(NA)}$ and $Y_{(NPQ)}$ (Fig. 5A), and between $Y_{(NA)}$ and $Y_{(ND)}$ (Fig. 5C) across species in each season, whereas $Y_{(i(i))}$ and $Y_{(i(i))}$ were significantly and positively correlated across species in each season (Fig. 5B).

**Discussion**

Chilling affects many components related to photosynthesis (*Allen et al. 2000*, *Allen and Ort 2001*), including nonstomatal regulation, i.e., electron transport between photosystems in thylakoid membrane and activities of CO$_2$ assimilation reactions of photosynthesis, photorespiration, and stomatal functioning. Both CO$_2$ fixation and PSII and PSI reactions of photosynthesis are inhibited by chilling. Seasonal chilling during winter induced photoinhibition of PSII in five of the six tropical tree species (not in *W. fruticosa*), which was evident from the significant reduction in F$_{v}$/F$_{m}$ and $Y_{i(i)}$ (Fig. 1, Table 1). However, the reduction of F$_{v}$/F$_{m}$ during chilling was less than 20% for all species, except for *I. chinensis*, which showed a 43% reduction (Fig. 1). This suggests that photoinhibition was moderate (*Huang et al. 2010a,b; 2016a,b*). On the other hand, maximum oxidizable P700 in PSI as indicated by $P_m$ was significantly higher across all species under chilling compared to summer. $Y_{i(i)}$ was reduced in five of the six species (not in *W. fruticosa*). Regardless of species, the magnitude of changes in F$_{v}$/F$_{m}$, $Y_{i(i)}$, $Y_{i(i)}$, and $P_m$ in adult trees corresponded to the response of seedlings of tropical tree species under artificially induced short-duration chilling (*Elsheery et al. 2007, 2008; Huang et al. 2010a,b; 2016a,b*). The higher F$_{v}$/F$_{m}$, $Y_{i(i)}$, and $Y_{i(i)}$ values and lower inhibition of CO$_2$ assimilation reaction parameters, particularly $P_m$ and CE, of *W. fruticosa* suggest that it is the most chilling-tolerant of the tested species (Fig. 1, Table 1). Hence, responses of the light-dependent and CO$_2$ assimilation reactions of photosynthesis in *W. fruticosa* during chilling enabled *W. fruticosa* to cope with chilling, as discussed by comparing it with the moderate and sensitive species included in this study.

Positive correlations of F$_{v}$/F$_{m}$ with $P_m$, $g_s$, and CE in both seasons (Fig. 2) revealed the strong coupling between the light-dependent and CO$_2$ assimilation reactions of photosynthesis. At the same time, relationships between these parameters were stronger during winter than summer. In contrast, there was no significant correlation between $P_m$ and the above-mentioned CO$_2$ assimilation reaction parameters (data not shown). Furthermore, all the above positive correlations (Fig. 2) also suggest that the initial target of chilling was CO$_2$ assimilation reactions of photosynthesis (evident from lower $P_m$, $g_s$, and CE; Figs. 1, 2) across all species and subsequently caused moderate photoinhibition of PSII and lower electron flow (evident from lower F$_{v}$/F$_{m}$ and LEF; Fig. 1). This was evident from the increased $C_i$ and reduced $g_s$ and the positive correlation of $g_s$ with F$_{v}$/F$_{m}$ (Fig. 2). An increase in $C_i$ occurred at the time of slower CO$_2$ fixation associated with the inactivation of CO$_2$ assimilation reactions (as discussed above) or nonstomatal limitation. Further, higher $C_i$ was associated with a partial closure of the stomata which suggests the simultaneous engagement of both stomatal and nonstomatal effects on $P_m$ under chilling (*Allen and Ort 2001, Raven 2014, Huang et al. 2016a,b; Jurczyk et al. 2019*). Partial closure of the stomata under chilling was evident from lower $g_s$ resulting from higher $C_i$.

Lower $P_m$ and reduction of LEF between photosystems led to the moderate photoinhibition of PSII across the species and *W. fruticosa* showed the smallest reduction in LEF in winter compared to summer (Table 1). The lower rate of LEF was due to the inhibition of electron transfer from plastocyanine (PQ) to plastocyanin (PC), resulting in the increased reduction of the PQ pool. The reduced PQ pool was caused by the lower CO$_2$ fixation known as photosynthesis control (West and Wiskich 1968, *Baker et al. 2007, Miyake 2020*). Over-reduction of the PQ pool, which induces an inhibition of the O-cycle in the cytochrome (Cyt) b/f complex and P700 oxidation is known as reduction-induced suppression of electron flow (RISE), which is also a part of photosynthesis control in cyanobacteria (*Shaku et al. 2016, Miyake 2020*). Photosynthesis control and RISE are important mechanisms to prevent deleterious photoinhibition of PSI by reducing the electron flow from PSII to PSI along with activation of alternative electron flows (CEF and WWC). Electron flow from PSII to PSI is a prerequisite for the photoinhibition of PSI and consequent secondary damages (*Sonoike 2011, Miyake 2020*). Because of photosynthesis control and RISE, the higher oxidation state of PSI (P700$^+$) as indicated by higher

In this study, we assumed the existence of RISE and confirmed the photosynthesis control in all tested species with higher $Y_{(ND)}$ (Table 1), from its strong negative correlation with $Y_{(II)}$, LEF, and $Y_{(CEF)}$ under winter compared with summer (Figs. 3, 4A). $Y_{(ND)}$ is the electron-donor side (plastoquinone; PQ) limitation of PSI. A negative correlation of $Y_{(ND)}$ with LEF and $Y_{(II)}$ under chilling implies that the $Y_{(ND)}$ has mediated reduced electron flow between photosystems and lower efficiency of PSII to utilize light energy for photosynthesis,
respectively. At the same time, a minor increase in Y\textsubscript{(ND)} was observed in the chilling-tolerant species \textit{W. fruticosa} (Table 1). Similar trends were observed in seedlings of tropical trees exposed to chilling temperatures (Huang \textit{et al.} 2010a, b, 2011).

A decrease in the ratio of oxidized PQ to total PQ [higher reduction of PQ (photosynthesis control and RISE) or higher Y\textsubscript{(ND)}] supports important alternative electron flow, i.e., CEF (Miyake \textit{et al.} 2005a, b; Kubo \textit{et al.} 2011). The correlation between Y\textsubscript{(ND)} and Y\textsubscript{(CEF)} was negative in the current study (Fig. 3C), which confirms the involvement of photosynthesis control and RISE in the activation or enhancement of CEF in \textit{W. fruticosa}, and maintenance of minimum CEF across all other species. Despite the varying degree of CEF during winter across the tree species, CEF allowed PSII to manage the electron load (Joliot and Johnson 2011) to a certain extent as evidenced by the positive correlation of Y\textsubscript{(CEF)} with Y\textsubscript{(II)} and LEF (Fig. 4).

Higher Y\textsubscript{(NA)} values imply that a portion of reduced electron carriers on the acceptor side of PSI could not be oxidized due to the limitation of CO\textsubscript{2} assimilation reactions, particularly caused by lower P\textsubscript{N} and higher C\textsubscript{i} (Shimakawa and Miyake 2018, Miyake 2020). While in this study, similar to the response of seedlings of tropical tree species observed in earlier studies by Huang \textit{et al.}
PHOTOSYNTHESIS IN TROPICAL TREE SPECIES UNDER CHILLING STRESS

(2010a,b; 2011), \( Y_{\text{NA}} \) was lower during winter than in summer (Table 1). This contradiction in \( Y_{\text{NA}} \) under chilling across species with lower \( P_n \) and higher \( C_i \) can be attributed to lower \( F_v/F_m \), higher \( Y_{\text{ND}} \), and CEF, which supported the oxidative status of P700 in PSI (P700'). In contrast to \( Y_{\text{ND}} \), there was no correlation between \( Y_{\text{NA}} \) and any of the other measured physiological parameters in both seasons, except \( P_a \) (Fig. 3).

A weak negative correlation between \( Y_{\text{ND}} \) and LFI, \( Y_{\text{CEF}} \) and \( Y_{\text{II}} \), during summer, compared to winter, was due to the favorable weather conditions, which enabled faster photosynthesis, thus leading to unenclosed electron flow between photosystems (Figs. 1B, 3; Table 1). On the other hand, lower \( Y_{\text{ND}} \) and \( P_a \) and higher \( Y_{\text{NA}} \) and CEF in four of the six species during summer (Table 1) can be attributed to the high ambient light intensity as PSI is susceptible to high light intensity in summer or due to the acclimation and higher PSII to PSI ratio (Fig. 1S) (Barth and Krause 1999, Miyake et al. 2004, 2005a,b; Kubo et al. 2011, Kono and Terashima 2016).

CEF may be negligible in favorable growth conditions (Kou et al. 2013, Kadota et al. 2019), under chilling greater significance of CEF was evident from the response of tolerant \( W. \) fruticosa compared with the other four moderately tolerant species, whereas no significant change in CEF was observed in the \( L. \) speciosa (Table 1). This is consistent with the previous finding of activation of CEF with increased photoinduction and limited photosynthesis (Miyake et al. 2004, 2005a,b; Takahashi et al. 2009, Agrawal et al. 2016, Neto et al. 2017). There are several main functions of CEF involved in the mitigation of the impact of stress, thereby preventing severe damage to PSI and ultimately PSI: (1) to synthesize ATP to support LEF and to maintain the balance of ATP and NADPH consumption; (2) to generate a higher proton gradient (\( \Delta \text{pH} \)) along with PsbS protein, which was not observed in \( L. \) speciosa. This can be due to the inadequacy of the \( \Delta \text{pH} \) created by the stimulation of CEF in \( L. \) speciosa to increase NPQ. Alternatively, it may be due to the conformational changes in PsbS protein structure that led to a lower degree of NPQ, thereby resulting in higher photoinhibition of PSI. Meanwhile, \( A. \) odorata showed an opposite trend to \( L. \) speciosa (Table 1). This species showed lower CEF, but higher \( Y_{\text{NPQ}} \) than \( W. \) fruticosa, with lower \( F_v/F_m, P_n, CE, Y_{\text{NPQ}} \) and \( Y_{\text{I}} \) and higher \( Y_{\text{II}} \). This might be explained by the higher level of structural damage in PSI in \( A. \) odorata which impaired efficient management of excess energy even after a higher activation of NPQ. Further studies are required to clarify these controversial results and to understand the mechanisms underlying photodamage.

In summary, a seasonal chilling event induced moderate photoinhibition of PSII in the majority of the tested tropical tree species. Stomatal and nonstomatal regulations on photosynthesis under chilling resulted in reduced fixation and light-dependent reactions, such as a slower rate of LEF by increasing photosynthesis control and thereby higher oxidation state of PSI that prevented photoinhibition of PSI. \( W. \) fruticosa was the least affected by chilling as demonstrated by a lower reduction of photosynthetic rate and photochemical efficiency of PSI and higher CEF and oxidation state of P700 in PSI compared with the other species. Correlation analysis suggested that the light-dependent and CO\(_2\) assimilation reactions of photosynthesis were closely coupled across all tree species in each season, with stronger coupling in winter. The tropical tree species demonstrated a range of strategies to regulate photosynthesis by rearranging the degree of photoprotection mechanisms according to seasonal meteorological conditions. The present results have implications for screening tropical plant species to improve planning for the management of urban landscapes based on future climatic predictions.

References


© The authors. This is an open access article distributed under the terms of the Creative Commons BY-NC-ND Licence.