Fluorescence Response of Greening Seedling of Four Tree Species to Low Temperature Stress

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ABSTRACT: Fluorescence indexes of *Cassia bakeriana*, *Syzygium hainanense*, *Ilex rotunda* and *Phoebe sheareri* seedlings were determined under simulated low temperature stress using artificial climate chambers. The results showed that with increasing low temperature time, the minimal initial fluorescence $F_o$ of *C. bakeriana* fluctuated, *S. hainanense* and *P. sheareri* decreased followed by an increase, *I. rotunda* decreased and then fluctuated; The biggest fluorescent $F_m$ of *C. bakeriana* and *I. rotunda* increased followed by a decrease and increased again, *S. hainanense* continuously decreased; the largest PSII photochemical efficiency $F_o/F_m$ of *C. bakeriana* and *I. rotunda* *S. hainanense* continuously decreased, *S. hainanense* increased followed by a decrease, whereas *P. sheareri* decreased followed by an increase; the PSII actual light quantum yield $Y(II)$ and photosynthetic electron transport rate $ETR$ of seedlings of the four tree species decreased; non-photochemical quenching of *C. bakeriana* and *S. hainanense* fluctuated, whereas that of *I. rotunda* and *P. sheareri* continuously decreased. Two days after low-temperature release, $F_o$ of seedlings of the four tree species decreased, $F_m$ of *C. bakeriana*, *S. hainanense* and *I. rotunda*, and $F_o/F_m$, $Y(II)$, $ETR$ and $NPQ$ of seedlings of the four tree species increased. The fluorescence parameters of seedlings of the four tree species was evaluated using principal component analysis, indicating that cold-resistance ability decreased in the order of *C. bakeriana* > *P. sheareri* > *I. rotunda* > *S. hainanense*

1 INTRODUCTION

With strengthening environmental protection, afforestation area expands rapidly with a surge in demand for green plants. Low temperature is an important factor limiting greening tree species growth and geographical distribution (Wang & Pang, 2007), but knowledge on related physiological and ecological theory and achievement is relatively rare. Consequently, cold-resistance ability is studied.
study of greening tree species becomes an urgent and important research subject. Although there were some studies on cold-resistance of herb (Wang, 2014), fruiter (Zheng et al., 2008) and some few tree species (Liao, 1997), we still know little about cold-resistance of greening tree species. *Cassia bakeriana, Syzygium hainanense, Ilex rotunda* and *Phoebe sheareri* are important greening and landscaping tree species in tropical and subtropical regions. Despite there were a few studies on these species, including photosynthetic properties of *P. sheareri* at room temperature (Chen, 2013), SO$_2$ resistance of *I. rotunda* (Wen et al., 2003), photosynthetic characteristics of *S. hainanense* under ozone and drought stress (Huang et al., 2006), we still know little about fluorescence characteristics of the four tree species under low temperature stress. This study is aimed at providing the reference for cold greening tree species selection from the view of their fluorescence characteristics.

2 MATERIALS AND METHODS

2.1 The testing site

Study was conducted in the building of College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou city (113°18′E, 20°6′N), belonging to subtropical monsoon climate with characteristics of warm and rainy and sufficient sunlight. The annual average temperature, the most cold month and the most warm month temperature are 21.8°C, 13.3°C and 28.1°C, respectively. Annual rainfall is 1714.4 mm, occurring mainly from April to September, and annual average relative humidity is 79%.

2.2 Experimental material

Confined One-year-old seedlings of *C. bakeriana, S. hainanense, I. rotunda* and *P. sheareri* came from Shenzhen Techand and Ecology & Environment CO. LTD. At the beginning of experiment, general characteristics of the seedlings were shown in Table 1.

<table>
<thead>
<tr>
<th>Tree species</th>
<th>The average diameter/cm</th>
<th>The average seedling height/cm</th>
<th>The average crown width/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cassia bakeriana</em></td>
<td>0.38±0.03</td>
<td>30.5±3.2</td>
<td>20.3±3</td>
</tr>
<tr>
<td><em>Phoebe sheareri</em></td>
<td>0.99±0.06</td>
<td>33.9±2.3</td>
<td>22.9±3.1</td>
</tr>
<tr>
<td><em>Ilex rotunda</em></td>
<td>0.5±0.01</td>
<td>34.5±2.5</td>
<td>14.5±2.6</td>
</tr>
<tr>
<td><em>Syzygium hainanense</em></td>
<td>0.56±0.2</td>
<td>54.9±1.3</td>
<td>25.9±0.2</td>
</tr>
</tbody>
</table>
2.3 Test method

The experiment seedlings with similar size were put into the RXZ intelligent artificial climate box. Lighting time was from 8:00 to 17:00 with a light intensity of 120 mmol(photons)·m$^{-2}$$\cdot$s$^{-1}$ and a relative humidity of 80% ~ 85%. The seedlings were treated with 6±0.5°C low temperature (low temperature treatment 0 d as control), temperature of intelligent artificial climate box was reduced with 6°C·h$^{-1}$. When the temperature dropped to 6°C, five leaves of each seedling (the third to eighth functional leaves from seedling top) were marked, each marked leaf was selected to determine chlorophyll fluorescence after low temperature treatments of 0 d, 2 d, 4 d and 6 d. Seedlings treated with low temperature treatment of 6 d were moved from intelligent artificial climate box to the outdoor to restore growth for 2 d, and then the chlorophyll fluorescence parameters of marked leaves was determined. Each fluorescence parameter was determined with triplicate.

2.4 Determination method

OS-1P pulse modulation fluorometer from Gao (2006) was used to determine chlorophyll fluorescence. The initial fluorescence yield ($F_0$), maximal fluorescence yield($F_m$), steady state fluorescence ($F'$), maximum fluorescence in light adapted state ($F_m'$) were measured and PSII maximum photochemical efficiency ($F_v/F_m$), variable fluorescence ($(F_v)=F_m'-F_o$), PSII actual photosynthetic efficiency [$Y(II)=F_m'-F'/F_m'$], electron transport rate [ETR=ΦPSII×1×0.084×0.5], nonphotochemical quenching ($NPQ=F_m'/F_m'$) were calculated.

2.5 Data analysis

All statistical analyses were used Excel 2010 and the Statistical Analysis System (SAS 8.1).

3 RESULTS

3.1 Minimal initial fluorescence ($F_0$)

With increasing stress time, the minimal initial fluorescence $F_0$ of C. bakeriana fluctuated, S. hainanense and P. shearerii decreased followed by an increase, and I. rotunda decreased then fluctuated (Figure 1). The $F_0$ of seedlings of the four species had a significant decreased 2 d after low-temperature release ($P<0.05$).
A. *Cassia bakeriana*; B. *Syzygium hainanense*; C. *Ilex rotunda*; D. *Phoebe sheareri*

Significant differences between treatments were indicated by different letters above bars, $P<0.05$.

Figure 1. Effect of low temperature stress on minimal initial fluorescence in seedling leaves of the examined plants.

3.2 Biggest fluorescence (Fm)

With increasing stress time, the biggest fluorescence $F_{m}$ of *C. bakeriana* and *I. rotunda* decreased followed by an increase and then decreased again, *S. hainanense* decreased continuously, and *P. sheareri* decreased followed by an increase (Figure 2). The $F_{m}$ of seedlings of four species had a slight increase except *P. sheareri* 2 d after low-temperature release.

Figure 2. Effect of low temperature stress on biggest fluorescence in seedling leaves of the examined plants.

3.3 PSII maximum photochemical efficiency

With increasing stress time, the PSII maximum photochemical efficiency $F_{v}/F_{m}$ of *C. bakeriana* and *I. rotunda* decreased consciously, *S. hainanense* increased followed by a decrease, whereas *P. sheareri* decreased followed by an increase (Figure 3). The $F_{v}/F_{m}$ of seedlings of
the four species had a significant increase ($P<0.05$) and higher than the controls 2 d after low-temperature release.

Figure 3. Effect of low temperature stress on PSII maximum photochemical efficiency in seedling leaves of the examined plants.

3.4 PSII actual photosynthetic efficiency

With increasing stress time, $PSII$ actual photosynthetic efficiency $Y(II)$ of seedlings of the four species decreased and then reached the minimum for 6 d after low temperature treatment (Figure 4). The $Y(II)$ of seedlings of the four species increased 2 d after low-temperature release ($P<0.05$).

Figure 4. Effect of low temperature PSII actual photosynthetic efficiency in seedling leaves of the examined plants.
3.5 Electron transport rate

With increasing stress time, electron transport rate $ETR$ of the seedlings of the four species decreased and then reached the minimum for 6 d after low temperature treatment (Figure 5). The $ETR$ of seedlings of the four species increased after 2 d low-temperature release ($P<0.05$).

![Graph of electron transport rate](image)

A. *Cassia bakeriana*; B. *Syzygium hainanense*; C. *Ilex rotunda*; D. *Phoebe shearerii*

Figure 5. Effect of low temperature stress on electron transport rate in seedling leaves of the examined plants.

3.6 Non-photochemical quenching

With increasing stress time, non-photochemical quenching $NPQ$ of *C. bakeriana* and *S. hainanense* fluctuated, *I. rotunda* and *P. shearerii* decreased continuously (Figure 6). The $NPQ$ of seedlings of the four species increased 2 d after low-temperature release ($P<0.05$).

![Graph of non-photochemical quenching](image)

A. *Cassia bakeriana*; B. *Syzygium hainanense*; C. *Ilex rotunda*; D. *Phoebe shearerii*

Figure 6. Effect of low temperature stress on non-photochemical quenching in seedling leaves of the examined plants.

3.7 A comparative evaluation for cold resistance of seedlings

Fluorescence indexes can be comprehensively and accurately analyzed using principal component analysis to get cold-resistance ability of seedlings. In this study, principal component anal-
ysis was used to evaluate cold-resistance ability of seedlings. The calculating score decreased in the order of *C. bakeriana > P. sheareri > I. rotunda > S. hainanense* (Table 2).

Table 2. Score of cold-resistance of seedling of the four species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Score</th>
<th>Ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. bakeriana</em></td>
<td>1.56</td>
<td>1</td>
</tr>
<tr>
<td><em>P. sheareri</em></td>
<td>1.37</td>
<td>2</td>
</tr>
<tr>
<td><em>I. rotunda</em></td>
<td>1.09</td>
<td>3</td>
</tr>
<tr>
<td><em>S. hainanense</em></td>
<td>0.48</td>
<td>4</td>
</tr>
</tbody>
</table>

4 DISCUSSION AND CONCLUSION

Under low temperature stress, the fluorescence parameters have different response. The decrease of $F_0$ means an increase in heat dissipation of antenna pigment, whereas the increase of $F_0$ indicates the destruction or reversible inactivation of PSII reaction center (Feng et al., 2011). During low temperature stress, the $F_0$ of *S. hainanense* and *I. rotunda* decreased, which was in favor of heat dissipation of antenna pigment, whereas fluctuation in $F_0$ of the other species had an adverse effect on activity of PSII reaction center (Zhong et al., 2010). $Fv/Fm$ represents potential activity of PSII, the lower value of $Fv/Fm$ represents photoinhibition occurred. In this study, $Fv/Fm$ of *S. hainanense*, *I. rotunda* and *P. sheareri* decreased, indicating that their PSII reaction center may not be seriously damaged.

The actual photosynthetic efficiency $Y(II)$ is actual original light-trapping efficiency (Xu et al., 2007). Under low temperature stress, decrease in $Y(II)$ of *C. bakeriana*, *I. rotunda* and *P. sheareri* indicated that their actual original light-trapping efficiency decreased, preventing the formation of plant assimilatory power and affecting plant fixed and carbon assimilation.

$ETR$ refers to the apparent photosynthetic electron transport rate and reflects the PSII reaction center of electron capture efficiency (Lu et al., 2014). In this study, $ETR$ of seedlings of the four species had a significant decrease under low temperature stress, which showed that low temperature caused change in light-harvesting and structure of PSII reaction center, preventing the PSII electron transfer and causing photoinhibition (Xu et al., 2012). Under low temperature stress, the $Y(II)$ of seedlings of the four species decreased, indicated that the actual photochemical efficiency declined, which prevented the formation of plant assimilatory power (NADPH and ATP) and affected carbon assimilation of plants (Liu et al., 2010).
Non-photochemical quenching (NPQ) represents the index of heat dissipation and reflects the heat dissipation ability after natural pigment of PSII reaction center absorbs excessive light energy (Yang et al., 2012). The decrease in NPQ of I. rotunda and P. shearer had an adverse effect on heat dissipation of PSII antenna pigment.

The two days after low-temperature release, the Y(II), ETR and NPQ of seedlings of the four species recovered to a certain degree, indicated that the cold harm to the seedlings was a reversible process. The six fluorescence parameters of seedlings of the four tree species was evaluated using principal component analysis, indicating cold-resistance ability decreased in the order of C. bakeriana>P. shearer>i. rotunda>S. hainanense, which suggested C. bakeriana and P. shearer being as the first-choice tree species growing in the cold regions.

REFERENCES


